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# INVESTIGATION OF PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF *ACACIA NILOTICA* FROM JHELUM, PUNJAB PAKISTAN

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## ABSTRACT

The phytochemical and antioxidant activities of leaf and bark of *Acacia nilotica* (Linn.) wild of family Fabaceae were evaluated to study ethnopharmacological importance.In phytochemical investigation qualitative and quantitative tests were performed. Qualitative tests were performed on ethanolic extract of leaves and bark of *Acacia nilotica.* Phytochemical analysis showed that most of the phytochemical contents were present in *Acacia nilotica* leaves and bark. Triterpenoids, Sterols, Proteins, Carbohydrate, Tannins, Glycosides were present in better quantity rather than the Alkaloids were only detected in leaves. Quantitative tests were performed to know the quantity of Alkaloids and Tannins in the bark and leaves of *Acacia nilotica*. 7.8% Alkaloids were present in leaves and Alkaloids were not detected in bark. 2.64% Tannins were present in leaves and 6.09% Tannins were present in bark of *Acacia nilotica.*Antioxidant effect was investigated by using two methods and maximum result of DPPH was detected by *A. nilotica* leaves 0.919±0.03 mm in ethanolic extract of leaves at 1000 concentration. Maximum result of DPPH was detected by *A. nilotica* bark 0.694±0.06 mm in ethanolic extract of bark at 1000 concentration. Total phenolic content of *A. nilotica* leaf extract of ethanol was 0.806±0.08 mm and Total phenolic content of *A. nilotica* bark extract of ethanol of 0.803±0.11 mm showed maximum value at 1000 concentration.

## INTRODUCTION

As the beginning of man on earth, plants are being used by human beings to complete their daily needs which are necessary to sustain life. Plant lives provide food and medicines for human beings and fodder for domestic animals, plants also provide materials for building of houses which are studied in a discipline known as ethnobotany. The plants are also used to make agricultural tools, crafts and several other goods like poisons, resins, paints and fuel (Ishtiaq *et al*., 2007a). Plants also play a great role in ritual characters and are also being used because of their hallucinogenic nature. In many parts of the world, several plants are used in veterinary therapeutics (Ishtiaq *et al*., 2006b).

The *Acacia nilotica* is common in Asia and Africa; it is also common in Kenya and Australia. It is present in Sudanian savannas to the southern Arabian Peninsula and in well-watered Sahelian, Gamibia and the East Africa, the Togo, Ghana, Nigeria and Sudan. It is found on lateritic soil in the Himalayan foothills in India and is broadly cultivated in the Indian subcontinent.*Anilotica* is a tree, generally medium-sized (2.5-25m) having a small, broad and cylindrical stem; the bark colour isblack, grey or reddish-brown, furrowed, rough. Its leaves are bipinnately-compound,alternate,axis fairly hairy, 5-15cm long, narrowly oblong, with 3-8 pairs of side axes (pinnae) 1-4cm long; blunt at the ends with tiny hairs along edges,leaflet 10-30 pairs on each side axis, small, 3-6mm long, grey-green. Flowers are stalk less, united at base, crowded, many, 6 mm long, many yellow,composed of 5-toothed corolla 3 mm long; threadlike stamens, dotlike anthers and threadlike style and pistil with slender ovary.Pods are flattened 8-17 x 1-2cm straight,narrow,mostly narrowed between seeds, long, mostly aromatic, short,beanlike,stalked at the base,rounded, pointed grey or black, breaking in segments;flattened,blackish-brown, not splitting open, seeds 8-15, 7-9mm in diameter. The blossoming phase of the *A. nilotica* is among November and March (Mann *et al*., 2003).

*A. nilotica* recommend a range of Phytochemicals for example glucopyranoside, ellagic acid, m-catechol, leucocyanadin, gallic acid, apigenin-6,8-bis-C-glucopyranoside, derivatives of (+)-catechin-5-gallate, kaempferol-7-diglucoside, isoquercitinandrutin. This plant also contains robidandiol (7,3,4,5-tetrahydroxyflavan-3-4-diol), gallic acid, catechin-5-galloyl ester,

androstene steroid,m-digallic acid, gallolyated flavan-3,4-diol, D-pinitol carbohydrate and chlorogenic acid (Singh *et al*., 2009a).

Water extracts of *Acacia nilotica* with lipid per oxidation test acquire peroxyl radical scavenging capability and it proves the antioxidant property of this plant. The powder of the stem bark ofthe plant extracts by different types of solvents established the scavenging action by means of maceration extraction (Del, 2009). According to a study natural antioxidants can also be obtained from *A. nilotica*, which are used to assist the treatment of those diseases which are mediated by free radicals for example inflammation, diabetes, cancer etc. Moreover, the *A. nilotica* shows high rummaging property due to the existence of hydroxyl groups in phenolic compounds which are used for free radical scavenging (Kalaivani and Mathew, 2010).

