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Phytochemical Investigation and Antioxidant Activity of Methanolic Extract of Betula Utilis Bark

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Abstract **- Antioxidant plays a major role in protecting our body from disease by reducing the oxidative damage to cellular component caused by Reactive Oxygen Species. The antioxidant activity of plant extracts was determined by different in vitro methods such as the DPPH free radical scavenging assay, SOS activity,hydroxyl radical scavenging and ferric thiocynate activity. Decreased absorbance of the reaction mixture indicates stronger scavenging activity. DPPH radical scavenging activity of Betula utilis ethyl acetate and methanolic extract exhibited percent inhibition 72% and its IC50 value was found to be 30.16 µg/ml and 83.86% and its IC50 value was found to be 27.62 µg/ml respectively. Ascorbic acid was used as a reference compound which exhibited percent inhibition 91.81% and showed IC50 value of 25.82 µg/ml. The Betula utilis ethyl acetate extract displayed SOS activity which exhibited percent inhibition of 72.49% and showed IC50 value of 44.47µg/ml. Similarly, SOS scavenger activity of Betula utilis methanol extract exhibited percent inhibition 81.49% and its IC50 value was found to be 20µg/ml. For SOS activity, Ascorbic acid was used as a reference compound which exhibited percent inhibition 86.72% and showed IC50 value of 12.01µg/ml. Hydroxyl radical scavenging activity of Betula utilis ethyl acetate and methanol extract exhibited percent inhibition 76.93 and 81.62% and its IC50 value was found to be 48.003 and 27.14µg/ml. Similarly, ferric thiocynate activity Betula utilis ethyl acetate and methanol extract exhibited percent inhibition 74.68 and 81.27% and its IC50 value was found to be 40.112 and 23.79µg/ml.The Betula utilis could be valuable natural source of antioxidants that could be further applied for the development of useful pharmaceutical products.**

Keywords— Methanolic extract, Ferric thiocynate activity, Betula utilis, Ascorbic acid

1. Introduction

The *Betula utilis* is one of the most important tree species across the high reaches of the Himalayas, growing at elevations upto 4,500 m (14,800ft). It belong to family Betulaceae. *Betula utilis* is a medium sized tree, which attains upto 20 m height.It is a multi-branched tree with usually irregular bole. Its shoot, young leaves and bracts are covered with short soft hairs. It has ovate and irregular serrate leaves, which are deciduous and are arranged in alternate fashion. It has reddish-white or white shining bark. Its outer bark has multiple smooth layers, which can be peeled in horizontal flakes. Its inner cortex is reddish.

Betula utilis possess various pharmacological activities like antimicrobial, anti-inflammatory, anticancer, antioxidant and anti-HIV activities. The plant possesses various alkaloids which have various therapeutic effects. *Betula utilis* bark is antiseptic and carminative. The specific epithet, utilis, refers to many uses of the different parts of the tree.

Free radicals donate to more than one hundred disorders in humans counting atherosclerosis, arthritis, ischemia and reperfusion damage of numerous tissues, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen, 1999; Cook and Samman, 1996). Free radicals due to ecological pollutants, radiation, chemicals, toxins, profound fried and spicy foods as well as corporeal stress, cause exhaustion of immune system antioxidants, modify in gene expression and persuade abnormal proteins. Oxidation development is one of the most imperative routs for producing free radicals in food,

drugs and still living systems. Catalase and hydroperoxidase enzymes change hydrogen peroxide and hydroperoxides to nonradical forms and purpose as natural antioxidants in human body. Owing to depletion of immune system natural antioxidants in dissimilar maladies, overwhelming antioxidants as free radical scavengers may be essential (Halliwell, 1994; Kuhnan, 1976; Kumpulainen and Salonen, 1999; Younes, 1981). At present available synthetic antioxidants similar to butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, have been supposed to cause or punctual negative health effects. Consequently, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Furthermore, these synthetic antioxidants also show low solubility and reasonable antioxidant activity (Barlow, 1990; Branen, 1975). Recently there has been an increase of interest in the therapeutic potentials of medicinal plants as antioxidants in dropping such free radical induced tissue injury. Polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Frankel, 1995). A number of confirmations suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski*et al.,* 1987). An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the occurrence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (Kolev*aet al.,* 2002).

2. Materials and Methods

A. Plant material collection

The bark of *Betula utilis* was collected from higher reaches of chakialpora mountains in the month of October and were identified by the courtesy of Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir and authenticated by a Botanist Akther H. Malik (Jr. Scientist and curator, Centre for Biodiversity and Taxonomy, University of Kashmir).The voucher specimen has been retained in the KASH Herbarium, University of Kashmir for future reference under voucher specimen No. 4310-KASH Herbarium, Centre for Biodiversity and Taxonomy, University of Kashmir.

