International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 603-610 www.ijprajournal.com ISSN: 2456-4494

A Comparitive Invitro Study on Antioxidant Properties of Selected Flowers

Kali shailaja*, S.Daniel sumanth[1], Kinjingi Prasanth[2], Nelluri Ajay Kumar[3], V.G.M. Pavan kalyan[4], D.Suryachaitanya varma[5].

** Assistant Professor, Department of Pharmacology , SIR C R Reddy College of Pharmaceutical Sciences, Eluru -534007*

[1,4,5][IV B.Pharmacy], SIR C R Reddy College of Pharmaceutical Sciences, Eluru -534007

[2,3][IV/VI Doctor of Pharmacy], SIR C R Reddy College of Pharmaceutical Sciences, Eluru -534007

Submitted: 10-07-2022 Accepted: 21-07-2022

ABSTRACT

Health is a central issue in today's life. Most illnesses are due to oxidative stress. Oxidation usually causes cytolysis. Plants such as Sesbaniagrandiflora, Spatodeacampanulata (African tulip),Allamandacathartica(golden trumpet) and Mirabilis jalapa (4 o'clock flower) are spreading in our environment. They are also used medicinally to treat various human illnesses. The antioxidant activity of the leaves of these plants was studied using aqueous extracts. The extract is subjected to phytochemical analysis. Sesbaniagrandiflorais known to contain alkaloids, saponins, carbohydrates, tannins, flavonoids and phenols. , Protein, terponoid.Spathodeacampanulateis known to contain alkaloids,saponins, glycosides and flavonoids. Allamandacathartica containssaponins, glycosides, tannins and flavonoids. Mirabilis jalapa contains alkaloids, saponins, glycosides, tannins, flavonoids, phenols andterpinoids. Two different in vitro methods, namely hydrogen peroxide capture activity, reducing power assay, were used to estimate antioxidant activity using a standard antioxidant (ascorbic acid). All four natural flower extracts showed antioxidant activity. However,Catharticaallamanda showed the highest activity. The results are roughly comparable to the standard drug ascorbic acid. Therefore, the flowers of these plants are useful as antioxidants

KEY WORDS : Antioxidant, Free radicals, Phenols, Flavanoids , Oxidative strees, cancer

I. INTRODUCTION

ANTI OXIDANT ACTIVITY

Antioxidants are natural and synthetic substances that can prevent cell damage from free radicals, unstable molecules produced by the body in response to the environment and other stresses. Free radicals are produced by humans during aerobic respiration, and free radicals produced by humans, plants and animals are inactivated by antioxidants.[5] Free radicals can be generated by physiological or biochemical processes that can react with membrane lipids, enzymes, proteins, nucleic acids, and some molecules to damage cells. "Antioxidants act as free radical scavengers" and protect the body. Free radicals have a short half-life, are highly reactive, and damage macromolecules such as DNA, proteins, and lipids. Oxidative damage to DNA causes premature aging and wrinkles. Since the skin is the largest organ in the human body, it is important to protect it. Antioxidants reduce free radicals, thereby limiting the signs of aging, wrinkles, inflammation, scars, and sunburn damage[8]. Naturally occurring antioxidants are rich in flavonoids, saponins, fresh fruits and vegetables, and whole grains are rich in antioxidant phytochemicals, alkaloids, tannins, phenols and ligans. Green leaves are the richest source of protein, vitamins and minerals. Plants are rich in free radical-removing antioxidants such as vitamins, phenolic acids, terpenoids, alkaloids, quinones, flavonoids, tannins, ligans, stilbens, amines, coumarins, betalains and other metabolites. Therefore, experts believe that antioxidants from foods are the healthiest[1]. Oxygen is essential for aerobic organisms, but can be toxic if taken in large amounts. Excessive intake of antioxidants can be harmful. For example, vitamin E supplements may increase the risk of bleeding in people taking anticoagulants, and high-dose beta carotene supplements increase the risk of lung cancer in smokers. The most important antioxidants are beta-carotene, lycopene, and some vitamins such as vitamin A, vitamin C, and vitamin E. Decades of nutritional research suggest that eating large amounts of antioxidant-rich foods may

