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ANTIOXIDANT AND ANTICANCER PROPERTIES OF *ACACIA NILOTICA* LEAF EXTRACT

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ABSTRACT
Medicinal plants are rich sources of herbal properties contributing in the discovery of new drugs towards various disorders, diseases including cancer without any toxic effects on the individuals treated. *Acacia nilotica* is widely used as tradition medicine such as for the treatment of venereal diseases, burns, wounds, stomachache etc. This study focuses on the antioxidant and anticancer properties of *Acacia nilotica* leaves methanolic extract. Two antioxidant assays i.e., DPPH assay and ABTS assay were assigned to study the antioxidant potential of the leaf extract of *Acacia nilotica*. The anticancer activity was also studies on the MCF 7 and 3T3 cancer cells through the MTT assay and dual acridine orange/ethidium bromide fluorescent staining. The studied leaf sample had a potential anti-oxidant and anti-cancer potential as it gave significant positive results for both the activities. Leaves part of *Acacia nilotica* had higher antioxidant activity at 90 µg/ml concentration in DPPH assay. In ABTS scavenging activity of leaves, highest activity showed at 100 µg/ml concentration. Accordingly, *Acacia nilotica* leaves extract was more effective to inhibit MCF 7 breast cancer cell line compared to 3T3 cell lines as the percent cell viability of MCF 7 breast cancer cell line at 10µg/ml leaves extract was recognized 92.48 % while it was 96.08 % cell viability at 25µg/ml for 3T3 cells. *Acacia nilotica* can be a better candidate for isolation of cytotoxic and anticancer compounds especially for methanol extract. Therefore, from the results obtained *Acacia nilotica* plays an important role in the prevention and management of various diseases. It can be used in future as herbal drugs with ecofriendly, non-expensive and without any side effects.

KEYWORDS: *Acacia nilotica*, Antioxidant, DPPH assay, ABTS assay, Anticancer, MCF7 cancer cells, 3T3 cell line

INTRODUCTION

Drastic changes in daily life over the past century are increasing the growing burden of chronic diseases, including atherosclerosis, hormone related and gastrointestinal cancers, osteoporosis, and type 2 diabetes mellitus (Omenn, 2010). These diseases share a high-degree of comorbidity and behavioral risk factors, are endemic to industrialized nations, and have been associated with a similar physiologic profile of metabolic and inflammatory dysregulation (Nicklas *et al.,* 2005; Shoelson *et al.,* 2007). However, over the long period, humans constantly created new

ways of living and eating, and actual physical or genetic changes evolved to minimize the effects of these diseases. From the point of view any shift in the physical makeup or behavior of human represents not only an obstacle but also a challenge to be overcome. As a result, new diseases emerged with each major change in the human way of life (Armelago *et al.,* 1996).

One such disease is cancer, which is essentially a problem of abnormal cell growth. Under the influence of various environment factors normal cells are transformed to cancer cells that divide in an uncontrolled manner (Nwafor *et al.,* 2001). Cancer is a major cause of morbidity and mortality in developing and developed countries (Ferlay *et al.,* 2012) in comparison to the other diseases. In many low-income and middle-income countries, including India, most of the population does not have access to a well-organized and well-regulated cancer care system (Mallath *et al.,* 2014). Estimated number of peoples are living with the disease around 2.25 million every year in India. Nearly 11.57 lakhs of cancer cases are diagnosed and about 7.84 lakhs die (Men: 4.13 lakhs, Women: 3.71 lakhs) (NICPR Report, 2018). In Rajasthan, nearly 82 thousand of cancer cases are diagnosed and about 41 thousand died in 2016 (NCRP Report, 2016).

Surgery, chemotherapy and radiotherapy are considered as the most common methods of cancer treatment, although all of these treatment methods are not always useful and the clinical results are not acceptable (Yang *et al.,* 2012). Although chemotherapy and radiotherapy are highly effective methods of cancer treatment, but these methods exert severe side effects in use (Qi *et al.,* 2010). One of the main problems in cancer treatment is gradual resistance of cancer cells against treatment (Wang *et al.,* 2012). Hence, achieving a new approach is one of the aims of immuno-pharmacological studies to improve cancer treatment results (Azadmehr *et al.,* 2011).

India has tremendous wealth of aromatic and medicinal plants. In current days medicinal plants are a pillar of traditional healthcare systems of medicine in many developing countries. Since from the ancient times, several drugs have been formulated using the bioactive compounds present in these medicinal plants (Rahmati *et al.,* 2015). According to World health organization (WHO) more than 80% world's population depends on medicines derived from these medicinal plants for primary health care needs. The use of medicinal plants as a source for relief from illness can be traced back over since before recorded history. These phytomedicines are safe and environment friendly (Bansod and Rai, 2008).

The anticancer efficacy of natural polyphenols has largely been attributed to their potent antioxidant and anti-inflammatory activities as well as their abilities to modulate molecular targets and signaling pathways, which were associated with cell survival, proliferation, differentiation, migration, angiogenesis, hormone activities, detoxification enzymes, immune responses, etc. (Li *et al.,* 2013a, b). Antioxidants can be described as oxidation inhibitors as they prevent the propagation of chain reactions involving oxidation of other molecules and thus help in scavenging the reactive molecules (Pokorny and Korczak, 2001).