The main objectives of the study are:

- To analyze the phytochemical nature of ethanolic extract of leaves and bark of *Acacia nilotica* (L.) by carrying different phytochemical group tests
- To determine the antioxidant activity by mean of different assay, that is determination of DPPH and total phenolic content

## MATERIALS AND METHOD

Present research work was conducted in Mirpur University of Science and Technology (Bhimber Campus) Pharmacognosy laboratory. This research was confined to the phytochemistry and antioxidant activity of *Acacia nilotica*.

### Collection of plant materials

The bark and the leaves of *Acacia nilotica* were gathered from District Jhelum. The materials of the plant were taxonomically identified and authenticated by comparing with herbarium specimens. The materials of plant were dried in shade till the vaporization of all the molecules of water and plant parts were dried well for grinding. Later the plant products were well ground using blender into very fine for later use.

### Preparation of Ethanolic Extract

The powders of both the leaves and bark of *Acacia nilotica* (L.) of 250g were dipped in 700mL of 90 percent for 7 days in a relative amount of 1:4. The dipped materials were shacked at distinct time intervals in order to facilitate suitable extraction. After 7 days the dipped materials were filtered first by muslin cloth and then the filtrates were further transferred by Whatmann filter paper to attain apparent filtrates. The extraction method was repeated three times to completely finish the plant material. The ethanolic extracts were dried up using the rotary evaporator at a temperature lower than 40 ºC. Dark green thick extract of leaves and dark brown extract of bark was got and these extracts were stored in sealed jars which was safe in refrigerator for further research (Gmaraldeen *et al.*, 2016).

## PHYTOCHEMICAL SCREENING

## QUALITATIVE ANALYSIS

The Qualitative analysis of phytochemicals was conducted to detect the phytochemical substances like teriterpenoids, sterols, proteins, carbohydrates, alkaloids, tannins and glycosides test by using the method of Ayoola *et al*., (2008).

## QUANTITATIVE ANALYSIS

### Alkaloids Determination

5 gram of crushed leaf concentrate (extract) was separated with 50ml of methanol. From that concentrate, 10ml was set in 250ml isolating pipe and 5ml of dilute $H_2SO_4$ and refined H2O was included. The concentrate was stunned two times with 10ml of chloroform. The consolidated chloroform-remove was moved to another separating funnel that contained 5ml of dilute $H_2SO_4$and 10ml of refined $H_2O$. The layer of chloroform was disposed of subsequent to shaking and the watery acidic layer was moved to the substance of the first isolating channel. The concentrate was basified by adding the solution and it was stirred for 30 seconds. The alkaloids were totally separated by progressive bits of chloroform. Then, the joined chloroform concentrate was mixed with 5ml of water and was gone through a fitting of cotton fleece recently dampened with chloroform. The substance was secured with a slight anhydrous sodium sulfate

that was washed with 5 ml chloroform. The remainder was then set into a 25ml cone shaped jar after which the chloroform was refined totally pursued by the expansion of 5ml of unbiased liquor, which was vanished on boiling water bath. The buildup was additionally warmed on a water bath for 15 minutes. The buildup was broken down in 2ml of chloroform and 20ml 0.02N $H_2SO_4$. The substance was warmed to evacuate chloroform. The abundance corrosive was titrated with 0.02N sodium hydroxide utilizing methyl red as an indicator; a shading change from pink to yellow was noticed. The accessible substance of the example was then determined utilizing the equation

Alkaloid content = (mls taken of 0.02N NaOH x 0.00578/10) = g% w/v (Okoro *et al.*, 2014).

**Tannins Determination**

From water concentrate of every example 5ml was set into a stoppered funnel shaped flagon pursued by 10 ml of 4 percent NaOH and 25ml of 0.1N iodine. The subsequent blend was kept in obscurity for 15 minutes. 10 ml of water was utilized to weaken the blend and fermented with 10ml 4% sulphuric corrosive. The blend was titrated with 0.1N sodium thiosulphate sol. and the solution of starch was utilized as marker. Titration worth relates to the aggregate of tannins and pseudo tannins fixation. Another 25ml of each water concentrate was put in a stoppered cone shaped flagon pursued by 15ml gelatin. The volume was made equal to 100ml with water and separated. The aliquot of 20ml was set in a volumetric flagon, 25ml of 0.1N iodine and 10ml of 4% NaOH were included blended and kept in obscurity for 15 minutes. The blend was weakened with 10ml of water and fermented with 10ml of 4% sulphuric corrosive. This was at last treated with 0.1N sodium thiosulphate utilizing starch as pointer. The titration esteem that was acquired compares just to the pseudo tannins focus B. The tannins and pseudo tannins substance of each example was then determined utilizing the equation underneath:

A = (Blank - Exp. A) x 0.029 x 100g %/5 (volume taken). B = (Blank - Exp. B) x 0.029 x 100g%/5 (volume taken). Where A = % of tannins and pseudo tannins, B = % of pseudo tannins just Therefore % of genuine tannins = A - B g% w/v (Okoro *et al.*, 2014).

**Detection of Antioxidant Activity**

Antioxidant investigation of concentrate was completed in Pharmacognosy laboratory of Botany department, Mirpur University of Science and Technology, Bhimber. Subsequent parameters (Rashid *et al.,* 2017) were utilized to assess the antioxidant action.

For determination of antioxidant actions subsequent procedures were utilized:

- DPPH radical scavenging assay
- Total Phenolic Content (TPC)

**Preparation of Stock Solution**

For assessment of antioxidant analysis, stock solutions of desiccated samples of plantwere prepared. The 0.02 g of desiccated materials of *Acacia nilotica* (L.) was weighted and it was broke down in 20 ml ethanol to form the stock solution of 1000µg/ml. From these stock solutions different focuses were set up as 60µg/ml, 125µg/ml, 250µg/ml and 500µg/ml (Singh *et al*., 2009a).

**Table 1: Preparations of Stock Solutions**

| Concentration required (µg/ml) | Stock solution used (µl) | Ethanol used (µl) | Final volume of Dilution (µl) |
|---|---|---|---|
| 500 | 500 | 500 | 1000 |
| 250 | 250 | 750 | 1000 |
| 125 | 125 | 875 | 1000 |
| 60 | 60 | 940 | 1000 |

**Total Phenolic Content**

The total soluble phenolic compounds in the ethanolic crude extracts and solvent fractions were determined by following parameters (Singh and Thakur, 2016):

**Preparation of plant stock solution**

Different dilutions from stock were prepared (Table 1).

**10% Sodium carbonate ($Na_2CO_3$) Preparation**

Weight 10 g Sodium carbonate and dissolved in modest quantity of distilled $H_2O$ completely, raising the volume up-to 100 ml with extra distilled $H_2O$.

**2N Folin-Ciocalteu reagent Preparation**

Industrially accessible brilliant yellow 2N FC reagent was utilized to explore. It is made out of hydrochloric acid (HCL), lithium sulfate ($Li_2SO_4.H_2O$), phosphoric corrosive ($H_3PO_4$), bromine (Br), sodium tungstate ($Na_2WO_4.2H_2O$), water and sodium molybdate ($Na_2MoO_4$).

**Standard Curve of Gallic Acid Preparation**

Various fixation (60, 125, 250, and 500 ml) of standard solution such as Gallic acid were set up by sequential dilution technique with exactly weigh the amount of solute with addition of methanol to raise the final volume via 1000 ml and along different concentrations.

**Principle**

The total phenolic contents were valuated with using of FC reagent, which has molybdenum oxides and tungsten. When the reaction occurs, the blue colour developed and absorbance calculated with UV- spectrophotometer at 725 nm. The standard curve was made on Microsoft excel 2007 with the values showed by UV-spectrophotometer and along different concentrations.

**Procedure**

The total phenolic content wasanalysed to adopting the strategy. All the test tubes were labelled correctly. Measured 100 ug/ml from plant prepared dilution, added 2.8 ml freshly prepared sodium carbonate (10%) and then added 0.1 ml FC reagent, so the test tubes were kept at the room temperature about 40 minutes. after passing the time of concentrations were measured at the 725nm.

**Analysis of Results**

All the values of phenolic contents were communicated in GAE µg/g

**DPPH Radical Scavenging Action**

Mukhanwati and Khabiruddin, 2017 methodology was followed for this screening.

**Arrangement of plant stock solutions**

Different dilutions from stock solutions were prepared (Table 1).

**Preparation of 0.1 mm DPPH Solution**

Precisely weigh 0.00394 g of DPPH, transfer it into a beaker and caused the volume to up to 100 ml via methanol. This arrangement can be stored 4 ºC.

**Formulations of BHT Dilutions**

Different concentrations of (60, 125, 250, and 500 ml) of standard solution of BHT were prepared by serial dilution method with exactly weight the amount of solute with the mixing of methanol to raise the volume up to 1000 ml.