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 603-610 www.ijprajournal.com ISSN: 2456-4494

help protect against illness. Much research has been done on antioxidant supplements for these discoveries. [9]Rigorous studies of antioxidant supplements in many people have not found that high-dose antioxidant supplements prevent the disease. This section provides preliminary findings, in vitro findings, and possible explanations for the differences between the findings.[2]

II. MATERIALS AND METHODS

EXTRACT PREPARATION:

Plants can be thought of as biosynthetic laboratories where various types of organic compounds such as carbohydrates, proteins, lipids and flavonoids are synthesized. [14]

It has been used as a bioactive ingredient since ancient times, such as glycosides, alkaloids, volatile oils, and tannins. However, the medicinal properties of herbs depend on the type of chemical component they contain and are called active ingredients. In this study, the active ingredients were extracted by ingesting medicinal herbal plants such as Cesbaniagrandiflora, Spatodeacampanulata (African tulip), Allamandacatarrica (golden trumpet), and Mirabilis jalapa (Oshiroibana).[11]

Solvent Purification: Distilled water obtained by distillation is used for water extraction of the powder drug substance.

Extract preparation:

The newly collected botanical material was obtained from the Botanical Gardens of Sir C.R. Reddy Pharmaceutical Science College, Eluru A.P. Collect, wash thoroughly with tap water, dry in the shade at room temperature (32 ° C), powder and use for solvent extraction. Plant samples were sequentially extracted with distilled water using a Soxhlet extractor. Prior to each extraction, the material was dried in a hot air oven at 40 ° C. The solvent was evaporated using a rotary vacuum evaporator at 50 ° C. Extract recovery was expressed as a percentage of the dry matter of the plant sample. The lyophilized extract thus obtained It was dissolved in an aqueous solvent at a concentration of 1 mg / ml and used to evaluate antioxidant capacity by various chemical assays.[18]

FIG-1

FIG-2

Qualitative phytochemical analysis.

Alkaloid test: a. Dragendorff's test: Add 1 ml of Dragendorff's reagent (potassium iodide bismuth solution) to 1 ml of extract. An orange-red precipitate indicates the presence of alkaloids.

Meyar test: Add 1 ml of Meyer reagent (mercury

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 603-610 www.ijprajournal.com ISSN: 2456-4494

iodide potassium solution) to 1 ml of extract. A whitish yellow or off-white precipitate indicates the presence of alkaloids.

Hager's test: Add 3 ml of Hager's reagent (saturated aqueous solution of picric acid) to 1 ml of extract. A yellow precipitate indicates the presence of alkaloids. That is, Wagner test: Add 2 ml of Wagner reagent (iodine in potassium iodide) to 1 ml of extract. The formation of a reddish brown precipitate indicates the presence of alkaloids.

Saponin test: Take a small amount of alcoholic extract and aqueous extract separately, add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes. The absence of a layer of foam indicates the absence of saponins.

Glycoside test:a.Legal test: Dissolve the extract in pyridine and make it alkaline with sodium nitroprussidesolution. The absence of pink to red formation indicates the absence of glycosides.

Baljet test:Add 1 ml of sodium picricate solution to 1 ml of test extract. Yellow to orange indicates the presence of glycosides.

Keller-Killiani test: Extract 1 g of powdered drug with 10 ml of 70% alcohol for 2 minutes, filter, add 10 ml of water and 0.5 ml of strong lead acetate solution to the filtrate, filter and shake the filtrate. increase. With 5 ml of chloroform. The chloroform layer was separated in a porcelain dish and gently evaporated to remove the solvent. Dissolve the cooled residue in 3 mL of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2 mL of concentrated sulfuric acid. Reddish brown layerFormed at the junction of two liquids, the upper layer slowly turns turquoise and darkens when standing.

Borntrager test: Add a few ml of diluted sulfuric acid to 1 ml of extract. The filtrate is boiled, filtered and extracted with chloroform. Treat the chloroform layer with 1 mL of ammonia. The red formation of the ammonia layer indicates the presence of anthraquinone glycosides.

