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STUDIES ON *IN VITRO* ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS OF *BUCHANIA LANZAN* LEAVES

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Abstract*:*
Background: Breast cancer is the most prevalent disease among women around the world, and is also the leading cause of death from cancer. Herbal medicine is an actual prehistoric form of healthcare known to mankind. Herbal medicine is being used by people belonging to all the cultures. The present study is based on the antioxidant and anti-cancerous potential of *Buchanania lanzan* which is also named as Chironji. *Buchanania lanzan* (chironji) is a tree species which belongs to the family *Anacardiaceae* and is commercially very useful.
Methods: *In vitro* Antioxidant Activity 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and reducing power assay
Result: The total antioxidant activity of *Buchanania lanzan*in leaves was assessed based on scavenging activity of DPPH free radicals, among them pet ether extract of leaves shows 25.54 % inhibition at conc. of 20 µg / ml and at 100 µg / ml it shows 38.95 % inhibition. Ethyl acetate extract at 20 µg/ml it shows 38.41% inhibition and at 100 µg/ml it shows 50.36% inhibition. In methanolic extract at 20 µg/ml it shows 34.42% inhibition and at 100 µg/ml it shows 57.43% inhibition. The IC_{50} value is defined as the concentration of the substrate that causes 50 % loss of the DPPH activity. Reducing power assay of Petroleum ether extracts, ethyl acetate extract and methanolic extract of *Buchanania lanzan* was studied at different concentrations and found to be increase with increase in concentration.
Conclusion: Therefore, present study concludes that *Buchanania lanzan* is an excellent medicinal plant which holds numerous bioactive phytochemicals and it can be used in prevention of cancer.

Key Words: **Herbal Medicines, *In vitro* Antioxidant Activity, Reducing Power Assay, *Buchanania Lanzan*.**

INTRODUCTION

An antioxidant can be defined as: “any substance that, when present in low concentrations compared to that of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate” (**Halliwell B et al 1995).** The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called “free radicals.” Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function (**Mark Percival).**
The physiological role of antioxidants is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals. In recent years, a substantial body of evidence has developed supporting a key role for free radicals in many fundamental

cellular reactions and suggesting that oxidative stress might be important in the pathophysiology of common diseases (**Young et al 2001).** Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases (**Sies, H. et al 1992).**
Buchanania Lanzan a widely used plant with a history of traditional medicinal use for the treatment of various diseases. It is used as decoction to treat intrinsic haemorrhage, blood-borne diarrhoea and as tonic **(Hung *et al*., 2011)**.

Present study established the antioxidant potential of *Buchanania Lanzan* studying the efficacy in preventing the *in-vitro* free radical using DPPH and reducing power assay.

MATERIALS AND METHODS

Authentication of Plant: The identification and authentication of plant was done by Dr. Saba Naaz, Botanist, from the Department of Botany, Saifia College of Science, Bhopal. A voucher specimen number 135/Saif. /Sci. /Clg/Bpl. was kept in Department of Botany, Saifia College of Science, Bhopal for future reference.

Collection of Plant: The plant material was selected on the basis of Ethano–botanical survey. Leaves of *Buchanania lanzan* were collected from the Pinnacle Biomedical Research Institute, Bhopal Campus.

Solvent Extraction: In present study, plant material was extracted by using cold maceration method. About 3kg of the powder was extracted with different organic solvent petroleum ether, ethyl acetate and methanol. Extract was transferred to beaker, evaporated & excessive moisture was removed and extract was collected in air tight container. Dimethyl sulfoxide (DMSO) was used to dissolve each extract and sterilized using 0.22 µm syringe filters (Axiva, Scichem Biotech) for further use.

In vitro Antioxidant Activity

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity:
Principle: The scavenging reaction between (DPPH•) and an antioxidant (H-A) was written as:

(DPPH•) + (H-A) ⟶ **DPPH-H + (A)**
(Purple **(Yellow)**

Antioxidant reacts with DPPH, which was a stable free radical and was reduced to DPPHH and as consequence the absorbance's decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in term of hydrogen donating ability. Then the scavenging ability was calculated using the following equation:

$$I\% = 100\times (A_{blank} - A_{sample}/ A_{blank})$$

Where, I (%) is the inhibition percent, a blank is the absorbance of the control reaction (containing all reagents except the test compound) and a sample is the absorbance of the test compound (**Braca *et al*., 2001**).