B. Preparation of extract

Plant material was extracted by using cold maceration method; plant samples were collected, washed, rinsed and dried properly. Powder form of plant sample was extracted with different organic solvents (petroleum ether, ethyl acetate, and methanol) and allows standing for 4-5 days each. The extract was filtered using filter paper to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. Extract was transferred to beaker and evaporated; excessive moisture was removed and extract was collected in air tight container (Kokate*et al.,* 2006). Extraction yield of all extracts were calculated using the following equation below:

$$\text{Percentage Yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

C. Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in extracts by using standard procedures (Kokate*et al*., 2006). The extracts were subjected to following tests:

Tests for carbohydrates:

- **Molisch test:** To 1ml of extract, 2-3 drops of alcoholic α-naphthol solution was added. Conc. sulphuric acid was added along the side of the test tube. The appearance of purple ring at the junction of two liquids was observed, which confirms the presence of carbohydrates in the test samples.
- **Fehling's test:** To 1 ml of extract, similar quantity of Fehling's solution A and B was added and heated on a water bath for few minutes. The development of brick red precipitate was observed.
- **Benedict's test:** Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.
- **Barfoed's test:** 1 ml of extract and Barfoed's reagent were mixed in a test tube and heated on water bath for 2 minutes. Red colour due to formation of cupric oxide indicates the presence of monosaccharide.

Test for alkaloids:

All the test extracts were first treated with dil. hydrochloric acid separately and filtered. The filtrate of all the test extracts was exposed to following tests:

- **Mayer's test:** To 2-3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of white or creamy precipitate indicates the presence of alkaloids.
- **Hager's test:** To 1-2 ml of filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow color precipitate indicates the presence of alkaloids.

- **Wagner's test:** To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish-brown precipitate indicates the presence of alkaloids.

Test for flavonoids:

- **Lead acetate test:** The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate may indicate the presence of flavonoids.
- **Alkaline reagent test:** The extract was treated with few drops of sodium hydroxide separately in a test tube. Formation of intense yellow color, which becomes color less on addition of few drops of dilute acid, indicate presence of flavonoids.
- **Shinoda test:**To the extract, 5 ml (95%) of ethanol was added. The mixture was treated with few fragments of magnesium turning, followed by drop wise addition of concentrated hydrochloric acid. Formation of pink color indicates presence of flavonoids.

Test for glycosides:

- **Borntrager's test:** To 3 ml of extract, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and shake it welled. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammonical layer indicates presence of anthraquinone glycosides.
- **Legal's test:** 1 ml of extract was dissolved in pyridine. 1 ml of sodium nitropruside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red color indicates the presence of cardiac glycosides.
- **Keller-Killiani test:** To 2 ml of extract, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presence of cardiac glycosides.

Test for protein and amino acids:

- **Biuret's test:** The extract was treated with 1 ml of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulphate solution was added to the above mixture. The formation of violet or pink colour indicates the presence of proteins.
- **Ninhydrin test:** 3 ml of the extractwas heated with 3 drops of 5% Ninhydrin solution in a water bath for 10 minutes. Formation of blue colour indicates the presence of amino acids.

Test for saponins:

- **Foam test:** 1 ml of extract was dissolved in 20 ml of distilled water and shacked for 15min in a graduated cylinder. Formation of persistent foam around 1cm layer was observed.

Test for triterpenoids and steroids:

- **Salkowski's test:** The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layers turn red, sterol are present. Presence of golden yellow layer at bottom indicates the presence of triterpenes.
- **Libermann-Burchard's test:** The extract was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two layers, if upper layer turned green, indicate presence of steroids and formation of deep red color indicate presence of triterpenoids.

Test for tannin and phenolic compounds:

- **Ferric chloride test:** Some amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.
- **Lead acetate test**: Some amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. Formation of white precipitate indicates presence of phenolic compounds.

D. In-vitro Anti-oxidant Activity

a) DPPH Radical Scavenging Activity

Preparation of DPPH reagent

0.1mM solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol was prepared.

Preparation of Sample/Standard

Freshly prepared 1 mg/ml methanol solution of all extracts (sample)/standard will be prepared. 1 mg of extracts/standard will be taken with methanol to make 1mg/ml stock solution. Different volumes of extracts/standard (20 – 100µl) will be taken

from stock solution in a set of test tubes and methanol will be added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent will be added mixed thoroughly and absorbance will be recorded at 517 nm after 30 minutes incubation in dark at room temperature.

Preparation of control

For control, Take 3 ml of 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control will be taken against methanol (as blank) at 517 nm (Athavale*et al.*, 2012).

Percentage antioxidant activity of sample/standard will be calculated by using formula:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

b) Superoxide anion radical scavenging activity

1 ml of nitrobluetetrazolium (NBT) (100 µl of NBT in 100 mM phosphate buffer, pH 7.4), 1 ml of NADH (468 µl in 100 mM phosphate buffer, pH 7.4), solution as well as varying volumes of extracts (*sample*) (20, 40, 60, 80 and 100 µg/ml), were mixed well with methanol. The reaction was started by the addition of 1 ml of phenazine methosulfate (PMS) (60 µl/100 mM phosphate buffer, pH 7.4). The reaction mixture was incubated at 30°C for 15 min. The absorbance was measured at 560 nm in a spectrophotometer. Incubation without the sample (extract) was used as a blank sample. Ascorbic acid was used as the standard in comparing the different sample. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity (Nishikimi*et al.*, 1972).The percentage scavenging was calculated by using the formula shown below:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

