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Cytotoxicity studies and antioxidant potential of *Acacia nilotica* roots

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Abstract

The antioxidant activity and cytotoxicity of *Acacia nilotica* roots extracts were evaluated in present reaesrch work. The antioxidant activity was evaluated by the measurement of total phenolic contents (TPC), total flavonoid contents (TFC), reducing power, DPPH radical scavenging activity and antioxidant activity in linolieic acid oxidation. The tested plant material contained the TPC (1.47-6.61 GAE mg/g of dry matter), TFC (2.31-6.42 CE mg/g of dry matter), DPPH radical scavenging activity the IC_{50} value (10.53-70.23) and % inhibition of linoleic acid oxidation (33.8-86.61%). Furthermore the antioxidant effectiveness of extracts and fractions were assessed using corn oil (CO) as the oxidation substrate. The oxidative alterations were evaluated by analysis of conjugated dienes (CD), conjugated trienes (CT), *p*-anisidine, free fatty acid (FFA) and peroxide (PO) values. The plant extract and fraction were assessed against human blood erythrocytes (RBCs) for cytotoxic studies by haemolytic activity and the percentage lysis was found to be in the range of 1.27-3.59%. It was concluded that plant roots may be used as a potential source of antioxidant agents.

Key words: *Acacia nilotica*, Antioxidants, Scavenging, Inhibition

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1. Introduction

The use of herbs in treatment of animal and human diseases has long been established. Most plant extracts are shown to have anti-microbial agents against micro organisms *in vitro* [1]. *Acacia nilotica* has been reported to be very useful in treating diarrhea and cough in human [2]. *Acacia* is a pantropical and subtropical genus with species abundant throughout Australia, Asia, Africa and America [3]. It belongs to the family Fabaceae. Source of natural antioxidants are primarily, plant phenolics that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and bark [4-5] . A lots of these antioxidant compounds have anti-inflammatory, atherosclerotic, anti-tumor, anti-carcinogenic, anti-bacterial or anti-viral activities to a greater or lesser extent [6]. *A. nilotica* is reported tohave habitual medicinal uses such as appetiteenhancer, strength and nutrient supplement, for sorejoints, stomach ache and clear out circumcision wounds.Antioxidants have been implicated in many diseases, malaria, cardiovascular disease, gastric ulcer, diabetes, malignant tumors, rheumatic joint inflammation, cataracts disease [7-8]. Natural antioxidants such as flavonoids, phenolics, tannins, curcumin and terpenoids are found in this plant [9]. Previously various authors have evaluated nutritional, antioxidant and biological studies of various plants of Pakistan [10-14]. As per our knowledge no literature has been available regarding the use of different polarity based solvents for the evaluation of *Acacia nilotica* roots for antioxidant and cytotoxicity studies.

2. Materials and methods

2.1. Plant material

The roots of the plant *Acacia nilotica* were collected from botanical garden University of Agriculture, Faisalabad (Pakistan) and were further identified by Taxonomist of Botany Department, University of Agriculture, Faisalabad.

2.2. Extraction of plant material

The plant roots were washed with distilled water and then shade dried. The grinded fine powder (1kg) of plant roots was extracted with petroleum ether at room temperature. After filtering the extract was concentrated through rotary vacuum evaporator. This process was repeated thrice to obtain a (24.7g) sufficient quantity of Petroleum ether extract. The remaining plant residue was

further extracted with other different polarity based solvents and obtained successively Petroleum ether: Chloroform (1:1) (35g), Chloroform (22.5g), Chloroform: ethyl acetate (1:1) (23g), Ethyl acetate (72g), Ethanol (39g), absolute methanol extracts (78g).

2.3. Phytochemical analysis

Powdered roots of plant were subjected to qualitative and quantitative phytochemical screening for the presence of alkaloids, tannins, saponins, steroids, using standard phytochemical procedures described by [15]. The different assays were employed for evaluation of antioxidant activity of plant. The total phenolic contents were calculated using the Folin–Ciocalteu reagent method as already reported [16] The total flavonoid contents were determined by procedure as described [17-18].

2.4. Antioxidant activity

The reducing power of plant was determined according to the procedure described [18-19]. The 1,1-diphenyl-1-picrylhydrazyl radical (DPPH) assay was carried out spectrophotometrically as described by [20-21]. % inhibition by linoleic acid peroxidation of sample extract was determined the method described [22-23].