**Principle**

DPPH is constant as well as the nitrogen based natural radical containing of nitrogen bases at the lambda concentrated of 515 nm. It has mixed with DPPH its purple goes to lighter and it reveals the antioxidant potential.

**Procedure**

The DPPH radical scavenging movement of selected plant concentrates extracts was inspected. Each DPPH response tube was set up by including 1000µl of plant test into test tube and mixed it with 2.5 ml of recently arranged DPPH solution. The blend was brooded at the room temperature for 45-60 min. During incubation the decrease response reaction of DPPH reagent had occurred and shading had progressively changed from purple color to yellow denoting the end purpose of response. After brooding, absorbance was taken at a wavelength of 515 nm by utilizing the spectrophotometer. BHT(Butylated Hydroxytoluene) was utilized as standard and methanol was utilized as clear. Colour had gradually changed from purple to yellow marking the end point of reaction. After incubation, absorbance was taken at the wavelength of 515 nm by using a spectrophotometer. BHT(Butylated Hydroxytoluene) was used as standard and methanol was used as a blank.

**Result Analysis**

The percentage of DPPH radical scavenging activity was calculated by following rule:

$$\%\text{age DPPH} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \text{ X } 100$$

Spectrophotometer at the 515 nm.

**Statistical Study of the Estimation**

All the parameters were applied on leaves and bark of the *Acacia* nilotica. In three times. Values were achieved by the mean and with standard error. All the measurable examination was done on Microsoft excel (Mukhanwati and Khabiruddin, 2017).

## RESULTS

The extracts of *Acacia nilotica* were prepared in the ethanol for the investigation of antioxidant activity and phytochemical study in the Pharmacognosy Laboratory of Department of Botany (Bhimber Campus), Mirpur University of Science and Technology.

**Percentage Yield**

The %age yield of concentrates of *Acacia nilotica* bark and leaves were measured as a proportion of proficiency of the dissolvable utilized during maceration. The percentage yield of leaves and bark extracts determined before exposing them to facilitate examination. Ethanolic concentrates of leaves and bark of *Acacia nilotica* indicated most extreme % yield i.e., 4.88 and 5.28 separately.

**Table 2: Percentage yield of the leaves and the bark of *Acacia nilotica***

| Plant part | Solvent | %age yield |
|---|---|---|
| Leaf | Ethanol | 4.88 |
| Bark | Ethanol | 5.28 |

**Physical properties**

The physical properties of the *Acacia nilotica* extract showed that the colour of leaf extract of *Acacia nilotica*was dark green and the colour extract of bark of *Acacia nilotica*was dark brown. Appearance of both the extracts of leaves and bark of *Acacia nilotica*was sticky. The extracts of both the parts had smooth texture.

**Table 3: Physical properties of *Acacia nilotica* extracts**

| Plant parts | Extract | Colour | Appearance | Texture |
|---|---|---|---|---|
| Leaf | Ethanol | Dark Green | Sticky | Smooth |
| Stem Bark | Ethanol | Dark Brown | Sticky | Smooth |

**Phytochemical Investigation**

**Qualitative investigation**

Qualitative phytochemical investigation of ethanolic extract of bark and leaves of *Acacia nilotica* exposed the existence of different secondary metabolites. Phytochemical analysis showed that phytochemical contents were present in this plant. Triterpenoids, Sterols, Proteins, Carbohydrates, Tannins, Glycosides were present in both the leaves and bark of *Acacia nilotica*but Alkaloids were only present in leaves.

**Table 4: Qualitative investigation of phytochemicals present in *Acacia nilotica***

| Groups | Name of test | Leaves | Bark |
|---|---|---|---|
| **Teriterpenoids** | Salkowaski Test | + | + |
| **Sterols** | Salkowaski Test | + | + |
| **Proteins** | Millon's Test | + | + |
| **Carbohydrates** | Benedict's Test | + | + |
| **Alkaloids** | Mayer's test | + | – |
| **Tannins** | Ferric chloride Test | + | + |
| **Glycosides** | Bromine water Test | + | + |

- Absence + Presence

**Quantitative Investigation**

Quantitative phytochemical investigation of the extracts of *Acacia nilotica* leaves and bark showed that percentage weight of tannins in leaves of *Acacia nilotica* was 2.53 and in bark was 5.10.

Quantitative phytochemical analysis of the extracts of *Acacia nilotica* leaves and bark showed that percentage weight of alkaloids in leaves of *Acacia nilotica* was 6.12 and in bark it was absent.

