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Evaluation of antioxidant activity of crude extracts and different fractions of stem bark of *Acacia nilotica*

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Abstract

Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias. The crude methanolic extract of Acacia nilotica bark with different soluble partitionates were subjected to investigate for the evaluation of analgesic, hypoglycemic, CNS depressant and antidiarrheal activity on mice and thrombolytic, antihelmentic, antimicrobial, antioxidant along with cytotoxicity different in vivo experiment. Dichloromethane fraction and methanolic extract of bark of Acacia nilotica showed moderate antioxidant activity with the IC_{50} values 157.41 µg/ml and 136.79 µg/ml respectively.

Keywords: Antioxidant activity, Crude extracts, Fractions, Stem bark, *Acacia nilotica.*

Introduction

In living system, free radicals are generated as part of the body's normal metabolic process and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxidase activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics. In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting, can result in increased radical activity and damage. Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders. Oxygen free radical can initiate per-oxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long-term complication of diabetes[1].

Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegeneration[2], Parkinson's diseases[3], mongolism, ageing process and perhaps dementias. Antioxidant based drugs/formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease[4], and cancer have appeared during the last 3 decades. This has attracted a great deal of research interest in natural antioxidants. The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, betacarotene, and tocopherol are known to possess antioxidant potential.

In recent times, medicinal plants occupy a considerable position for being the paramount sources of drug discovery irrespective of its categorized groups- herb, shrub or tree[5]. With this background and abundant source of unique active components harbored in plants, the present study was taken up on the medicinal plant namely *Acacia nilotica.*

The plant family: Fabaceae[6] (a,b)

The plant under investigation is *Acacia nilotica* belongs to the family Fabaceae. The Fabaceae, also called Leguminosae or bean and pea family, is the third largest family in terms of agricultural and economic importance. Legumes includes a large number of domesticated species harvested as crops for human and animal consumption as well as for oils, fiber, fuel, fertilizers, timber, medicinals, chemicals, and horticultural varieties[7]. In addition, the family includes several species studied as genetic and genomic model systems.

Growth Pattern

Legumes vary in habit from annual and perennial herbs to shrubs, trees, vines/lianas, and even a few aquatics. Ranging in size from some of the smallest plants of deserts and arctic/alpine regions to the tallest of rain forest trees, legumes are a conspicuous, and often dominant, component of most of the vegetation types distributed throughout temperate and tropical regions of the world[8]. Legumes are particularly diverse in tropical forests and temperate shrub lands with a seasonally dry

or arid climate. This preference for semi-arid to arid habitats is related to a nitrogen demanding metabolism. While many species have the ability to colonize barren and marginal lands because of their capacity to "fix" atmospheric nitrogen via a symbiotic association with root-modulating bacteria, this is just one of several ways in which legumes obtain high levels of nitrogen to meet the demands of their metabolism[9].

Over the past 30 years, the study of legume classification and biology has benefitted from major advances in understanding of the morphology, evolution and systematics, and ecology of the family[10].

Characteristics: Morphologically, Fabaceae is characterized by leaves simple to compound (pinnate, rarely palmate, or bipinnate), unifoliate, trifoliate (*Medicago, Trifolium*), sometimes phyllodic (many species of *Acacia*), or reduced to a tendril (as in *Lathyrus*), spirally arranged, with stipules present that are sometimes large and leaf-like (*Pisum*) or developed into spines (*Prosopis, Robinia*).

Flowers are usually regular or irregular (i.e., actinomorphic to zygomorphic in symmetry, respectively), bisexual, with a single superior carpel (hypogynous to perigynous), pentamerous, arranged singly or in racemes, spikes, or heads. The principal unifying feature of the family is the fruit, the legume[10].

With a few exceptions, legumes are typically one-chambered pods (one locule), with parietal placentation along the adaxial suture, ovules 2 to many, in two alternating rows on a single placenta, typically dry and dehiscent along one or both sutures (legume).

Taxonomy[11]: Taxonomically, Fabaceae has been traditionally divided into three subfamilies: i. Caesalpinioideae, ii. Mimosoideae, iii. Papilionoideae.

The recognition of three subfamilies is based on characteristics particularly of the flower, aestivation of petals, sepals (united or free), stamen number and heteromorphy, pollen (single or polyads), leaf complexity, and presence of root nodules. Differences in these characteristics led to the view that the Mimosoideae and Papilionoideae are unique and distinct lineages in the family which arose independently within a paraphyletic "basal" caesalpinioid assemblage.

Agricultural & Economic Importance of Legumes

Legumes have demonstrated agricultural importance for thousands of years, beginning with the domestication of lentils (*Lens esculenta*) in Iran dating to 9,500 to 8,000 years ago, their use as a food source during the prehistory of North and South America (beans, more than 3,000 years ago), and their use by the Roman Empire as a food source and for soil improvement[12]. Today legumes are an increasingly invaluable food source not just for humans, accounting for 27% of the world's primary crop production, but also for farm animals[12]. Legumes were grown on more than 13% of the total arable land under cultivation in the world in 2004[14]. Grain legumes alone contribute 33% of the dietary protein nitrogen needs of humans, while soybeans (*Glycine max*) and peanut (*Arachis hypogeae*) provide more than 35% of the world's processed vegetable oil and a rich source of dietary protein for the poultry and pork industries[12].

While they produce nitrogen-containing protein in abundance, legumes are deficient in sulfur containing amino acids and other nutrients needed by people and animals. For this reason, legumes and cereal crops are often raised together to account for the amino acids and other elements they are each deficient in[13]. The primary dietary legumes grown, such as bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), chickpea (*Cicer arietinum*), broad bean (*Vicia faba*), pigeon pea (*Cajanus cajan*), cowpea (*Vigna unguiculata*), and lentils (Graham and Vance, 2003), include representatives of each of the four clades within papilionoids, the genistoids, dalbergioids, Hologalegina, and phaseoloid/millettioids.

Industrially, legumes have many uses in making biodegradable plastics, oils, dyes, and biodiesel fuel. Legumes are used traditionally in folk medicines, but also demonstrate importance in modern medicine. Isoflavones commonly found in legumes are thought to reduce the risk of cancer and lower cholesterol and soybean phytoestrogens are being studied for use in postmenopausal hormone replacement therapy. Legumes also produce a hypoglycemic effect when eaten, making them a recommended food for diabetics[13].

The plant: Acacia nilotica[14-17]: *Acacia nilotica* is also known as Gum Arabic tree, Babul, Egyptian thorn, or Prickly Acacia is multipurpose nitrogen fixing tree legume. It occurs from sea level to over 2000 m and withstand at extreme temperature (>50^{o}C) and air dryness. It is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India.

Synonyms: ACAR11 - *Acaciaarabica* (Lam.) Willd, MINI2 - *Mimosanilotica* L.

