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Potential Source of Antioxidants : Buchanania Lanzan Spreng

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ABSTRACT

The term 'antioxidant' referes to any molecule capabale of stabilizing or deactivating free radicals before they attack cells. Humans have evolved highly complex antioxidant systems (enzymic and nonenzymic), which work synergistically and in combination with each other to protect the cells and organ systems of the body against free radical damage. The antioxidants can be endogenous or obtained exogenously e.g., as a part of diet or as dietary supplements. Endogenous antioxidants play a crucial role in maintaining optimal cellular functions and thus systemic health and well-being. However, under conditions, which promote oxidative stress, endogenous antioxidants may not be sufficient and dietary antioxidants may be required to maintain optimal cellular functions. The most efficient enzymatic antioxidants involve glutathione peroxidase, catalase and superoxide dismutase (Mates et al 1999).

Keywords: Antioxidant, Drug Toxicity, Antioxidative Effect, Amny Spices, Buchanania Lanzan

I. INTRODUCTION

Investigations of natural antioxidants and bioactive compounds for the preservation of traditional medicines and use in treating certain human diseases have received much attention (Lin Y W et. al., 2010). Phenolic antioxidants can inhibit free radical formation and interrupt propagation of auto oxidation. Vitamin E and vitamin C are both effective in appropriate matrix. Plant extracts, generally used for their flavoring characteristics, often have strong H-donating activity thus making them extremely effective antioxidants. The antioxidant activity is most often due to phenolic acids, phenolic diterpenes, favonoids and volatile oils (Brewer M S et. al., 2011). Reactive oxygen species (ROS) effect oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA (Farber JL et. al., 1994). This oxidative damage is a critical ethological factor implicated in several chronic human diseases including cardiovascular dysfunctions, atherosclerosis, inflammation, carcinogenesis, drug toxicity, neurodegenerative diseases and cancer. Recently, traditional medicine plants have received much attention as sources of biological active substances including antioxidants that might serve as leads for the development of novel drugs (Silvaa CG et. al., 2009).

Spices have been investigated for their antioxidant properties for last 50 years. As early as 1952, amny spices were examined and 32 spices were found to retard the oxidation. Many spices have been shown to impart an antioxidative effect in foods. Spices and herbs have been used for flavour, colour and aroma for more than 2000 years. Due to the tremendous phytochemicals, the spices have been used as preservatives in foods and beverages. The antioxidants in spices and herbs are very effective because they possess excellent antioxidant activity. The spices and herbs have been used as antioxidants as whole or ground spice/herb, extracts, encapsulated or as emulsions (Milda E.Embuscado, 2015). Several studies also showed that black pepper, clove, cinnamon and coriander exhibited antioxidant properties. In recent decades, a number of phenolic substances were isolated from a variety of spice sources, including phenolic acids (e.g., Gallic acid, Caffeic acid, etc), flavonoids (eg. quercetin, rutin, myricetin, luteolin, naringenin and silybin), phenolic diterpenes and volatile oils. Considering all these aspects relevant to spices and antioxidants, the current study has been formulated to investigate the treasure of antioxidants from *Buchanania lanzan* Spreng.

Buchanania lanzan, commonly called as Chironji (belongs to family Anacardiaceae), is an evergreen tree with a straight cylindrical trunk up to 15m height, commonly found in dry deciduous forest upto an altitude of 1200m and in the sub Himalayan tract up to 900m. This plant is reported to possess cardio tonic, astringent, antioxidant activity and is also used in treatment of skin diseases (Y. Dai, W. C. Ye et. al., 2002). The parts of the plant are used for the treatment of various disorders. The oil from the seeds is used to reduce the swelling of the neck (T. Horio and A. A. Gohar K Ye et. al., 1997). Antistress activity of the methanolic extract of *Buchanania lanzan* in-vivo in both normal and stress induced rats following a biochemical approach was evaluated (Kapoor et. al., 2011). The present work is therefore channelized towards the analysis of phenols and flavonoids found in *Buchanania lanzan.* Oxygen free damage induce damage due to peroxidation to bio-membranes and also to DNA, which leads to tissue damage, thus cause occurrence of a number of diseases. Antioxidants neutralize the effect of free radicals through different ways and may prevent the body from various diseases. Antioxidants may play vital role in the metabolic disorders. Indian stands with highest percentage of people with diabetes, hypertension and cardiovascular disorders among the world. This may be due to the life style, ethnicity and improper food habits. Hence, the search for effective, non-toxic natural compounds with anti-oxidative potentials has been identified in recent years. In this study, antioxidant activity of methanolic, ethanolic and aqueous extracts of *Buchanania lanzan* Spreng. was evaluated. Along with phenols and flavonoids, sterols and thiamine were also considered to be one of the perspectives of the study. Subsequently, the study is aimed at questing and analyzing the immense antioxidant properties of *Buchanania lanzan*.

