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In Vitro Antioxidant Activity of Bioactive Flavonoids identified from *Crateva adansonii* DC Bark Extracts

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ABSTRACT: In the present study *Crateva adansonii* bark extracts were known to possess *In vitro* antioxidant activity, which is subsequently evidenced by the presence of bioactive flavonoid in bark extracts of the plant analyzed by TLC. Qualitative phytoconstituents screening of bark extracts revealed that all the major phytocompounds were present in both extracts. Quantitative phytoconstituents analysis of the plant extracts revealed that methanolic bark extract contains the highest amount of flavanoid, tannin and total phenol 11.29±0.34 mg of QE/g of bark extract, 3.27±0.41 mg of GAE/g of bark extract and 47.20±1.27 mg of GAE/g of bark extract whereas chloroform bark extract contain highest alkaloidal content 6.32±0.59 mg of AE/g of bark extract. Determination of *In vitro* antioxidant activity by four different assays such as DPPH, ABTS, Phosphomolybdate and Hydrogen peroxide radical scavenging assay revealed that among two bark extracts methanolic bark extract possess lowest IC50 value (152.25, 228.50, 168.60 and 127.50 µg) for four assay respectively having highest and prominent *In vitro* antioxidant activity. Further TLC analysis of bark extract confirms the presence of bioactive flavonoids on the basis of Rf value of the bark extracts. The results clearly indicates that bark extracts of *Crateva adansonii* contain bioactive flavonoids with varied medicinal properties that can be exploited for the treatment of many diseases.

Keywords: *Antioxidants; Bioactive compounds; Crateva adansonii; In vitro; Phytoconstituents;*

1. INTRODUCTION

The medicinal value of plants has assumed more important dimensions in the past few decades moreover most of modern drugs are derived from the traditional plant sources only. Currently, about one third of the active component of the modern drug was identified from plants that are used as prescribed medicines [1]. Vast number of medicinal plants has been investigated for their *in vitro* free radicals scavenging effects to find out antioxidant potential of the plant extracts. Natural antioxidants from medicinal plant origin either in the form of raw plant extracts or their pure chemical constituents are very effective to prevent the destructive processes caused by oxidative damage [2]. There is an increasing interest in natural antioxidants present in medicinal plants, which might help prevent oxidative damage. Different parts of the plants such as seeds, leaf and bark of stem and root known to possess substantial amounts of phytoconstituents such as phenolics, flavonoids, tannins having the ability to inhibit the free radicals that are excessively produced, hence can act as antioxidants [3]. The continued search among plant secondary metabolites for natural antioxidants has gained more attention in recent years because of the increasing awareness of herbal remedies as potential sources of antioxidants to treat various disease conditions [4].

A free radical is a chemical compound that contains one or more unpaired electrons in atomic or molecular orbitals [5]. Accumulation of free radicals leads to the generation of reactive oxygen species (ROS) which can damage the cells of all organisms. During oxidation, ROS are produced at low levels in normal physiological conditions, which are necessary for maintaining normal cell functions and the endogenous antioxidant defense systems of the body have the capacity to avert any harmful effects. However, ROS are extremely harmful to organisms at high concentrations. When the level of ROS exceeds the defense mechanisms, , they can affect many cellular functions by damaging nucleic

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acids, oxidizing proteins, and causing lipid peroxidation. Accumulated reactive oxygen species (ROS) and other oxidants cause numerous disorders and diseases. The role of free radical reactions in disease pathology is well established and is found to involved in many acute and chronic disorders in human beings such as diabetes, atherosclerosis, aging, immunosuppressive and neurodegenerative disorders [6]. Thus Neutralizing of free radicals by an antioxidant agent is important for cell protection against oxidative stress.

Crateva adansonii DC belonging to the family *Capparidaceae* is commonly known as "garlic pear" in English [7]. Different parts of the plant are extensively used in folkloric medicine for the cure of many disease conditions. The leaf extracts was used to relieve pain in joints, ear ache, toothache and eye infections. The powdered bark is used in the treatment of in rheumatism, itch, epilepsy, asthma and urinary, renal tubules, gastrointestinal, and uterine infection [8,9]. Perusal of literature reveals that no earlier reports available regarding the detailed bioactive compounds analysis of *Crateva adansonii* bark extracts. Therefore, the aim of the present study is to screen phytoconstituents and *In vitro* anti-oxidant activity of *Crateva adansonii* bark extracts and also identify bioactive flavonoids of bark extracts responsible for In vitro anti-oxidant activity..

2. MATERIALS AND METHODS:

2.1 Collection and processing of plant material

The bark of *Crateva adansonii* collected from Salem District, Tamil Nadu, India. The bark were cleansed, grounded into a fine powder and preserved for further use.

2.2 Preparation of plant extract

50g of fine bark powder of *Crateva adansonii* was packed with Whatman No 1 filter paper and placed in soxhlet apparatus along with 300 ml of methanol. Then the sample was boiled for ten soxhlet cycles to obtain methanolic bark extract of *C. adansonii* and then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. The above mentioned procedure was repeated with chloroform solvent to obtain chloroform bark extract of *C.adansonii*. Then the dried bark extracts were labeled and stored in sterile screw capped bottles at 5°C in the refrigerator for further use.

2.3 Preliminary Qualitative Phytoconstituents Screening

The freshly prepared bark extracts of *Crateva adansonii* was tested for the presence of particular bioactive phytoconstituents by using following standard methods.

2.3.1 Test for Alkaloids

To 0.4 g of bark extract was vortexed with 8 ml of 1% HCl and the mixture was boiled and filtered [10]. 2 ml of filtrate was treated separately with (a) with few drops potassium mercuric iodide (Mayer's reagent) and (b) potassium bismuth (Dragendroff's reagent). Turbidity or precipitation with either of these reagents was taken as evidence for existence of alkaloids.

2.3.2 Test for Flavonoids

To 50 mg of bark extract was mixed with 100 ml of distilled water and filtered to get the filtrate. 5 ml of dilute ammonia solution was added to 10 ml of filtrate followed by the addition of few drops of concentrated H_2SO_4. Presence of flavonoids was confirmed by yellow coloration [11].