**Table 5: Quantitative determination of phytochemical present in *A. nilotica***

| Plant Part Extract | Percentage weight of Tannins/5ml of extract | Percentage weight of Alkaloids/5g of extract |
|---|---|---|
| **Leaves** | 2.53 | 6.12 |
| **Bark** | 5.10 | - |

**Antioxidant Activity**

The assessment of antioxidant value of the extract of plant is as yet an irritated. To appraise the complete potential of the plant single technique is inadequate because of the differences in investigational situations, existence of vigorous values showed responsibility for antioxidant strength in the different elements of plants, the chemicals and their nature of antioxidants. Various strategies were used for the calculation of antioxidant contents of the *Acacia nilotica*, DPPH method, Total Phenolic contents.

DPPH is steady and it is industrially abused nitrogen based natural radical which have lambda most extreme 515nm. On decline the purple shading blurs away that demonstrates the antioxidant capability of the example. *A. nilotica* (L.) had demonstrated much ability to neutralize the DPPH radicals. The ability to neutralize the DPPH radical was appeared by ethanol concentrate of Leaves and Bark.

**Table 6: Free radical scavenging activity and absorbance of *A. nilotica* by DPPH assay**

| Absorbance at different concentration (µg/ml) | | | | | | |
|---|---|---|---|---|---|---|
| Plant part | Fraction | 60 | 125 | 250 | 500 | 1000 |
| Leaves | Ethanol | 0.37±0.01 | 0.63±0.05 | 0.654±0.00 | 0.731±0.09 | 0.919±0.03 |
| Bark | Ethanol | 0.391±0.01 | 0.64±0.06 | 0.677±0.08 | 0.685±0.04 | 0.694±0.06 |
| BHT | | 0.45±0.01 | 0.66±0.03 | 0.845±0.02 | 1.38±0.4 | 2.437±0.09 |

**The outcome revealed were kept running in triplicates and expressed as Mean ± Standard error**

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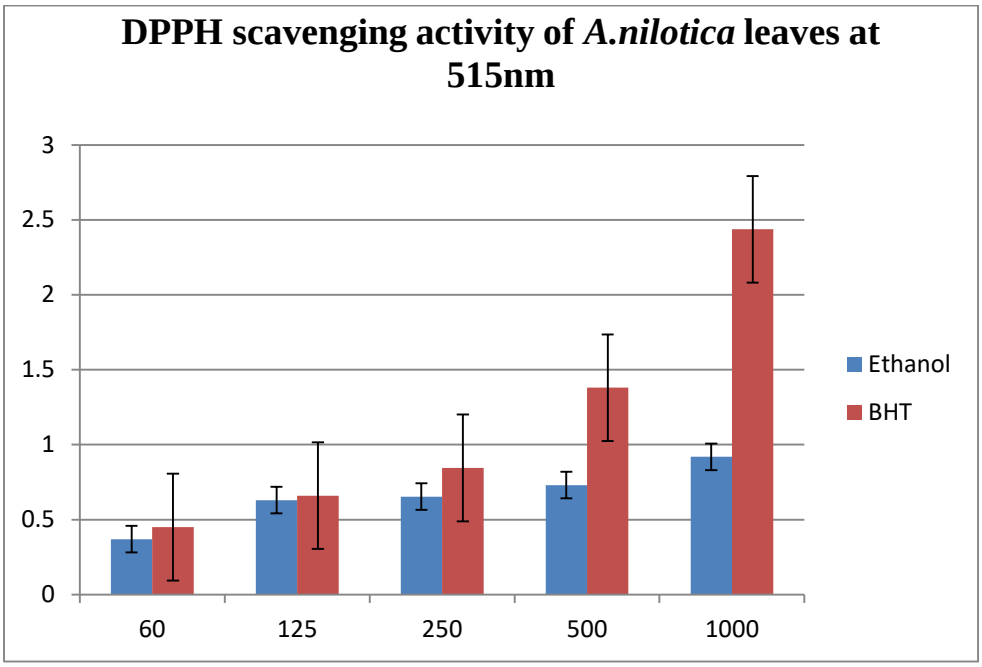