Taxonomical classification: Kingdom - Plantae – Plants, Subkingdom *Tracheobionta*– Vascular plants, Superdivision - *Spermatophyta*– Seed plants, Division - *Magnoliophyta*– Flowering plants, Class - *Magnoliopsida*– Dicotyledons, Subclass - *Rosidae*, Order - *Fabales*, Family - *Fabaceae*– Pea family, Genus - *Acacia* Mill. – acacia, Species - *Acacianilotica* (L.) Willd. ex Delile – gum arabic tree.

Plant Description: *Acacia nilotica* is a single stemmed plant with a well-developed deep root system.

Height: The average height of the plant has been 15-18 m in height and 2-3 m in diameter.

Pods and Seeds: Pods are 7-15 cm long, green and tomentose (when immature) or greenish black (when mature), indehiscent, deeply constricted between the seed giving a necklace appearance. Seeds are 8-12 per pod, compressed, ovoid, dark brown shining with hard testa[18].

Leaves: The leaves are bipinnate, pinnate 3-10 pairs, 1.3- 3.8 cm long, leaflets 10-20 pairs, and 2-5mm long[19].

Flowers: Flowers are globular heads, 1.2-1.5 cm in diameter of a bright golden yellow color, develop either in axillary or whorl pattern on peduncles 2-3 cm long located at the end of branches[20].

Stem: Stems are usually dark to black colored, deep longitudinal fissured, grey-pinkish slash, exuding a reddish low quality gum[20].

Bark: The bark a tinge of orange and/or green (young tree), but older trees have dark, rough bark and tend to lose their thorns[21].

Thorns: Thorns are thin, straight, light grey exist in axillary pairs (usually 3-12), 5-7.5 cm long in young trees.

Root: Root is generally of brown color in older and whitish in younger regions.

Gum: The gum varies in color from very pale yellowish brown to dark reddish brown depending on the quantity of tannins in the sample. The lighter, more highly valued gums are soluble in water and very viscous; the tannins in the darker gum reduce the solubility. The gum has a moisture content of about 13% and is slightly dextrorotary[22].

Growth pattern and germination

Acacia nilotica is a tropical species found throughout India and occurs from sea-level to over 2000 m altitude. Prickly Acacia germinates in rainfall in the wet season. But some seeds may still germinate up to 15 years after seed drop. Seedlings grow rapidly near water but more slowly in open grasslands. It grows in average annual temperatures range from 15–28°C, being frost sensitive when young and withstanding daily maximum temperatures of 50°C[23]. The mean maximum temperature of the hottest month is 25–42ºC and the mean minimum temperature of the coldest month 6–23°C. Babul plant prefers dry conditions, with an annual rainfall of (100–) 250–1500(–2300) mm. This subspecies is commonly found on soils with high clay content, but may grow on deep sandy loam in areas of higher rainfall. It commonly grows close to waterways on seasonally flooded river flats and tolerates salinity well[24]. Trees can flower and fruit two to three years after germination, but after high rainfall it is more quickly, usually between March and June[25]. Pods are formed between July and December. Most leaf fall between June and November and seed pods drop during October to January[26]. Seeds are very simple. Inner integument degenerates completely and the testa is formed by the outer integument[27,28]. Meharia has observed that A. nilotica is more productive than A. tortilis after slat treatment. It grows well in two types of soils i.e. riverian alluvial soil and black cotton soil[29].

Distribution

The native distribution of *Acacia nilotica* includes much of Africa and the Indian subcontinent[16]. From the GRIN database, the native distribution includes[30]:

Africa: Algeria, Angola, Botswana, Egypt, Ethiopia, Gambia, Ghana, Guinea-Bissau, Kenya, Libya, Malawi, Mali, Mozambique, Niger, Nigeria, Senegal, Somalia, South Africa, Sudan, Tanzania, Togo, Uganda, Zambia, Zimbabwe
Asia: Iran, Iraq, Israel, Oman, Saudi Arabia, Syria, Yemen, India, Nepal, Pakistan.

In Bangladesh it is found in Bogra, Faridpur, Jessore, Kushtia, Pabna, Rajshahi also planted by the road sides and embankments throughout the country.

Table-1: Some common medicinal uses of different parts of *Acacianilotica*.

Part used	Uses
Root	The roots are used against cancers and/or tumors (of ear, eye, or testicles), tuberculosis and indurations of liver and spleen[31].
Leaf	Chemoprventive, anitmutagenic, anti bacterial, anticancer, astringent, anti microbial activity Tender leaves are used to treat diarrhea, Aphrodisiac, dressing of ulcers, anti-inflammatory and Alzheimer's diseases[32].
Gum	Astringent, emollient, liver tonic, antipyretic and antiasthmatic[33].
Stem bark	Anti bacterial, antioxidant, anti-mutagenic, cytotoxic bark is used as astringent, acrid cooling, styptic, emollient, anthelmintic, aphrodisiac, diuretic, expectorant, emetic, nutritive, in hemorrhage, wound ulcers, leprosy, leucoderma, small pox, skin diseases, biliousness, burning sensation, toothache, leucoderma, dysentery and seminal weakness. The trunk bark is used for cold, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leukoderma[34].
Seeds	Spasmogenic activity and antiplasmodial activity[35].
Pods	Anti hypertensive and antispasmodic, anti-diarrhoerial, astringent, anti-fertility and against HIV-1 PR, Inhibited HIV-1 induced cythopathogenicity, antiplatelet aggregatory activity and anti oxidant[36].

Figure-1: Flower of *Acacia nilotica*.

Figure-2: Bark of *Acacia nilotica.*

Figure-3: Whole tree of *Acacia nilotica.*

Materials for partitioning and extract preparation

Table-2: List of glass wares.

Materials	Source
Distilled machine	BDH Laboratory Equipments
Conical flasks (250 ml)	BDH Laboratory Equipments
Beakers (100 ml, 500 ml)	BDH Laboratory Equipments
Test tubes	BDH Laboratory Equipments
Funnels	BDH Laboratory Equipments
Measuring cylinders	BDH Laboratory Equipments
Pipettes	BDH Laboratory Equipments
Automatic pipette puller	Bel-Art Products, USA

Table-3: List of solvents.

Materials	Source
n-Hexane	Merck
Carbon tetrachloride (CCl_4)	Merck
Dichloromethane (CH_2Cl_2)	Merck
Ethyl acetate ($CH_2CH_3OOCCH_3$)	Merck
Methanol	Scharlau
Acetic acid	Merck
Ethanol	Merck
Distilled Water	-

Table-4: List of filter aid

Filter aids
Filter Paper (Whatman no. 1)
Normal Cotton

Table-5: List of equipments.