c) Hydroxyl radical scavenging activity

Deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium. The reaction mixture containing FeCl3 (100 µM), EDTA (104 µM), H2O2 (1 mM) and 2-deoxy- D-ribose (2.8 mM) were mixed with or without extract at various concentrations (20-100 µg) in 1 ml final reaction volume made with potassium phosphate buffer (20 mM, pH 7.4) and incubated for 1 hr at 37 ^{0}C. The mixture was heated at 95 ^{0}C in water bath for 15 min followed by the addition of 1 ml each of TCA (2.8%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.02% BHA). Finally the reaction mixture was cooled on ice and centrifuged at 5000 rpm for 15 min. Absorbance of supernatant was measured at 532 nm. All readings were corrected for any interference from brown color of the extract or antioxidant by including appropriate controls. The negative control without any antioxidant or CPLL was considered 100% deoxyribose oxidation. The % hydroxyl radical scavenging activity of test sample was determined accordingly in comparison with negative control. Ascorbic acid was taken as the positive control (Ramakrishnaet al., 2012).

% inhibition = ($A_{control} - A_{sample}$/ A control) × 100

d) Ferric thiocynate assay

In this assay, hydroperoxide produced by linoleic acid added to the reaction mixture, which has been oxidized by air during the experimental period, is indirectly measured. An assay mixture of 2 mL sample [or methanol (as blank) or BHA/vitamin E (as reference)], 2.05 mL of 2.51% linoleic acid in 99.8% ethanol, 4 mL of 0.05 mol/L phosphate buffer (pH 7.0) and 1.95 mL of distilled water was placed in an Erlenmeyer flask in a rotary incubator (150 r/min, 40 C) in a dark place. To measure the antioxidant activity, 0.1 mL of the reaction mixture was transferred into a test tube. Then, 9.7 mL of 75% ethanol was added to it, followed by 0.1 mL of 30% ammonium thiocyanate, and 0.1 mL of 2 £ 10¡2 mol/L ferrous chloride in 3.5% hydrochloric acid. Three minutes after the addition of ferrous chloride to the reaction mixture, the absorbance was measured at 500 nm. Measurements were taken every 24 h until the absorbance of the control reached its maximum value. This mixture was also prepared without linoleic acid as a negative control **(Ghaima et al., 1930).** Vitamin E and BHA were used as positive controls. Antioxidant activity was calculated using the following equation:

% inhibition = ($A_{control} - A_{sample}$/ A control) × 100

Where $A_{control}$ is the absorbance of the control reaction and $_{Asample}$ is the absorbance of the tested extract samples.

3. Results & discussion

A. Percentage yield

Table 1: Percentage yield of *Betula utilis*

S. No.	Solvent	Color of extract	Theoretical weight (gm)	Yield in gms	% Yield
1.	Pet. Ether	Brown	322.02	0.28	0.087
2.	Ethyl acetate	Brown	315.35	26.88	8.523
3.	Methanol	Brown	292.29	41.45	14.18

B. Solubility determination

Table2: Solubility Determination of *Betula utilis* extract

S. No	Solvent	Pet. Ether	Ethyl acetate	Methanol
1.	Water	Insoluble	Insoluble	Slightly soluble
2.	Ethanol	Insoluble	Insoluble	Soluble
3.	Chloroform	Slightly soluble	Soluble	Slightly soluble
4.	DMSO	Soluble	Soluble	Soluble
5.	Pet. Ether	Soluble	Slightly soluble	Insoluble

C. Qualitative Phytochemical Analysis of Betula utilis extracts

Table 3: Phytochemical analysis of *Betula utilis* extracts

		Result		
S. No.	**Experiment**	**Pet. Ether**	**Ethyl acetate**	**Methanol**
Test for Carbohydrates				
1.	Molisch's Test	-	-	+
2.	Fehling's Test	-	-	+
3.	Benedict's Test	-	-	+
4.	Bareford's Test	-	-	+
Test for Alkaloids				
1.	Mayer's Test	-	-	+
2.	Hager's Test	-	-	+
3.	Wagner's Test	-	-	+
4.	Dragendroff's Test	-	-	+
Test for Terpenoids				
1.	Salkowski Test	-	+	+
2.	Libermann-Burchard's Test	-	+	+
Test for Flavonoids				
1.	Lead Acetate Test	-	+	+
2.	Alkaline Reagent Test	-	+	+
3.	Shinoda Test	-	+	+
Test for Tannins and Phenolic Compounds				
1.	$FeCl_3$ Test	-	+	+
2.	Lead Acetate Test	-	+	+
3.	Gelatine Test	-	+	+
4.	Dilute Iodine Solution Test	-	+	+
Test for Saponins				
1.	Froth Test	+	-	-
Test for Protein and Amino acids				
1.	Ninhydrin Test	-	-	-
2.	Biuret's Test	-	-	-
3.	Million's Test	-	-	-
Test for Glycosides				
1.	Legal's Test	-	-	+
2.	Keller Killani Test	-	-	+
3.	Borntrager's Test	-	-	+

D. Antioxidant activity

- **DPPH radical scavenging activity**

Table 4: DPPH radical scavenging activity of Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.447	46.97
40	0.383	54.56
60	0.215	74.49
80	0.137	83.74
100	0.069	91.81
Control	0.843	
IC50		25.82

