# Evaluation of Aitchisonia rosea for oxidative stress and its protective effect on $H_2O_2$ induced oxidative damage on DNA and RBCs

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**Article**

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# Abstract

### Background

*Aitchisonia rosea* Hemsl. ex Aitch (Family Rubiaceae) has been used traditionally for the treatment of different diseases involving oxidation reactions. The current work was carried out to evaluate anti-oxidant potential of various organic fractions and essential oils from *Aitchisonia rosea* and to study their protective effect on $H_2O_2$ induced oxidative damage on pBR322 DNA and RBC cellular membrane.

### Methodology

The in-vitro assays were performed with different concentrations of plant extract, its various fractions and essential oil. The essential oil was isolated, its GCMS analysis was performed and chemical constituents were identified in essential oil. Moreover, DPPH scavenging assay and ferric reducing anti-oxidant power assay was performed. Additionally, the DNA protection assay and RBC cellular membrane protection assay were performed.

### Results

The main components identified by GCMS in essential oil of plant were germacrene (18.43%), carvacrol (12.80%) and linalool (2.67%). The results clearly indicated that methanol extract, ethyl acetate, *n*-butanol fractions and essential oil exhibited very promising radical scavenging effect and reducing power anti-oxidant activity. They also contained higher contents of phenolic compounds. The protective effect of plant on pBR322 DNA with $H_2O_2$ treatment showed that at concentration 1000 µg/ml, it converted the open circular damaged form of pBR322 DNA to super coiled safe form. The plant also protected RBC cellular membrane and thus safe for human use.

### Conclusion

The results proved *Aitchisonia rosea* to be a valuable anti-oxidant that protects pBR322 DNA and RBC cellular membrane from free radical induced oxidative damage. Furthermore, it could be further develop as powerful and new anti-oxidant.


# 1. Background

Free radicals are the unpaired electron-containing molecules, which have the potential to scavenge electrons from other sources to get stabilized. In this process, another radical is released, which causes the release of thousands of free radicals within seconds. Harming the DNA occurs from both of reactive oxygen species (ROS) and free radicals by generating oxidized forms of purine and pyrimidine bases causing the breakage of a single strand of DNA. The oxidation-mediated damage of macromolecules like DNA, proteins, and lipids leads to various pathological conditions including cancer and aging. A typical example of ROS is hydrogen peroxide ($H_2O_2$), which has been involved in the synthesis of hydroxyl radicals leading to DNA damage [1]. Various valuable compounds, known as anti-oxidants, reduce the

free radicals formation that will lead to the prevention of oxidative processes [2]. Eating foods that have plentiful of phytochemicals like phenolics and flavonoids have been linked to a decreased risk of cancer due to anti-oxidant property. It has renewed the attention towards diets rich in plants [3].

Aitchisonia rosea Hemsl. ex Aitch is a plant belonging to the Rubiaceae family, with a distribution mainly in the hilly regions of Pakistan's Baluchistan and Afghanistan, and in Iran. Aitchisonia is a genus consisting of only one species, Aitchisonia rosea [4]. The plant was first collected by Aitchison back in 1882 [5]. The species is known to have medicinal properties and is used to treat certain infectious skin diseases in Baluchistan area. A research study isolated and identified and were isolated two glycosides; Aitchisonides A and Aitchisonides B, from the n-butanol fraction of Aitchisonia rosea, along with deacetylasperulosidic acid and nepetanudoside B. Additionally, anthraquinone derivatives named Rosenones A and B were separated and identified for the first time from the ethyl acetate fraction of A. rosea using liquid column chromatography examined using PTLC. The study also isolated another compound, 1,3,6-trihydroxy-2-methylanthraquinone, from this Aitchisonia species [7].

The extensive folklore uses of *A. rosea* for the treatment of different diseases have led to ensure its anti-oxidant potential. It is revealed from literature survey that *A. rosea* has not been so far evaluated for anti-oxidant property. In the present study, anti-oxidant potential and safety profile of this plant specie were evaluated which might be utilized to standardize the plant for therapeutic purposes.

## 2. Materials and Methods

## 2.1. Plant material and extraction

The plant species *A. rosea* was collected from the hilly regions of Quetta, Balochistan, Pakistan. The plant specimen was then identified and confirmed to be authentic by Taxonomists, namely Prof. Dr. Rasool Bakhsh Tareen from the Department of Botany at the University of Baluchistan, Quetta and Prof. Dr. Zaheer Ahmad Khan from the Department of Botany at GC University, Lahore, Pakistan. To serve as a point of reference, voucher specimen number 1911 was deposited in the Sultan Ayoub Herbarium at GC University Lahore. The plant name has been checked with http://www.theplantlist.org.