2.5. Cytotoxicity studies

The cytotoxicity studies was carried out by haemolytic activity assay as method described by [24].

2.6. Determination of antioxidant activity using crude corn oil as oxidation substrate

The crude concentrated extract of *A. nilotica* was independently added into the refined, bleached and deodorizer corn oil at concentration of 300 ppm. The oil sample was stirred for 30 minutes at 50°C for uniform mixing. Control oil (without antioxidant) and stabilized corn oil samples (100 mL) were placed in dark brown airtight glass bottles with narrow necks and subjected to accelerated storage in an electric hot air oven (IM-30, Irmeco, Germany) at 60 °C for 28 days.All oil samples were examined in triplicate. A corn oil sample, without antioxidant, was used as the control. Oil samples were analyzed after every seven days interval.

2.7. Stabilization of corn oil

The oxidation of the corn oil was determined by the peroxide value (PV), free fatty acid (FFA), conjugated trienes (CT), conjugated dienes (CD) and *para*-anisidine values assays. The PV and FFA of the stabilized and control sunflower oil samples were measured following the AOCS Official Method Cd 8-53 and F 9a-44, respectively [25]. *para*-Anisidine value was determined following the IUPAC method II.D.26 [26]. The conjugated dienes and trienes were analyzed by following the IUPAC Method II.D.23 [26]. The absorbance was noted at 232 and 268 nm, respectively.

2.8. Statistical analysis

All the experiments were conducted in triplicate unless stated otherwise and statistical analysis of the data was performed by analysis of variance (ANOVA), using STATISTICA 5.5 (Stat Soft Inc, Tulsa, Oklahoma, USA) software. A probability value of difference $p \leq 0.05$ was considered to denote a statistically significance All data were presented as mean values ± standard deviation (SD).

3. Results and Discussion

3.1. Yield of extract

The yield of plant root extracts was in the range of 22.25 to 78 g/Kg of dry plant. The maximum yieldwas observed with methanol while minimum with petroleum ether and chloroform. The yield of extract in methanol, ethanol, ethyl acetate, ethyl acetate: chloroform (1:1), petroleum-ether: chloroform (1:1), petroleum-ether and chloroform was found to be 78, 39, 72, 24, 35, 23 and 22.5 g/Kg respectively. No earlier reports have been available for comparison of yield by different polarity of solvents. The extraction yield depends on extraction solvent and also on the chemical nature of the sample. As per literature review the solvent used and the nature of sample are the two most important factors for the extraction of plant material [27].

3.2. Phytochemical studies

The qualitative phytochemical analysis of plant indicated the presence of alkaloids, tannins, flavonoids, saponins, terpenoids. As per earlier reports the *Acacia nilotica*contains tannins and alkaloids reported by [28].The existence of terpenoids, alkaloids, saponins and glycoside was also reported by [29]. Total phenolic, total flavoniod contents and antioxidants activities of all the tests are documented in table 1. Total phenolic contents (TPC) obtained from the plant rootswere in the range of 1.47 to 6.61 to GAE mg/g. The maximum yield of TPC obtained from methanolic extract and lowest in petroleum ether. The amounts of TPC in methanol, ethanol, ethyl acetate, ethyl acetate: chloroform (1:1), petroleum-ether: chloroform (1:1), petroleum ether and chloroform was found to be 6.61, 5.51, 5.01, 2.32, 3.52, 1.47 and 2.25 GAE mg/g respectively. As per earlier reports the total phenolics content were found to be in 4.45, 12.7 and 16.7 GAE mg/g in ethyl acetate, methanol and ethanol extracts of plants [17] the results are somewhat comparable with our results. Total flavonoid contents of plant rootextracts were ranged from 2.01 to 6.42 CE mg/g respectively. The TFC obtained in methanol was found to be the highest and lowest TFC obtained in ethyl acetate: chloroform (1:1). The amount of TFC in methanol, ethanol, ethyl acetate, ethyl acetate:chloroform (1:1), petroleum ether: chloroform (1:1), petroleum ether and chloroform was found to be 6.42, 4.51, 4.02, 2.01, 3.01, 2.47 and 3.25 CE mg/g of dry extract respectively. Furthermore Shahidi and Wanasundara reported that phenolics contents found in plants behave as antioxidants, due to the reactivity of the phenolics functional group [30]. As per earlier reports the polyphenolic constituents have inhibitory effects on carcinogenesis in human beings [31]. Consequently the antioxidant activities of plant and herb extracts are often explained by their total phenolic contents and total flavonoids content.