Equipments	Source
Rotary vacuum evaporator	-
Electronic balance	Denver Instruments M-220
Table-top UV detector (252 & 366 nm)	*CAMAG*
Grinding machine	-
Oven (0^0C-210^0C)	Gallen Kamp Hotbox
Solvent distillation plant	University Instruments Lab
Distilled water plant	University Instruments Lab

Collection and preparation of plant material

Plant sample (bark) of *Acacia nilotica* was collected from Pabna, Bangladesh in April 2012. Then proper identification of plant sample was done by an expert taxonomist. The bark was sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature for better grinding. The dried bark was then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Faculty of Pharmacy; University of Dhaka.

Extraction of the Plant Material

About 950 gm of the powdered material was taken in separate clean, round bottomed flask (4.5 liters) and soaked in 5 liter of methanol. The container with its content was sealed by cotton plug and aluminum foil and kept for a period of 21 days accompanying occasional shaking and stirring.

The whole mixture was then filtered through cotton followed by Whatman No.1 filter paper and the filtrate thus obtained was concentrated at 39ºC with a Heidolph rotary evaporation. The concentrated extract was then air dried to solid residue. The weight of the crude methanol extract obtained from the powdered whole plant was 22 gm.

Solvent-solvent partition of crude extract: Solvent-solvent partitioning of crude methanolic extract was done following Modified Kupchan Partition[37].

Preparation of mother solution

5 gm of methanol extract was triturated with 90 ml of methanol containing 10 ml of distilled water. The crude extract was dissolved completely. This was the mother solution which was partitioned off successively by four solvents of different polarity. In subsequent stages each of the fractions was analyzed separately for the detection and identification of compounds having antibacterial, cytotoxic, antioxidant and other pharmacological properties.

Partition with n-hexane

The mother solution was taken in a separating funnel. 100 ml of the n-hexane was added to it and the funnel was shaken and then kept undisturbed. The organic portion was collected. The process was repeated thrice (100 ml×3). The n-hexane fraction was then air dried for solid residue.

Partition with carbon tetrachloride

To the mother solution left after partitioning with n-hexane; 12.5 ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with carbon tetrachloride (CCl_4). The process was repeated thrice (100 ml×3). The carbon tetrachloride fraction was then air dried for solid residue. The aqueous fraction was preserved for the next step.

Partition with dichloromethane

To the mother solution that was left after partitioning with petroleum ether and carbon tetrachloride; 16 ml of distilled water was added and mixed uniformly. The mother solution was then taken in a separating funnel and extracted with dichloromethane (CH_2Cl_2) (100ml×3). The dichloromethane soluble fractions were collected together and air dried. The aqueous fraction was preserved for the next step.

Partition with ethyl acetate

To the mother solution that was left after washing with petroleum ether, carbon tetrachloride and dichloromethane; was then taken in a separating funnel and extracted with ethyl acetate (100 ml×3). The ethyl acetate soluble fractions were collected together and air dried.

After evaporation the weight of the different fractions obtained are as follows:

Table-6: Amount of fractions after fractionation of crude methanolic extract.

Fraction	**Weight**
n-Hexane soluble fraction	0.50 g
Carbon tetrachloride soluble fraction	0.90 g
Dichloromethane soluble fraction	1.25 g
Ethyl acetate soluble fraction	1.80 g

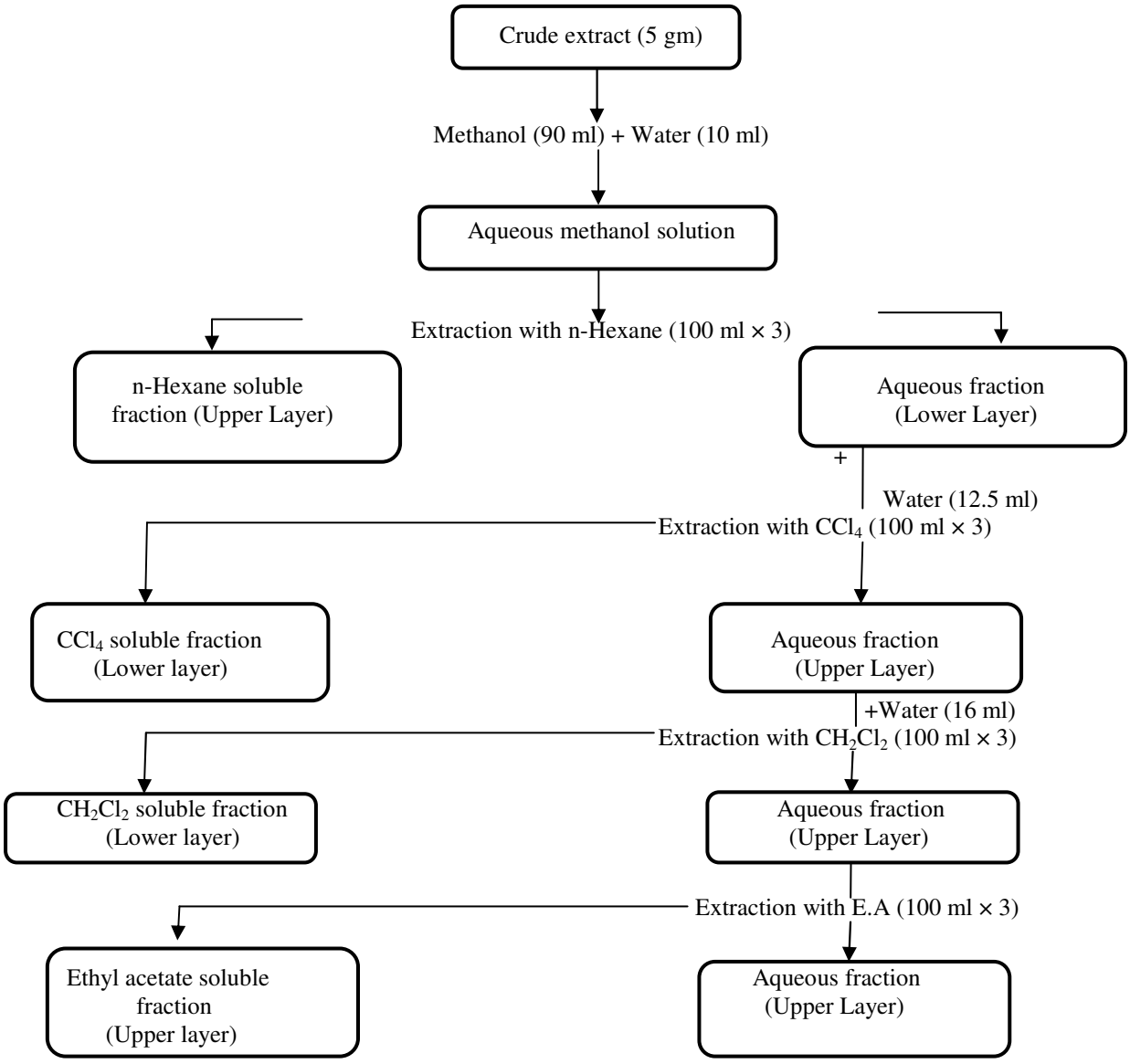

Figure-4: Schematic representation of the modified Kupchan Partitioning of methanolic crude extract of *Acacia nilotica.*

Principle of DPPH method

The *in vitro* free radical scavenging activity of *Acacia nilotica* (bark) was carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH) by the method of Brand-Williams *et al.*[38]. 2.0 ml of a methanol solution of the extract at different concentration were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of *tert*-butyl-1-hydroxytoluene (BHT) by UV spectrophotometer.