Stems and branches of plant were air dried under the shade for several days at room temperature an electrical mill was used to pulverize the plant into fine powder. The powdered plant material (10 kg) was extracted thrice with methanol (3×12 L) by dipping for seven days. The methanol extract was concentrated to dryness under reduced pressure using a rotary evaporator (Heidolph, model Laborata 4000, Schwabach, Germany). The yield of methanol extract was 27.0%. Following the initial methanol extraction, the plant material was fractionated using a successive solvent extraction method with solvents of varying polarity including n-hexane, chloroform, ethyl acetate, and n-butanol respectively. The resulting fractions were then subjected to concentration using rotary evaporator and further air dried to yield dry samples. The methanol extract was further fractioned using a successive solvent extraction method with different polarity based solvents such as *n*-hexane, chloroform, ethyl acetate and *n*-butanol.

After fractionation, samples were concentrated to dryness under reduced pressure. The yields of *n*-hexane, chloroform, ethyl acetate and *n*-butanol fractions were 3.0, 5.4, 6.0 and 8.0%, respectively. They were stored in a refrigerator at 4°C, until used for analysis [8].

## 2.2. Phytochemical screening

Upon conducting several qualitative phytochemical analysis, it was found that the methanol extract of A. rosea and its various fractions contain several chemical constituents, including alkaloids, glycosides, tannins, saponins, terpenoids, fats, phenols, and flavonoids [9, 10].

### 2.2.1. Isolation of essential oil

500 g powder of the plant was passed through hydro-distillation for 4 h by using a Clevenger-type apparatus as described by earlier workers [11, 12]. The percentage yield of essential oil was determined. It was collected, dried over anhydrous sodium sulphate, filtered and stored at 4°C until evaluated.

### 2.2.2. GC-MS analysis of essential oil

For analysis of essential oil, GC 6850 network system equipped with a 7683B series auto injector and 5973 inert mass detector (Agilent Technologies, Wilmington, DE, USA) was used. An HP-5 MS capillary column with a 5% phenyl polysiloxane stationary phase (30.0 m × 0.25 mm, film thickness 0.25 µm) was employed to separate the compounds. Initially, after maintaining the oven temperature (OT) at 45°C for 5 min, OT was increased to 150°C at a rate of 10°C per min. Then, OT was increased to 280°C at a rate of 5°C per min and lastly; OT was increased to 325°C at a rate of 15°C per min and maintained this temperature for 5 min. At a pressure of 60 KPa and linear velocity of 38.2 cm/sec, helium gas was flown at a rate of 1.1 ml/min for monitoring the components in scanning mode from 40 to 550 *m/z* [13].

### 2.2.3. Identification of components in essential oil

Based on retention index (RI), the components were identified by comparison with reference alkane series ($C_9$-$C_{24}$). The compound identification was further verified by comparing their MS profile with NIST 05 Mass Spectral Library and published mass spectra. To obtain the quantitative data, the FID area percentage approach was adopted.

## 2.3. Anti-oxidant activity by DPPH scavenging assay

The DPPH free radical scavenging approach was adopted to determine the anti-oxidant activity of the plant methanolic extract, its various organic fractions and essential oil by following the same method as previously described by Sukanya et al., [14]. A 10 µl (mg/ml) of test solution was added to each well of 96-wells plate, and 90 µl of 100 µM of the methanolic DPPH solution was added to make a total volume of 100 µl. Both solutions were mixed and the plate was incubated at 37 $^{o}$C for 30 min. After incubation, absorbance was measured at 517 nm to find out reduction of samples by using a micro plate reader. Ascorbic acid served as standard antioxidant agent. The experiments were performed thrice to avoid percentage error. The $IC_{50}$ values were determined by assessing all samples at various dilutions i.e., 0.5,

0.25, 0.125, 0.0625, 0.0313, and 0.015 mM to monitor the influence of increasing concentrations on the inhibition values of samples. The data recorded were computed with the Ez-fit software (Perella Scientific Inc. Amherst, USA). The reduction in absorbance value indicated increased radical scavenging mechanism that can be calculated by equation given below:

Inhibition (%) = (Ac – As) /Ac × 100

Where Ac is the absorbance of control and As is the absorbance of sample.

## 2.4. Ferric reducing anti-oxidant power assay (FRAP)

The anti-oxidant potential of samples was also examined through the reducing power assay approach [15]. Different concentrations of the test plant extract, fractions and essential oil as well as standard ascorbic acid were dissolved in 1 ml of deionized water to give final concentrations of 62.5–1000 µg/ml. After mixing these solutions with 2.5 ml of each 200 mM/L sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide, the incubation of this mixture was carried out at 50 $^{o}$C for 20 min. This material system was then mixed with 2.5 ml of 10% trichloroacetic acid, followed by centrifugation of the resultant material at 3000 rpm for 5 min. After removing upper layer, an aliquot volume (2.5 ml) from this supernatant layer was added to 3 ml of solution containing distilled water: 0.1% ferric chloride (1: 2 v/v). The absorbance of this solution was determined at 700 nm using UV-visible spectrophotometer. The higher absorbance represented higher reducing power of samples analysed [16].