2.3.3 Test for Tannins

To 50 mg of bark extract was boiled with 20 ml of distilled water, filtered and the filtrate was collected. A few drops of 0.1% $FeCl_3$ was added in filtrate and observed for color change; brownish green or a blue-black coloration was taken as evidence for the presence of tannins [11].

2.3.4 Test for Saponins

The ability of saponins to produce emulsion with oil was used for the saponins screening test in plant extracts [10]. 20 mg of bark extract was boiled with 20 ml of distilled water in a boiling water bath for five min and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for froth formation. 3 drops of olive oil were mixed with froth, shaken vigorously and observed for emulsion development.

2.3.5 Test for Terpenoids

Presence of terpenoids in bark extracts was carried out by taking 5 ml (1 mg/ml) bark extract and mixed with 2 ml of chloroform followed by 3 ml of concentrated H_2SO_4. A reddish brown coloration of the interface confirmed the presence of terpenoids in pant extract [10].

2.3.6 Test for Phenol

To 10mg of bark extract was treated with few drops of ferric chloride solution. Formation of bluish black color indicates that the presence of phenol [10].

2.3.7 Test for Anthraquinones

To 200 mg of bark extract was boiled with 6 ml of 1% HCl and the mixture was filtered to collect the filtrate. Then the filtrate was shaken with 5 ml of benzene, filtered and 2 ml of 10% ammonia solution was added to the final filtrate. The mixture was shaken and the presence of a pink, violet or red colour in the ammonical phase indicated the presence of free hydroxyl anthraquinones [12].

2.3.8 Test for Cardiac Glycosides

To 5 ml (10 mg/ml in methanol) of bark extract was mixed with 2 ml of glacial acetic acid having one drop of $FeCl_3$ solution. To the mixture obtained 1 ml of concentrated H_2SO_4 was added to form a separate layer. The presence of brown ring of the interface indicated deoxy sugar

characteristic of cardiac glycosides [12].

2.3.9 Test for Coumarins

In a small test tube, 300 mg of bark extract was covered with filter paper moistened with 1 N sodium hydroxide. The test tube was placed for few minutes in a boiling water bath. After removing the filter paper it was examined under UV light, formation of yellow florescence indicated the presence of coumarins [12].

2.3.10 Test for Phlobatannins

To 80 mg of bark extract was boiled with 1% aqueous hydrochloric acid; the deposition of a red precipitate indicated the presence of phlobatannins [12].

2.3.11 TEST FOR STEROIDS

2.3.11.1 Liebermann's test

Bark extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice and concentrated H_2SO_4 was added to the mixture. A color change from violet to blue or green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside characteristics of steroids [10].

2.3.11.2 Salkowski's test

Bark extract was mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown color formation indicated the presence of steroidal ring, i.e., glycone portion of the glycoside [10].

2.3.12 TEST FOR CARBOHYDRATES

2.3.12.1 Fehling's test

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars [11].

2.3.12.2 Molisch's test

To 5ml of bark extract was mixed with 2ml of Molisch's reagent and the mixture was shaken gently. After that, 2ml of concentrated H_2SO_4 was added carefully along the side of the test tube. Appearance of a violet ring at the junction of two liquids indicated the presence of carbohydrate [11].

2.3.13 TEST FOR PROTEINS

2.3.13.1 Millon's test

To 5ml of bark extract was mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirms the presence of protein [11].

2.3.13.2 Ninhydrin test

To 5ml of bark extract boiled with 2ml of 0.2% Ninhydrin solution, appearance of violet color indicating the presence of amino acids and proteins [11].

2.4 QUANTITATIVE PHYTOCONSTITUENTS ANALYSIS

2.4.1 Determination of Alkaloid

The bark extract (1mg) of the plant was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2 N HCl and filtered. Then the filtrate solution was transferred to a separating funnel, to this 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. Then the mixture was shaken vigorously with chloroform and collected in a 10-ml volumetric flask and diluted upto the volume with chloroform. A set of reference standard solutions of atropine were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV-Visible spectrophotometer. All the determinations were carried out in triplicate. The total alkaloid content was expressed as mg of Atropine Equivalent per gram of bark extract [13, 14].

2.4.2 Determination of Total flavonoid content

Total flavonoid content was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1 ml of bark extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To this add 0.3ml of 5 % sodium nitrite and after 5 minutes 0.3 ml of 10 % aluminium chloride was mixed. After 5 minutes 2 ml of 1M sodium hydroxide was added and diluted up to 10 ml with distilled water. A set of reference standard solutions of quercetin at different concentrations (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV-Visible spectrophotometer. The total flavonoid content was expressed as mg of Quercetin Equivalents per gram of bark extract [15-18]. All the experiments were performed in triplicate.

2.4.3 Determination of tannin Content

The tannins were determined by Folin - Ciocalteu method. About 0.1 ml of the bark extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteuphenol reagent, 1 ml of 35 % sodium carbonate solution and dilute to 10 ml with distilled water. Then the mixture was shaken well and kept at room temperature for 30 minutes. A set of reference standard solutions of gallic acid acid at different concentrations (20, 40, 60, 80 and 100 µg/ml) were also prepared in the similar manner as described earlier. Absorbances for test as well as standard solutions were measured against the blank at 725 nm with an UV-Visible spectrophotometer. The tannin content was expressed in terms of mg of Gallic Acid

equivalent per gram of bark extract [19-22]. All the determinations were carried out in triplicates.

2.4.4 Determination of Total Phenolic Content

The concentration of phenolics in plant extracts was determined using spectrophotometric method [23]. Folin-Ciocalteu assay was used for the determination of total phenolic content of plant extracts. Briefly, the reaction mixture consists of 1 ml of bark extract and 9 ml of distilled water was taken in a 25ml volumetric flask. 1ml of Folin-Ciocalteu phenol reagent was added to the reaction mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate (Na_2CO_3) solution was treated to the mixture. Then the final volume was made up to 25 ml with distilled water. A set of standard solutions of gallic acid with different concentrations (20, 40, 60, 80 and 100 μg/ml) were prepared in the similar manner as described earlier. Finally all the tubes were incubated at room temperature for 90 minutes and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm using UV-Visible spectrophotometer. The results were expressed in mg of gallic acid equivalent per gram of bark extract. All determinations were carried out in triplicates [24, 25].