Acacia nilotica

Acacia nilotica L. (RUBL-211715) belongs to the kingdom Plantae and family *Fabaceae* (Rather and Mohammad, 2015). It is the second-largest genus of the family *Fabaceae*, with about 1350 species. *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 (Tyagi *et al.,* 2016). There is total nine sub species in the *Acacia nilotica*. It is medium in size and thorny and has height approximately around 21-24 meters and will not grow properly if suitable conditions are not given to it. *Acacia nilotica* and its chief phytoconstituents play a pivotal role in several therapeutic strategies. Approximately all parts of *Acacia nilotica* such as the leaves, roots, seeds, bark, fruits, flowers, gum and immature pods act as anti-cancer, antimutagenic, spasmogenic, vasoconstrictor, anti-pyretic, anti-asthamatic, cytotoxic, anti-diabetic, anti-platelet agregatory, anti-plasmodial, molluscicidal, anti-fungal, inhibitory activity against Hepatitis C virus (HCV) and human immunodeficiency virus (HIV)-I and antioxidant activities, anti-bacterial, antihypertensive and anti-spasmodic activities, and are also engaged for the treatment of different ailments in the indigenous system of medicine. *Acacia nilotica* has effect on central nervous system activities (Alzheimer's disease) due to potent Acetylcholin esterase inhibitory activities (Kalaivani *et al.,* 2011).

Several studies have been done on whole plant and every part of the *Acacia nilotica* plant, but the leaf is the less touched area of research. So, this study has been designed to focus on leaf part of the *Acacia nicotila* and study the antioxidant and *in vitro* anti-cancer activities. This can lead to a new direction in the field of research.

MATERIALS AND METHODS

1) Collection of plant material

The experimental plant material i.e., *Acacia nilotica* leaf was collected from the campus of SPNKS Govt. PG. College, Dausa whose plant part leaves were used for the evaluation process.

2) *In vitro* antioxidant activity

The free radical scavenging activity of the *Acacia nilotica* plant parts was determined by using various *in vitro* assays such as catalase activity, superoxide dismutase activity, glutathione peroxidase activity, DPPH assay and ABTS assay.

a) DPPH assay

1, 1 Diphenyl 2- Picrylhydrazyl (DPPH) is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity.

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability by identifying using this protocol (Brand-Williams *et al*., 1995).

Procedure

100 ml concentrated methanol was added to 10 gm sample (10:1) and incubated at 37°C temperature for 24 hours After this the above sample was filtered in a petri-plate for drying. Methanol was added in dry crude extract plate according to 1mg/ml and was collected in microtubes. Different concentration was taken ranged from 10 to 100 µl of sample and final volume was made up to 1 ml with methanol.

To the above solution 1 ml of DPPH was added (0.01gm in 100 ml methanol) in all series one by one and was mixed properly, and incubated in dark for 30 min. Blank used was 1.5 ml methanol and Control used was methanol + DPPH (1:1) and absorbance was taken at 517 nm. Quercetin was used as standard. The DPPH scavenging activity was calculated in percentage by following formula:

$$\% \text{ inhibition of DPPH} = \{(A_B - A_S)/A_B\} \times 100$$

Where, A_B was the absorbance of the blank and A_S was the absorbance of sample.

b) ABTS assay

The ABTS (2, 2′-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid) assay measures the relative ability of antioxidants to scavenge the ABTS generated in aqueous phase. The ABTS is generated by reacting with a strong oxidizing agent (eg. potassium permanganate or potassium persulfate) with the ABTS salt. The reduction of the blue-green ABTS radical by hydrogen-donating antioxidants is measured by the 734 nm of its characteristic long wave absorption spectrum. For ABTS assay, the procedure followed was the method of Zheleva-Dimitrova *et al*., 2010; Roberta *et al.,* 1999 with some modifications.

Procedure

ABTS was dissolved in water to make a concentration of 7 mmol/l. $ABTS^+$ was produced by reacting the ABTS stock solution with 2.45 mmol/l potassium persulfate (final concentration) and the mixture was left in the dark at room temperature for 12–16 hours before use. The $ABTS^+$ stock solution was diluted with 80 % methanol to an absorbance of 0.70 ± 0.02 at 734 nm. 1 ml of diluted $ABTS^+$ was added into 10-100 µg/ml samples. The absorbance reading was taken at 6 min after the initial mixing. Gallic acid was used as positive controls. The activities of the samples were evaluated by comparison with a control (containing 1 ml of ABTS solution and 1ml methanol). $ABTS^+$ scavenging activity was calculated by the following formula:

$$ABTS^+ \text{ scavenging activity } (\%) = [(A_c - A_s)/A_c] \times 100$$

Where A_C is the absorbance value of the control and A_S is the absorbance value of the added samples test solution.

3) Anticancer activity

MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed to detect anticancer activity using MCF7 cancerous cell lines and 3T3 fibroblast cell lines (Lovitt *et al.,* 2015).

a) Reagent Preparation

- **MTT Solution**

MTT was dissolved in Dulbecco's Phosphate Buffered Saline (DPBS, pH 7.4) according to 5 mg/ml. This MTT solution was filter-sterilized through a 0.2 µM filter into a sterile, light protected container. The MTT solution was stored at 4°C for frequent use or at -20°C for long term storage and was protected from light.

