2011 International Conference on Bioscience, Biochemistry and Bioinformatics
IPCBEE vol.5 (2011) © (2011) IACSIT Press, Singapore

Antibacterial and Antioxidant potential of White and Pink Nelumbo Nucifera Gaertn Flowers

Brindha Venkatesh
Department of Biochemistry
PSG College of Arts and Science
Coimbatore-641 014, TamilNadu, India
E-mail: brindhavenkatesh@ymail.com

Arthi Dorai
Department of Biochemistry
PSG College of Arts and Science
Coimbatore-641 014, TamilNadu, India
E-mail: arthidorai@yahoo.com

Abstract—**The recent interest on alternative medicine has taken up great dimensions in changing the health care scenario across the globe. The worldwide interest in medicinal plants reflects recognition of the validity of studying the antimicrobial and antioxidant activity. Hence the present study was carried out to explore antibacterial and antioxidant potential of hydroethanolic extract of both white and pink flowers of Nelumbo nucifera Gaertn (Nelumbonaceae) flower in vitro. The antibacterial activity was screened against different bacterial strains by detecting zone of inhibition and minimum inhibitory concentration (MIC). The zone of inhibition and MIC values were compared with control compared with standard antibiotic disc suggesting their potential as alternatives to orthodox antibiotics in the treatment of infectious caused by these microorganisms. Total antioxidant potential was evaluated in hydroethanolic extract of white Nelumbo nucifera (HEWNN) flower and hydroethanolic extract of pink Nelumbo nucifera (HEPNN) flower by: Ferric reducing antioxidant power (FRAP), Hemoglobin glycosylation,Reducing power and Phosphomolybdenum and compared with the standard ascorbic acid in dose dependent manner. Both HEWNN and HEPNN flower extracts showed maximum activity 16.53 mg and 14.21 mg at 1000µg/ml concentration in FRAP assay. Significantly high antioxidant activity (55.5% & 41.6%) was noticed in haemoglobin glycosylation followed reducing power (0.52 & 0.45 Abs). The results suggest that alkaloids, phenols and flavonoids in flowers provide considerable antioxidant activity. However, in comparison with HEPNN flower, HEWNN flower extract exerted effective antibacterial and potent antioxidant activity which can be used as a lead compound for drug development in the future.**

Keywords-Nelumbo nucifera Gaertn; antibacterial activity; Minimum Inhibitory Concentration (MIC); antioxidant; agar disc diffusion.

I. INTRODUCTION

Medicinal plants are gifts of nature used to cure number of human diseases. To promote the proper use and to determine their potential as sources for new drugs, it is essential to study the medicinal plants [1]. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of phytomedicine to act against microbes [2]. Therefore, researchers are increasingly turning their attention to folk medicine to develop better drugs against microbial infections [3].

A perusal of literature revealed that the flower parts of *Nelumbo nucifera* had not been subjected to screening for antibacterial properties so far. From this viewpoint the present study was carried out to evaluate the antibacterial activity of hydroethanolic extract of white (HEWNN) and pink (HEPNN) *N. nucifera* flowers.

Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals [4]. The natural antioxidant mechanism is inefficient and hence dietary intake of antioxidant compounds is important [5]. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants [6]. Thus the present study was focused to further probe the new safe and effective *in vitro* antioxidant agents in white and pink *N.nucifera* flower extracts.

Nelumbo nucifera Gaertn (Family: Nelumbonaceae) commonly known as Indian lotus, one of the oldest perennial aquatic herb consumed throughout Asia. Pharmacological studies of the plant revealed that the whole plant possess antidiabetic, antipyretic, anti-inflammatory, anticancerous, antimicrobial, antiviral and anti-obesity properties [7]. Furthermore, *N.nucifera* flower has considerable reputation as a potent adjunct in the treatment of various ailments such as cancer, hypertension, diarrhea, fever, weakness, infection and body heat imbalance [8].

The major constituents isolated from the lotus plant are alkaloids (liensinine, neferine, nuciferine, remrefidine and isoliensinine) and flavonoids ((+)-1(R)-coclaurine, (-)-1(S)-norcoclaurine and quercetin 3-O-b-D-glucuronide). Several previous reports suggested that seed could suppress cell cycle progression, cytokine genes expression and cell proliferation in human peripheral blood mononuclear cells. Recently, the leaf of *N.nucifera* showed the hypotensive effects that were mediated by vasodilatation *via* nitric oxide [9] and betulinic acid isolated from rhizomes and used as anti-tumoral and melanoma specific cytotoxic agent.

However, scientific evidence on antioxidant potential in hydroethanolic extract of white and pink *N.nucifera* is still unknown. Therefore, our study has been focused to gain extensive knowledge regarding the power of antioxidants from both white and pink *N.nucifera* flowers and to tap their potential.