2.5 IN VITRO ANTIOXIDANT ACTIVITY

2.5.1 DPPH Radical Scavenging Assay

The antioxidant activity was measured in terms of radical scavenging ability using the stable free radical DPPH. DPPH radicals scavenging ability of plant extracts were carried out according to the method described by Blois [26] with a slight modification [27]. The reduction of the DPPH radical is followed by a decrease in the absorbance at 517 nm. A volume of 2 ml of different concentrations of bark samples (50-250μg) was taken in a separate test tubes and 2 ml of 1 mM DPPH solution was added to this. Then the tubes were covered with parafilm and kept in the dark for 1 hour. Absorbance for test and standard solutions were measured at 517 nm using UV-Visible spectrophotometer. A set of reference standard solutions of gallic acid with different concentrations (50-250 μg/ml) were prepared in the same manner as described earlier. All the tests were performed in triplicates and represented as mean ± SE. The percentage inhibition of the DPPH radical was calculated using the following formula:

$$\text{DPPH Radical Scavenging Effect } (\%) = \left(\frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}}\right) X\ 100$$

2.5.2 ABTS Radical Scavenging Assay

The ability of *Crateva adansonii* bark extracts to scavenge the free radical 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were determined by the method described by Re et al. with minor modifications [28] . ABTS radical cation ($ABTS^+$) was produced by reacting ABTS solution with 2.45 mM ammonium persulphate and the mixture were allowed to stand in dark at room temperature for 12-16 hours before use. Different concentrations (50-250 μg/mL) of the bark extracts and reference standard gallic acid (0.5 ml) were added to 0.3 ml of ABTS solution and 50% methanol to make 1ml. Absorbance for test and standard solutions were measured at 745 nm using UV-Visible spectrophotometer. Each assay was carried out in triplicate and represented as mean ± SE. The percentage inhibition of the ABTS radical was calculated using the following formula:

$$\text{ABTSRadical Scavenging Effect } (\%) = \left(\frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}}\right) X\ 100$$

2.5.3 Phosphomolybdate Assay

The total antioxidant capacity of the *Crateva adansonii* bark extracts was determined by phosphomolybdate assay using gallic acid as a reference standard [29]. An aliquot of 0.1 ml of bark sample solution was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 minutes. After that the samples were cooled to room temperature, the absorbance of the mixture was measured at 765 nm against a blank. A typical blank contained 1 ml of the reagent solution and the appropriate volume of the solvent and incubated under the same conditions. A set of reference standard solutions of gallic acid with different concentrations (50-250 μg/ml) were prepared in the similar manner as described earlier. Each assay was carried out in triplicate and represented as mean ± SE. The antioxidant capacity was estimated using following formula:

$$\text{Scavenging Effect } (\%) = \left(\frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}}\right) X\ 100$$

2.5.4 Hydrogen Peroxide Radical Scavenging Assay

Hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 ml) of different fractions was transferred into the test tubes and their volumes were made up to 0.4 ml with 50 mM phosphate buffer (pH 7.4) After addition of 0.6 ml hydrogen peroxide solution, tubes were vortexed and absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank [30]. Gallic acid was used as reference standard. Each assay was carried out in triplicate and represented as mean ± SE. The abilities to scavenge the hydrogen peroxide were calculated using the following equation:

$$\text{Hydrogen peroxide Scavenging Effect } (\%) = \left(\frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}}\right) X\ 100$$

Statistical Analysis

All the determinations of *In vitro* antioxidant activity were carried out in triplicates and the results were expressed as Mean ± SD. The difference between experimental groups was compared by One- Way Analysis Of Variance (ANOVA) followed by Post Hoc Turkey HSD multiple comparison test (control Vs test) using the soft ware SPSS and the values were considered significantly at $P < 0.05$.

2.6 Thin Layer Chromatography (TLC) Analysis

Thin Layer Chromatography (TLC) was performed on the 20 × 20 cm plates precoated with microcrystalline silica gel (Merck). A volume of 10µl of two bark extract extracts was spotted on the silica gel coated TLC plates. TLC analysis was performed with n-Butanol: Acetic acid : Water in the volume ratio of 4:1:5 as mobile phase. Obtained color spots were observed under UV light [31, 32]. The movement of the active compound was expressed by its retardation factor (Rf), values were calculated for different bands of samples.

$$Retardation\ factor = \frac{Distance\ travelled\ by\ the\ solute}{Distance\ travelled\ by\ the\ solvent}$$

3. RESULTS & DISCUSSION

3.1 Preliminary Phytoconstituents Analysis

The major phytoconstituents present in two different bark extracts (Methanol and Chloroform) of the medicinal plant *Crateva adansonii* were examined and the results were presented in Table1. Alkaloid, flavonoid, phlobatannin and protein were present in moderate concentration in methanol and chloroform bark extracts. Tannin, phenol and steroid were present high concentration in methanol, whereas in chloroform it is present in moderate concentration. Flavanoid, terpenoid,coumarins were present in low concentration in methanol, whereas in chloroform it is present in moderate concentration.Tannin, Phenol and steroid were present high concentration in methanol, whereas in chloroform it is present in moderate concentration. Saponin and cardiac glycoside were present in low concentration in both methanol and chloroform bark extract. Terpenoid and coumarin were present in low concentration in methanolic extract whereas in chloroform extract it is present in moderate concentration. Carbohydrate present in moderate concentration in methanol whereas in chloroform it is present in low concentration. Anthraquinone present in moderate concentration in methanol whereas it is absent in chloroform extract.