- **Solubilization Solution**

Firstly, 40% (v/v) dimethylformamide (DMF) was prepared in 2% (v/v) glacial acetic acid. To this 16% (w/v) sodium dodecyl sulfate (SDS) was added and dissolved, at pH 4.7. The solution was stored at room temperature to avoid precipitation of SDS. If a precipitate forms, warm to 37°C and mix to solubilize SDS. All the work was done in a ventilated fume hood and selection of appropriate solvent resistant container was done.

b) Extraction Process

Dried and powdered *Acacia nilotica* leaves were placed in a conical glass percolator, submerged in 100% methanol, and kept at room temperature for 20 hours. The extraction procedure was repeated four times and the percolate were collected and filtered. The distillation of methanol was occurred from pooled percolate using a rotavapour under reduced pressure at 50°C. The final drying was done initially in vacuum desiccators and finally lyophilized. The dried extracts were scrapped off and transferred to a wide mouth glass container. Nitrogen was blown in the container before capping and stored at -20°C in a desiccator.

c) MTT Assay Protocol

All the cells and test compounds were prepared in 96-well plates containing a final volume of 100µl/well. Incubation for desired period of exposure was carried out and 10 µl MTT solution was added per well to achieve a final concentration of 0.45 mg/ml. Incubation was done for 1 to 4 hours at 37°C. To this, 100 µl Solubilisation solutions were added to dissolve formazan crystals to each well and were mixed thoroughly for complete solubilisation. Absorbance was taken at 570 nm. Cisplastin was used as standard.

d) Dual acridine orange/ethidium bromide fluorescent staining protocol for MCF7 cancerous cell lines

This dual staining was carried to check apoptotic or necrotic nature of the cells. Dual fluorescent staining solution (1µl) contains 100µg/ml acridine orange (AO, Sigma) and 100µg/ml ethidium bromide (EB, Sigma).

Procedure

The cells were treated with test compound at IC_{50} concentration and incubated for 24 hours in CO_2 incubator at 37°C. The cells were removed by trypsination and collected by centrifugation including the non-adherent cells. The cell pellet was resuspended in medium and cell suspensions (25 µl) were transferred to glass slides. Dual fluorescent staining solution was added to each suspension and then covered with a cover slip. The morphology of apoptotic cells was examined and counts the cells within 20 minutes using a fluorescent microscope.

4) Statistical analysis

Statistical analysis is based on biological studies (Epstein and Polly, 1970). Each experiment was done in triplicate form and then all results were presented as mean± Standard Error (SE). Standard Error (SE) was calculated as follows:

$$SE = SD / \sqrt{N}$$

Here, SD represents standard deviation and N denotes number of observations.

RESULTS AND DISCUSSION

1) Antioxidant activity

Leaf part of *Acacia nilotica* was taken for identified antioxidant activity using DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay.

a) DPPH Assay

The DPPH assay constitutes a quick and low-cost sensitive method where the decolorization of the radical solution from purple to light yellow is evaluated in the presence of the plant extract at 517 nm using UV spectroscopy (Molyneux, 2004). The antioxidant activity of the leaves of *Acacia nilotica* depends on the high percentage of scavenging activity. The percentage (%) scavenging of DPPH free radical was found to be concentration dependent, i.e., concentration of the extract between 10-100 µg/ml greatly increasing the inhibitory activity. On comparing, it is found that the scavenging activity of the *Acacia nilotica* leaves is less than that of standard at all concentration but is fairly close as shown in Table 1 and Graph 1. Similarly, in 2018 Yadav *et al.* studied different extracts of *Acacia nilotica* leaves and tested for antioxidant activities. Antioxidant potential was determined using DPPH free radical scavenging assay in which methanol extract exhibited maximum antioxidant activity (94.3 %)

followed by the ethyl acetate extract (90.7 %). Also, in the study conducted by Hussain *et al.,* in 2019 the IC_{50} value found from for *Acacia nilotica* is 39.62µg/ml. In DPPH scavenging assay, *Acacia nilotica* extract exerts far better antioxidant activity. Ahmed *et al.,* (2022) estimated the anti-oxidant activity of *Acacia jacquemontii* Benth, through DPPH assay and observed a strong antioxidant potential in dried methanolic extract of 154.04 ± 2.47. In the study of Abduljawad (2020) the ethanol extract of *Acacia* leaves showed a powerful antioxidant efficacy in 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay (IC_{50} = 75.2 µg/ml) plus a significant hydroxyl radical scavenging effect (IC_{50} = 159.6 µg/ml). There total antioxidant capacity of the extract equal to 152.8 µg/ml ascorbic acid. It was also found that the methanol extract of *Acacia* leaves exerted a powerful antioxidant capacity (94.3%) in DPPH. Also, in 2021 Prayogo *et al.* their study aimed to select extracts of *Acacia* heartwood on the basis of their pharmacological and phytochemical profiles and identify their bioactive compounds. The anti-oxidant activity was measured using DPPH assay that ranged from 1.36 to 1.96 mmol TE/g DE respectively. The LC_{50} of the five extracts ranged from 566.10 to 2390.40 µg/ml, reflecting the toxicity levels of the extracts. The *Acacia decurrens* and *Acacia mangium* extracts had the lowest and highest LC_{50} values, respectively. In comparison, the antioxidant capacity trend of these assays was relatively different. However, the *Acacia crassicarpa* extract exhibited the highest capacity, which was significantly different from other extracts ($p < 0.05$).