Procedure: The DPPH free radical scavenging ability of the extracts was assessed. 0.1mM DPPH solution (4mg/100ml) prepared in methanol. Samples were prepared to get concentration of 1mg/ml in methanol, various concentrations of sample solution is further diluted with methanol to 2ml than added 1ml of DPPH solution incubated at room temperature for 10 min absorbance was measured at 517 nm against blank (Gulçin et al., 2006). The free radical scavenging activity was expressed as the percentage inhibition which was calculated by using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance}_{\text{Blank}} - \text{Sample Absorbance})}{\text{Absorbance}_{\text{Blank}}} \times 100$$

The inhibition concentration (IC_{50}) value was determined from extrapolating the graph of % Inhibition versus the concentration of extract (using linear regression analysis), which is defined as the amount of antioxidant necessary to reduce the initial radical concentration by 50%. Lower the IC_{50} value higher the antioxidant effects **(Chew *et al*., 2012).**

Reducing Power assay

Principle: This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanides (Fe^{2+}), which then react with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples

Potassium ferricyanide + Ferric chloride $\xrightarrow{\textbf{Antioxidant}}$ **Potassium ferrocyanide + ferrous chloride**

Ascorbic acid (20µg/ml) was used as standard. A blank was prepared without adding standard or test compound. Increased absorbance of the reaction mixture indicates increase in reducing power **(Singhal *et al*., 2011)**

% increase in reducing power = A test – A blank/A blank

Where, A test is absorbance of test solution,
A blank is absorbance of blank

Procedure: Different concentrations of test samples were prepared. To 0.5 mL of different sample concentrations, 0.2 M Phosphate buffer (pH 6.6) and 0.5 mL Potassium ferricyanide (1% w/v) were added and incubated at 50^{o}C for 20 min. After cooling, 1.5 mL Trichloroacetic acid (10% w/v) added to reaction mixture to terminate the reaction. To this, 0.5mL Ferric chloride (0.1% w/v) was added and absorbance measured at 700 nm using ascorbic acid as reference standard. A curve was then plotted between absorbance and concentration. Increased absorbance of the reaction mixture indicates increase in reducing power **(Jain and Jain, 2011).**

RESULTS:

DPPH Radical Scavenging Activity

The DPPH Radical Scavenging Activity (DPPHRSA) method is based on the measurement of the scavenging capacity of antioxidants towards it. Upon receiving a hydrogen atom from antioxidants to the related hydrazine, the odd electron of nitrogen atom in DPPH is reduced, 2,2-diphenyl-1-picrylhydrazyl (DPPH) is characterized as a stable free radical by the advantage of delocalization of the spare electron over the molecule as a whole, resulting in a

dark violet color with a methanol solution absorption of approximately 517 nm. When mixing DPPH solution with a substance which can donate a hydrogen atom, the reduced form gives rise to pale yellow with the loss of violet color. The DPPH method can be used in organic aqueous and non-polar solvents and can be used to study antioxidants both hydrophilic and lipophilic.

The scavenging activity of extracts and standard on the DPPH radical expressed as IC_{50} value of methanol was 73.18, ethyl acetate was 101.7, Petroleum Ether was 168.12 and Ascorbic acid was 25.07. IC_{50} value of Methanolic extract was effective and close to ascorbic acid which is a well-known antioxidant.

Table 1: DPPH radical scavenging activity of Std. Ascorbic acid

Ascorbic acid (std.)			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	**20 µg/ml**	0.291	47.28
2.	**40 µg/ml**	0.253	54.17
3.	**60µg/ml**	0.217	60.69
4.	**80µg/ml**	0.172	68.84
5.	**100µg/ml**	0.144	73.91
IC_{50}		**25.07**	