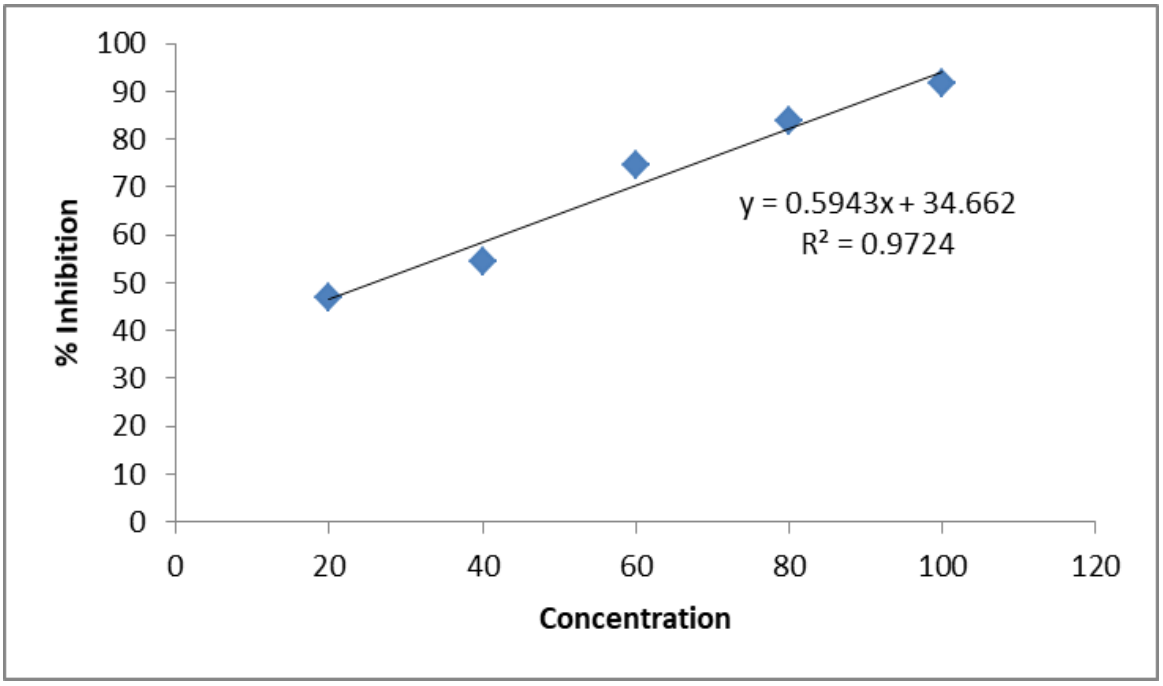

Fig.1: Bar Graph represents the Percentage Inhibition *Vs* Concentration of Ascorbic acid

Table 5: DPPH radical scavenging activity of Ethyl acetate *extract of Betula utilis*

Concentration	Absorbance	% inhibition
20	0.437	48.16
40	0.398	52.78
60	0.367	56.46
80	0.281	66.66
100	0.236	72.00
control	0.843	
IC50		**30.16**

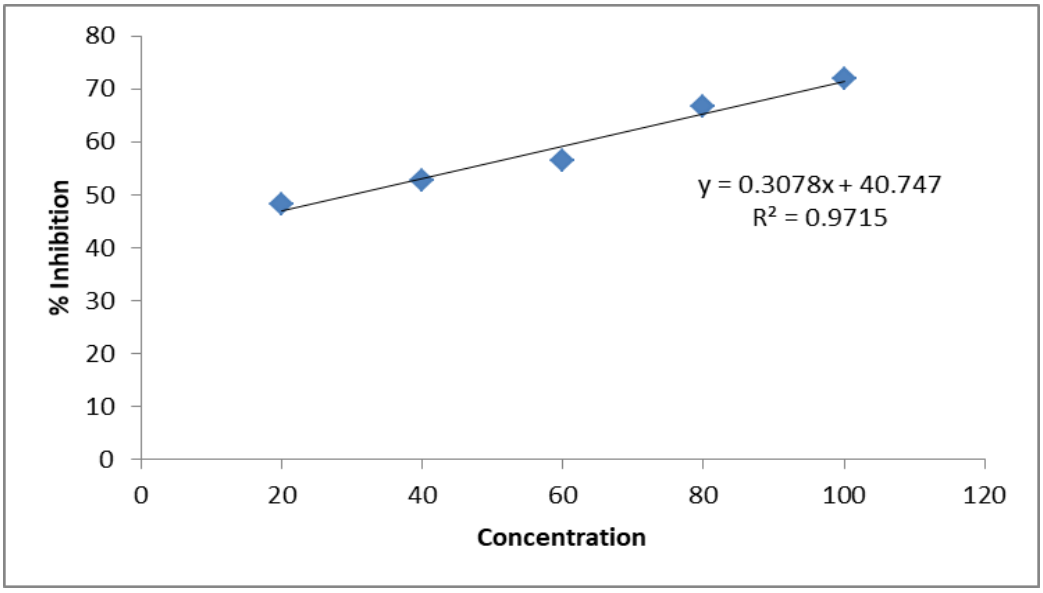

Fig.2: Bar Graph represents the Percentage Inhibition *Vs*Concentration of *Betulautilis*(Ethyl acetate)