Table 1: DPPH scavenging activities of the *Acacia nilotica* leaf and standard

S.No.	Concentration in µg/ml	% Inhibition of sample	% Inhibition of standard (Quercetin)
1.	10	33.09±3.78	49.02±4.56
2.	20	51.05±4.47	53.21±0.95
3.	30	78.94±3.44	85.15±2.65
4.	40	91.31±2.48	92.32±5.06
5.	50	91.48±3.11	92.11±4.78
6.	60	92.07±2.65	92.59±1.30
7.	70	93.05±3.33	93.23±4.48
8.	80	93.71±4.92	94.07±3.40
9.	90	94.03±1.04	96.14±2.99
10.	100	94.19±2.67	96.03±2.85

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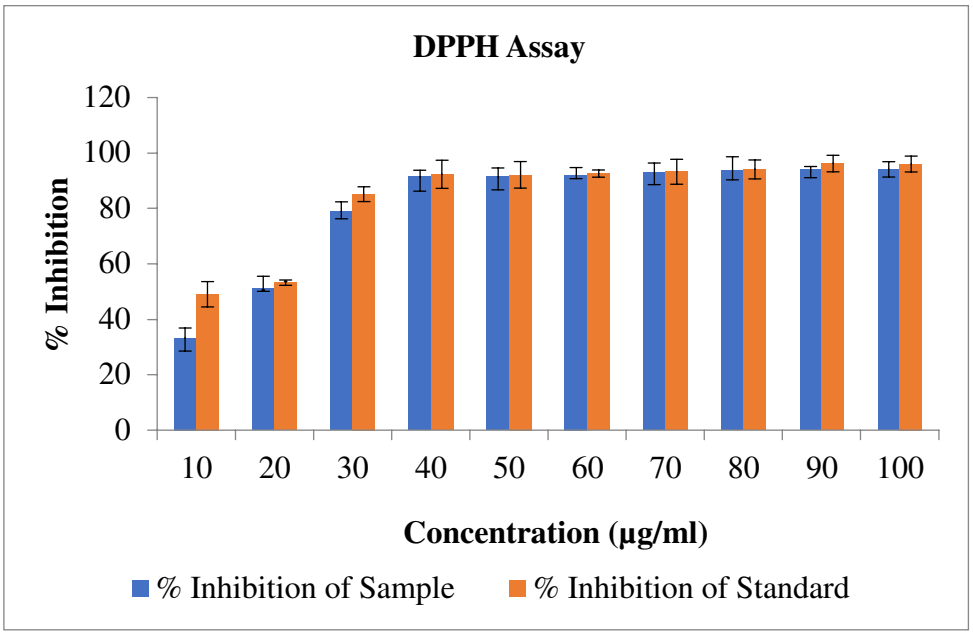

Graph 1: DPPH scavenging activities of the *Acacia nilotica* leaf and standard

(ii) ABTS Assay

The percentage (%) scavenging of ABTS free radical was found to be concentration dependent, i.e., concentration of the extract between 10-100 µg/ml greatly increasing the inhibitory activity. Gallic acid was used as a standard. On comparing it is found that the scavenging activity of the methanol extract of leaves of given sample plant is less than that of standard at all concentration but is quite close to that of standard as described in Table 2 and Graph 2. ABTS scavenging activity of *Acacia nilotica* with ascorbic acid standard was at 25 µg/ml concentration. The IC_{50} value found to be 19.48µg/ml in *Acacia nilotica* (Agrawal *et al.,* 2010). Similarly, Ahmed *et al.,* (2022) estimated the anti-oxidant activity of *Acacia jacquemontii* Benth, through ABST assay and observed a strong antioxidant potential in dried methanolic extract of 122.36 ± 0.80 mg Trolox/g of dry extract. Also, in 2021 Prayogo *et al.* study aimed to select extracts of *Acacia* heartwood on the basis of their pharmacological and phytochemical profiles and identify their bioactive compounds. The anti-oxidant activity was measured using ABST assay that ranged from 2.16 to 5.40 mmol TE/g DE respectively. The LC_{50} of the five extracts ranged from 566.10 to 2390.40 µg/ml, reflecting the toxicity levels of the extracts. The *Acacia decurrens* and *Acacia mangium* extracts had the lowest and highest LC_{50} values, respectively. In comparison, the antioxidant capacity trend of these assays was relatively different. However, the *Acacia crassicarpa* extract exhibited the highest capacity, which was significantly different from other extracts ($p < 0.05$).

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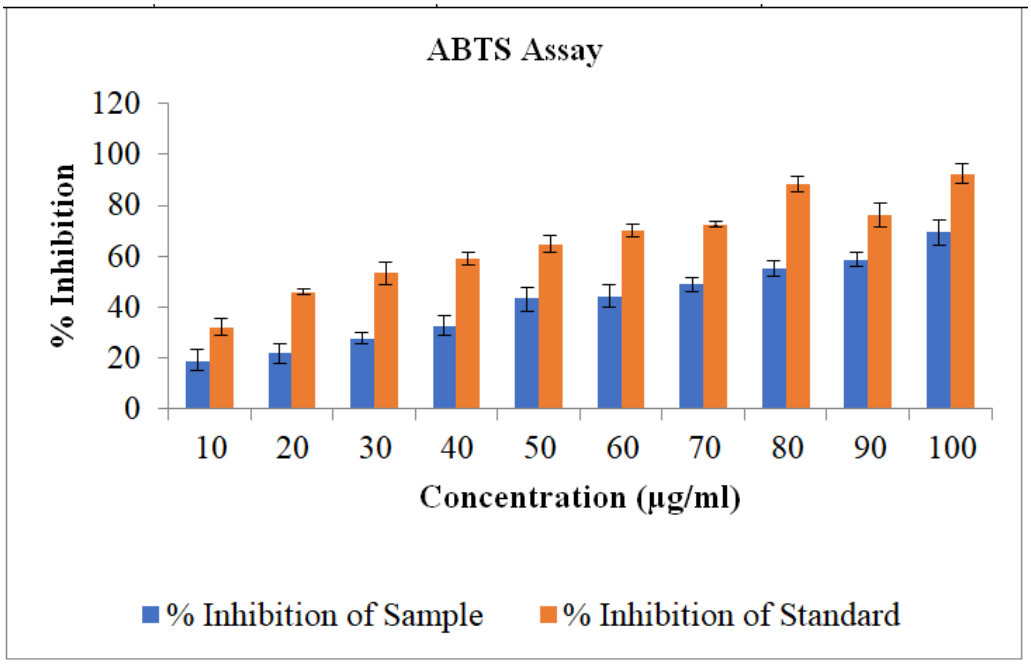
Table 2: ABTS scavenging activities of the *Acacia nilotica* leaf and standard