Table 1 Preliminary phytoconstituents screening of *Crateva adansonii* bark extracts

Test/Plant Extract	Methanol	Chloroform
Alkaloid	++	++
Flavonoid	++	++
Tannin	+++	++
Saponin	+	+
Terpenoid	+	++
Phenol	+++	++
Anthraquinone	++	-
Cardiac Glycoside	+	+
Coumarins	+	++
Phlobatannins	++	++
Steroid	+++	++
Carbohydrate	++	+
Protein	++	++

Note:

+++: Present in high concentration,

++: Present in moderate concentration,

+: Present in low concentration

-: Absent

3.2 Quantitative Phytoconstituents Analysis

Quantitative phytoconstituents analysis of two different bark extracts (methanol & chloroform) of *Crateva adansonii* was performed as per the standard methods for each phytoconstituents (Table 2 & Figure 1). Methanolic bark extract contains the highest amount of phytoconstituents such as flavanoid, tannin and total phenol (11.29 mg QE /g, 3.27 mg GAE/g and 47.20 mg GAE /g respectively) content expressed as mg/g of dry weight of bark extracts. Whereas the highest alkaloid content was measured in chloroform bark extract than the methanolic bark extract (6.32 mg AE/g & 4.64 mg AE/g respectively).

Table 2 – Quantitative Phytoconstituents analysis of *Crateva adansonii* bark extracts

Plant Extracts	Alkaloid mg AE/g of extract	Flavanoid mg QE/g of extract	Tannin mg GAE/g	Total Phenolic Content mg GAE/g of extract
Methanol Extract	4.64±1.45	11.29±0.34	3.27±0.41	47.20±1.27
Chloroform Extract	6.32±0.59	8.77±1.72	2.16±0.61	41.20±0.26

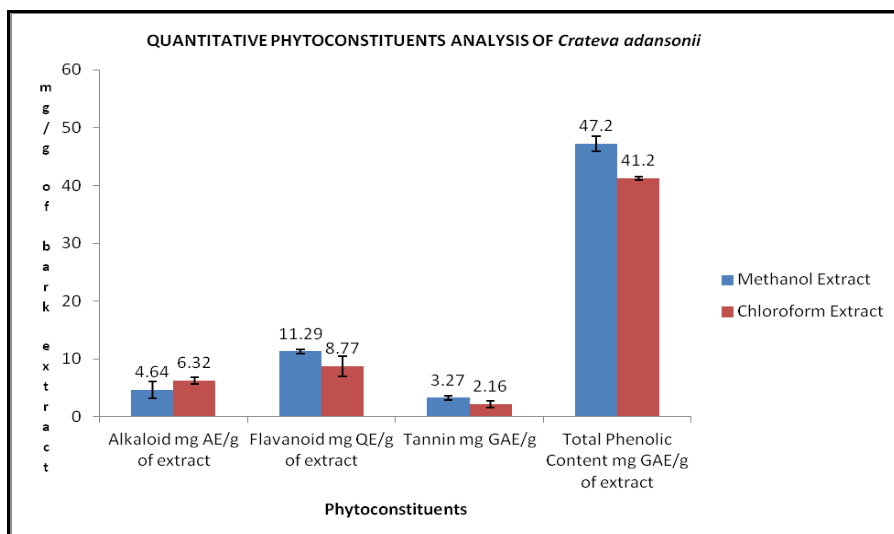

Fig. 1: Quantitative Phytoconstituents analysis of *Crateva adansonii* bark extracts

3.3 In Vitro Antioxidant Activity

3.3.1 DPPH Radical Scavenging Assay

The results of DPPH radical scavenging assay of two different bark extracts of *Crateva adansonii* as well as reference standards was shown in Table 3 and Figure 2. The scavenging effect of bark extracts on the DPPH free radicals were expressed as percentage (%) of scavenging effect and they were compared with reference standard gallic acid. All the three extracts showed a dose dependent scavenging activity of DPPH comparable to reference standard. Maximum percentage of scavenging effect was measured in methanolic bark extract among two extracts of the plant. The IC50 value for reference standard gallic acid 53.70 µg/ml as well as methanol and chloroform bark extracts was found to possess prominent antioxidant potential with IC50 value of 152.25 & 248.20 µg/ml respectively. From the results it is known that *Crateva adansonii* bark extracts possess free radicals scavenging effect.

Furthermore, it was noticed that the bark extract has least pronounced scavenging activity than that of the standard gallic acid. DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of the plant extract [33]. Antioxidants either transfer an electron or a hydrogen atom to DPPH radical, thus neutralizing its free radical character [34]. The DPPH assay has been used as a quicker, reliable and reproducible parameter for *in vitro* antioxidant acitivity of pure compounds as well as plant extracts [35]. The therapeutic potential of natural medicinal plants as an antioxidant in reducing free radical can be therapeutically useful to treat various free radicals related disease conditions [36].

Table 3 – DPPH Radical Scavenging Assay of *Crateva adansonii* bark extracts

Concentration (µg/mL)	Scavenging Effect %		
	Gallic Acid	Methanolic Extract	Chloroform Extract
50	48.18±0.47^{a}	36.42±0.21^{b}	28.74±1.37^{d}
100	59.37±0.51^{b}	43.39±1.28^{a}	33.36±0.42^{d}
150	64.82±0.27^{c}	49.24±1.72^{a}	39.34±1.48^{a}
200	74.71±1.47^{b}	54.47±0.62^{d}	44.41±0.91^{a}
250	81.29±0.78^{c}	62.36±1.25^{d}	51.72±0.78^{b}
IC50	53.70	152.25	248.20

Data are presented as the mean ± SD (n=3) and values were analysed by one- way ANOVA (Tukey-HSD multiple range *post hoc* test, Different superscript letters a-d indicate values within a column significantly different from each other, $p < 0.05$).