II. MATERIALS AND METHODS

The plant material of *Buchanania lanzan* Spreng. was collected from Mumbai region where the plant has been naturally inhabited. The plant was identified and authenticated with the help of wealth of India and flora of Bombay presidency.

Preparation of plant extracts:
The plant part used for the analysis was fruit pulp of *Buchanania lanzan*. The slices of dried fruits of the plant were successively extracted using methanol, ethanol and aqueous separately. The collected extracts were concentrated by evaporation under room temperature and used for the study. They were further screened for the flavonoids, phenols, sterols and thiamine by using standard protocols.

Determination of total flavonoid content: spectrophotometric aluminium chloride method was used for determination of flavonoids (Ebrahimzadeh et al, 2009 b). Total flavonoid contents were calculated as quercetin from a calibration curve. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 – 100 mg/ml.

Determination of total phenol content: Total phenol content was determined by Folin-Ciocalteau method (Nabavi et al, 2008b). Standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg/ml solutions of gallic acid in methanol:water (50:50 v/v). Total phenol values are expressed in terms of gallic acid equivalent (GAE) [mg/g of dry mass], which is a common reference compound.

TLC method for flavonoids: Minute quantity of seeds are measured in 2M hydrochloric acid and heated at 100˚ C for 30-40 minutes. Extract is cooled, filtered and extracted with ethyl acetate. It may be re-extracted with a small volume of amyl alcohol. The amyl alcohol extract is concentrated to dryness, taken up in a few drops of 1% methanolic HCL and aliquots chromatographed one-dimensionally in Forestal solvent (Acetic acid-conc. HCL- water; 30:3:10).

TLC method for phenols: The thin layer chromatographic method was done for revealing the presence of different flavonoids in the plant material. Four different liquid phases have been used to prove the particular phenolic acids present. These moving phases involve: a. CHCL3: Acetic acid (9:1); b. Acetone: Toluene: Acetic acid (50:40:20); c. Benzene: methanol: acetic acid (79:14:7); CHCL3: methanol: H2O (65:45:12) (Nedime D UR UST et. al., 1999). 0.2M sodium acetate was used as an absorbent.

Extraction and isolation of phenols: 2M HCL was added to known amount of finely ground fruits and hydrolysed by heating for 1 hour. After filteration the aqueous acidic solution was extracted with ethyl acetate. Ethyl acetate extract was treated with 5% $NaHCO_3$. The organic acids in the aqueous phase were extracted with ethyl acetate again and p-hydroxybenzoic acid, caffeic acids and p-coumaric acid were isolated from this extract and there presence was observed in chromatogram.

Estimation of sterols: It was done by Liebermann-Burchard method (J.B. Harborne et al., 1973). In this reaction the Acetic anhydride in the Liberman – Burchard regent is reacted with the sterol in the sample which gives a Green colour, whose absorbance can be determined by UV-visible spectrophotometer. Total sterol contents were calculated as cholesterol from a calibration curve. Pipette out standard cholesterol solution as 0.5, 1.0, 1.5, 2.0, 2.5 ml in five test tubes whereas tube 6 was kept blank and marked as S1, S2, S3, S4, S5 and S6. Then, 2 ml of the Liberman-Burchard regent was added to all six tubes and final volume of 5ml was made equal in each test tube by adding chloroform**.** The test tubes were covered with carbon black paper and kept in dark for 15 minutes in ice bucket. Then taken base line on spectrophotometer with blank (S6) at λmax 640nm. The absorbances of all standards (six tubes) were determined on spectrophotometer and standard graph was plotted.

Reducing power determination: 1 ml of different concentrations of the extract fractions was mixed with potassium ferricyanide and 2.5 ml of phosphate buffer. The mixture was incubated at 50^0 C for 20 min. 2.5 ml TCA (10%) was added to it and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was taken and 2.5 ml wate and 0.5 ml $FeCl_3$ (0.1%) were added to it. The absorbance was measured at 700nm. Higher absorbance of the reaction mixture indicated higher reducing power.

III. RESULT AND DISCUSSION

Determination of total flavonoid content: The present investigation showed the values of total flavanol compound for aqueous, methanolic and ethanolic extract to be 60 ug/ml, 57ug/ml and 27ug/ml respectively. This indicates the efficiency of aqueous extract of *Buchanania lanzan*. Thus the concentration of flavonoids obtained is **Aqueous > Methanol > Ethanol**. Thus, *Buchanania lanzan* contain good amount of flavonoids which can be used as medicinal foods (John Bradley Morris, 2008) and thus proving its beneficence for human as potential nutraceutical. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (Min and Chun-Zhao, 2005).