The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The olor turns from purple to yellow as the molar absorption of the DPPH radical at 517 nm reduces when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH (DPPH-H). DPPH radical scavenging activity is described as IC_{50} which is the concentration of samples to produce 50% reduction of the DPPH.

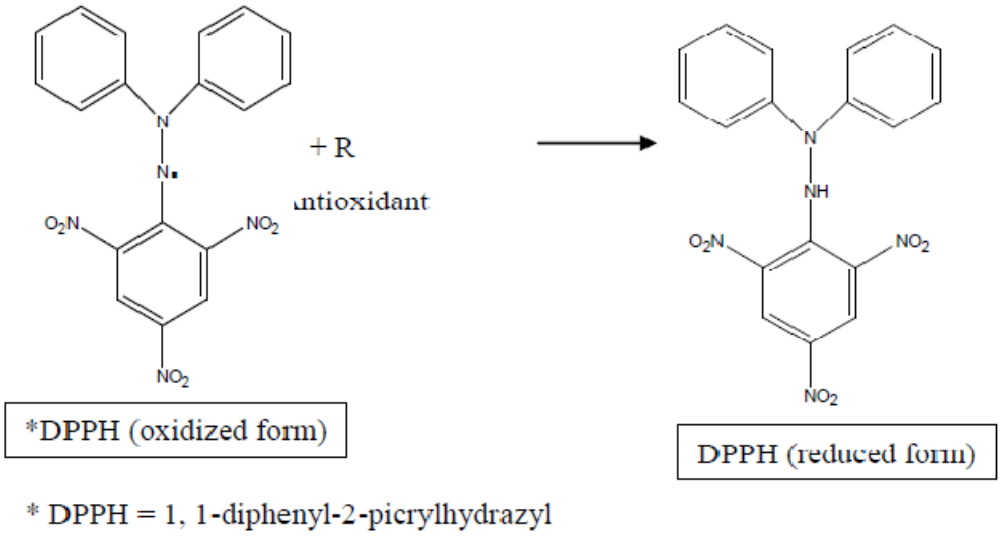

Figure-5: Mechanism of free radical scavenging activity. Evaluation of antioxidant activity of crude extracts and different fractions of stem bark of *Acacia nilotica.*

Preparation of test samples

All the test samples (2mg) were taken and dissolved in 200 µl of distilled methanol in vials to get stock solutions. Then 100 µl of solution was taken in test tube each containing 2ml of distilled methanol. Thus, final concentration of the prepared solution in the first test tube was 500 µg/ml. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. In each case 100 µl sample was added to test tube and fresh 100µl methanol was added to vial. Thus the concentrations of the obtained solution in each test tube were 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.625 µg/ml, 7.8125 µg/ml, 3.90625 µg/ml, 1.9531µg/ml and 0.9766 µg/ml for 10 dilutions.

Preparation of control sample

Control groups are used in antioxidant study to validate the test method and ensure that the results obtained are only due to the activity of the test agent and the effects of the other possible factors are nullified. Usually two types of control groups are used - i. Positive control, ii. Negative control

Preparation of positive control sample

Positive control in an antioxidant study is a widely accepted antioxidant agent and the result of the test agent is compared with the result obtained for the positive control. In the present study *tert*-butyl-1-hydroxytoluene (BHT) is used as the positive control. Measured amount 2 mg of the BHT is dissolved in 200 µl methanol to get an initial concentration of 500 µg/ml from which serial dilutions are made using methanol to get concentrations of 250µg/ml, 125µg/ml, 62.5µg/ml, 31.25µg/ml, 15.625µg/ml, 7.8125µg/ml, 3.90625µg/ml, 1.9531µg/ml and 0.9766 µg/ml for 10 dilutions.

Preparation of negative control sample

80 mg of DPPH was weighed and dissolved in 200 ml methanol to make 0.004% (w/v) solution. To dissolve homogeneously magnetic stirrer was used.

Table-7: Test samples used in the evaluation of antioxidant activity of crude extract and different fractions of bark of *Acacia nilotica.*

Test samples	Purpose	Measured Amount
DPPH in methanol	Negative Control Group	80 mg in 200 ml
BHT	Positive Control Group	2mg in 200 µl
BEAN	Test sample	2mg in 200 µl
DCMAN	Test sample	2mg in 200 µl
CTAN	Test sample	2mg in 200 µl
HXAN	Test sample	2mg in 200 µl
EAAN	Test sample	2mg in 200 µl

BE = Bark Extract, DCM= Dichloro Methane, CT= Carbon tetrachloride EA = Ethyl acetate fraction of *Acacia nilotica.*

Procedure: i. At first 11 test tubes were taken for each sample to make aliquots of 10 concentrations, one being kept as negative control. ii. Plant extracts and BHT were weighed 3 times and dissolved in methanol to make the required concentrations by dilution technique. iii. DPPH was weighed and dissolved in ethanol to make 0.004% (w/v) solution. To dissolve homogeneously magnetic stirrer was used. iv. DPPH solution was kept in dark place. v. After making the desired

concentrations 3 ml of 0.004% DPPH solution was applied on each test tube by pipette which contained 1ml of stock solution of desired concentration. vi. The test tubes were stirred well on a magnetic stirrer to complete the reaction. vii. The test tubes were covered with foil and then kept in 30 minutes incubation period. viii. After 30 min incubation period at room temperature the absorbance was read against negative control at 517 nm. ix. Inhibition free radical DPPH in percent (I%) was calculated as follows: (I%) = (1 – A_{sample}/A_{blank}) X 100, where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material). x. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration. xi. The result was compared to that of the positive control. xii. The test was carried out in triplicate and average value was taken.

Result of test sample

The methanolic extract and it's different partitionate of bark of *Acacia nilotica* were subjected to free radical scavenging activity using ter-butyl-1-hydroxytoluene (BHT) was used as reference standard. The results of the assay are presented in Table-8 to 14 and graphically represented in Figure-6 to 12.