S. No.	Concentration in µg/ml	% Inhibition of sample	% Inhibition of standard (Gallic acid)
1.	10	19.11±4.17	32.22±3.16
2.	20	21.79±3.77	46.12±1.32
3.	30	27.76±2.08	53.36±4.32
4.	40	32.83±3.93	59.17±2.62
5.	50	43.28±4.68	64.81±3.32
6.	60	44.47±4.30	70.03±2.57
7.	70	48.95±2.98	72.64±1.02
8.	80	55.22±3.25	88.32±3.01
9.	90	58.81±2.61	76.32±4.59
10.	100	69.25±4.91	92.55±3.69



Graph 2: ABTS scavenging activities of the *Acacia nilotica* leaf and standard

B. Anticancer activity

The natural products having anticancer property has broad spectrum than the synthetic compound having market value around 63% among all drugs available (Cragg *et al.,* 2009). It is observed that the existing anticancer drugs have a limited selectivity and are highly toxic. Further, the secondary metabolites in medicinal plants are low molecular weight compounds, exhibiting a potential anticancer activity. The present study revealed the anticancer and cytotoxic potential of methanol extract of leaves of *Acacia nilotica* on breast cancer cells MCF 7 and the report was compared with cisplastin at various concentrations. The IC_{50} value of 198 µg of methanol extract of *Acacia nilotica* leaf was found to MCF 7 breast cancer cell line as shown in Figure 3. It was noticed that leaves extract with a concentration ranging from 10 to 500µg/ml resulted in dose dependent decrease in cellular viability of cancer cells where the percent cell viability of MCF 7 breast cancer cell line reduced from 92.48% at 25µg/ml to 43.44% at 500 µg/ml (Table 4 and Graph 4) which was higher when compared to cisplastin (10.53% at 500µg/ml) as shown in Table 3 and Graph 3. The variation between the positive drug and samples was because the positive drug was a pure and so it is required lower concentration to inhibit the growth of cancer cells. Alternatively higher concentration of samples resulted in 56.56% inhibition of MCF7 breast cancer cell line which revealed the fact that it is toxic at increasing concentration of test sample. Screening of cytotoxicity of methanol extract of leaves of *Acacia nilotica* on 3T3 cell line revealed that 96.08 % cell viability was observed at 25µg/ml and 66.48% at 500µg/ml consequently it was marginally toxic to cells even at higher concentration which proved its non-toxicity. Overall *Acacia nilotica* leaves extract was more effective to inhibit MCF-7 breast cancer cell line compared to 3T3 cell lines as shown in Table 3-5, Graph 3-5 and Figure 1-2.

Acridine orange is a vital dye and will stain both live and dead cells. Ethidium bromide stains only cells that have lost membrane integrity. Live cells will appear uniformly green. Early apoptotic cells will stain green and contain bright green dots in the nuclei as a consequence of chromatin condensation nuclear fragmentation. Late apoptotic cells will also incorporate ethidium bromide and therefore stain orange, but in contrast to necrotic cells, the late apoptotic cells will show condensed and often fragmented nuclei. Necrotic cells stain orange, but have a nuclear morphology resembling that of viable cells, with no condensed chromatin. Thus, the dual staining report revealed presence of intact live cells in control wells as shown in green color fluorescence, while the wells treated with test compound shows apoptotic cells as reddish orange color fluorescence (Figure 3).

Meena *et al.* (2006) reported the anticancer effect of the aqueous extract of gum, flowers, and leaves of *Acacia nilotica* against 7, 12-Dimethylbenz[a]anthracene produced skin papillomagenesis in mice. Medication with the aqueous extract (800 mg/kg orally) for 15 days was the most effective. Treatment with various extracts resulted in decreased tumor load, tumor incidence, and a cumulative number of skin papilloma. The latency of the tumor in the groups treated with leaf and flower extracts was prolonged. In another study, it was found that the ethanol extract of *Acacia nilotica* leaves had more effective cytotoxic activity against two cell lines. IC_{50} value was found to be 53.6 µg/ml for Vero cell lines and 28.9 µg/mL for Hela