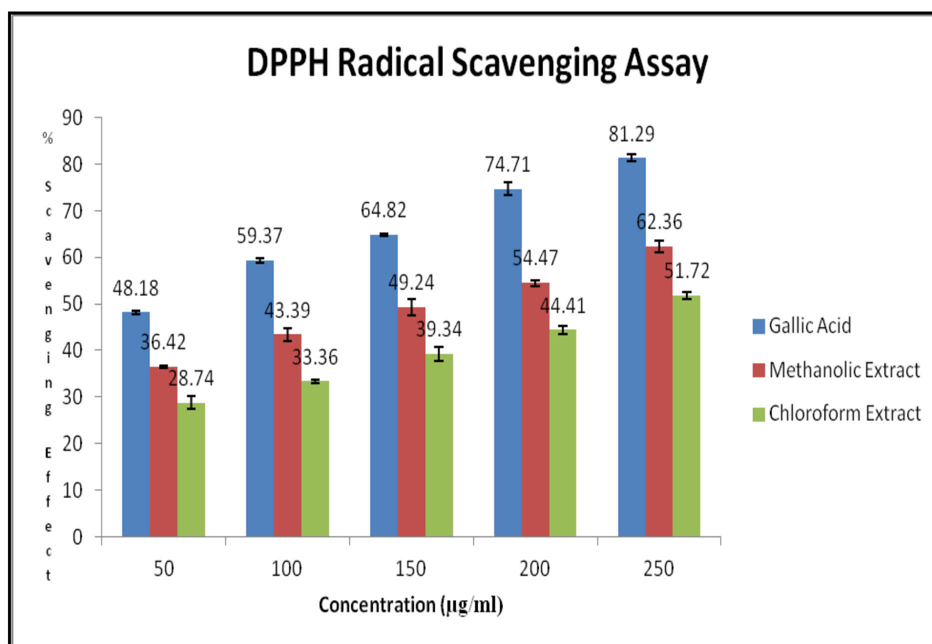

Fig. 2: DPPH Radical Scavenging Assay of *Crateva adansonii* bark extracts

3.3.2 ABTS Radical Scavenging assay

ABTS, a prorogated radical has characteristic absorbance maximum at 745nm which decreases with the scavenging of the proton radicals. ABTS was generated by incubating ABTS [2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)] with potassium persulfate. The occurrence of chemical compounds in the tested extracts that inhibit the potassium persulfate activity may reduce the production of ABTS. The present study reports that the methanolic bark extract among two extracts of *Crateva adansonii* shown the highest total antioxidant activity 53.21±0.35 followed by chloroform bark extract with 50.21±1.23, which indicates that both extracts of the plant have significant radical scavenging activity. Actually, the ABTS radical cation scavenging activity also reflects hydrogen donating ability.

Though the antioxidant potential of bark extract was found to be low ($P < 0.05$) than those of standard gallic acid, the study revealed both methanolic and chloroform bark extracts have prominent antioxidant activity; the presence of phenolic compounds (containing phenolic hydroxyls) are mainly found in these two extracts and could be attributable to the observed high antiradical properties of *C. adansonii* bark extracts and the results matches well with previous study results [37].

Table 4 – ABTS Radical Scavenging Assay of *Crateva adansonii* bark extracts

Concentration (µg/mL)	Scavenging Effect %		
	Gallic Acid	Methanolic Extract	Chloroform Extract
50	32.34±1.30^{b}	27.62±0.81^{a}	21.27±0.45^{a}
100	39.18±0.84^{a}	32.37±1.42^{b}	28.18±0.49^{b}
150	48.82±0.72^{b}	38.42±0.83^{d}	34.39±0.81^{d}
200	62.76±0.57^{c}	45.49±0.64^{b}	41.46±0.25^{d}
250	73.32±0.46^{a}	53.21±0.35^{c}	50.21±1.23^{b}
IC50	154.25	228.50	248.70

Data are presented as the mean ± SD (n=3) and values were analysed by one- way ANOVA (Tukey-HSD multiple range post hoc test,, Different superscript letters a-d indicate values within a column significantly different from each other, $p < 0.05$).

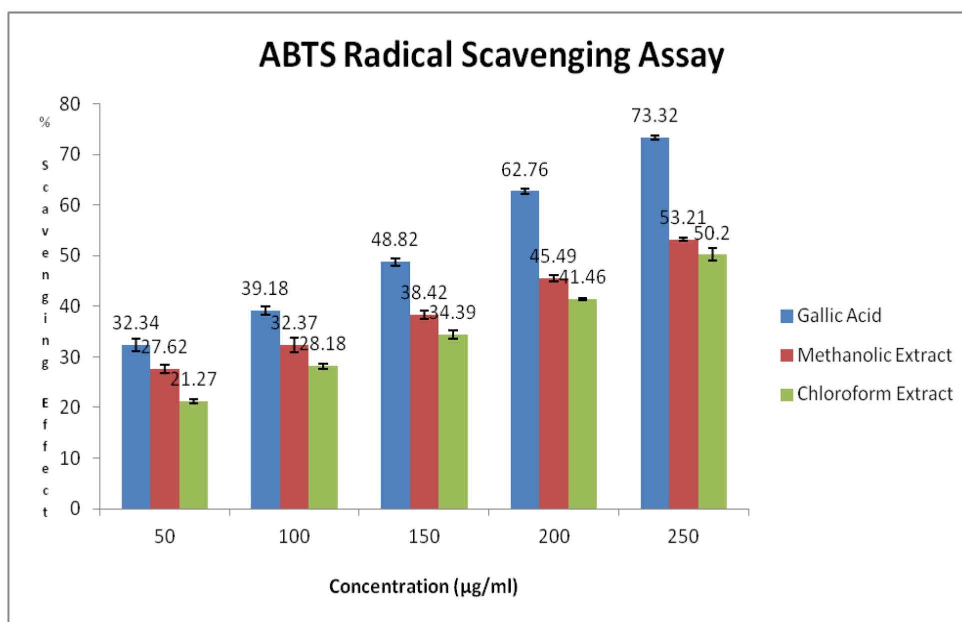

Fig. 3: ABTS Radical Scavenging Assay of *Crateva adansonii* bark extracts

3.3.3 Phosphomolybdate Assay

Total antioxidant capacity of *Crateva adasonii* bark extracts was investigated by phosphomolybdate assay and the results were expressed as percentage of scavenging effect shown in Table 5 and Figure 4. The maximum percentage of antioxidant effect was measured at 250µg/ml concentration in reference standard gallic acid 71.13±0.26 than the methanol and chloroform bark extracts 59.47±0.87 & 51.56±0.87 respectively.