II. Materials And Methods

A. Plant material

The flowers of *N.nucifera* were collected from different localities of Coimbatore District and authenticated by Botanical Survey of India (BSI) in "Tamil Nadu Agriculture University" Coimbatore, Tamil Nadu, India. A voucher specimen (No.BSI/SC/5/23/09-10/Tech.279) has been deposited at the Herbarium of the Botany department of "Tamil Nadu Agriculture University" for future reference.

B. Plant Extraction

The air-dried and powdered white and pink flowers (100g of each) were cold macerated with 50% ethanol for 3 days, with occasional stirring. After 3 days, the suspension was filtered through a fine muslin cloth and was evaporated to dryness at low temperature (< 40ºC) under reduced pressure in a rotary evaporator. Dark brown colored crystals of approximately 8g was stored in air-tight desiccators and used for further analysis.

C. Antibacterial Assay

Antibacterial activity was determined using the agar disc diffusion method described by Parekh [10]. Each bacterial inoculum was incubated in 2.5ml Mueller-Hinton broth at 37°C for 18 hours. Every inoculum was spread over plates containing Mueller-Hinton agar. Five millimeter discs containing 500µg/ml and 1000µg/ml of extract were placed on cultured pathogenic bacteria on agar plates and incubated at 37°C. The plates were checked for bacterial growth after a minimum of 16 hours and occasionally till 24 hours. The diameter of the zone of inhibition was then measured. Commercial disc of Chloramphenicol (30µg) was used as positive control and experiment was done thrice for each extract.

D. Determination of Minimum Inhibitory Concentration (MIC)

MICs are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. MIC of antibiotics was evaluated using standard microbroth dilution method [11].

E. Antioxidant activity

The air-dried and powdered flowers (100g of each) were cold macerated with 50% ethanol for 3 days, with occasional stirring. After 3 days, the suspension was filtered through a fine muslin cloth and was evaporated to dryness at low temperature (<40ºC) under reduced pressure in a rotary evaporator. Dark brown colored crystals of approximately 8g was stored in air-tight desiccator and used for further analysis.

F. Chemicals

Ammonium thiocyanate, ferrous chloride, ferric chloride, ammonium molybdate, potassium ferricyanide, tripyridyltriazine (TPTZ) and ascorbic acid were purchased from Hi-media, Mumbai, India. All other chemicals and reagents used were of analytical grade and were purchased from Hi-media, Mumbai, India.

In FRAP assay, when a ferric tripyridyltriazine (Fe^{III} - TPTZ) complex is reduced to the ferrous (Fe^{II}) form, an intense blue colour with an absorption maximum at 593 nm develops and measured at low pH by the method of Benzie.F.F. and J.J.Strain [12]. Degree of non-enzymatic haemoglobin glycosylation was measured by Pal & Dutta method [13]. The reducing power was measured according to the method of Oyaizu [14]. Antioxidant capacity was measured by phosphomolybdenum method [15].

G. Statistical Analysis

The mean zones of inhibition and MIC was calculated from the values of the three experiments for each isolates and reported as final results. Antioxidant activity results are expressed as mean ± SD. values.

III. Results And Discussion

The antibacterial activity of the hydroethanolic extract of both white and pink *N.nucifera* flower extracts were evaluated at two different concentrations (500 µg & 1000µg) against five bacterial strains by the disk diffusion method and the results were summarized in Table I. The antibacterial activity of both *N.nucifera* flower extracts was found to be increased in dose dependent manner. The maximum zone of inhibition was exhibited by both white and pink *N.nucifera* flowers against *Escherichia coli* (16mm & 14mm), *Bacillus Subtilis* (15mm & 13mm) and

TABLE I. Antibacterial Activity Of Both White And Pink *Nelumbo Nucifera* Flowers By Disc Diffusion Method

Microorganism	Inhibition Zone (mm)*				
	White *Nelumbo nucifera* flower		Pink *Nelumbo nucifera* flower		Chloramphenicol (Control)
	1000 µg/ml	500µg /ml	1000µg /ml	500µg/ml	30µg/ml
Escherichia coli	16	11	14	9	30
Klebsiella pneumonia	12	6	10	8	22
Pseudomonas aeruginosa	9	7	8	5	29
Bacillus Subtilis	15	9	13	5	26
Staphylococcus aureus	13	10	11	7	27

* mean of three replicates

TABLE II. Antioxidant Activity Of Both Hewnn And Hepnn Flower Extract By Frap Assay

S.No	Concentration (µg/ml)	White *Nelumbo nucifera* flower (mg)	Pink *Nelumbo nucifera* flower (mg)
1	200	3.165	2.861
2	400	8.612	6.327
3	600	10.18	7.562
4	800	14.22	12.57
5	1000	16.53	14.21

Staphylococcus aureus (13mm & 11mm). The moderate zone of inhibition was found in both white and pink flower extracts against *Klebsiella pneumonia* (12mm & 10mm) *and Pseudomonas aeruginosa* (9mm & 8mm).