3.3. Antioxidant activity

The antioxidant activity was evaluated by DPPH scavenging assay. The IC_{50} values of extracts ranged from 53.12 to 70.23 µg/mL (Table 1). We found IC_{50} values in

Table 1: The total phenolic contents (TPC), total flavonoid contents (TFC) and antioxidant potential of *Acacia nilotica* roots

Samples	TPC (GAE mg/g)	TFC (CE mg/g)	DPPH(IC_{50}) (µg/mL)	% Inhibition of oxidation in linoleic acid
Petroleum ether	1.47±0.01	1.07±0.01	70.23±0.91	33.8±0.19
Chloroform	2.25±0.02	3.25±0.01	65.25±0.71	38.91±0.38
Methanol	6.61±0.06	6.42±0.04	53.12±0.81	66.35±0.49
Ethanol	5.51±0.05	4.51±0.03	59.12±0.60	51.23±0.48
Ethyl acetate	5.01±0.03	4.02±0.06	55.25±0.50	45.82±0.44
Ethyl acetate:Chloroform (1:1)	2.32±0.02	2.01±0.03	69.52±0.72	48.51±0.42
Petroleum ether:Chloroform (1:1)	3.52±0.03	3.01±0.03	60.12±0.70	45.11±0.45
BHT	-	-	10.53±0.21	86.61±0.52

The results are the average of triplicate samples (n=3) ± S.D., ($p < 0.05$)

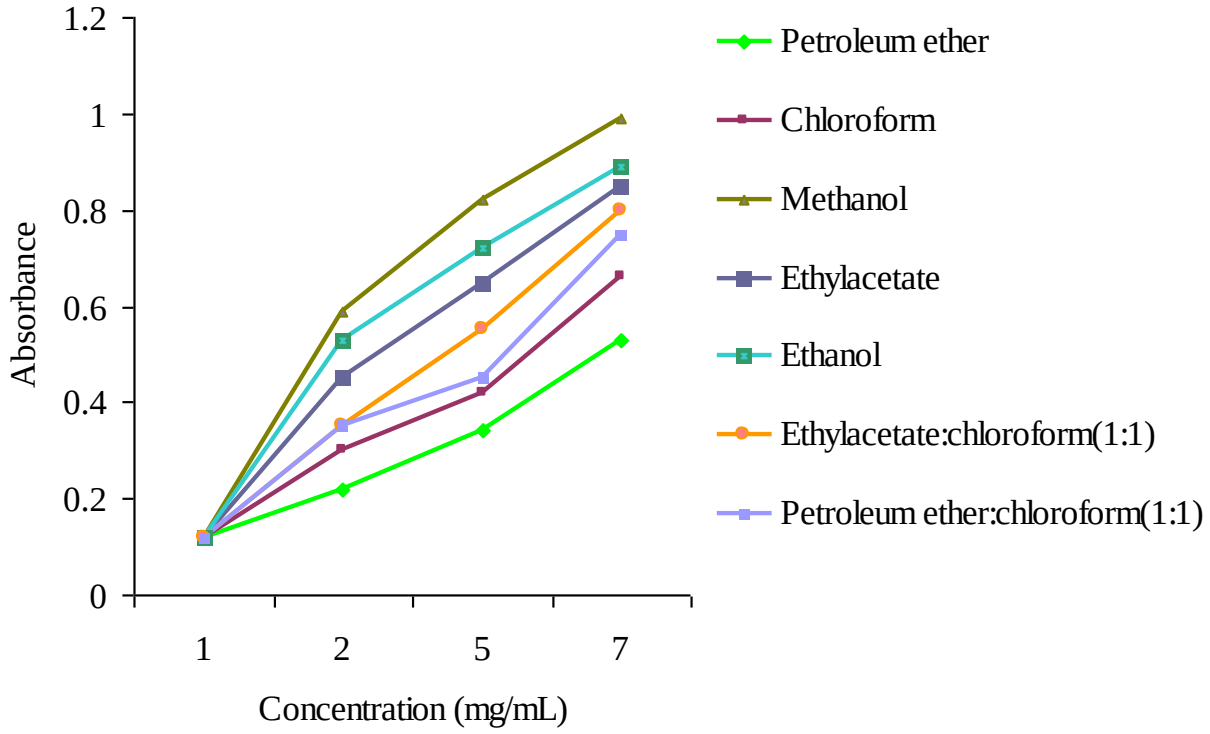

Fig. 1. Reducing power values of *Acacia nilotica* root extracts at different concentration