Table-8: IC_{50} value of ter-butyl-1-hydroxytoluene (BHT)

SL	Absorbance of blank	Concentration (µg/ml)	Absorbance of sample	%inhibition	IC_{50}
1	0.284	500	0.016	94.37	33.69
2		250	0.067	76.41	
3		125	0.098	65.49	
4		62.5	0.134	52.82	
5		31.25	0.16	43.66	
6		15.625	0.174	38.73	
7		7.813	0.207	27.11	
8		3.906	0.224	21.13	
9		1.953	0.239	15.85	
10		0.977	0.257	9.51	

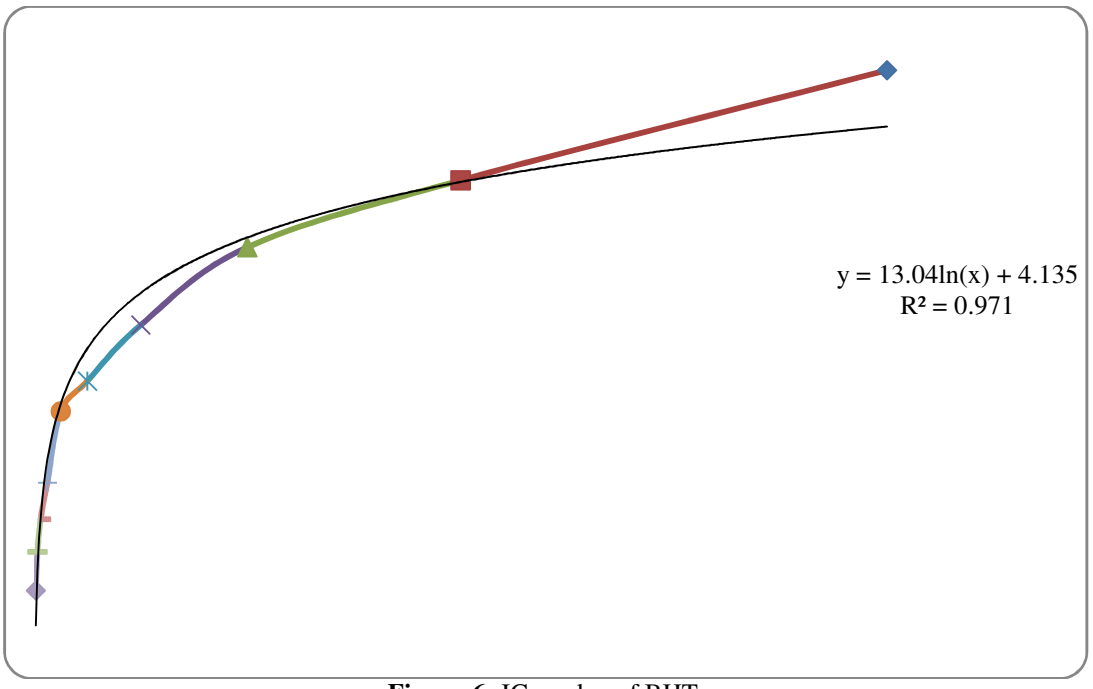

Figure-6: IC_{50} value of BHT.

Table-9: IC_{50} value of Methanolic crude extract of *Acacia nilotica bark (MEAN)*.

SL	Absorbance of blank	Concentration (µg/ml)	Absorbance of sample	%inhibition	IC_{50}
1	0.284	500	0.131	53.87	136.79
2		250	0.134	52.82	
3		125	0.141	50.35	
4		62.5	0.151	46.83	
5		31.25	0.16	43.66	
6		15.625	0.184	35.21	
7		7.813	0.194	31.69	
8		3.906	0.146	48.59	
9		1.953	0.221	22.18	
10		0.977	0.243	14.44	

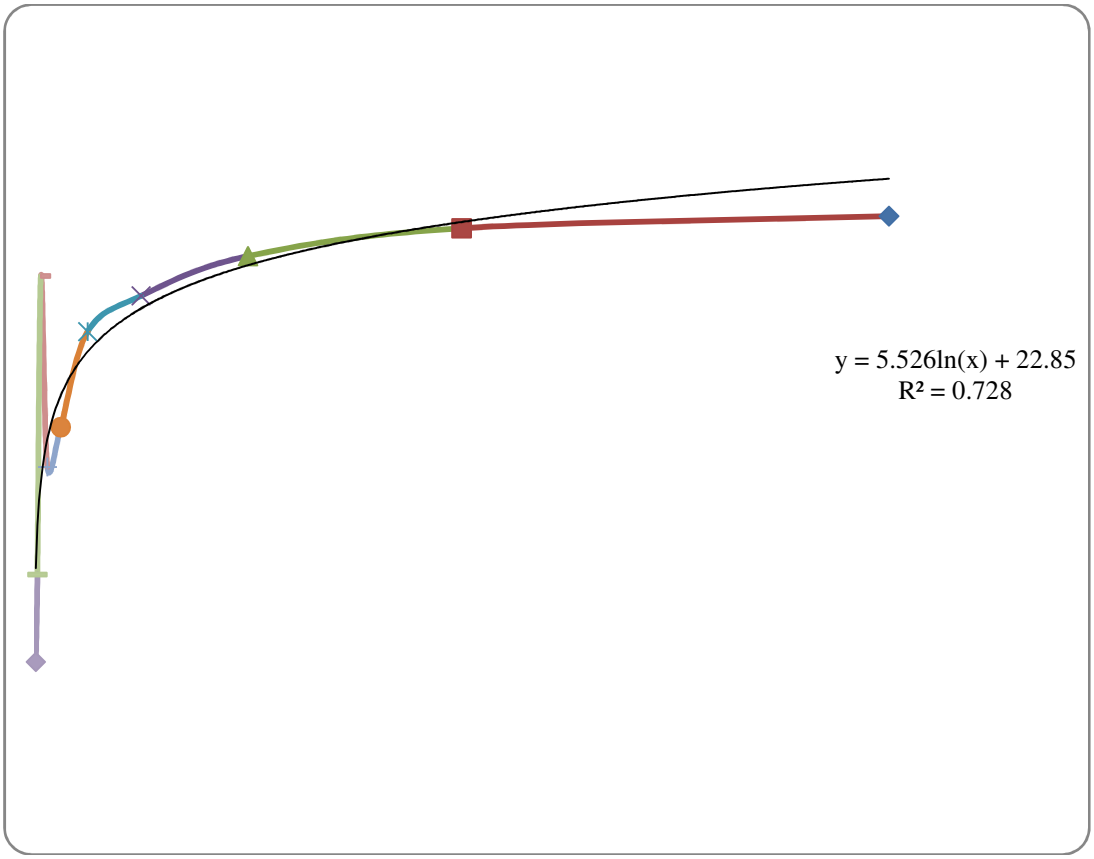

Figure-7: IC_{50} value of methanolic extract of *Acacia nilotica.*

Table-10: IC_{50} value of carbon tetrachloride fraction of *Acacia nilotica bark (*CTAN).