Phosphomolybdate Assay method is based on the reduction of Mo (VI) complex occurs to Mo (V) with the maximal absorption at 765nm. Electron transfer from antioxidants to Mo (VI) complex occur in phosphomolybdenum assay. Previous study report stated the antioxidant activity of different extracts may depend on the presence of polyphenols which may act as reductones [38]. The result shown that the methanolic bark extract (59.47% antioxidant effect with IC50 value of 168.60 µg/ml) of *Crateva adansonii* possess maximal absorption with good phosphomolybdenum reduction capacity than chloroform bark extract (51.56% antioxidant effect with IC50 value of 234.70 µg/ml) of the plant. A similar report was given by earlier study made in *Rhodiola imbericata* rhizome extracts [39].

Table 5 – Phosphomolybdate assay of *Crateva adansonii* bark extracts

Concentration (µg/mL)	Antioxidant Effect %		
	Gallic Acid	Methanolic Extract	Chloroform Extract
50	44.21±0.58^{a}	36.16±1.22^{c}	27.29±0.89^{b}
100	57.47±0.95^{b}	41.28±0.57^{b}	35.48±1.34^{a}
150	64.39±0.87^{c}	47.45±0.74^{b}	37.27±0.69^{d}
200	69.25±0.92^{b}	52.69±1.34^{c}	46.76±1.29^{a}
250	71.13±0.26^{d}	59.47±0.87^{a}	51.56±0.87^{d}
IC50	70.60	168.60	234.70

Data are presented as the mean ± SD (n=3) and values were analysed by one- way ANOVA (Tukey-HSD multiple range *post hoc* test,Different superscript letters a-d indicate values within a column significantly different from each other, $p < 0.05$).

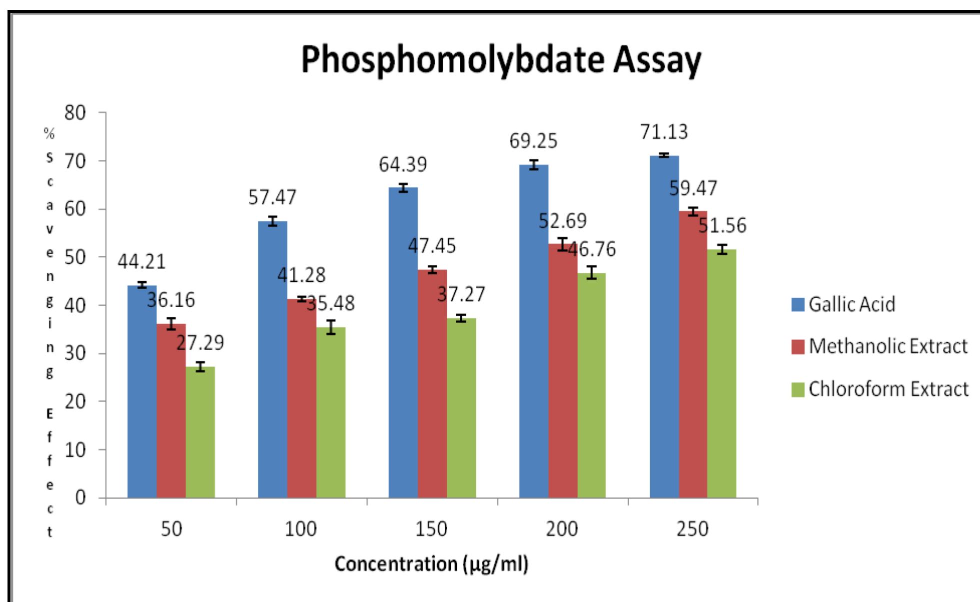

Fig. 4: Phosphomolybdate assay of *Crateva adansonii* bark extracts

3.3.4 Hydrogen Peroxide Radical Scavenging Assay

The results of hydrogen peroxide radical scavenging assay were expressed as percentage of scavenging effect shown in Table 6 and Figure 5. The highest hydroxyl radical scavenging effect was measured at 250µg/ml in standard gallic acid than the methanolic and chloroform bark extract samples (81.60, 64.23 & 59.72%) respectively.

Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells. Thus, the removing of hydrogen peroxide is very important for antioxidant defense in cell or food systems [40]. From these reports it has been concluded extract of *Crateva adasonii* methanolic bark extract (64.23%) have a good hydrogen peroxide scavenging activity than the chloroform extract (59.72% inhibition).It was observe that good correlation exist between phenolic, flavonoid quantitative phytoconstituents analysis and *in vitro* anti-oxidant activity of methanolic bark extracts of *C. adansonii*, similar previous study result report was also made in *Eclipta alba* [41].

Table 6 - Hydrogen peroxide radical scavenging assay of *Crateva adansonii* bark extracts

Concentration (µg/mL)	Scavenging Effect %		
	Gallic Acid	Methanolic Extract	Chloroform Extract
50	49.29±0.63^{b}	41.20±1.35^{d}	38.24±1.37^{a}
100	58.32±0.76^{a}	47.58±0.87^{b}	42.38±0.69^{b}
150	67.49±1.58^{d}	51.45±1.32^{a}	48.72±0.78^{d}
200	74.29±1.28^{a}	58.69±0.67^{c}	52.37±1.41^{a}
250	81.60±0.47^{c}	64.23±0.89^{b}	59.72±0.83^{b}
IC50	52.70	127.50	197.30

Data are presented as the mean ± SD (n=3) and values were analysed by one- way ANOVA (Tukey-HSD multiple range *post hoc* test, Different superscript letters a-d indicate values within a column significantly different from each other, $p < 0.05$).