Gram-negative bacteria were more susceptible to the *N.nucifera* flower extracts than gram-positive bacteria which contradict the previous reports that plant extracts are more active against gram-positive bacteria than gram-negative bacteria. However, the results revealed that the hydroethanolic extract of white *N.nucifera* flower showed effective antibacterial activity when compared to pink *N.nucifera* flower which may be due to its variation in phytochemical constituents like flavonoids, alkaloids and tannins which was also reported by Bose *et al*., [16] and these results were compared with the standard antibiotic chloramphenicol (30µg/ml). Similar work by Rogger *et al*., [17] showed that antibacterial effect of *Tithonia diversifolia* against *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli* suggesting that the plant can be used in the treatment of gastrointestinal infection and diarrhea in human.

The minimum inhibitory concentration for white *N.nucifera* flower extract against *Escherichia coli* and *Staphylococcus aureus* was found to be 430µg and 450µg respectively and pink flower showed 480µg and 490µg respectively (Fig. 1 and 2). The lowest MIC was exhibited by white *N.nucifera* flower extract against both the microorganisms when compared to pink *N.nucifera* flower.

Ushimaru and Silva, [18] have reported that medicinal plants like *Allium sativum, Zingiber officinale* have antibacterial activity against *Salmonella typhimurium, Staphylococcus aureus* at minimum inhibitory concentration of 450µg/ml.The findings of this study confirmed the therapeutic potency of both white and pink *N.nucifera* flowers used in traditional medicine. These results offer promising lead for the discovery of potent antibacterial compounds in therapeutic and dietary use globally.

The antioxidative phytochemicals in grains, vegetables and fruits have received increasing attention recently for their potential role in prevention of human diseases as well as in food quality improvement [19]. Lotus has been used both as food and medicine in Asia, particularly in India [20]. The antioxidant activity of plant extracts vary with methods [21]. Therefore, a single assay

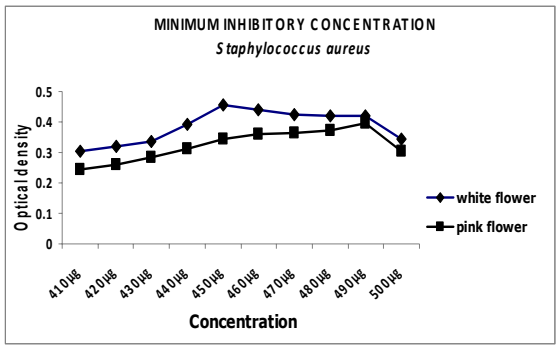

Figure 1. Minimum inhibitory concentration of both white and pink *Nelumbo nucifera* Flowers against *Escherichia coli*

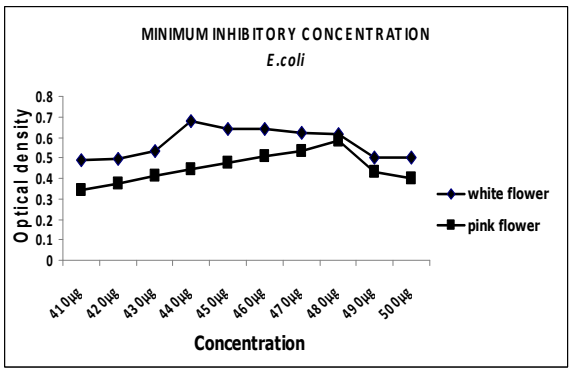

Figure 2. Minimum inhibitory concentration of both white and pink *Nelumbo nucifera* Flowers against *Staphylococcus aureus*

may be inadequate. For this reason, we cross checked antioxidant activities of both white and pink lotus flower extracts with different assays.

FRAP assay is presented as a novel method of assessing total antioxidant capacity and is considered as a useful indicator of the body's antioxidant status to counteract the oxidative damage due to ROS. The advantage of the FRAP assay is in being fast, easy to handle, with highly reproducible results [22]. Antioxidant activity by this method was evaluated on the basis of alkaloid and element content where ferric to ferrous ion reduction at low pH causes a ferrous-tripyridyl-triazine complex which has absorption at 593nm [23]. Both HEWNN and HEPNN flower extracts showed concentration dependent increase in their ferric reducing capacities. However, HEWNN flower extract was more potent than HEPNN flower which may be due to the presence of different levels of active constituents in both flowers (Table 2). The maximum antioxidant capacity of both HEWNN and HEPNN flower extract was found to be 16.53mg and 14.21mg at 1000µg/ml concentration in FRAP assay (Table 2).