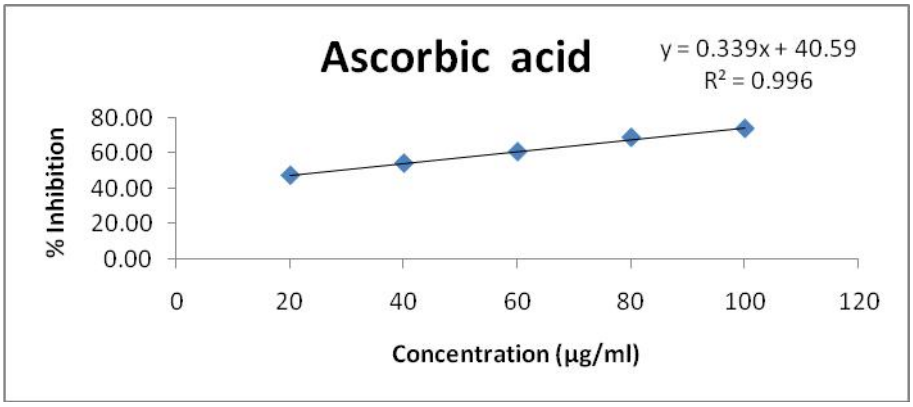


Table 2: DPPH radical scavenging activity of Petroleum ether extract of *B. lanzan*

Petroleum ether extract of *B. lanzan*			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	**20 µg/ml**	0.411	25.54
2.	**40 µg/ml**	0.399	27.72
3.	**60µg/ml**	0.385	30.25
4.	**80µg/ml**	0.358	35.14
5.	**100µg/ml**	0.337	38.95
IC_{50}		**168.12**	

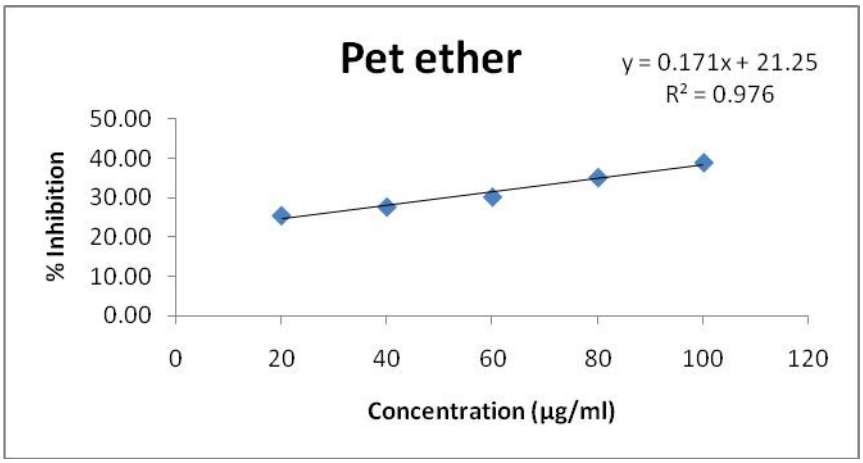


Table 3: DPPH radical scavenging activity of Ethyl acetate extract of *B. Lanzan*

Ethyl acetate extract of *B. lanzan*			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	**20 µg/ml**	0.34	38.41
2.	**40 µg/ml**	0.323	41.49
3.	**60µg/ml**	0.306	44.57
4.	**80µg/ml**	0.299	45.83
5.	**100µg/ml**	0.274	50.36
IC_{50}		**101.7**	

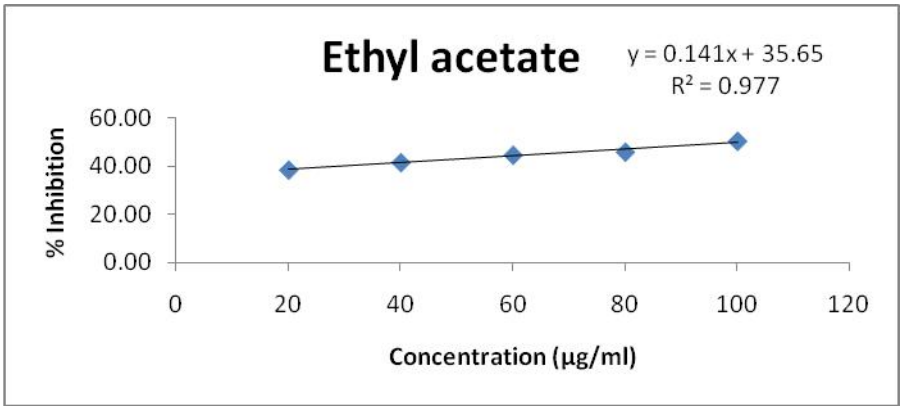
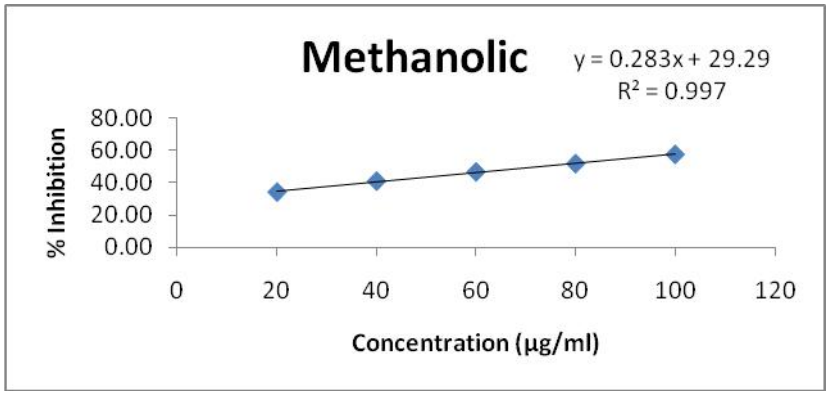