SL	Absorbance of blank	Concentration (µg/ml)	Absorbance of sample	%inhibition	IC_{50}
1	0.284	500	0.188	33.80	11388.48
2		250	0.195	31.34	
3		125	0.208	26.76	
4		62.5	0.218	23.24	
5		31.25	0.223	21.48	
6		15.625	0.234	17.61	
7		7.813	0.247	13.03	
8		3.906	0.256	9.86	
9		1.953	0.267	5.99	
10		0.977	0.276	2.82	

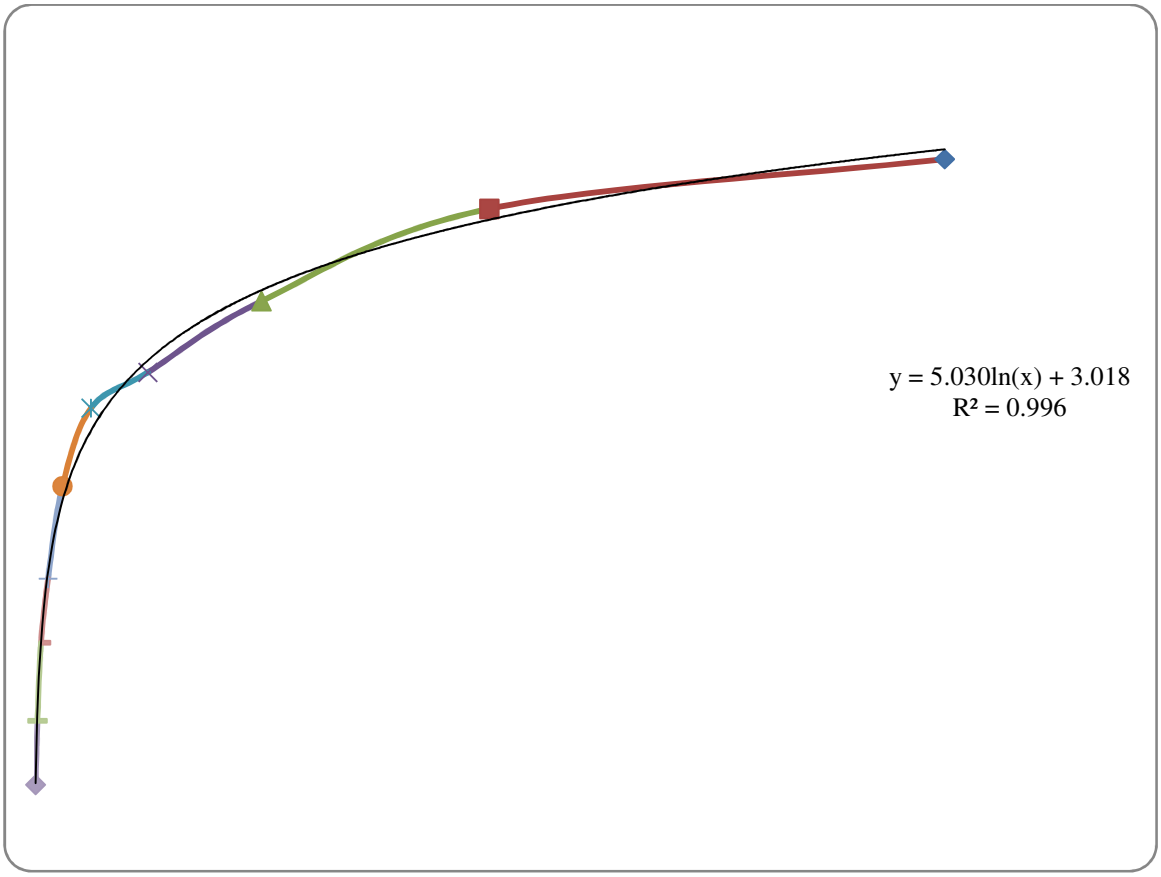

Figure-8: IC_{50} value of CTAN.

Table-11: IC_{50} value of dichloromethane fraction of *Acacia nilotica bark (*DMAN).

SL	Absorbance of blank	Concentration (µg/ml)	Absorbance of sample	%inhibition	IC_{50}
1	0.284	500	0.135	52.46	157.41
2		250	0.138	51.41	
3		125	0.145	48.94	
4		62.5	0.155	45.42	
5		31.25	0.164	42.25	
6		15.625	0.188	33.80	
7		7.813	0.198	47.18	
8		3.906	0.15	20.77	
9		1.953	0.225	13.03	
10		0.977	0.247	13.03	

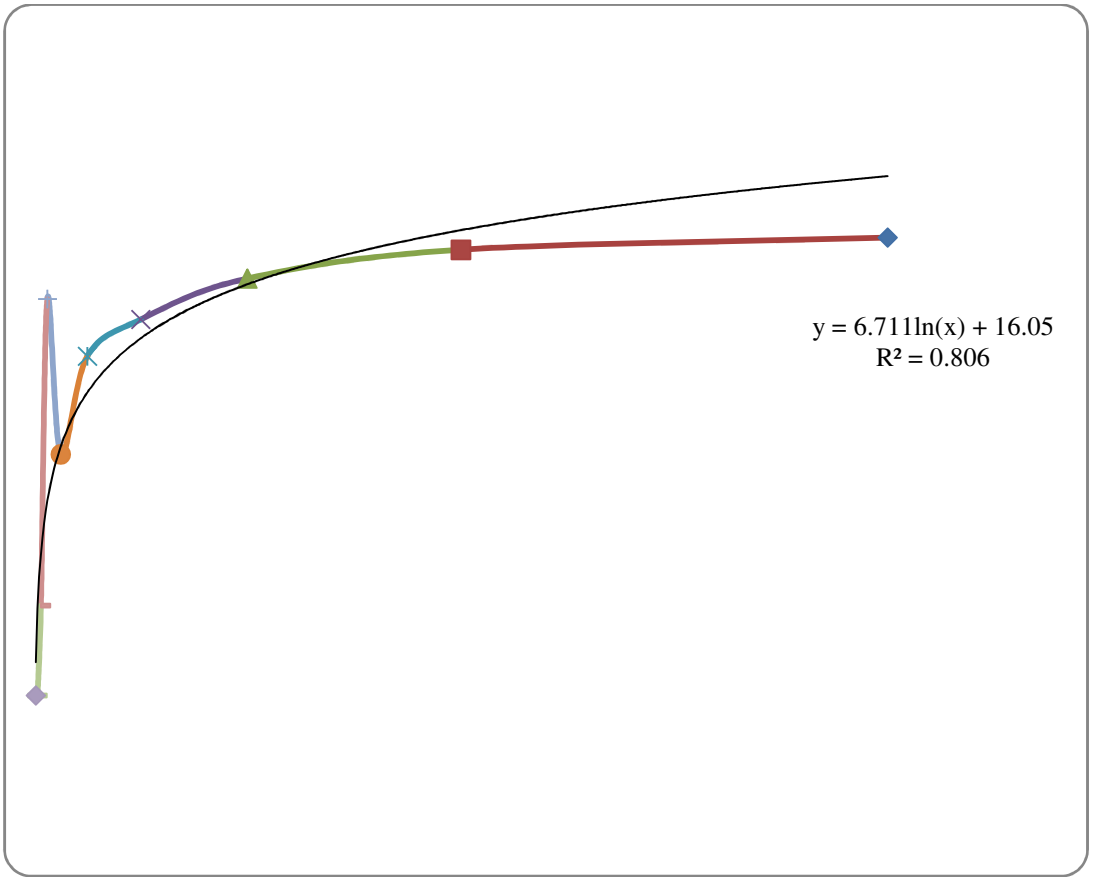

Figure-9: IC_{50} value of DMAN.