## 2.5. Determination of total phenolic contents (TPC)

The total phenolic contents of plant material were determined by Folin-Ciocalteu colorimetric method using gallic acid as a standard [17]. The 0.1 ml (1 mg/ml of methanol) of each extract and fractions and 0.5 ml of Folin–Ciocalteu reagent were dissolved in distilled water (7.5 ml) in a test tube. The mixture was incubated at room temperature for 10 min. After incubation, 1.5 ml of 20% $Na_2CO_3$ (w/v) was added to this mixture. Afterward, the mixture was heated at 40°C for 20 min on water bath. It was cooled using ice bath and this solution was then used for determination of absorbance at 725 nm. The control solution contained all the above reagents except extracts and fractions. Subsequently, the calibration curve for gallic acid absorbance was constructed by linear dose response regression on excel sheet using 10, 20, 40, 80, 100 and 120 µg/ml concentrations of gallic acid. Total phenolic contents were described as GAE mg/g, i.e., milligrams of gallic acid equivalents (GAE) per gram of samples.

## 2.6. DNA protection assay

A previously reported approach was slightly modified for DNA protection study [18]. In this assay, plasmid pBR 322 DNA with $H_2O_2$ and UV light was studied as a control. The reaction mixture was prepared by diluting 0.5 µg of plasmid pBR 322 DNA with 3 µl of 50 mM sodium phosphate buffer having pH 7.4. After treating 3 µl of this diluted pBR 322 DNA with 5 µl of plant methanol extract, 4 µl of 30% $H_2O_2$ was also added to this mixture in the presence and absence of various concentrations of methanolic extract like 10, 100 and 1000 µg/mL. The DNA protective effect was best studied for the 1000 µg/ml

concentration, which was further processed for the organic fractions and essential oil. Afterwards, sodium phosphate buffer (pH 7.4) was added to the reaction mixture to increase its volume up to 15 µl. After incubating the reaction mixture in a dark place at 37 $^{O}$C for one hour, the plasmid DNA was run on 1% agarose gel (prepared by dissolving 1 g agarose in 100 ml of TBE buffer (Tris boric acid EDTA) and subjected to heat in a microwave oven resulting in a homogeneously mixed material. This material was then allowed to cool. Subsequently, ethidium bromide was added in a volume of 20 µl. The obtained mixture was spontaneously changed into solid form after 20 min. In due course, TBE was added in a volume sufficient to achieve the immersion of the electrodes). The reaction mixtures as well as controls were run horizontally at 100 volts for 1 h in an electrophoresis apparatus. The documentation of gels was carried out by using a syngene model gene unit. For each run, a molecular marker, a control, hydrogen peroxide as well as various organic fractions of the plants wa loaded. Finally, the migration pattern of the native DNA was compared to that of oxidized DNA to determine the difference.

## 2.7. RBC cellular membrane protection assay

A slightly modified haemolytic approach was adopted to study the cytotoxicity of test plant crude extract, its subsequent fractions and essential oil [19]. A volume of 3 ml of heparinized human blood (freshly obtained) was poured into 15 ml polystyrene tube, followed by centrifugation for 5 min. After draining off the upper layer, RBCs washing was conducted in triplicate using 5 ml of chilled (4°C) sterile isotonic PBS (phosphate-buffered saline) solution each time. Subsequently, pH adjustment of this solution is made equal to 7.4. Then cells were counted on haemacytometer. The erythrocyte count was maintained to $7.068 \times 10^{8}$ cells/ml for each test. An aliquot of 20 µl (1 mg/ml of DMSO) of plant extract, its various fractions and essential oil was taken in 2.0 ml Eppendorf's tubes aseptically and 180 µl of diluted RBCs suspension was added to them. The positive and negative control for this test was 0.1% Triton X-100 and PBS, respectively. After incubating the samples at 37°C for 35 min, the tubes were transferred to the ice-pot. After 5 min, the tubes were centrifuged for 5 min. 100 µl of supernatant obtained after centrifugation was diluted with 900 µl of chilled PBS solution in Eppendorf's tubes. The tubes were then placed in the ice-pot. After this 200 µl mixture from each tube was transferred in 96 well microtiter plates. The experiments were performed in thrice. Absorbance was measured at 576 nm using BioTek, µ Cuant™ instrument. The %age lysis of RBCs was calculated by using the following equation

RBC lysis (%) = (As / At) × 100

Where As and At are the absorbance of sample and triton X-100, respectively.

## 2.8. Statistical analysis

Results were presented as mean ± S.E.M. Statistical significance between the groups was analysed by means of analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). P values less than 0.05 were considered as significant.

# 3. Results and Discussion

### *3.1.Phytochemical screening*

The qualitative phytochemical analysis showed the presence of different chemical constituents known to be present in the plant like alkaloids, glycosides, tannins, saponins, terpenoids, fats, phenols and flavonoids in methanol extract and its various fractions.