Carbohydrate test:

Molisch's test: Add 1 ml of naphthol solution to 2 ml of extract and add concentrated sulfuric acid from the side of the test tube. The purple or purplish red color at the junction of the two liquids indicates its presence. Fehling's test: Add equal volumes of Fehling's solutions A and B to 1 ml of extract and heat to form a red brick-colored precipitate, indicating the presence of sugar. Benedict test: Add 1 ml of extract to 5 ml of Benedict reagent, boil for 2 minutes and allow to cool. The formation of red precipitates indicates the presence of sugar.

Tannin test: a. Take a small amount of the test solution and mix it with the basic lead acetate solution. The formation of white precipitates indicates the presence of tannins. When ferric chloride solution is added to 1 ml of the extract, the formation of a dark blue or greenish black product indicates the presence of tannins.The little quantity of the extract is treated with potassium ferric cyanide and ammonia solution. A deep red color indicates the presence oftannins.To the test extract, add strong potassium dichromate solution, a yellow color precipitate indicates the presence of tannins and phenolicscompounds.

Test for Flavonoids:

The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light; formation of fluorescence indicates the presence offlavonoids**.** Littlequantity of extractistreated with amyl alcohol,sodiumacetateandferricchloride.A yellow color solution formed, disappears on addition of an acid indicates the presence of flavonoids.

Shinoda's Test: The alcoholic extract is treated with magnesium foil and concentrated HCl give intense cherry red color indicates the presence of flavonones or orange red color indicates the presence offlavonols. The extract is treated with sodium hydroxide; formation of yellow color indicates the presence offlavones. The extract is treated with concentrated H_2SO_4, formation of yellow or orange color indicatesflavones. The alcoholic and aqueous extract is treated with 10% sodium chloride; formation of yellow color indicates the presence ofcoumarins.

Test for Steroids:

Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform,3mlofaceticanhydride,3mlofglacialaceticacidwereadded,warmedandcooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green color shows the presence ofsterols.

Salkowski test: Dissolve the extract in chloroform and add equal volume ofconc.H_2SO_4 .Formation of bluish red to cherry color in chloroform layer

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 603-610 www.ijprajournal.com ISSN: 2456-4494

and green fluorescence in the acid layer represents the steroidal components in the tested extract.

Test for Proteins:

Biuret test: Add 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color is produced, and then add to the 1ml of the extract. Formation of pinkish or purple violet color indicates the presence ofproteins.

Ninhydrin test: Add two drops of freshly prepared 0.2% ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. Development of blue color reveals the presence of proteins, peptides or aminoacids.

Xanthoproteic test: To 1ml of the extract, add 1ml of concentrated nitric acid. A white precipitate is formed, it is boiled and cooled. Then 20% of sodium hydroxide or ammonia is added. Orange color indicates the presence of aromatic aminoacids.

Millon's test: 1ml of test solution is made acidify with sulphuric acid and add Millon's reagent and boil this

Noller's test: Dissolve two or three granules of tin metal in 2ml thionyl chloride solution. Then add 1ml of the extract into test tube and warm, the formation of pink color indicates the presence oftriterpenoids.

Test for Gums:

Add about 10ml of aqueous extract slowly to 25ml of absolute alcohol with constant stirring. Filter the precipitate and dry in air. Examine the precipitate for its swelling properties and for the presence of carbohydrate

Methods used for determining antioxidant activity.

Hydrogen peroxide scavengingactivity

A solution of 40 mM hydrogen peroxide was prepared with sodium phosphate buffer. Natural extracts of various concentrations such as 50, 100, 150, 200, 250, 300 µg / ml were added to 3 ml of 40 µm hydrogen peroxide to make 10 ml.Take 40 mM H2O2 as a control, set aside for 10 minutes, and measure the absorbance at 240 nm after 20 minutes for a phosphate buffer blank containing no H2O2. The absorbance of hydrogen peroxide at 240 nm was measured after 10 minutes. [18]

% inhibition of H2O2 = (AC – AS) / AC × 100

where

AC = control absorbance

AS = absorbance of the sample tested.