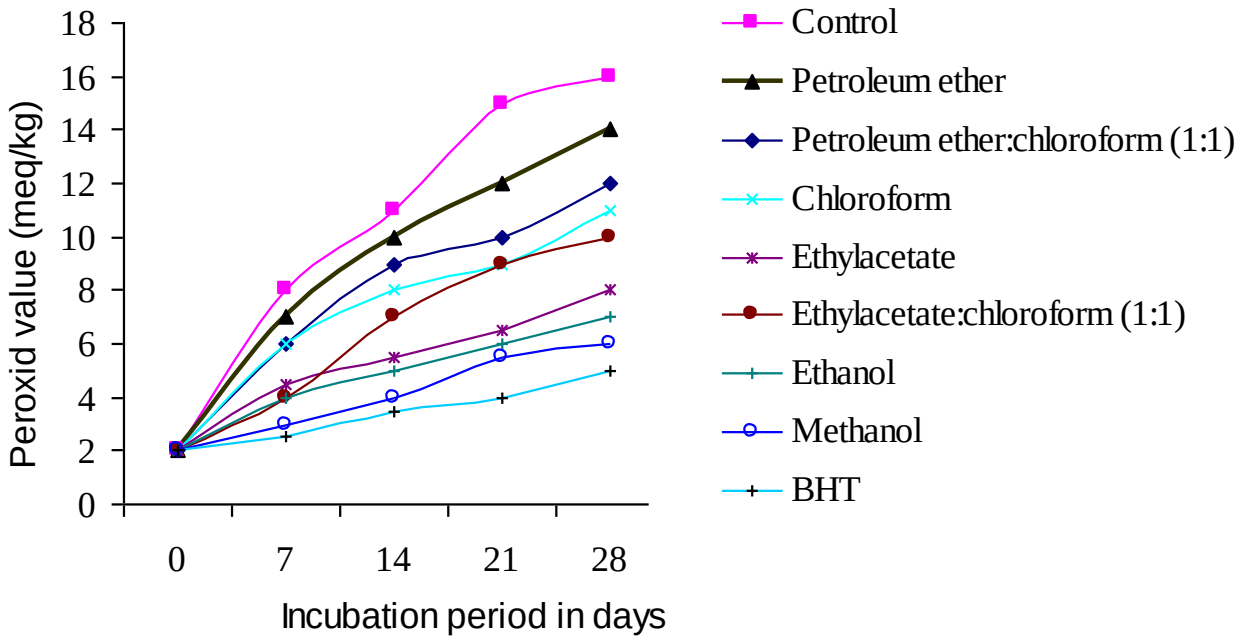

Fig. 2. Peroxide values of corn oil stabilized with *Acacia nilotica* root extracts