### *3.2 Percentage yield of essential oil*

The percentage yield of essential oil obtained from *A.rosea*was 0.27% (w/w of dry plant material).

### *3.3 GCMS analysis of essential oil*

The components identified in the essential oil of test plant using GCMS technique are depicted in Table 1. The GCMS chromatogram of essential oil is presented in Fig. 1. The main chemical constituents detected in the essential oil of *A. rosea* were germacrene (18.43%), octadecanoic acid methyl ester (14.43%), carvacrol (12.80%), β-caryophyllene (5.89%), linalool (2.67%) and β-elemene (2.66%).

### *3.4 DPPH scavenging effect*

The DPPH scavenging assay was used to determine the anti-oxidant activity. Vitamin C, used as a standard. The $IC_{50}$ values exhibited by methanolic extract, its fractions and essential oil is depicted in Table 2. Principally, the anti-oxidant activity study by DPPH assay is characterized by a change of the violet colour DPPH solution to yellow; this colour change represents the scavenging of free radicals by the anti-oxidant molecules [20]. In the DPPH test, the anti-oxidant agents connect with the stable DPPH free radical having violet colour and convert it into a yellow colour. The degree of discolouration demonstrated the scavenging ability of plant extracts, fractions and volatile oils [21]. According to prior reports, antioxidant agents give protons to DPPH free radicals, thus causing the decrease in absorption of test samples which has been utilized to gauge the extent of anti-oxidant to scavenge the free radicals [22]. The smaller the value of $IC_{50}$, the greater is the anti-oxidant activity and *vice versa*. The anti-oxidant potential of methanol extract, ethyl acetate and *n*-butanol fractions and essential oils (Table 2) was due to the presence of phenolics, flavonoids and other phytochemicals [23]. The number of reduced DPPH molecules has been found to be directly proportional to the number of hydroxyl groups in the tested samples [24].

### *3.5 Reducing power anti-oxidant activity*

For reducing power anti-oxidant activity, the test plant was evaluated at different concentrations ranging from 62.5 to 1000 µg/ml. The anti-oxidant activity of each fraction at all concentrations was examined by comparing the mean absorbance of compounds with the standard, i.e., ascorbic acid. The methanolic extracts of both test plants showed significant ($p<0.05$) anti-oxidant activity at all the concentrations compared to all other fractions while the ethyl acetate, *n*-butanol fractions and essential oils also presented comparable anti-oxidant activity. The *n*-hexane fraction was found to be a poor anti-oxidant (Fig. 2).

### *3.6 Total phenolic contents (TPC)*

The total phenolic contents were analysed in plant extract, various organic fractions and essential oil and it was observed that they contained high contents of phenolic components (Fig. 3). The methanol penetrates the cellular membrane easily and extracts the intracellular ingredients like phenolics and flavonoids from the plant material [25]. The methanol has been attributed as a valuable solvent to extract the phenolic and flavonoid components [26] supporting the present findings. The phenolics have been described as to remove the free radicals due to the existence of the hydroxyl group [27]. The phenolic compounds have a significant role in the maintenance of lipid and fat oxidation. The polyphenols also have been linked with antioxidant activity [28]. Tanaka and coworkers defined that phenolic compounds might have the inhibitory effects on carcinogenesis and mutagenesis in human beings when taken from a food such as vegetables and fruits [29]. It was also revealed from the literature that the phenolic contents of the plants varied with the nature of organic solvents resembling with our findings [30].

The methanol extract, ethyl acetate, *n*-butanol fractions and essential oil exhibited very promising anti-oxidant effects due to the abundance of phenolics in them. The earlier reports showed that the phenolic compounds present in various plant extracts play a vital role in their anti-microbial, anti-oxidant and other activities [31]. The anti-oxidant potential of essential oils is due to the presence of phytoconstituents. Derwich and coworkers previously reported that 1, 8-cineole, germacrene, limonene, pulegone, β-pinene and α-pinene found in essential oils are all good antioxidants [32]. Thymol also behaves as an anti-oxidant [33]. It is well documented that some volatile components such as linalool, β-pinene and α-pinene in essential oil have in antioxidant and anti-microbial properties [34].

### *3.7 DNA protection effect*

The methanolic extract, ethyl acetate, *n*-butanol fractions and essential oil of *A. rosea* were found to possess good anti-oxidant activity. Due to the reason, these were selected for DNA protection assay to ascertain their further safety. The results represented in Fig. 4 clearly showed the changes in the extent of the protective influence against $H_2O_2$-induced oxidative damage on pBR322 DNA after treatment with methanol extracts, fractions and essential oils at 1000 µg/ml concentration. In Fig. 4, Lane 1 illustrates the control, i.e. super-coiled plasmid pBR322 DNA without treatment. Lane 3 describes the $H_2O_2$-induced damaged pBR322 DNA in open linear form. Fifth, sixth and seventh lane shows the protective effect of *n*-butanol, ethyl acetate fractions and essential oil on DNA.