FIG-3

Reducing power assay

Required concentration of sample + 2.5 ml Phosphate buffer 6.6 + 2.5 ml Potassium ferric cyanide (1%) Next, incubate in water at 50 ° C for 10 minutes. Add 2.5 ml of trichloroacetic acid (10%) .To the above solution. The mixture was centrifuged at 800 rpm for 10 minutes at 10 ° C. Take 2.5 ml supernatant + 2.5 ml distilled water + 0.5 ml ferric chloride (0.1%). Measure the absorbance at 700 nm for the blank.[22]

% reduction = (AC-AS) / AC x 100

where

AC = control absorbance

AS = absorbance of the sample tested.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 603-610 www.ijprajournal.com ISSN: 2456-4494

FIG-4

III. RESULTS AND DISCUSSION

TABLE -1 Qualitative phytochemical analysis of aqueous extract of the powdered medicinal crude drugs

Phytoconstituents	**Sesabinagrandiflora**	**Mirabilis jalapa**	**Allamandacathartica**	**Spathodeacampanulat a**
Alkaloids	+	+	–	+
Saponins	+	+	+	+
Glycosides	–	+	+	+
Carbohydrates	+	–	–	–
Tannins	+	+	+	–
Flavonoids	+	+	+	+
Proteins	+	–	–	–
Terpenoids	+	+	–	–
Phenols	+	+	+	+

TABLE-2 Hydrogen peroxide scavengingactivity

S.no	conc µg/ml			**Sesbania Grandiflora**		**Cathartica Allamanda**		**Spathodeacampanulata**		**Mirabilis Jalapa**	
		Abs	%Rd	Abs	%Rd	Abs	%Rd	Abs	%Rd	Abs	%Rd
1	100	0.057	43%	0.059	41%	0.056	44%	0.059	41%	0.055	45%
2	200	0.040	60%	0.049	51%	0.051	49%	0.051	49%	0.048	52%

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 603-610 www.ijprajournal.com ISSN: 2456-4494

3	300	0.034	66%	0.042	58%	0.047	53%	0.042	58%	0.041	59%
4	400	0.028	72%	0.037	63%	0.041	59%	0.036	64%	0.031	69%
5	500	0.022	78%	0.028	72%	0.036	64%	0.030	70%	0.023	77%
6	600	0.015	85%	0.023	77%	0.029	71%	0.025	75%	0.014	86%

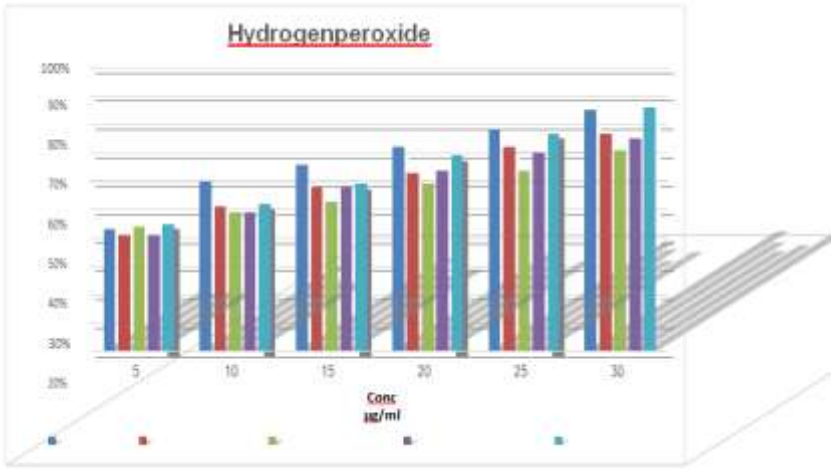

FIG-5

TABLE -3**Reducing Power Assay**

S.no	conc µg/ ml			SesbaniaGrandiflora		CatharticaAllamanda		Spathodea campanulata		Mirabilis Jalapa	
		Abs	%Rd	Abs	%Rd	Abs	%Rd	Abs	%Rd	Abs	%Rd
1	100	0.055	93.1 25	0.091	11	0.111	13	0.171	20	0.131	15
2	200	0.032	22	0.172	20	0.182	21	0.313	37	0.181	21
3	300	0.028	28	0.233	28	0.253	30	0.424	50	0.302	36
4	400	0.016	37	0.273	32	0.303	36	0.514	61	0.362	43
5	500	0.011	44	0.354	42	0.364	43	0.605	72	0.413	49
6	600	0.006	53	0.435	51	0.455	54	0.685	81	0.434	51