Table 4: DPPH radical scavenging activity of Methanolic extract of *B. Lanzan*

Methanolic extract of *B. lanzan*			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	**20 µg/ml**	0.362	34.42
2.	**40 µg/ml**	0.325	41.12
3.	**60µg/ml**	0.294	46.74
4.	**80µg/ml**	0.266	51.81
5.	**100µg/ml**	0.235	57.43
IC_{50}		**73.18**	



DPPH radical scavenging activity is one of the simplest and most widely used methods for screening the antioxidant activity of natural products. The antioxidant activity was assessed using DPPH radical scavenging method. The control, ascorbic acid showed 25.07µg/ml antioxidant activity at 1 mg/ml concentration. In the same concentrations, the methanolic extract of leaves produced 73.18 µg/ml antioxidant activity, ethyl acetate and petroleum ether extract showed 101.7 and 168.12 µg/ml antioxidant activity.

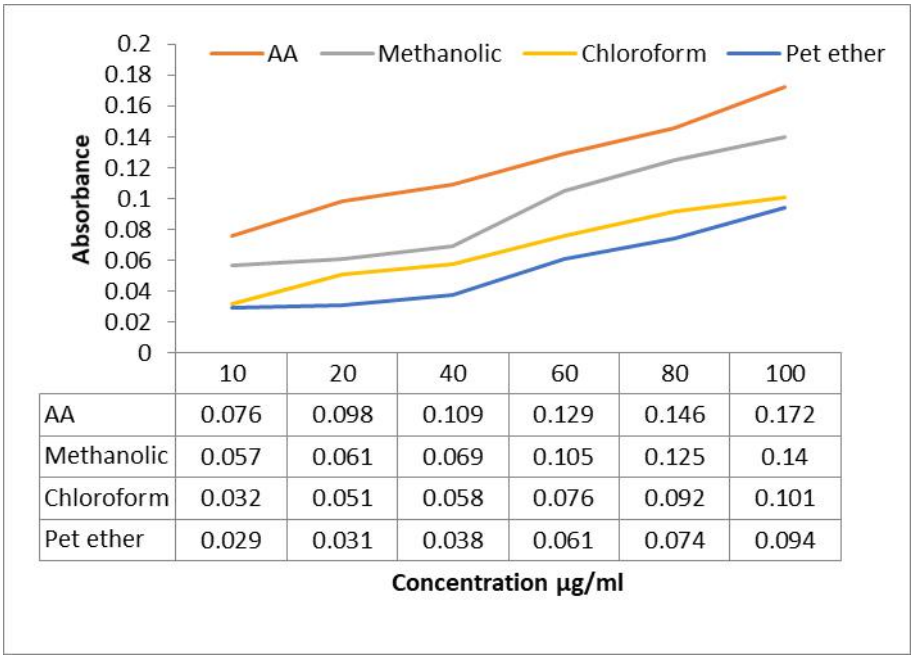
Reducing Power Assay

Reducing Power Assay (RPA) measures the reductive ability of antioxidant to transform Fe^{+3} to Fe^{+2}. The reducing properties are commonly correlated with reductones which have been shown to provide antioxidant activity through splitting the free radical chain through hydrogen-donating ability. The reducing power of extracts is shown graphically by depicting

absorbance as a function of concentration. The reducing power of all the extracts increased with increase in concentration. Reducing power of methanol extract is highest which is comparable to standard compound ascorbic acid.