It was observed that the DNA was protected from nicking with these samples. This DNA protective activity of the samples could be due to higher concentrations of phenolic and flavonoid compounds in them and also their potential to capture the free radicals and oxidation products [35]. The free radicals especially hydroxyl radicals have been involved in out breaking the DNA double strands converting them into single strand i.e., open circular form. The phytochemicals such as phenolics and flavonoids offer an excellent contribution in quenching the free radicals and thus decrease the DNA damage [36].

### *3.8 RBC cellular membrane protectioneffect*

*In-vitro* RBCs cellular membrane hemolytic effect of the test plant extract, its various fractions and essential oil was carried out by adopting the hemolytic method against human red blood cells (RBCs). Triton X-100 served as a standard hemolytic agent. The extract, various fractions and essential oil of *A. rosea* presented the hemolytic effect ranging from 1.88% to 9.36% (Fig. 5).

The plant's essential oil demonstrated a minimal hemolytic effect. The mechanical stability of the red blood cell membrane is crucial in reducing the cytotoxic effects of various compounds. Thus, the low in-vitro cytotoxicity of different plant extracts and fractions is essential for their safe usage in treating various diseases, except for cancer and tumors [37].

The observations collected from this study showed a high safety profile of the tested samples in human because the *in-vitro* hemolytic activity of all these samples was found to be in a safe range, i.e., below 10.0% (Fig. 5). Therefore, it could be expected that the extracts, fractions and essential oils have shown minor cytotoxicity. The plant extracts or fractions having minor cytotoxicity could be safely utilized as herbal medicines [19].

# 4. Conclusion

This was concluded that the anti-oxidant potential of tested extract, various organic fractions and essential oil of *A.rosea* was due to the presence of phytoconstituents which might protect the DNA and RBC cell membrane from damage and thus help the human body to reprieve from oxidative stress. It would help in the development of a new alternative medicine system, which has fewer side effects. This would also open the possibility of finding new clinically effective anti-oxidant compounds.

# Abbreviation

*A. rosea*, *Aitchisonia rosea;* ROS, reactive oxygen species; TPC, total phenolic contents; GC-MS, gas chromatography mass spectrometry; FRAP, ferric reducing anti-oxidant power assay; DPPH, 2,2-diphenyl-1-picrylhydrazyl; RBC, red blood cell

# Declarations

**Ethics approval and consent to participate**:

Plants studied in this article are in compliment with institutional, national, and international guidelines and legislation.

**Consent for publication:** Not Applicable.

**Availability of data and materials**:

Voucher specimen number 1911 was deposited in the Sultan Ayoub Herbarium, GC University Lahore for further reference. All data are available upon request from corresponding author.

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### Authors' contributions

Conceptualization, S.R.; methodology, S.R. and M.A.; investigation, S.R. and M.A.; resources, S.R. and M.A.; writing—original draft preparation, S.R. and M.A.; writing—review and editing, S.R. and M.A.; supervision, S.R. and M.A..; project administration, S.R. All authors have read and agreed to the published version of the manuscript.

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### Conflict of interest

Authors have no conflict of interest.

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# Tables

**Table 1** Chemical compounds in essential oil of *A. rosea* analysed by GCMS

| Retention Index (RI) | Compound | Area (%) |
|---|---|---|
| 940 | *α*-pinene | 0.79 |
| 953 | Camphene | 2.48 |
| 970 | *β*-pinene | 1.27 |
| 979 | Myrcene | 0.73 |
| 1025 | Cymene | 1.50 |
| 1028 | Limonene | 0.65 |
| 1030 | Cineole | 1.76 |
| 1033 | *β*-phellandrene | 0.78 |
| 1098 | Linalool | 2.67 |
| 1138 | Carveol | 0.80 |
| 1204 | verbenone | 0.98 |
| 1291 | Thymol | 2.96 |
| 1293 | undecanone | 0.73 |
| 1297 | Carvacrol | 12.80 |
| 1392 | *β*-elemene | 2.66 |
| 1411 | Eugenol | 2.33 |
| 1411 | *α*-copaene | 1.72 |
| 1417 | *β*-caryophyllene | 5.89 |
| 1483 | germacrene | 18.43 |
| 1493 | valencene | 2.78 |
| 1513 | Cadinene | 3.02 |
| 1577 | Spathulenol | 3.66 |
| 1647 | *α*-muurolol | 3.75 |
| 1654 | *α*-cadinol | 1.43 |
| 1962 | hexadecanoic acid methyl ester | 1.82 |
| 2000 | Eicosane | 2.35 |
| 2124 | octadecanoic acid methyl ester | 14.43 |