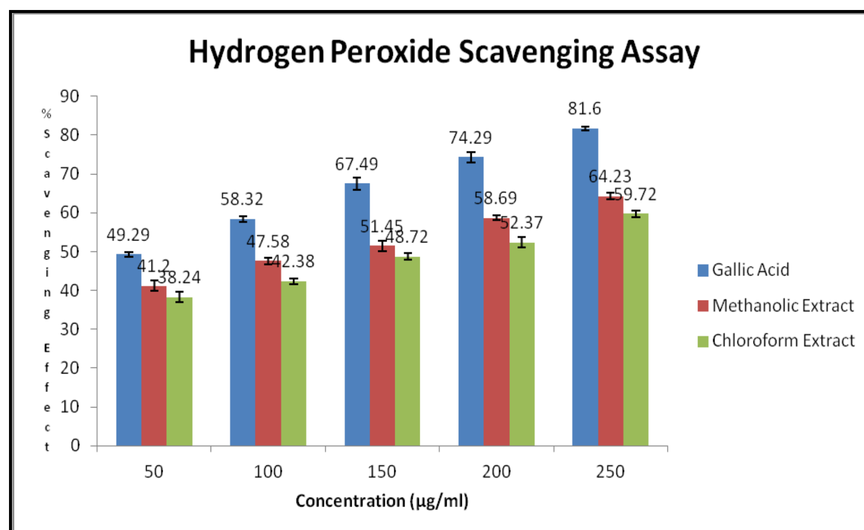

Fig. 5: Hydrogen peroxide radical scavenging assay of *Crateva adansonii* bark extracts

3.4 Thin Layer chromatography Analysis

TLC analysis of methanolic and chloroform bark extract shown six and four bands (Lane 1 & Lane 2) respectively. The yellow and green coloured spots were visualized under UV light. Rf values are calculated for each color band of bark extract. The Rf values for methanolic bark extract was 0.31, 0.47, 0.52, 0.71,0.82, 0.93 whereas Rf value for chloroform bark extract was 0.47, 0.52, 0.82, 0.93. Previous similar study [42] result revealed that Rf value for two important flavonoids such as quercetin and kaempferol in silica gel and n- Butanol: Acetic acid: Water solvent system was found to be 0.83 and 0.64 are matched with Rf values of two bark extracts of the plant and it confirmed the abundance of flavonoids in bark extract of the plant *Crateva adansonii*.

Fig. 6: TLC Chromatogram of *Crateva adansonii* bark extracts (Lane 1- Methanolic bark extract, Lane 2- Chloroform bark extract).

4. CONCLUSION

The results of the present study revealed that methanol & chloroform bark extracts of *Crateva adansonii* possess bioactive phytoconstituents responsible for *in vitro* antioxidant activity. Further it was confirmed that the presence of polyphenolic and flavonoid compounds of *Crateva adansonii* bark extracts by TLC analysis. The present study gives an idea of about further fruitful logic towards isolation and characterization of such novel anti-oxidant phytocompounds from the *Crateva adansonii* in future studies.

REFERENCES

[1] Gill, N. S., Bajwa, J., Sharma, P., Dhiman, K., Sood, S., Sharma, P. D., ... & Bali, M. (2011). Evaluation of antioxidant and antiulcer activity of traditionally consumed Cucumis melo seeds. *J Pharmacol Toxicol*, *6*, 82-89.

[2] Zengin, G., Aktumsek, A., Guler, G. O., Cakmak, Y. S., & Yildiztugay, E. (2011). Antioxidant Properties of Methanolic Extract and Fatty Acid Composition of Centaurea urvillei DC. subsp. hayekiana Wagenitz. *Records of Natural Products*, *5*(2).

[3] Samatha, T., Shyamsundarachary, R., Srinivas, P., & Swamy, N. R. (2012). Quantification of total phenolic and total flavonoid contents in extracts of Oroxylum indicum L. Kurz. *Asian Journal of Pharmaceutical and Clinical Research*, *5*(4), 177-179.

[4] Aliyu, A. B., Ibrahim, H., Musa, A. M., Ibrahim, M. A., Oyewale, A. O., & Amupitan, J. O. (2010). In vitro evaluation of antioxidant activity of Anisopus mannii NE Br. *African journal of biotechnology*, *9*(16), 2437-2441.

[5] Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford University Press, USA.

[6] Harman, D. (1988). Free radical theory of aging: current status. *Lipofuscin-1987: State of the art*, *1*, 3-21.

[7] Igoli, N. P., Gray, A. I., Clements, C. J., Igoli, J. O., Nzekwe, U., & Singla, R. K. (2012). Scientific investigation of antitrypanosomal activity of Crateva adansonii DC leaves extracts. *Indo Glob. J. Pharm. Sci*, *2*, 226-229.

[8] Sivarajan, V. V., & Balachandran, I. (1994). *Ayurvedic drugs and their plant sources*. Oxford and IBH publishing.

[9] Gitte, T. A., Kare, M. A., & Deshmukh, A. M. (2012). Ethno-medicinal studies on barks of some medicinal plants in Marathwada (MS), India â€"I. *Recent Research in Science and Technology*, *4*(10).

[10] Harborne, J. (1998). Phytochemical methods: a guide to modern techniques of plant analysis. Londres, Royaume-Uni.

[11] Sofowora, A. (1982). *Medicinal plants and traditional medicine in Africa*. John Wiley and sons LTD.

[12] Trease, G. E., & Evans, W. C. (1987). A text book of pharmacognosy. ELSB Baillere Tindal.

[13] Shamsa, F., Monsef, H., Ghamooshi, R., & Verdian-rizi, M. (2008). Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Thai J Pharm Sci*, *32*, 17-20.

[14] Rao, T. M., Rao, B. G., & Rao, Y. V. (2012). Antioxidant activity of Spilanthes acmella extracts. *Inter Jour of Phytopharm*, *3*(2), 216-20.

[15] Kaviarasan, S., Naik, G. H., Gangabhagirathi, R., Anuradha, C. V., & Priyadarsini, K. I. (2007). In vitro studies on antiradical and antioxidant activities of fenugreek (Trigonella foenum graecum) seeds. *Food chemistry*, *103*(1), 31-37.