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 603-610 www.ijprajournal.com ISSN: 2456-4494

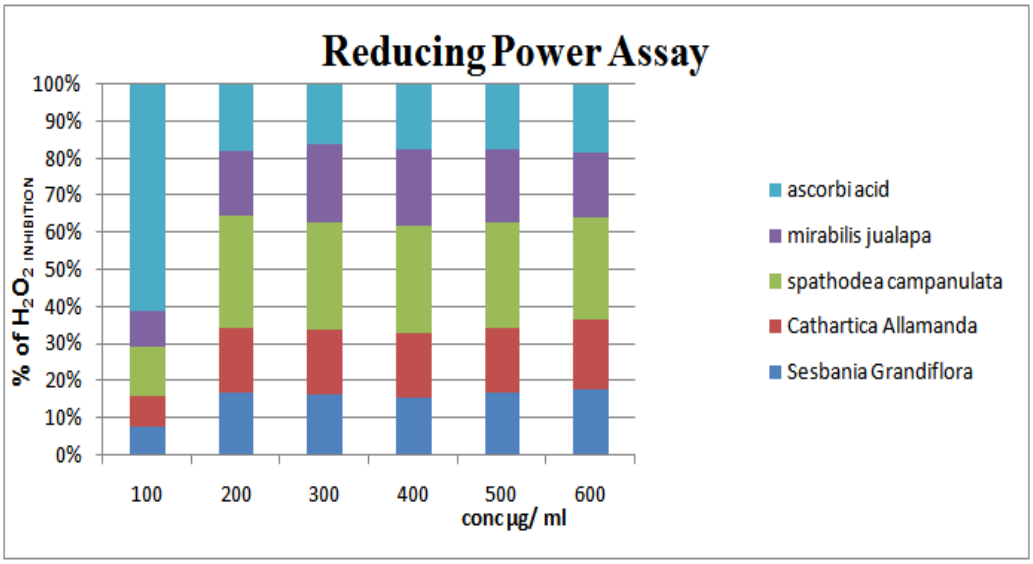

FIG-6

IV. DISCUSSION

The results obtained indicate that the selected flower extract has antioxidant activity comparable to ascorbic acid. The presence of strong antioxidant capacity can be attributed to the regulation of free radical production, thereby protecting cellular components from their harmful effects. Flower extracts contain high concentrations of phenolic and other flavonoid compounds that have been shown to have strong free radical trapping capacity and provide associated antioxidant capacity. Dark flower extract is rich in phenolic compounds, flavones and flavanols and has high antioxidant capacity

V. CONCLUSION

The results obtained in our study show that extracts of various flowers (Cesbaniagrandiflora, catarrticaallamanda, spatodeacampanulate, mirabilis jalapa) have antioxidant properties. The antioxidant activity of various flower extracts is almost comparable to standard ascorbic acid in the order Catharticaallamanda>Spatodeacampanulate>Sesba niagrandiflora> Mirabilis jalapa. Hydrogen peroxide suppression rate Next, the iron ion reduction rate is in the order of Sesbaniagrandiflora>Spathodeacampanulate> Mirabilis jalapa>Catharticaallamanda. This may be due to high levels of phenol and flavonoids in the flower extract. Finally, we conclude that due to its antioxidant properties, flower extracts can be used to protect us from cell damage caused by oxidative stress. However, further research is needed on its therapeutic effect in humans and its stability when used in the formulation.

REFERENCES

[1] Ahmad I, Beg AZ.”Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens”. Journal of Ethnopharmacology.2001, 74(2):113–124.

[2] Gilbert DL. Fifty years of radical ideas. Ann NyAcadSci, 2000, 14:899.

[3] Kolayli S. Kucuk M. Yaylachi F. Dural N. and Vsta M. “Comparison of in vitro antioxidant properties of trunk, bark of some tree species”, proceedings of ICNP, 2002, 133- 139.