**Table 2** DPPH scavenging effect of *A. rosea*

| Sr. No. | Sample tested | Concentration (mg/ml) | Inhibition (%) | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|
| 1 | Methanol | 5 | 52 ± 3.31 | 0.0047 ± 0.69[a] |
| 2 | *n*-butanol | 5 | 90 ± 2.54 | 0.00099 ± 1.15 |
| 3 | Ethyl acetate | 5 | 80 ± 3.00 | 0.0025 ± 1.25 |
| 4 | Chloroform | 5 | 60 ± 3.07 | 0.0038 ± 0.17 |
| 5 | *n*-hexane | 5 | 22 ± 1.30 | - |
| 6 | Essential oil | 5 | 82 ± 0.37 | 0.0024 ± 0.07 |
| 7 | Vitamin C[b] | 0.5mM | 90 ± 0.11 | 0.08 ± 1.14 µM |

[a]Results are presented as Mean ± SEM, (n=3)

[b]Standard anti-oxidant

# Figures

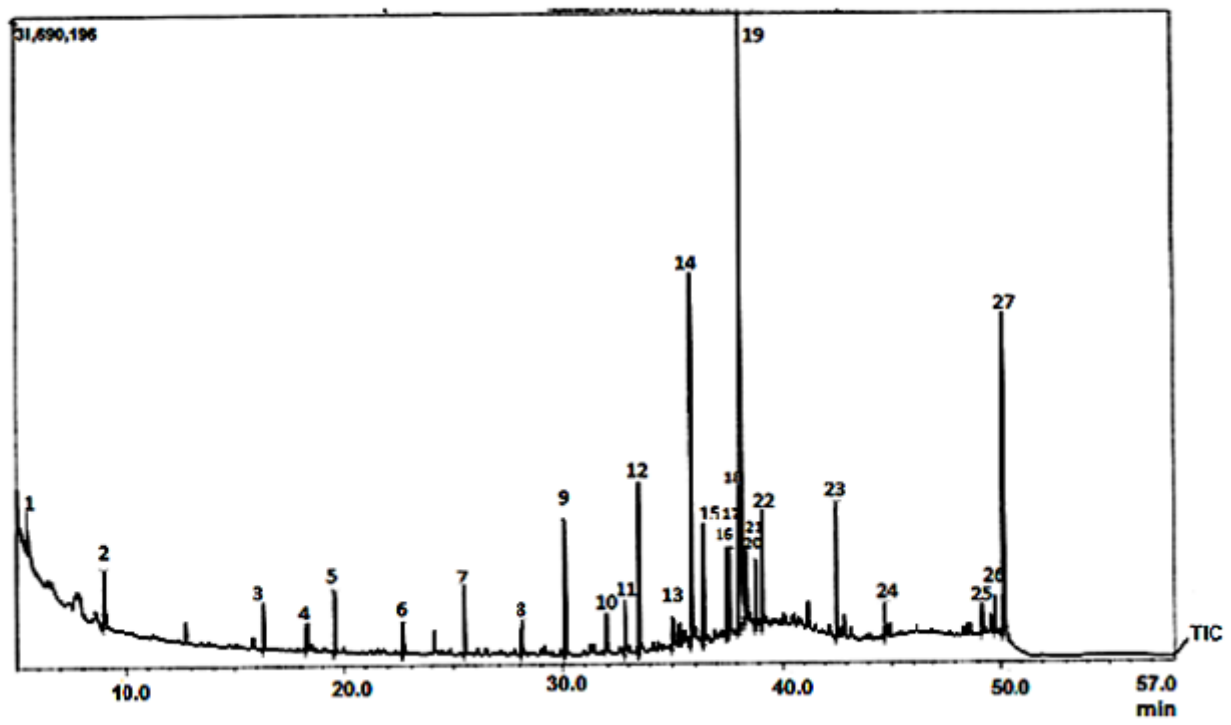
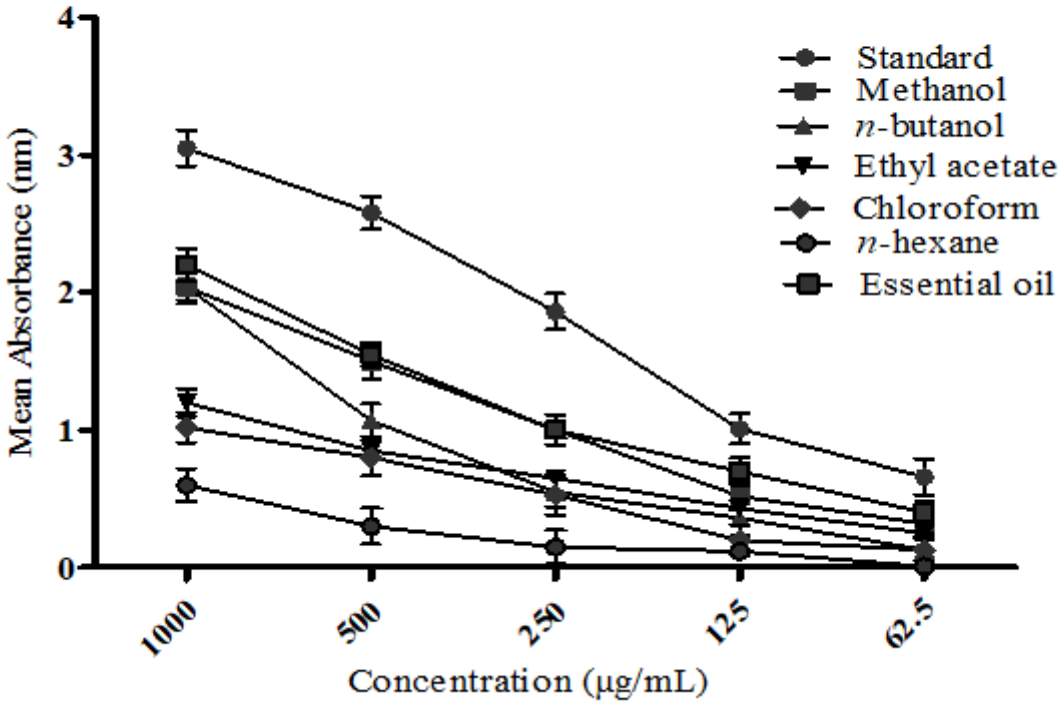