Table-12: IC_{50} value of ethyl acetate fraction of *Acacia nilotica bark* (EAAN).

SL	Absorbance of blank	Concentration (µg/ml)	Absorbance of sample	%inhibition	IC_{50}
1	0.284	500	0.153	46.13	620.42
2		250	0.159	44.01	
3		125	0.166	41.55	
4		62.5	0.176	38.03	
5		31.25	0.19	33.10	
6		15.625	0.199	29.93	
7		7.813	0.213	25.00	
8		3.906	0.225	20.77	
9		1.953	0.239	15.85	
10		0.977	0.257	9.51	

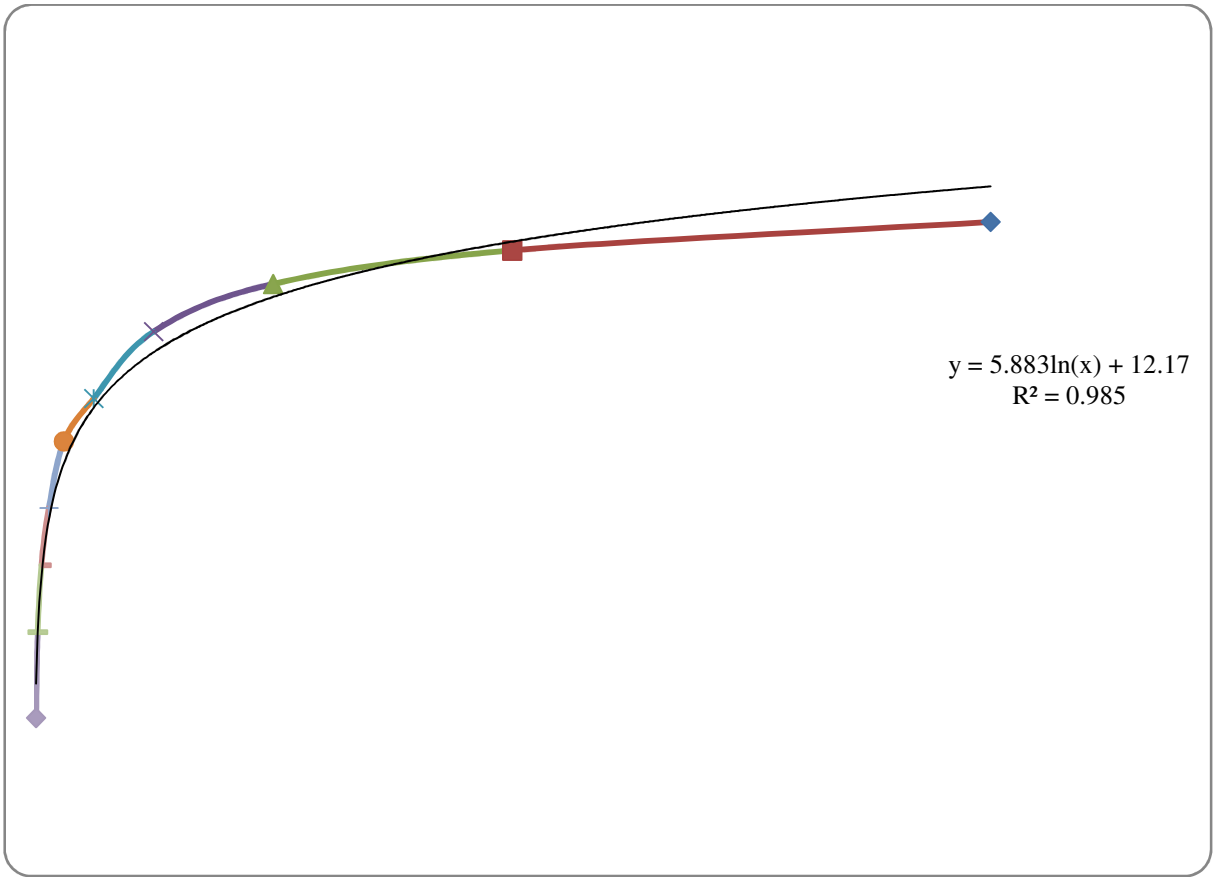

Figure-10: IC_{50} value of EAAN.

Table-13: IC_{50} value of hexane fraction of *Acacia nilotica bark (*HXAN).

Sl	Absorbance of blank	Concentration (µg/ml)	Absorbance of sample	%inhibition	IC_{50}
1	0.284	500	0.174	38.73	11388.48
2		250	0.185	34.86	
3		125	0.192	32.39	
4		62.5	0.203	28.52	
5		31.25	0.215	24.30	
6		15.625	0.225	20.77	
7		7.813	0.251	11.62	
8		3.906	0.256	9.86	
9		1.953	0.267	5.99	
10		0.977	0.281	1.06	