TLC method for flavonoids: In *Buchanania lanzan,* **Apigenin (0.83)** flavonoid was detected.

Determination of total phenol content: Phenolics are ubiquitous secondary metabolites in plants and possess a wide range of therapeutic uses such as antioxidant, antimutagenic, anticarcinogenic and also decrease cardiovascular complications (Yen G., Duh P. 1993). Total phenols determination in current study showed 150ug/ml, 50ug/ml and 190ug/ml total phenols in aqueous, methanolic and ethanolic extracts respectively. Thus the concentration of flavonoids obtained is **Ethanol > aqueous > Methanol**.

TLC method for phenols: In present investigation, the phenolic compounds were identified by TLC method with four different solvent systems and two spraying agents. In *Buchanania lanzan*, three phenolic compounds were detected. p-OH benzoic acid was obtained in solvent system C and D with $FeCl_3$ reagent. p-Coumaric acid was obtained in system A and B with $FeCl_3$ reagent and Vanillic acid was obtained in solvent system A with Folin reagent. **This shows the abundance of p-OH benzoic acid and p-Coumaric acid in *Buchanania lanzan*.**

Estimation of sterols: The present investigation showed the ethanolic extract of *Buchanania lanzan* with maximum amount of sterols (105ug/ml) followed by methanolic extract (100 ug/ml) followed by aqueous extracts (35ug/ml). Thus the concentration of sterols obtained is **Ethanol > Methanol > Aqueous**.

Reducing power determination: Reducing capacity was determined with aqueous extract at different concentrations. In present work, the concentrations of phytoactive chemicals were found to be **Phenols > Flavonoids > Sterols. Interestingly sterols**

concentration was found to be highest in *Buchanania lanzan*.

IV. CONCLUSION

Considering the overview of present study, *Buchanania lanzan* showed presence of flavonoids, phenols, sterols which are the potential antioxidants. The present work also corroborates the justification of increase in concentrations proportionately rising with increase in reducing capacity. *Buchanania lanzan* is a rich source of phenolic compnds which have been linked to most of the pharmacological activities. Thus, the plant should be exploed further as alternative source of medicine. Further in vivo antioxidant activities and in different antioxidant mechanisms are needed. The spices screened for phytochemical constituents seems to have the potential to act as a source of useful drugs and also to improve health status of the consumers as a result of the presence of various compounds that are vital for good health.

V. REFERENCES

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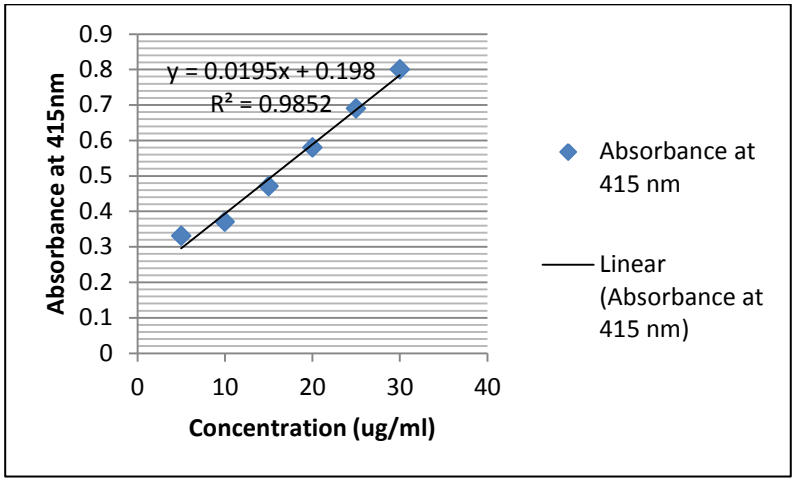
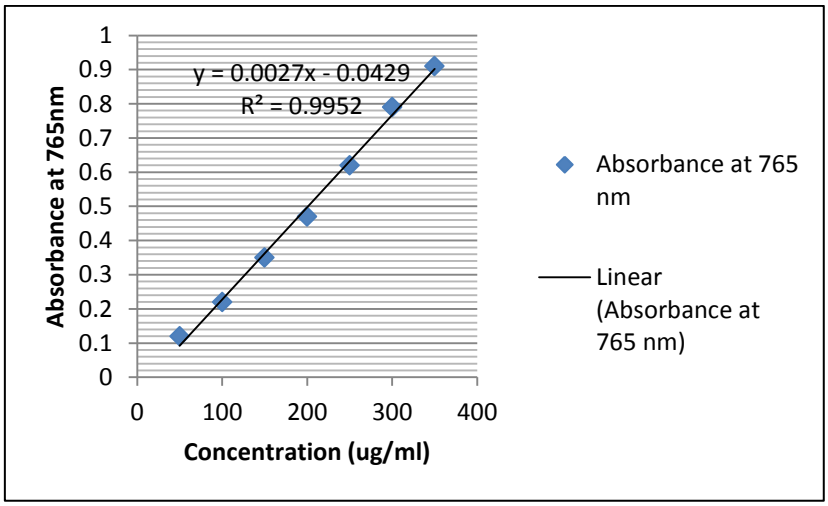
Tables and Graphical Representation:

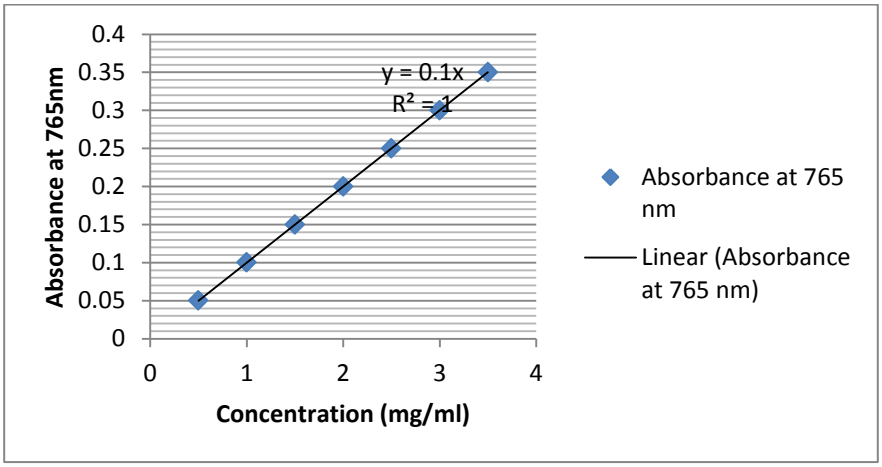
1. **Standard graph for Quercetin (Flavonoid)**:



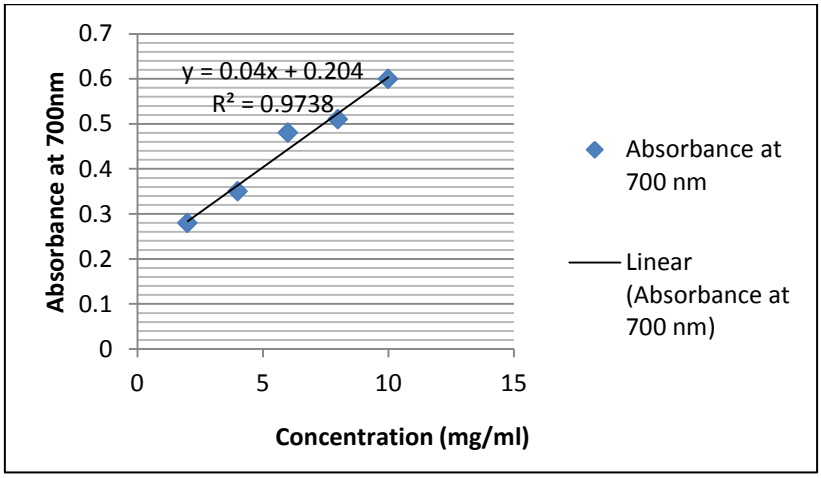
2. **Standard graph for Gallic acid (Phenol)**:



3. **Standard graph for Cholesterol (Sterol):**



4. Graph for reducing capacity:



5. Chromatographic results for Flavonoids:

SR. NO.	Flavonoids	Standard Rf Values	Rf values of flavonoids obtained
1	Apigenin	0.83	0.84

6. Total contents estimation:

Sr. no.	Extracts	Total Phenol content (ug/ml)	Total Flavonid content (ug/ml)	Total Sterol content (ug/ml)
1	Aqueous	150	60	35
2	Methanol	50	57	100
3	Ethanol	190	27	105

7. Chromatographic results for Phenols:

SR. NO.	Phenolic acids	Solvent systems and Rf values				Colours and spray reagent
		1	2	3	4	
1	P-Hydroxybenzoic acid	-	-	0.673	0.958	Yellow ($Fecl_3$)
2	P- Coumaric acid	0.51	0.905	-	-	Orange ($Fecl_3$)
3	Caffeic acid	-	0.851	-	-	Bluish grey ($Fecl_3$)
4	Vanillic Acid	0.82	-	-	-	Blue (Folin)