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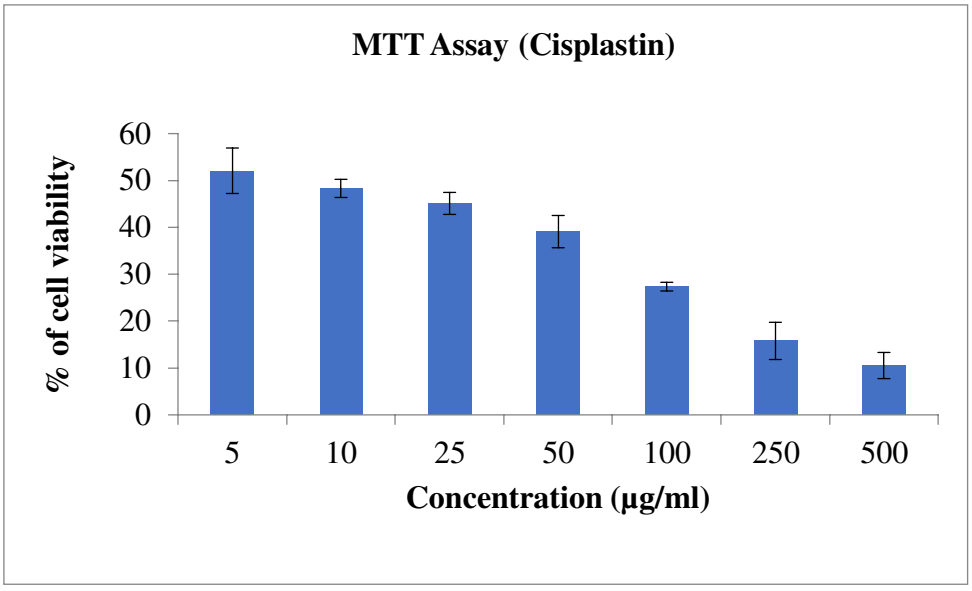
cell lines in cytotoxicity assays and the extract did not show any toxicity towards the erythrocytes in humans or rats (Kalaivani *et al.,* 2011). *In vitro* experiments of Diab *et al.,* 2015 showed that the selected plant extracts exhibited variety of cytotoxicity against the examined cancer cell lines. Among the examined cell lines, K562 cell lines were the most resistant toward the extracts with IC_{50} value in the range of 42.8-50 µg/ml.

Table 3: Percent cell viability of standard (Cisplastin) (MCF7 breast cancer cell line)

Concentrations(µg/ml)	% of cell viability
5	52.066±4.85
10	48.323±1.92
25	45.124±2.34
50	39.106±3.43
100	27.364±0.92
250	15.781±3.95
500	10.532±2.77



Graph 3: Percent cell viability of standard (Cisplastin) (MCF7 breast cancer cell line)

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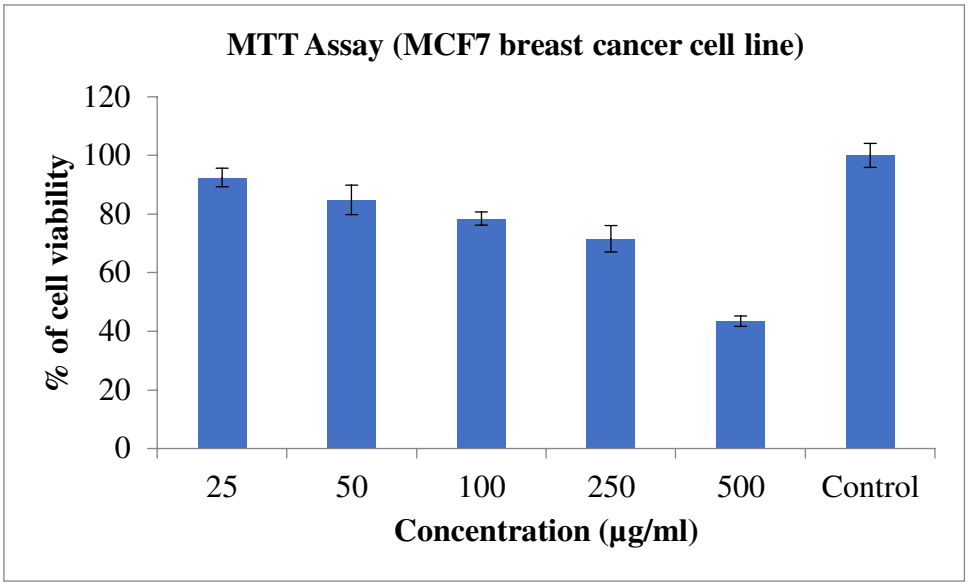
Table 4: Percent cell viability of tested sample against MCF7 breast cancer cell line

Tested concentrations (µg/ml)	% of cell viability
25	92.488±3.17
50	84.801±5.04
100	78.472±2.25
250	71.544±4.52
500	43.445±1.78
Control	100±4.10



Graph 4: Percent cell viability of tested sample against MCF7 breast cancer cell line

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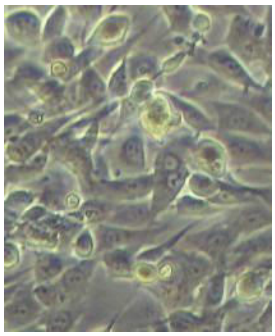
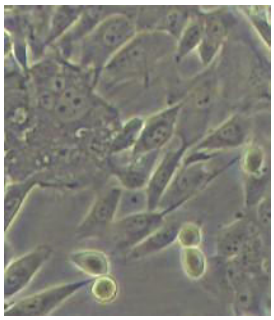
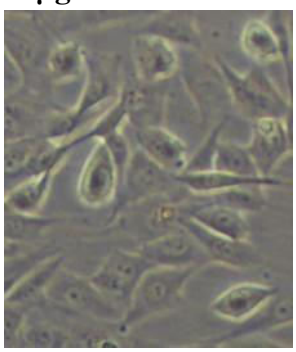
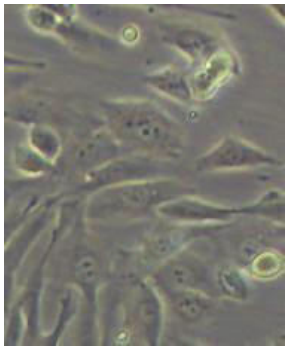
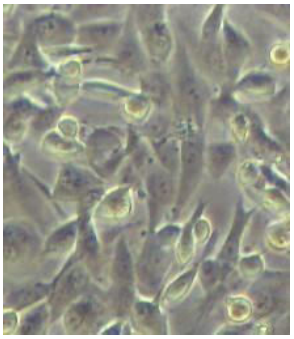
Cisplastin	**Methanol extract of *Acacia nilotica* leaf**
Control 	
5µg 	
10µg 	**10µg**
25µg 	**25µg** 