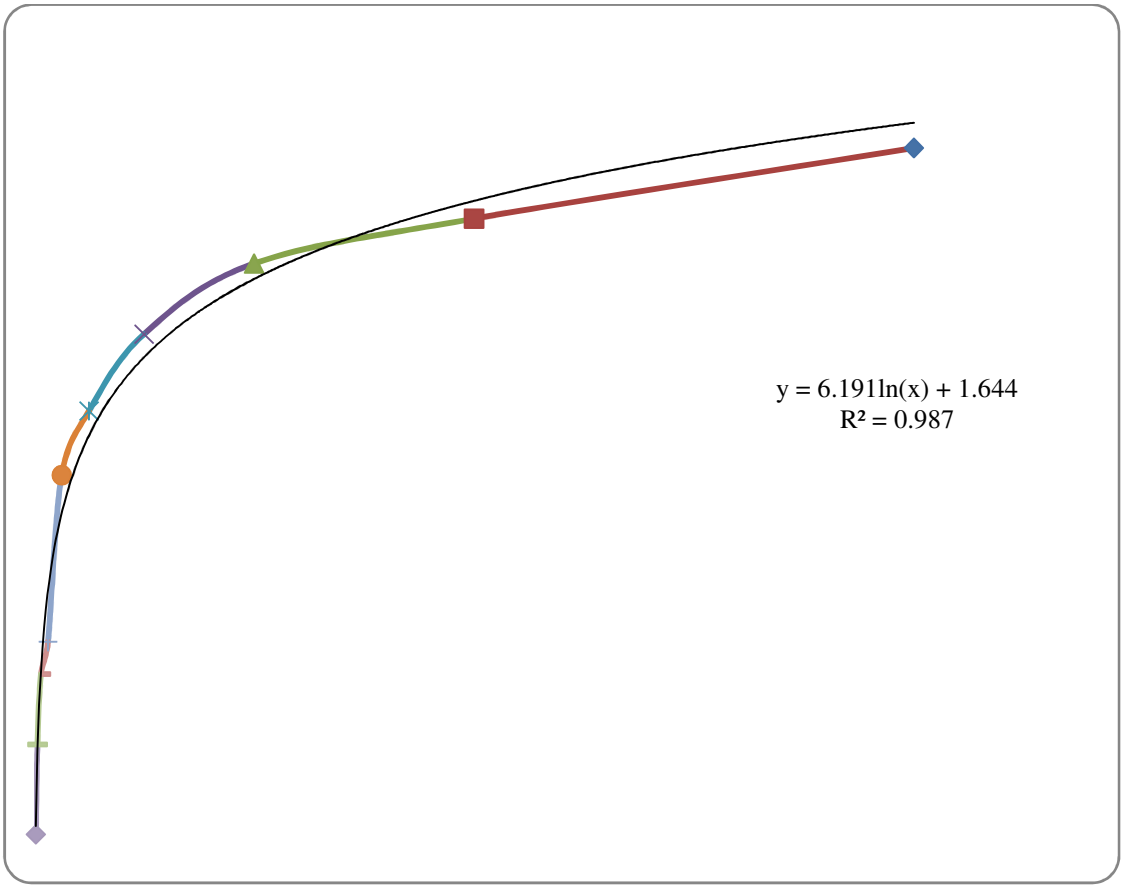

Figure-11: IC_{50} value of HXAN.

Table-14: IC_{50} values of the standard and different fractions of bark of *Acacia nilotica.*

Code	Sample	IC_{50}
BHT	Ter-butyl-1-hydroxytolune (STD)	33.69
MEAN	Methanolic extract of bark of Acacia nilotica	136.79
CTAN	CCL4	11388.48
DMAN	Dichloromethane fraction	157.41
EAAN	Ethyl acetate fraction	620.41
HXAN	Hexane fraction	11388.48

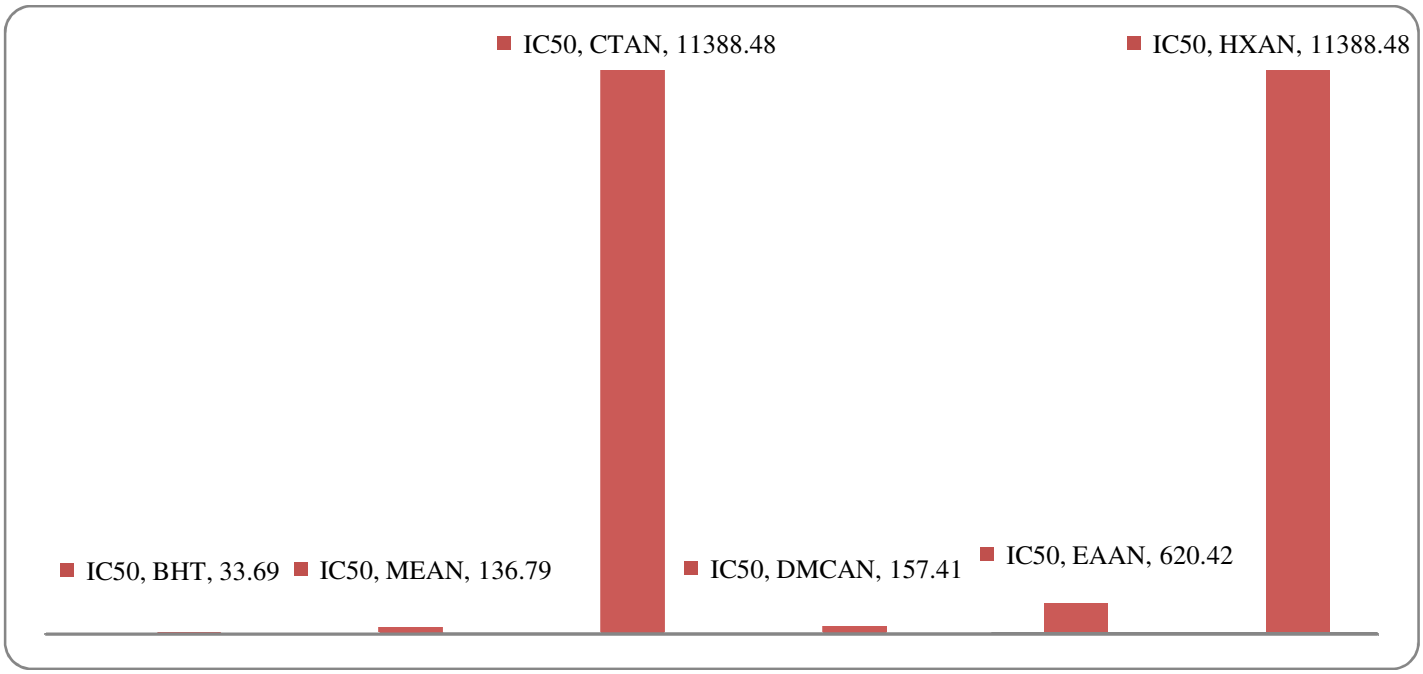

Figure-12: IC_{50} values of the standard and different fractions of bark of *Acacia nilotica* bark.

Discussion: DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of anti-oxidant, is a direct and reliable method for determining radical scavenging action. BHT was chosen as the reference for the absorbance at 515 – 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The IC_{50} values of the methanolic extract and its fractions have been furnished in the Table-14. Highest scavenging was observed with methanolic crude extract with an IC_{50} value of 136.79 micro gram/ml as opposed to the IC_{50} value of BHT 33.69 micro gram/ml, which is a well-known antioxidant. Scavenging of DPPH radical was found to rise with increasing concentration of the extract.

It has been determined that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins and phenolic terpenes.

The antioxidant activity of phenolic compounds is due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxide.

Conclusion

From the DPPH assay, it was observed that the Methanolic crude extract (136.79 µg/ml) and Dichloromethane (157.41 µg/ml) fractions of Acacia nilotica have shown moderate free radical scavenging activity compared to standard BHT (33.69 µg/ml); whereas Carbon tetrachloride (11388.48 µg/ml), Ethyl acetate (620.41 µg/ml) and Hexane (11388.48 µg/ml) fraction has no free radical scavenging activity compared to BHT (31.21 µg/ml).

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