Table 6: DPPH radical scavenging activity of Methanolic*extract of Betulautilis*

Concentration	Absorbance	% inhibition
20	0.458	45.67
40	0.361	57.17
60	0.298	64.65
80	0.21	75.08
100	0.136	83.86
Control	0.843	
IC50		**27.62**

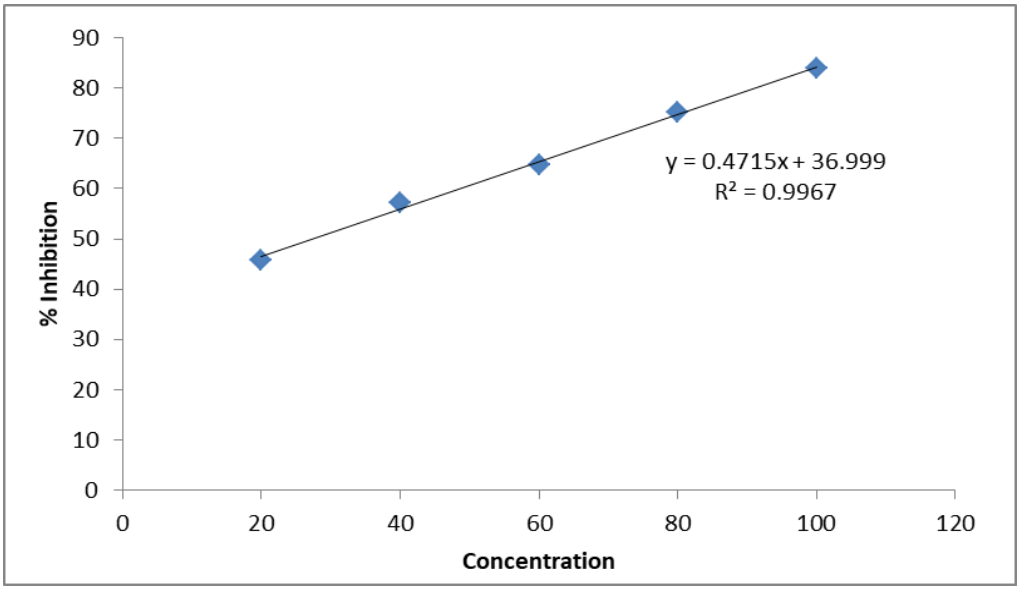

Fig.3: Bar Graph represents the Percentage Inhibition *Vs*Concentration of *Betulautilis*(Methanol)

- **Superoxide scavenging activity**

Table 7: SOS activity of Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.386	55.06
40	0.332	61.35
60	0.278	67.63
80	0.239	72.17
100	0.114	86.72
Control	0.859	
IC50		**12.01**

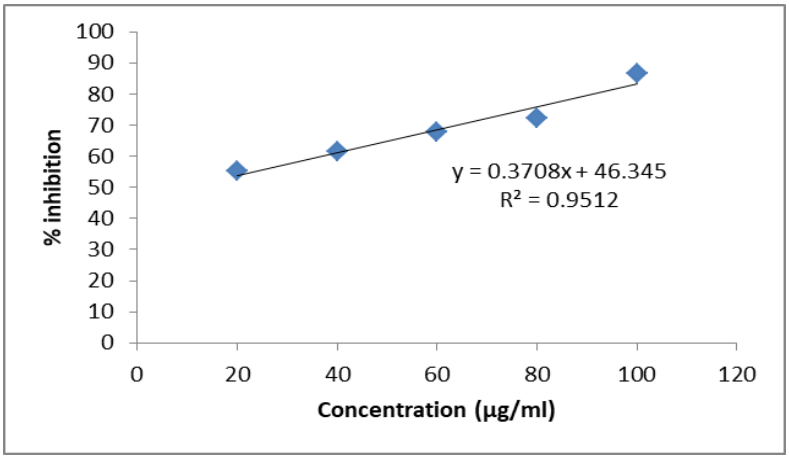

Fig.4: Bar Graph represents the Percentage Inhibition *Vs* Concentration of Ascorbic acid

Table 8: SOS activity of Ethyl acetate extract of *Betulautilis*

Concentration	Absorbance	% inhibition
20	0.463	41.31
40	0.401	49.17
60	0.365	53.73
80	0.293	62.86
100	0.217	72.49
Control	0.789	
IC50		**44.47**

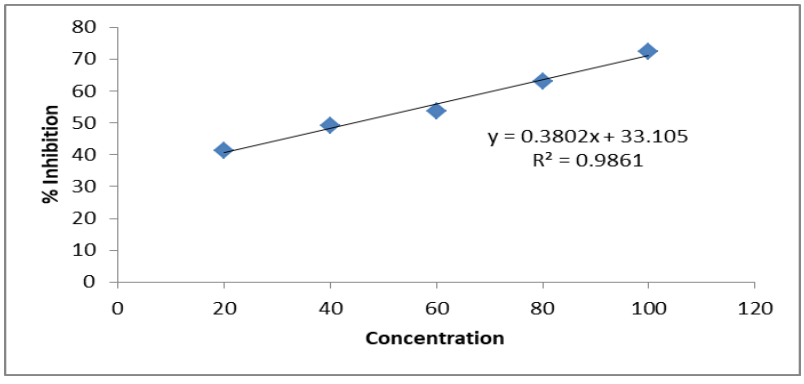

Fig.5: Bar Graph represents the Percentage Inhibition *Vs*Concentration of *Betulautilis*(Ethyl acetate)