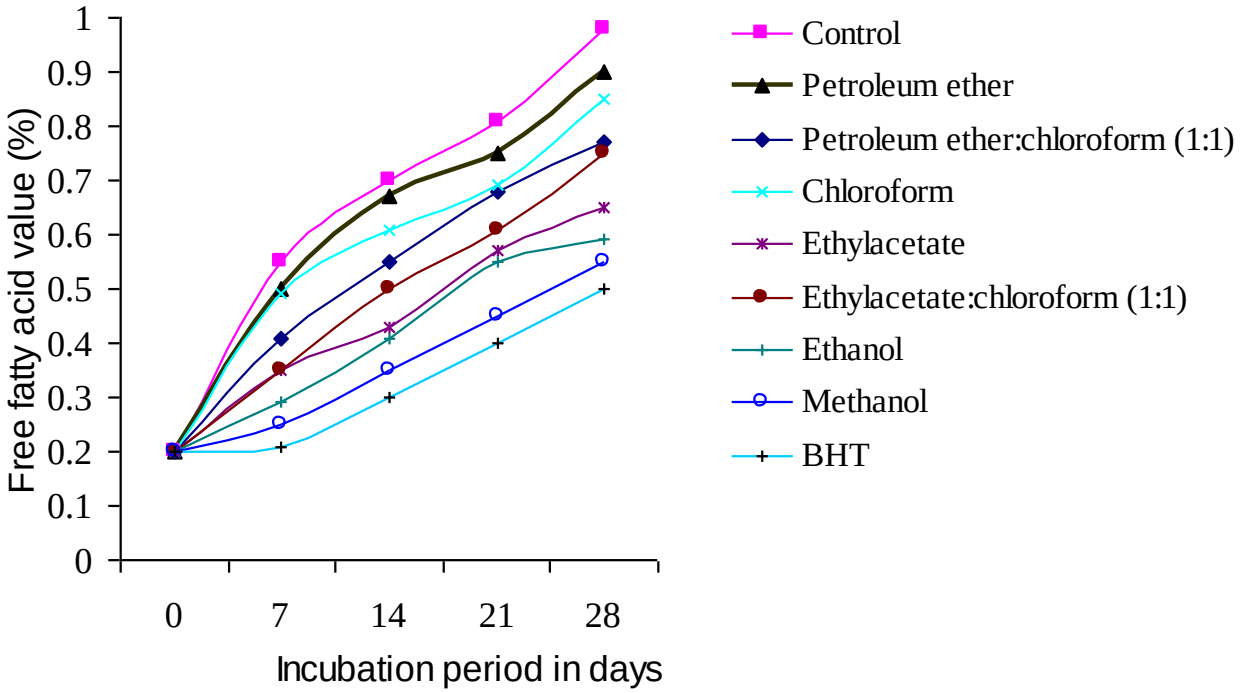

Fig. 3. Free fatty acid values of corn oil stabilized with *Acacia nilotica* root extracts

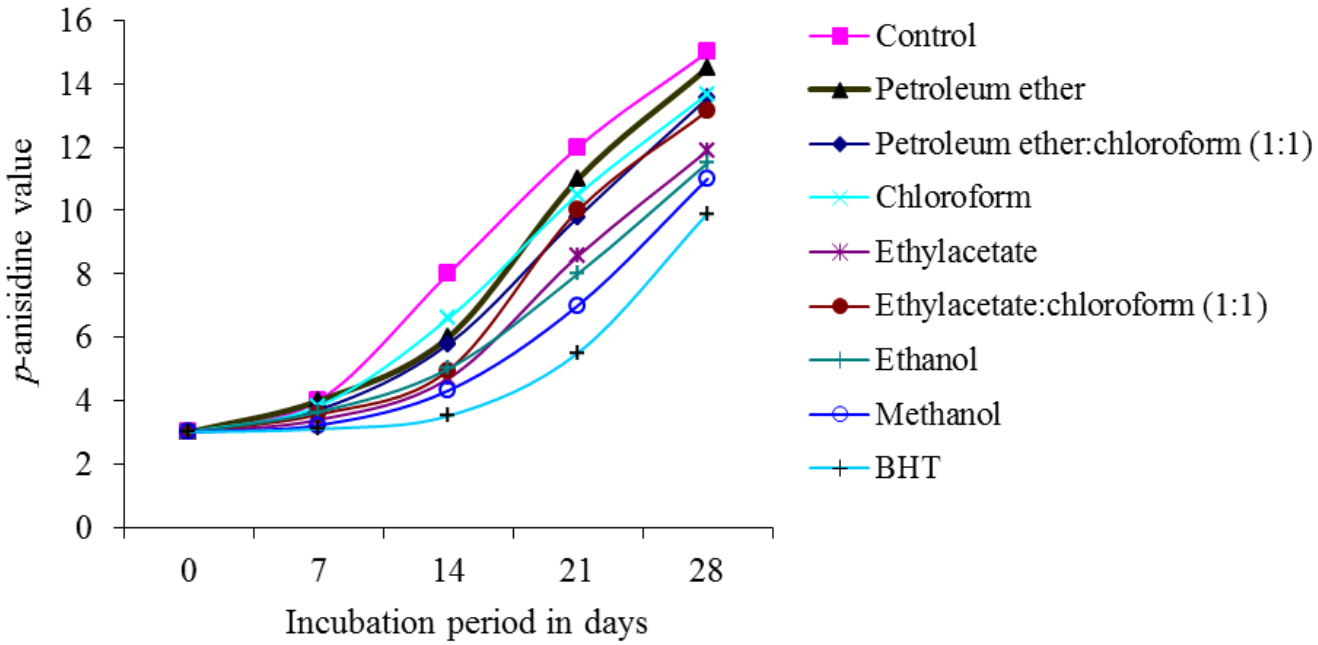

Fig. 4. *p*-ancidene values of corn oil stabilized with *Acacia nilotica* root extracts