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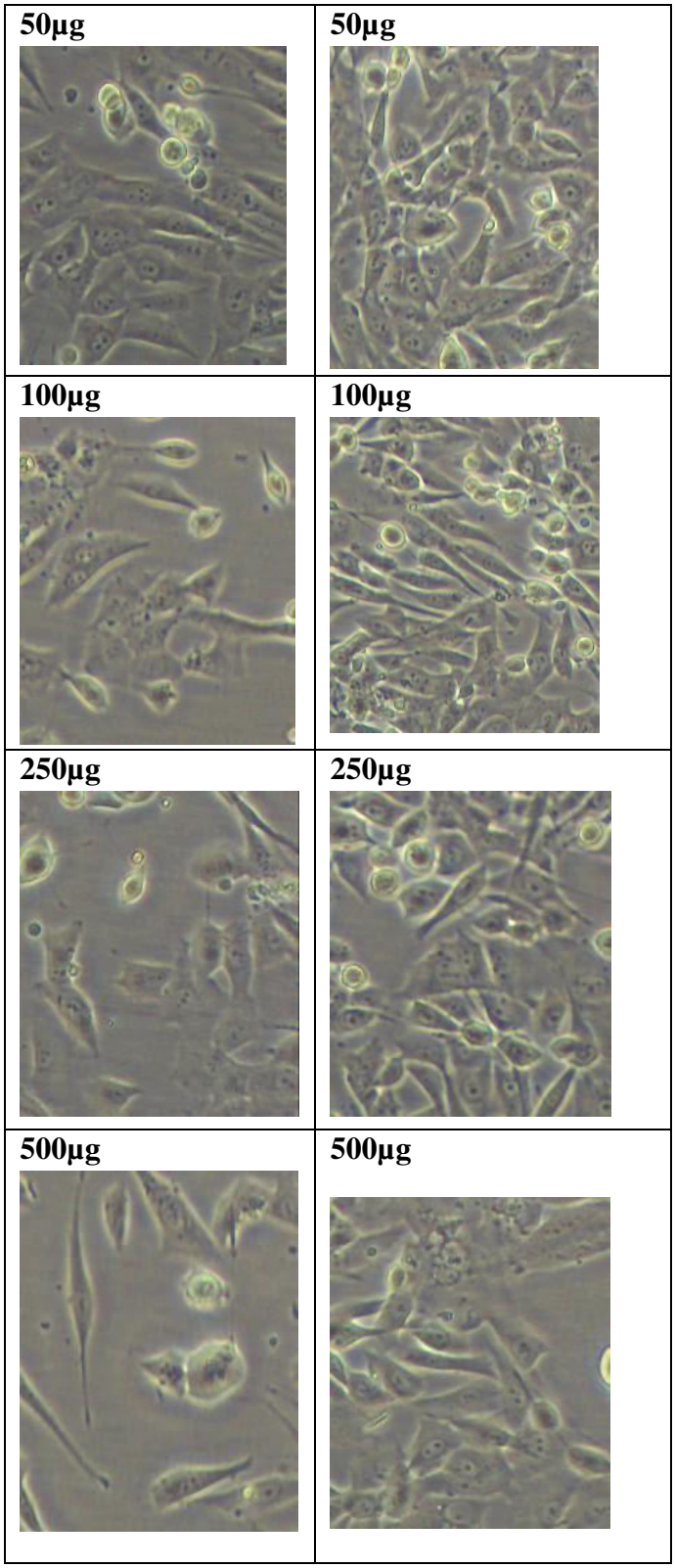

Figure 1: Cell viability of MCF 7 cell lines against methanol extract of *Acacia nilotica* leaf