Table 9: SOS activity of Methanolic extract of *Betulautilis*

Concentration	Absorbance	% inhibition
20	0.401	49.17
40	0.324	58.93
60	0.267	66.15
80	0.193	75.53
100	0.146	81.49
Control	0.789	
IC50		**20**

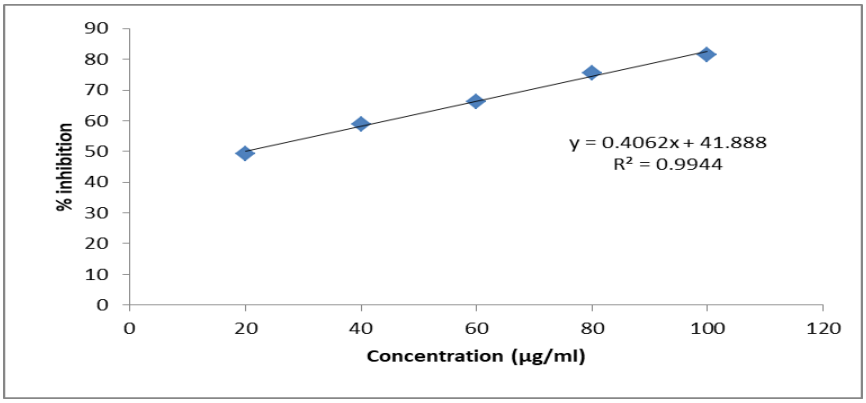

Fig.6: Bar Graph represents the Percentage Inhibition *Vs* Concentration of *Betul autilis*(Methanol)

- **Hydroxyl radical scavenging activity**

Table 10: Hydroxyl radical scavenging activity of Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.357	54.75285171
40	0.289	63.37135615
60	0.245	68.94803549
80	0.187	76.2991128
100	0.091	88.46641318
Control	0.789	
IC50		**9.33**

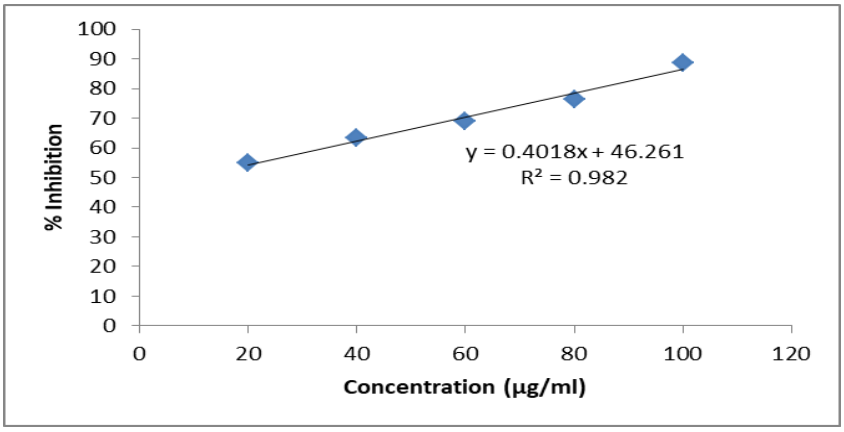

Fig.7: Bar Graph represents the Percentage Inhibition *Vs* Concentration of Ascorbic acid

Table 21: Hydroxyl radical scavenging activity of Ethyl acetate extract of *Betulautilis*

Concentration (µg/ml)	Absorbance	% inhibition
20	0.503	36.24841572
40	0.453	42.58555133
60	0.337	57.28770596
80	0.241	69.45500634
100	0.182	76.93282636
control	0.789	
IC50		**48.003**

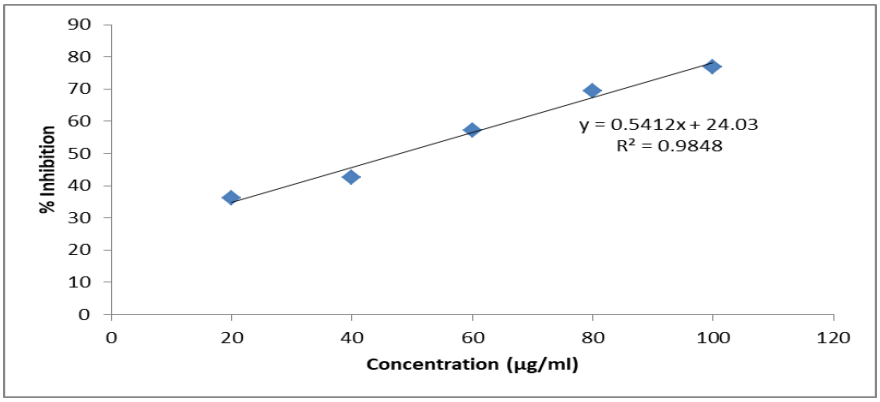

Fig.8: Bar Graph represents the Percentage Inhibition *Vs*Concentration of *Betulautilis*(Ethyl acetate)