Table 5: Absorbance of different extracts with different Concentration

Reducing Power Assay					
S. No.	**Concentration**	**Absorbance of Ascorbic acid**	**Absorbance of Petroleum ether extract**	**Absorbance of Ethyl acetate extract**	**Absorbance of Methanolic extract**
1.	**20 µg/ml**	0.076	0.029	0.032	0.057
2.	**40 µg/ml**	0.098	0.031	0.051	0.061
3.	**60µg/ml**	0.109	0.038	0.058	0.069
4.	**80µg/ml**	0.129	0.061	0.076	0.105
5.	**100µg/ml**	0.146	0.074	0.092	0.125



	10	20	40	60	80	100
AA	0.076	0.098	0.109	0.129	0.146	0.172
Methanolic	0.057	0.061	0.069	0.105	0.125	0.14
Chloroform	0.032	0.051	0.058	0.076	0.092	0.101
Pet ether	0.029	0.031	0.038	0.061	0.074	0.094

DISCUSSION

In vitro Antioxidant Activity

Free radical scavenging activity plays a vital role in a biological system. Many secondary metabolites which include flavonoids, phenolic compounds etc serves as sources on antioxidants and exhibited scavenging activities **(Ghasemi *et al*., 2009)**. Antioxidants (free radical scavengers) are chemicals that attach and neutralize the biological system's free radicals, thereby preventing them from causing cell damage **(Diplock *et al*., 1998)**.

DPPH Radical Scavenging Assay

DPPH is a stable, free radical that requires a radical electron or hydrogen to convert into a neutral diamagnetic molecule. Antioxidant on contact with DPPH, passing electron or

hydrogen atom to DPPH and thus neutralizing the free radical character and reducing it to 1,1-diphenyl-2-picryl hydrazine and the degree of discolouration suggests the drug's scavenging function **(Ogawa *et al.*, 2008)**. The degree of absorption of radical DPPH caused by antioxidant is due to the reaction between antioxidant molecules and radical progress that results in the scavenging of the radical by donation of hydrogen. This is visually visible from purple to yellow as a transition of color. Consequently, DPPH is generally used as a substance for evaluating antioxidant activity **(Noipa *et al*., 2011)**.

The total antioxidant activity of *Buchanania lanzan* in leaves was assessed based on scavenging activity of DPPH free radicals, among them pet ether extract of leaves shows 25.54 % inhibition at conc. of 20 µg / ml and at 100 µg / ml it shows 38.95% inhibition. Ethyl acetate extract at 20 µg/ml it shows 38.41% inhibition and at 100 µg/ml it shows 50.36% inhibition. In methanolic extract at 20 µg/ml it shows 34.42% inhibition and at 100 µg/ml it shows 57.43% inhibition. The IC_{50} value is defined as the concentration of the substrate that causes 50 % loss of the DPPH activity. Its value for ascorbic acid was found to be 168.12 for Pet. ether extract, 101.7 for ethyl acetate extract and 73.18 for methanolic extract. These results indicated that methanolic extract of *Buchanania lanzan* have a noticeable effect on scavenging free radical. Data of comparative analysis of antioxidant activities of samples are presented in Table 1-4.

Reducing Power Assay

The reducing power of a compound is related to the ability to transfer electron, and thus can serve as an important indicator of its potential antioxidant activity. However, plant extracts antioxidant activity may be due to various mechanisms such as chain initiation prevention, transition metal ion catalyst binding, peroxide decomposition, prevention of continued abstraction of hydrogen, reductive capacity and radical scavenging capacity **(Yildirim *et al*., 2001)**. In reducing capacity assessment, Fe^{3+}/ferric cyanide complex is reduced to ferrous form by antioxidants and can be monitored by measuring the formation of blue color at 700 nm **(Athukorala *et al*., 2006)**. Reducing power assay of Petroleum ether extracts, ethyl acetate extract and methanolic extract of *Buchanania lanzan* was studied at different concentrations and found to be increase with increase in concentration. The results are given in the table 5.

CONCLUSION

Therefore, present study concludes that *Buchanania Lanzan* is an excellent medicinal plant which holds numerous bioactive phytochemicals and it can be used in prevention of cancer. In modern scientific literatures, plant extracts have been reported to have potential efficacy against hypertension, diabetes, epilepsy, cancer, inflammation, ulcer, etc. Various plant parts have been found to possess biological activity more specifically towards overcoming metabolic ailments.

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