**Figure 1**

The GC-MS chromatogram of *A. rosea* essential oil




## Figure 2

Reducing power by *A. rosea* extract, fractions and essential oil

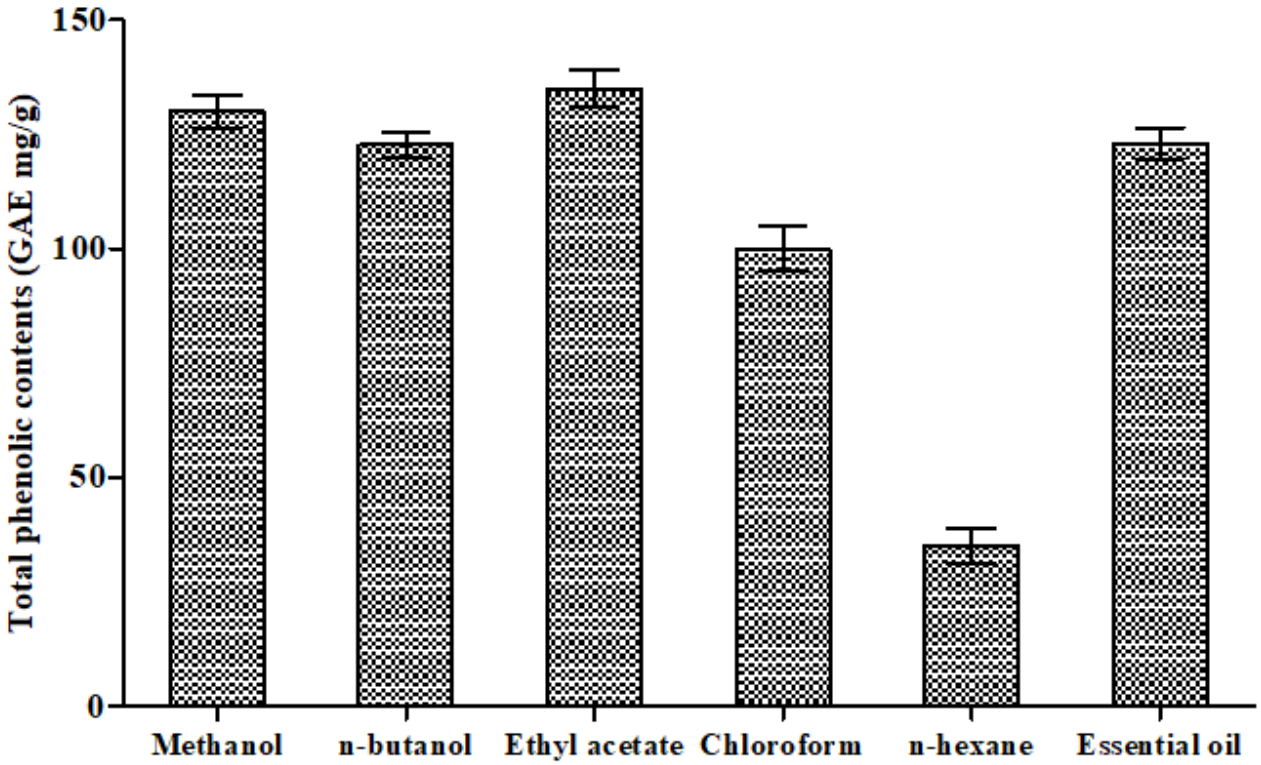



**Figure 3**

Total phenolic contents by *A. rosea* extract, fractions and essential oil

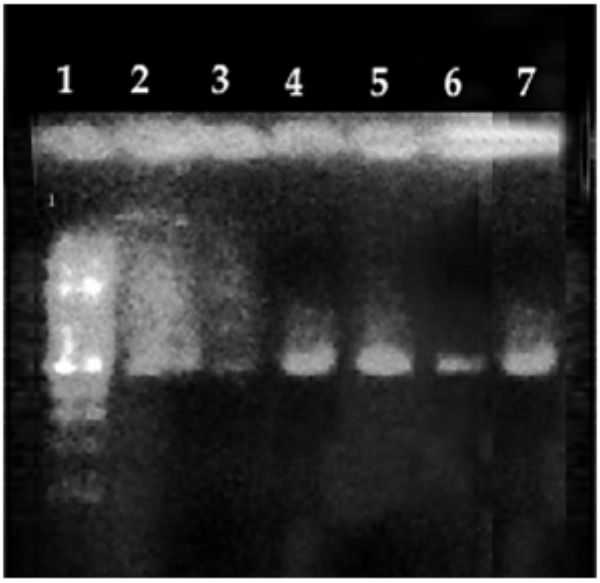



**Figure 4**

Electropherogram showing DNA protection effect by *A.rosea* extract, various organic fractions and essential oil with $H_2O_2$ induced oxidative damage on pBR322 DNA.