Table 32 Hydroxyl radical scavenging activity of Methanolic extract of *Betulautilis*

Concentration	Absorbance	% inhibition
20	0.412	47.78200253
40	0.355	55.00633714
60	0.289	63.37135615
80	0.225	71.48288973
100	0.145	81.62230672
control	0.789	
IC50		**27.14**

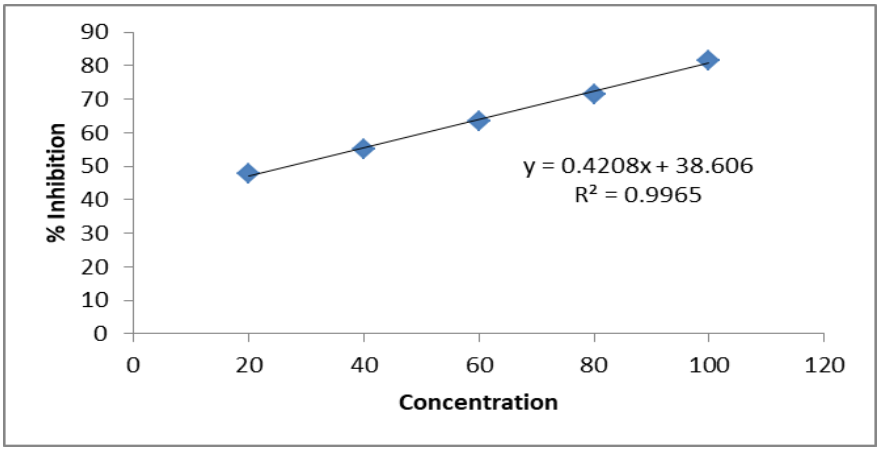

Fig.9: Bar Graph represents the Percentage Inhibition *Vs*Concentration of *Betulautilis*(Methanol)

- **Ferric thiocynate assay**

Table 43: Ferric thiocynate activity of Ascorbic acid

Concentration (µg/ml)	Absorbance	% inhibition
1 day	0.403	54.25652667
2 day	0.381	56.75368899
3 day	0.295	66.5153235
4 day	0.201	77.18501703
5 day	0.122	86.15209989
Control	0.881	
IC50		**16.85**

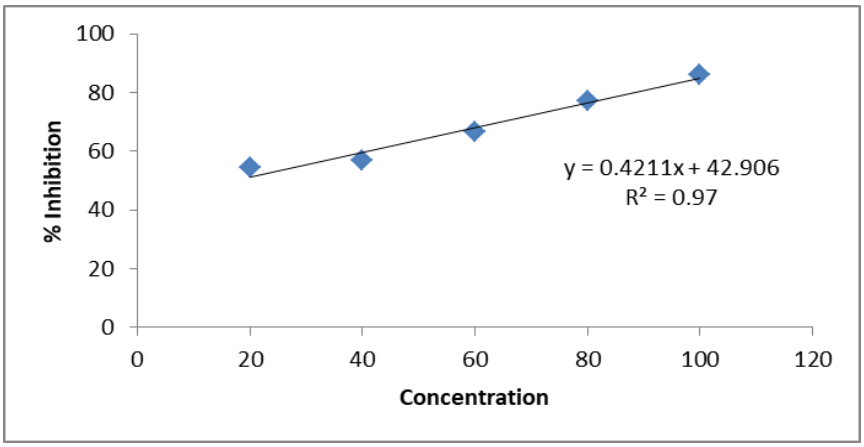

Fig.10: Bar Graph represents the Percentage Inhibition *Vs* Concentration of Ascorbic acid

Table 54: Ferric thiocynate activity of Ethyl acetate extract of *Betulautilis*

Concentration (µg/ml)	Absorbance	% inhibition
1 day	0.516	41.43019296
2 day	0.443	49.71623156
3 day	0.361	59.02383655
4 day	0.294	66.62883087
5 day	0.223	74.68785471
control	0.881	
IC50		**40.112**

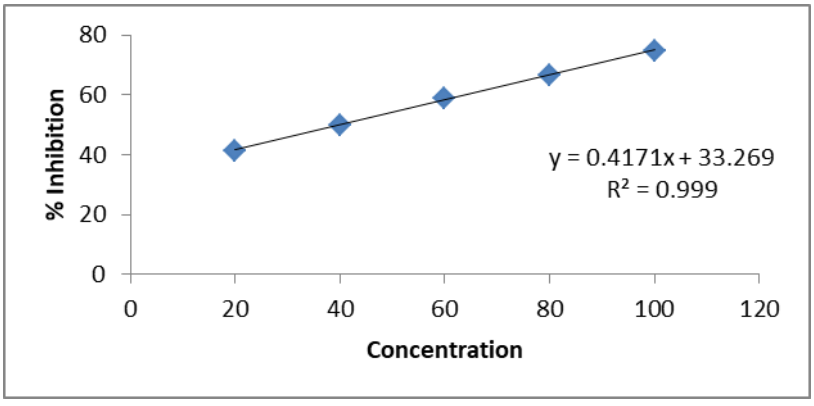

Fig.11: Bar Graph represents the Percentage Inhibition *Vs* Concentration of *Betula utilis* (Ethyl acetate)