**Fig. 1: Graphical portrayal of DPPH radical scavenging activity of leaf extract**

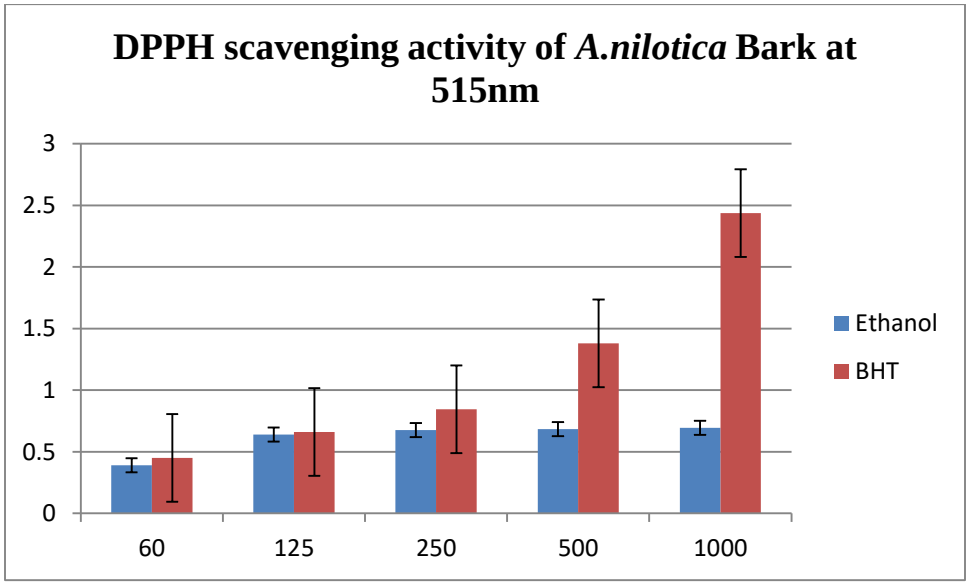


**Fig. 2: Graphical portrayal of DPPH radical scavenging activity of bark extract**

The total phenolic contents of *A. nilotica* (L.) leaves and bark was measured by using folin-ciocalteu reagent. Moreover, ethyl acetate had macerated more phenolic substances with the efficiency diminishing in ethanol. The assessment principle of total phenolic substances is that, in the essential circumstances, Polyphenols often ascent to phenolate anion which has capacity to decrease the Folin-ciocalteu reagent. A blue hued compound is shaped among Folin-ciocalteu reagent and phenolate. Gallic acid is utilized as standard in this examine and the results are communicated in Gallic acid reciprocal. It is felt that phenolic mixes can ascribe to the antioxidant activity.

**Table 7: Total phenolic contents in the leaves and the bark of *A. nilotica***

| Absorbance at different concentrations (µg/ml) | | | | | | |
|---|---|---|---|---|---|---|
| **Plant part** | **Fraction** | **60** | **125** | **250** | **500** | **1000** |
| Leaves | Ethanol | 0.220±0.00 | 0.369±0.03 | 0.735±0.02 | 0.799±0.07 | 0.806±0.08 |
| Bark | Ethanol | 0.263±0.02 | 0.424±0.03 | 0.774±0.05 | 0.803±0.09 | 0.803±0.11 |
| GAE (Gallic Acid) | | 0.27±0.03 | 0.54±0.04 | 0.79±0.02 | 1.44±0.04 | 2.84±0.06 |