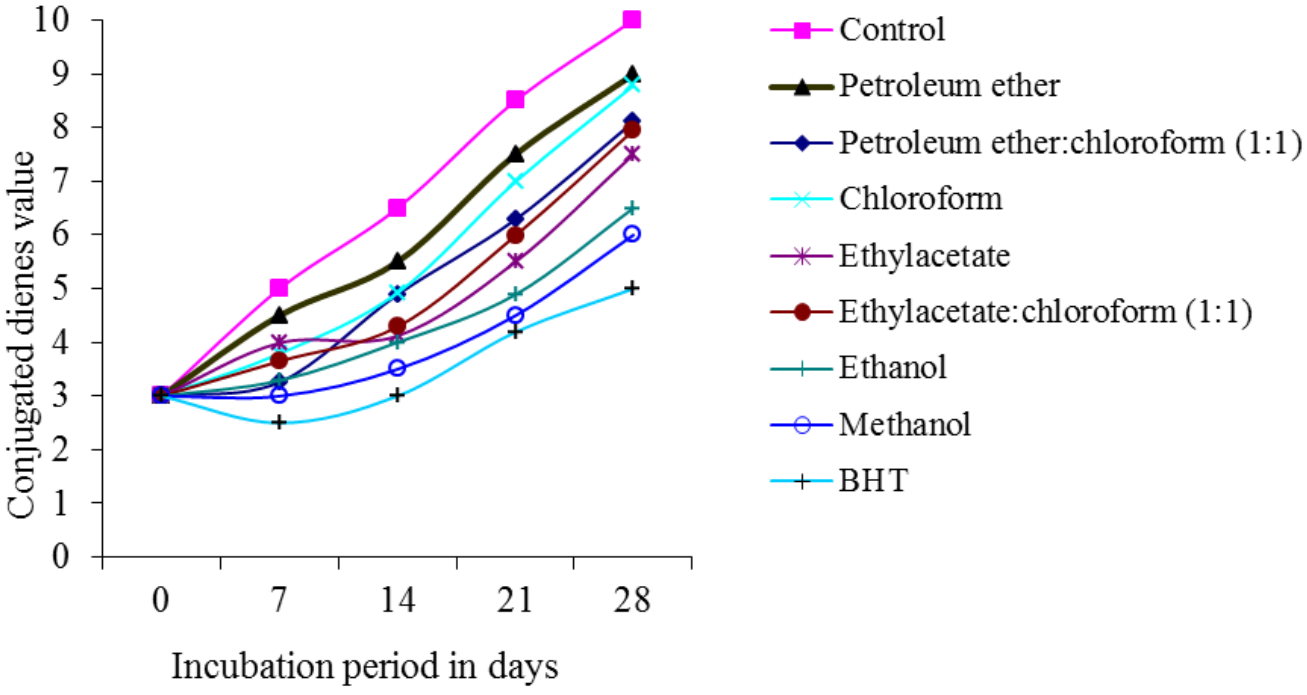

Fig. 5. Conjugated diene values of corn oil stabilized with *Acacia nilotica* root extracts