[4] Nakayama T. Yamada M. Osawa T. and Kawakishi S. “Suppression of active oxygen induced cytotoxicity by flavonoids”. Bio ChemPharmacol, 1998, 45:265-267.

[5] Kikuzaki H. and Nakatani N. An effect of some ginger constituents. J Food Chem, 1993, 57: 2144-2155.

[6] Chen H.M. Muramoto K. Yamavchi F.S. and Nokihara K. “Antioxidant activity of design peptides based on antioxidative peptide isolated form digest of a soybean protein”. J Agric Food Chem 1996, 44:2619-2623.

[7] Halliwell B. and Gutteridge J.M.C. Free radicals in Biology and Medicine. Claredon press, Oxford England, 1989,550-610.

[8] Dhiman A.K. 2003. Sacred plants and their medicinal uses. Daya publishing house Delhi, 172.

[9] Devdatta and Appanna. Nutritive value of Indian foods. Indian Acad. Sci, 1954, 398: 297.

[10] Sadasivam S. and Manickam A. Determination of total carbohydrate by anthrone method. In: Bochemical methods. Siddhuraju P. and Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three agro climatic origins of drumstick free (Moringaoleifera Lam) Leaves. J Agric Food Chem, 2003, 57: 2144-2155.

[11] Siddhuraju P. and Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three agro climatic origins of drumstick free (Moringaoleifera Lam) Leaves. J Agric Food Chem, 2003, 57: 2144-2155.

[12] Siddhuraju P. Mohan P.S. and Beaker K. Studies on the antioxidant activity of Indian Laburnum (Cassia fistula L.). A preliminary assessment of crude extracts from stem, bark, leaves and fruit pulp. Food chem, 2002, 79:61-67.

[13] Blios M.S. "Antioxidant determinations by the use of a stable free radical''. Nature 1958; 26: 1199-1200.

[14] Kikuzaki H. and Nakatani N. An effect of some ginger constituents. J Food Chem 1993, 57:2144-2155.

[15] Jayaprakasha G.K. Singh R.P. and Sakariah K.K. Antioxidant activity of grape seed (Vitisvinifera) extracts on peroxidation models in vitro, Food Chem, 2001, 73:285-290.

[16] Kolayli S. Kucuk M. Yaylachi F. Dural N. and Vsta M. Comparison of in vitro antioxidant properties of trunk, bark of some tree species, proceedings of ICNP, 2002, 133- 139.

[17] Zinol M.K, Abd-Hamid A. Yusof S. and Muse R. "Antioxidant activity and total phenolic compounds of leaf, root, petiole of four accessions of Centellaasiatica" (L.). Urban Food chemistry. 2003,81:575-581.

[18] Velioglu Y.S. Mazza G. Gao L. and Oomah. "Antioxidant activity and total phenolics in selected fruits and vegetables .J. Agric and Food Chem", 1998, 46:4113.

[19] Wang H. Nair MG. Strasburg GM. BoorenAM. and Gray J.I. "Antioxidant polyphenol from that cherry"(Peybyacerasus). J Agric Food Chem, 1999, 47:840-844.

[20] Hertog MGL. Hollman PCH. Vennema DP. Optimization of the quantitative HPLC Determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J Agric Food Chem, 1992, 40:1591-1598.

[21] Amarowicz R. Pegg RB. Rahimi-moghaddam P. Barl B. Weir JA. "Free radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies", Food Chem, 2004, 84:551-562.

[22] Chung, Y.C, Chang, C.T., Chao, W.W., Lin, C.F., and Chou, S.T, 2002. "Antioxidative activity and safety of the 50% etanolic extract from red bean fermented" by Bacillus subtilis IMR-NK 1, J Agric Food Chem, 50,2454-2458.

[23] Yamaguchi T. Takamura H. Matoba T. and Terao J. "HPLC method for evaluation of the free radical scavenging activity of foods by using 2, 2-diphenyl-1-picryl hydrazyl. Bio SciBiotechnol Bio Chem", 2002. 62,1201-1204