**The outcome revealed were kept running in triplicates and expressed as Mean ± Standard error**

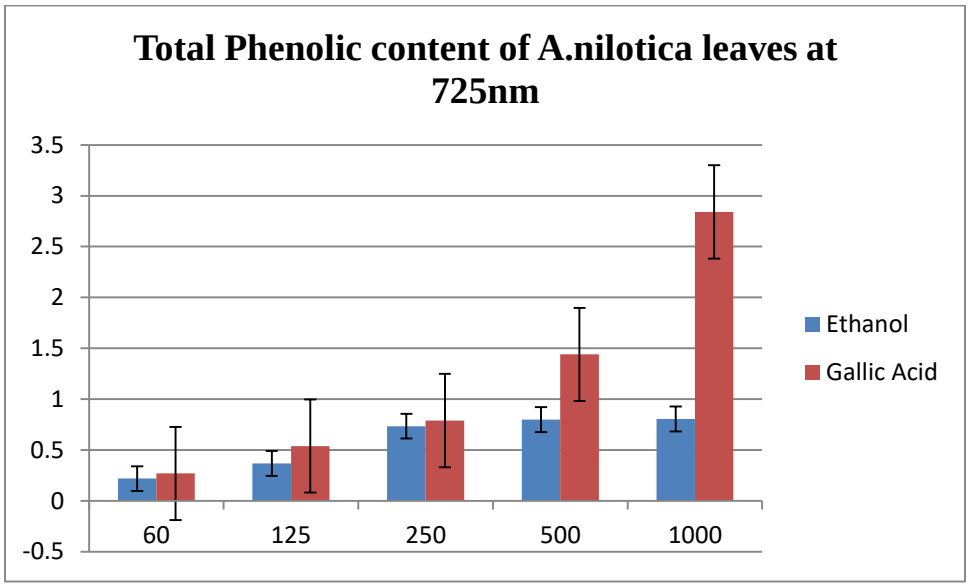


**Fig. 3: Graphical portrayal of total phenolic content of *A. nilotica* leaves extract**

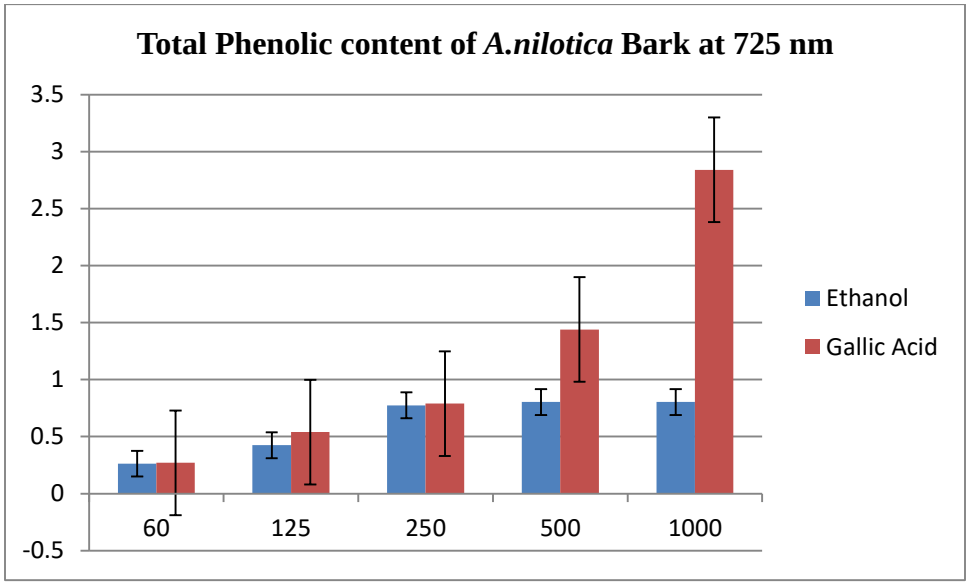


**Fig. 4: Graphical portrayal of total phenolic content of *A. nilotica* bark extract**

## DISCUSSION

Everywhere throughout the world the restorative plants are utilized with incredible interests and are dynamic members in the exchange and economy of the nation. In China

upwards of 2394 conventional Tibetan drugs are utilized all from plants (1106), creatures (448) and normal minerals (840). A significant number of the significant restorative plants are sold at more expensive rates in the market. As Hemamalini *et al.*, (2013) announced that yearly world market estimation of the meds got from the therapeutic plants by the indigenous individuals is US $ 43 billion. A large portion of the plants utilized by the nearby individuals are not preserved but rather are over misused. It is accordingly important to discover the methods for advancing the neighborhood individuals towards preservation as Ali *et al.*, (2012) recommended that ethnobotany is the study of recording the conventional learning on the utilization of plants by the indigenous individuals and for further surveying human associations with the regular habitat.

The current examination was intended to assess the phytochemical and antioxidant activity of *Acacia nilotica*. Plants are the wealthy wellsprings of drug and are fundamental to continue life as they yield bioactive combinations. Gigantic numbers of medications created currently are utilized in allopathy & homeopathy which are achieved from plant life. It is customary technique to monitor dynamic phytochemical items.

Qualitative phytochemical screening of ethanolic extract of the bark of *A. nilotica* uncovered that the bark of plant contains teriterpenoids, sterols, flavonoids, protein, sugar, tannin, glycosides and saponins (Table 5). Negative outcomes were recorded for alkaloids which affirm the nonappearance of this dynamic standard in bark (Table 5). This is like report of Banso (2009), that ethanolic concentrate of *Acacia nilotica* stem bark contain dynamic standards for example teriterpenoids, tannins, flavonoids and so on.

Subjective phytochemical screening of ethanolic concentrate of leaves of *Acacia nilotica* uncovered that the plant leaves contain triterpenoids, sterols, flavonoids, protein, sugar, tannin, saponins and glycosides (Table 5). This is like report of Okoro *et al*., (2014) that ethanolic concentrate of *A. nilotica* leaves contain dynamic standards for example alkaloids, tannin, sterols and so on.

Quantitative phytochemical screening of the leaves of *A. nilotica* uncovered that 2.53% of tannins were available in leaves of *Acacia nilotica* per 5ml of concentrate and 5.10% of tannins were available in bark of *Acacia nilotica* per 5ml of concentrate. The rate weight of

alkaloids in ethanolic leaf concentrate was 6.12%. Alkaloids were not recognized in the bark of this plant. This work was comparable with crafted by Okoro *et al*., (2014) on phytochemical screening of *Acacia nilotica*. 0.9% and 0.15% of tannins were available in the leaf and bark concentrates of *Acacia nilotica* separately (Table 6).