[16] El Hajaji, H., Lachkar, N., Alaoui, K., Cherrah, Y., Farah, A., Ennabili, A., ... & Lachkar, M. (2010). Antioxidant properties and total phenolic content of three varieties of carob tree leaves from Morocco. *Records of Natural Products*, *4*(4), 193.

[17] Xu, B. J., & Chang, S. K. C. (2008). Total phenolic content and antioxidant properties of eclipse black beans (Phaseolus vulgaris L.) as affected by processing methods. *Journal of food science*, *73*(2), H19-H27.

[18] Har, L., & Intan, S. I. (2012). Antioxidant activity, total phenolics and total flavonoids of Syzygium polyanthum (Wight) Walp leaves. *International Journal of Medicinal and Aromatic Plants*, *2*(2), 219-228.

[19] Marinova, D., Ribarova, F., & Atanassova, M. (2005). Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *Journal of the university of chemical technology and metallurgy*, *40*(3), 255-260.

[20] Singh, R., Verma, P. K., & Singh, G. (2012). Total phenolic, flavonoids and tannin contents in different extracts of Artemisia absinthium. *Journal of Intercultural Ethnopharmacology*, *1*(2), 101-104.

{21] Afify, A. E. M. M., El-Beltagi, H. S., El-Salam, S. M. A., & [Omran, A. A. (2012). Biochemical changes in phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties. *Asian Pacific Journal of Tropical Biomedicine*, *2*(3), 203.

[22] Miean, K. H., & Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *Journal of agricultural and food chemistry*, *49*(6), 3106-3112.

[23] Ghasemzadeh, A., Jaafar, H. Z., & Rahmat, A. (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (Zingiber officinale Roscoe). *Molecules*, *15*(6), 4324-4333.

[24] Rasool, N., Rızwan, K., Zubaır, M., Naveed, K. U. R., Imran, I., & Ahmed, V. U. (2011). Antioxidant potential of different extracts and fractions of Catharanthus roseus shoots. *International journal of phytomedicine*, *3*(1), 108-114.

[25] Stankovic, M. S. (2011). Total phenolic content, flavonoid concentration and antioxidant activity of Marrubium peregrinum L. extracts. *Kragujevac J Sci*, *33*(2011), 63-72.

[26] Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199.

[27] Gardeli, C., Vassiliki, P., Athanasios, M., Kibouris, T., & Komaitis, M. (2008). Essential oil composition of Pistacia lentiscus L. and Myrtus communis L.: Evaluation of antioxidant capacity of methanolic extracts. *Food chemistry*, *107*(3), 1120-1130.

[28] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, *26*(9-10), 1231-1237.

[29] Umamaheswari, M., & Chatterjee, T. K. (2008). In vitro antioxidant activities of the fractions of Coccinia grandis L. leaf extract. *African Journal of Traditional, Complementary and Alternative Medicines*, *5*(1), 61-73.

[30] Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, *10*(6), 1003-1008.

[31] Martínez-Cano, E., Martínez-Cano, S. M., Avalos-López, K. I., & González-Simental, J. A. (2017). Preliminary phytochemical study and TLC analysis of the fruit, leaves and flowers of Citrus limetta Risso. *Journal of Pharmacognosy and Phytochemistry*, *6*(5), 594-599.

[32] Prabha D.S, Elamparithi D, Moorthy V, Dahms H.U, Nagaraja Suryadevara (2018). Identification of Phenolic compounds from degraded Prosopis juliflora by Oscillatoria laetevirens. *International Journal of Current Research in Science, Engineering and Technology*. *1(1)*:39-45.

[33] Bhuiyan, M. A. R., Hoque, M. Z., & Hossain, S. J. (2009). Free radical scavenging activities of Zizyphus mauritiana. *World journal of agricultural sciences*, *5*(3), 318-322.

[34] Thirumalaisamy Rathinavel, Subramanian Ammashi and Govarthanan Muthusamy, (2018), Screening of anti-inflammatory phytocompounds from Crateva adansonii leaf extracts and its validation by In Silico modeling. *Journal of Genetic Engineering and Biotechnology* (2018), https://doi.org/10.1016/j.jgeb.2018.03.004.

[35] Koleva, I. I., Van Beek, T. A., Linssen, J. P., Groot, A. D., & Evstatieva, L. N. (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, *13*(1), 8-17.

[36] Kanatt, S. R., Chander, R., & Sharma, A. (2007). Antioxidant potential of mint (Mentha spicata L.) in radiation-processed lamb meat. *Food Chemistry*, *100*(2), 451-458.

[37] Uttara, B., Singh, A. V., Zamboni, P., & Mahajan, R. T. (2009). Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Current neuropharmacology*, *7*(1), 65-74.

[38] Jayaprakasha, G. K., Selvi, T., & Sakariah, K. K. (2003). Antibacterial and antioxidant activities of grape (Vitis vinifera) seed extracts. *Food research international*, *36*(2), 117-122.

[39] Senthilkumar, R., Parimelazhagan, T., Chaurasia, O. P., & Srivastava, R. B. (2013). Free radical scavenging property and antiproliferative activity of Rhodiola imbricata Edgew extracts in HT-29 human colon cancer cells. *Asian Pacific journal of tropical medicine*, *6*(1), 11-19.

[40] Serhat Keser, Sait Celik, Semra Turkoglu, Okkes Yilmaz and Ismail Turkoglu. (2012). Hydrogen Peroxide Radical Scavenging and Total Antioxidant Activity of Hawthorn. *Chemistry Journal*, 2(1), 9-12.

[41] Priya G, Thirumalaisamy Rathinavel. (2017). Evaluation of antioxidant activity in relation with their phenolic and flavanoid content of eclipta alba l leaf collected from five different geographic regions of Tamil nadu, Int J Adv Interdis Res., 4 (4), 10-16 .

[42] Preetpal Kaur and Navdeep Singh Bains. (2012). Extraction of Flavonoids from In Vivo and In Vitro Tissue Culture of Some Important Halophyes of Western Rajasthan. *International Journal of PharmTech Research, 4(3),* 1167-1171.