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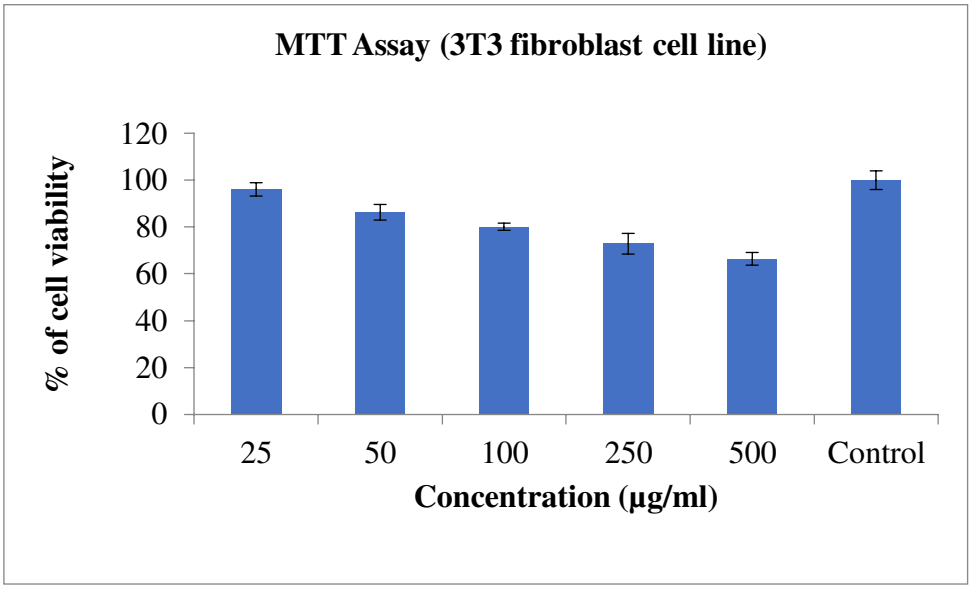
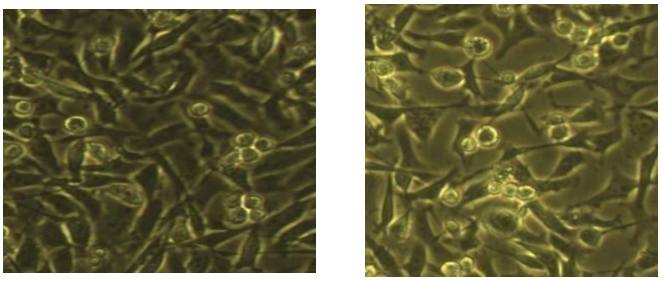
Table 5: Percent cell viability against 3T3 fibroblast cell line

Tested concentrations (µg/ml)	% of cell viability
25	96.082±2.84
50	86.336±3.32
100	80.113±1.53
250	72.889±4.40
500	66.482±2.69
Control	100±3.97



Graph 5: Percent cell viability of tested sample against 3T3 fibroblast cell line



Control **25 µg**

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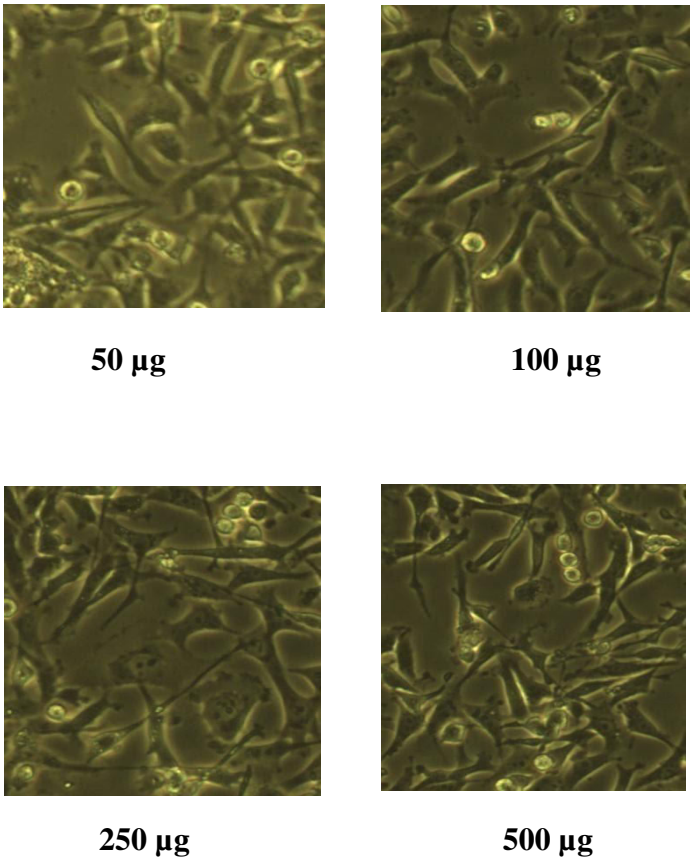
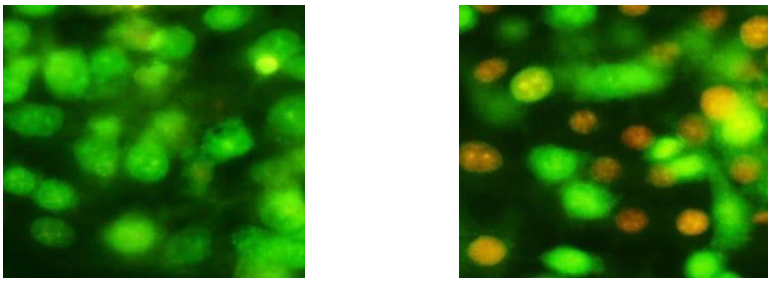


50 µg **100 µg**

250 µg **500 µg**

Figure 2: Cell viability of 3T3 against Methanol extract of *Acacia nilotica* leaf



Control **Methanol extract of *Acacia nilotica* leaf**

IC_{50} 198µg

Figure 3: Dual Acridine orange/Ethidium bromide fluorescent staining (MCF 7)

CONCLUSION

In the present study, the main objective was to gain understanding of the biological tendencies such as antioxidant and anti-cancer activity of *Acacia nilotica,* a wild plant also known as babool and traditionally been used due to its medicinal properties. During the investigation, anti-oxidant and anti-cancer activities have been performed. The *Acacia nilotica* leaf extract showed positive results to all the activities which can be due to the presence of various bioactive phytochemical compounds. The results of the present research investigation suggest that the 70% methanol leaves extract of *Acacia nilotica* posse high significant antioxidant and anti-cancer potential. All the studies were carried out *in vitro* and gave a significant result for the use in medical field. *Acacia nilotica* is a promising medicinal plant and further *in vivo* studies should be carried out to gain a better understanding of it to the future use of it in pharmacological and biomedical industries.

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