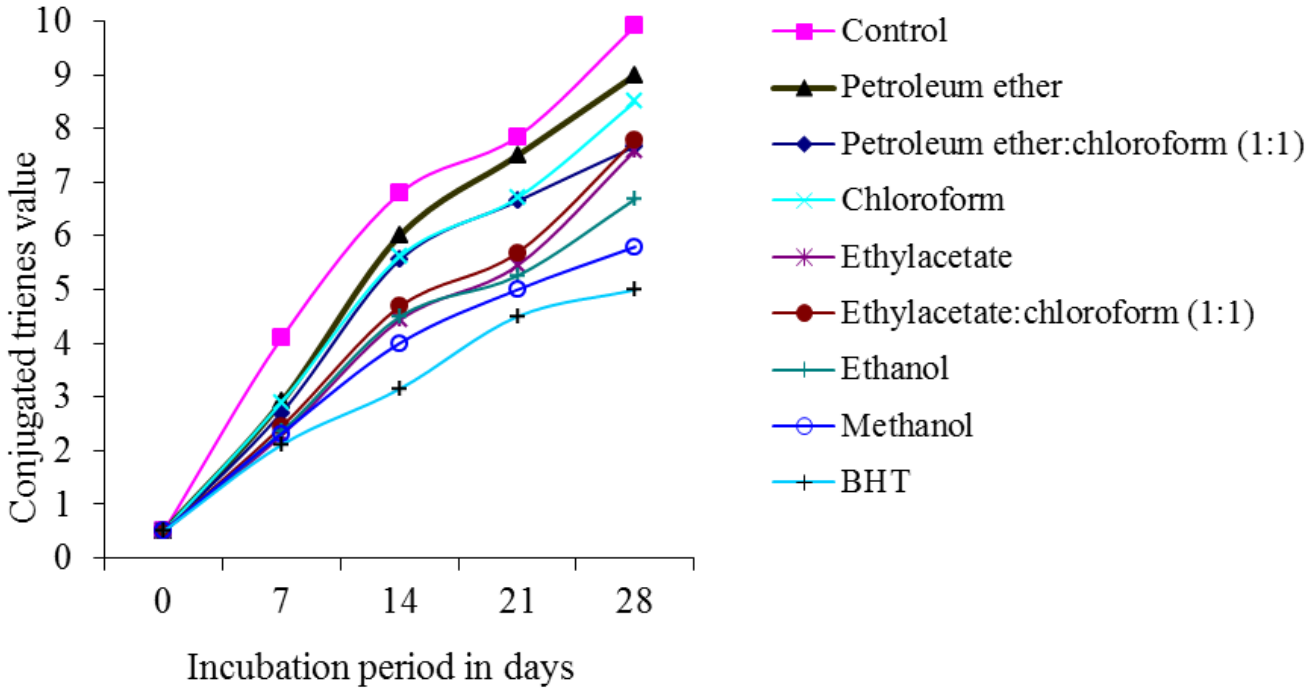

Fig. 6: Conjugated trienes values of corn oil stabilized with *Acacia nilotica* root extracts

methanol, ethanol, ethyl acetate, ethylacetate: chloroform (1:1), petroleum ether: chloroform (1:1), petroleum ether and chloroform was 53.12, 54.12, 55.25, 69.52, 60.12, 70.23, 65.25 µg/mL respectively. The extracts showed % inhibition in linoleic acid ranged from 33.8 to 56.3%. Petroleum ether showed minimum inhibition and maximum inhibition by methanol. Whereas, synthetic antioxidant (BHT) used as a standard has shown 86.6% inhibition of peroxidation in linoleic acid. The results of different solvents extract of reducing power ranged of different extracts are shown in (Fig.1). The maximum reducing power was shown by methanol and lowest by petroleum ether extracts. As per review of literature the antioxidant activity of plant extracts might be related to their phenolic contents [32]. The results indicated that the plant extracts have good antioxidant potential.

3.4. Cytotoxicity studies

The cytotoxicity studies of plantextracts evaluated by haemolytic activity at 1 mg/mL concentration of extracts were evaluated. The % haemolysis for phosphate buffer saline (PBS) used a negative control showed zero % lysis of RBCs while Triton X-100 used a positive control showed 99.6±1.123. The % lysis of RBCs by plant extracts were in ranged from 1.27-3.59%. The highest value of petroleum-ether and lowest was ethyl acetate.The toxicity values of different solvents extracts is given as, the value of petroleum-ether (3.59), chloroform (2.12), ethyl acetate (1.27), methanol (2.40), ethanol (1.79), ethyl acetate: chloroform 1:1 (1.40) and petroleum ether: chloroform 1:1 (2.68)%, respectively.

4. Conclusions

From complete investigation about antioxidant and cytotoxic studies of *Acacia nilotica* roots it can be recommended that extracts could be used as a easily available foundation of natural antioxidants, which can be used as supplement to aid the therapy of free radical mediated diseases such as cancer, diabetes swelling. Phytochemical studies of *Acacia nilotia* roots extracts revealed that this plant contains terpenoids, saponins, phenols, alkaloids and tannins which are very importance for pharmaceutical uses. From the result of different antioxidant estimations and different oxidation parameters of corn oil treatments, it is comprehensible that different extracts of *Acacia nilotica* exhibited good antioxidant activity. However the antioxidant activity of methanol, ethanol and ethyl acetate extracts were originate to be extensively higher than other extracts in stabilization of corn oil which might be recognized as high polarity of solvents. The values of control oil sample were higher than other sample and the value of BHT was lower than polar and non-polar sample solvents extract.

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