(Lane 1 = Plasmid pBR322 DNA without treatment (Super coiled); Lane 2 = Plasmid pBR322 DNA and dye), Lane 3 = Plasmid pBR322 DNA; treated with $H_2O_2$ (open circular or damaged); Lane 4 = Plasmid pBR322 DNA treated with methanol extract + $H_2O_2$; Lane 5 = Plasmid pBR322 DNA treated with *n*-butanol fraction + $H_2O_2$; Lane 6 = Plasmid pBR322 DNA treated with ethyl acetate fraction + $H_2O_2$; Lane 7 = Plasmid pBR322 DNA treated with essential oil + $H_2O_2$)

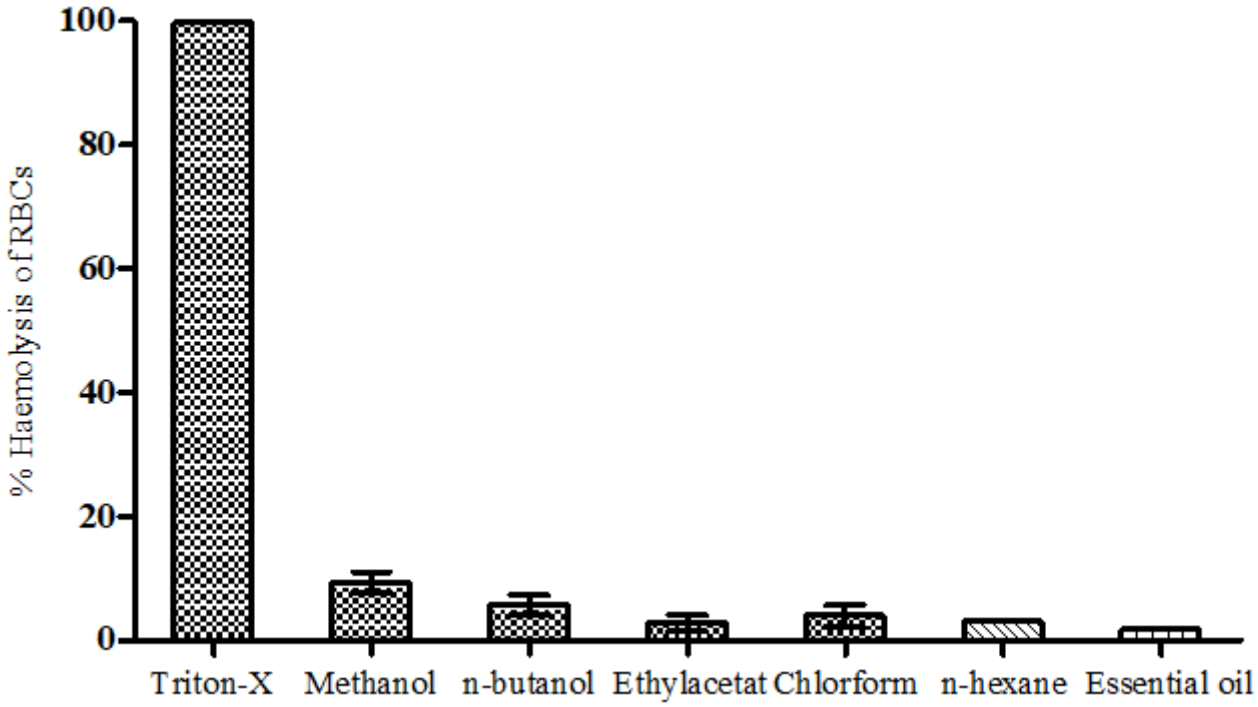



**Figure 5**

*In-vitro* hemolytic activity of *A. rosea*