Table 65: Ferric thiocynate activity of Methanolic extract of *Betulautilis*

Concentration (µg/ml)	Absorbance	% inhibition
1 day	0.446	49.37570942
2 day	0.397	54.93757094
3 day	0.303	65.60726447
4 day	0.243	72.41770715
5 day	0.165	81.27128263
control	0.881	
IC50		**23.79**

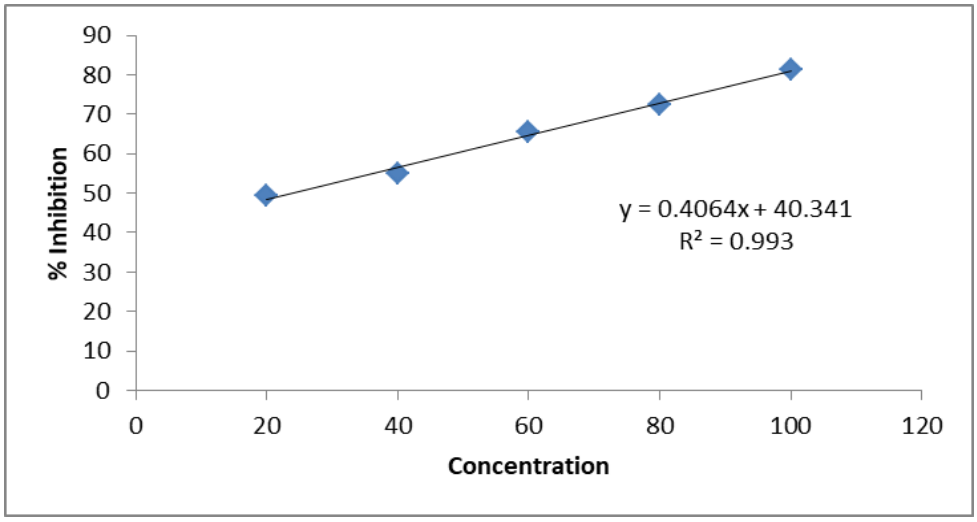

Fig.12: Bar Graph represents the Percentage Inhibition *Vs*Concentration of *Betulautilis*(Methanol)

The Qualitative analysis is very essential to identify the phytochemical constituents present in medicinal plants. The medicinal value of plants is due to the presence of particular bioactive constituents.Antioxidant plays a major role in protecting our body from disease by reducing the oxidative damage to cellular component caused by Reactive Oxygen Species. Recent investigations suggest that the plant origin antioxidants with free-radical scavenging properties may have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases, arthritis, and aging process.

The antioxidant activity of plant extracts was determined by different *in vitro* methods such as the DPPH free radical scavenging assay, SOS activity,hydroxyl radical scavenging and ferric thiocynate activity. Decreased absorbance of the reaction mixture indicates stronger scavenging activity. In this investigation, the in-vitro antioxidant effect of *Betula utilis* extracts was evaluated.

DPPH radical scavenging activity of *Betula utilis* ethyl acetate extract exhibited percent inhibition 72% and its IC_{50} value was found to be 30.16 µg/ml. Similarly, DPPH radical scavenging activity of *Betula utilis* methanol extract exhibited percent inhibition 83.86% and its IC_{50} value was found to be 27.62 µg/ml. Ascorbic acid was used as a reference compound which exhibited percent inhibition 91.81% and showed IC_{50} value of 25.82µg/ml.. The *Betula utilis* ethyl acetate extract displayed SOS activity which exhibited percent inhibition of 72.49% and showed IC_{50} value of 44.47µg/ml. Similarly, SOS scavenger activity of *Betula utilis* methanol extract exhibited percent inhibition 81.49% and its IC_{50} value was found to be 20 µg/ml. For SOS activity, Ascorbic acid was used as a reference compound which exhibited percent inhibition 86.72% and showed IC_{50} value of 12.01µg/ml.

Hydroxyl radical scavenging activity of *Betula utilis* ethyl acetate and methanol extract exhibited percent inhibition 76.93 and 81.62% and its IC_{50} value was found to be 48.003 and 27.14µg/ml.

Similarly, ferric thiocynate activity of *Betula utilis* ethyl acetate and methanol extract exhibited percent inhibition 74.68 and 81.27% and its IC_{50} value was found to be 40.112 and 23.79µg/ml.

4. Conclusion

The result of the current study showed that the extract of *Betula utilis*, exhibited the maximum antioxidant activity. The high scavenging property of *Betula utilis* may be due to hydroxyl groups accessible in the phenolic compounds' chemical structure that can offer the necessary component as a radical scavenger. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a probable anticipatory intervention for the diseases. All of the extracts in this research exhibited dissimilar amount of antioxidant activity. *Betula utilis* extract showed a higher potency than BHT in scavenging of DPPH free radical.

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