The work of Hemamalini *et al*., (2013) reported that the phytochemical examination of leaf concentrate of *Acacia nilotica* uncovered the nearness of a few organic dynamic mixes, for example, trans decalone,3-picoline-2-nitro, lavandulyl acetic acid derivation, 1-acetyl beta-carboline, hydroxycitronellal, propionic acid-2-chloro, D-glucuronicacid and ethyl ester by gas chromatography-mass spectrometry examination.

In plants as well as in human's oxidative stress might occur. Chlorophyll actions as the photo synthesizer forming oxygen and in plants, the wellspring of oxidative pressure is photosystem. The development of ROS may happen in the mitochondria, microsomes, peroxisomes and others. Under physiological conditions in human beings around-1 to 3 percent of the oxygen devoured by body is altered into superoxide and different ROS Singh and Thakur, (2016) during the study of plant stress, Avoidance, adaptation and defense in Plant Toxicology New York.

The activity of antioxidant was appraised by utilizing two measures, for example total phenolic substance and DPPH radical scavenging action. A few techniques have been accomplished for the appraisal of antioxidant competence in light of the fact that in the oxidative pressure process, numerous dynamic species and response systems are included, accordingly no unassuming strategy can quantify the antioxidant agent limit Frankel and Finley (2008) during the evaluation natural antioxidants.

The results shown by the DPPH radical scavenging activity had recognized that most extreme radical rummaging potential was seen by leaves of *A. nilotica* 0.919±0.03 at 1000 conc. in ethanol and maximum radical potential by bark of *A. nilotica* was observed 0.694±0.06 at 1000 conc. in ethanol. BHT was utilized as a standard to analyze the outcomes. Comparative outcomes have additionally been seen in various dissolvable concentrates of leaf and stem bark of *Cassia fistula*.

Reduction of F.C reagent is done to determine total phenolic content by phenolate anoin. The phenolic content analysis was executed for leaves and bark of *Acacia nilotica*. The results showed that the highest phenolic contents were given by ethanolic extract of leaves, i.e., 0.806±0.08µg/ml of GAE at 1000 concentration. The results for the bark showed that highest phenolic contents were given by ethanolic extract, i.e., 0.803±0.0108µg/ml of GAE at 1000 concentration.

The total phenol contents were surveyed by decrease of Folin-Ciocalteu reagent by phenolate anoin. The phenolic contents investigation was executed for bark and leaves of *Acacia nilotica*. The outcomes portrayed that the most noteworthy phenolic substance were given by ethanolic extracts of leaves, i.e., 0.806±0.08µg/ml of GAE at 1000 concentration. The outcomes for the bark delineated that most elevated phenolic content was given by ethanolic extract that is 0.803±0.0108µg/ml of GAE at 1000 concentration.

## CONCLUSION

The mankind immensely needs pharmacological association for the wellbeing office, especially against oxidative pressure, bacterial & contagious contaminations.This research work has established the ethno-pharmacological characters (Phytochemical and antioxidant) of ethanolic extract of *A. nilotica* a plant belonging to family Fabaceae. The qualitative phytochemical study observed the presence of almost all the tested secondary metabolites. Triterpenoids, Sterols, Proteins, Carbohydrate, Tannins, Glycosides were present in better quantity rather than the alkaloids were only detected in leaves not in bark. So from study it was concluded that this plant has medicinal values. 6.12% Alkaloids were present in leaves and Alkaloids were not detected in bark. 2.53% Tannins were present in leaves and 5.10% Tannins were present in bark of *Acacia nilotica.*Antioxidant effect was investigated by using two methods and maximum result of DPPH was detected by *A. nilotica* leaves 0.919±0.03 mm in ethanolic extract of leaves at 1000 concentration. Maximum result of DPPH was detected by *A. nilotica* bark 0.694±0.06 mm in ethanolic extract of bark at 1000 concentration. Total phenolic content of *A. nilotica* leaf extract of ethanol was 0.806±0.08 mm and Total phenolic content of *A. nilotica* bark extract of ethanol of 0.803±0.11 mm showed maximum value at 1000 concentration. Outcomes were compared with the standard drugs BHT and GAE. It was

concluded that this plant has essential phytochemicals and an impressive antioxidant activity. So it is highly recommended that instead of using allopathic medicines people should prefer herbal products for treatment to avoid side effects and other complications.

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