High degree of haemoglobin glycosylation (Fig. 3) activity was noticed in HEWNN (55.5%) and HEPNN (41.6%) at 500µg/ml concentration. This was compared to that of standard ascorbic acid (62.6%) at 300µg/ml concentration. HbA1c, or glycosylated hemoglobin, is formed through the

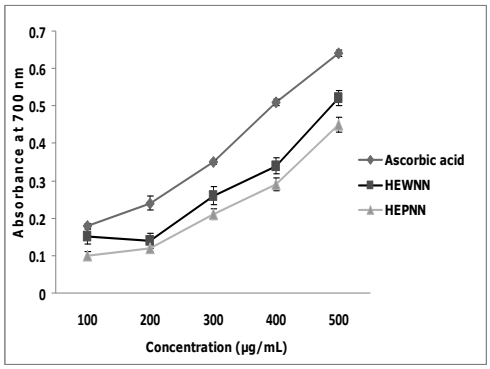

Figure 3. Antioxidant activity of HEWNN and HEPNN flowers by Haemoglobin glycosylation method. Values arthe average of duplicate experiments and represented as mean ± standard deviationnon-

enzymatic binding of circulating glucose to N terminal of beta chain of hemoglobin molecule (glycation) [24]. Degree of haemoglycosylation of HEWNN is much effective than HEPNN flower and exhibits increasing trend with the increasing concentration of plant extract. In figure 4, the reducing power increased with increasing concentration of plant extract and maximum reducing power of HEWNN, HEPNN and ascorbic acid at 700 nm were found to be 0.52, 0.45 and 0.64 absorbance respectively. These results clearly revealed that HEWNN flower extract have effective antioxidant activity than HEPNN flower extract, which may be due its phytochemical constituents.

The reducing ability of a compound generally depends on the presence of reductants (antioxidants) [25], which have been exhibited antioxidant potential by breaking the free radical chain by donating a hydrogen atom [26]. The presence of reductants in *N.nucifera* extract causes the reduction of the Fe^{3+}/Ferricyanide complex to the ferrous form. The reducing power of HEWNN flower extract was very potent and had effective antioxidant activity than HEPNN extract.

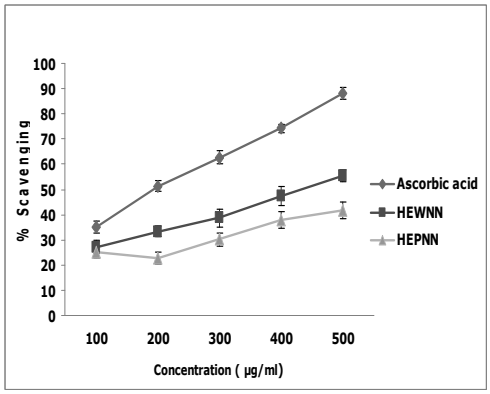

Figure 4. Reducing power of HEWNN and HEPNN flowers. Values are the average of duplicate experiments and represented as mean ± standard deviation

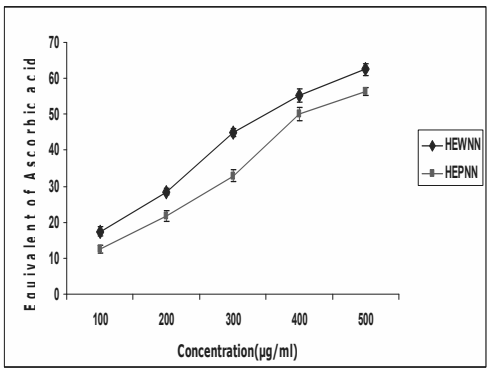

Figure 5. Antioxidant activity of HEWNN and HEPNN flowers Phosphomolybdenum method. Values are the average of duplicate experiments and represented as mean ± standard deviation

The maximum values of both white and pink N.nucifera flower extract were found to be 62.5mg and 56.3mg ascorbic acid equivalent at 500µg/ml in Phosphomolybdenum assay. Our findings are in good accordance with the observation made by Nagarajan *et al*., 2008 [27] who reported that the alcoholic extract of *Wrightia tomentosa* showed antioxidant activity.

Phosphomolybdenum assay used to determine the total antioxidant capacity based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo(V) complex at acidic pH. This assay has been successful in the quantification of vitamin E antioxidant capacity and being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extracts [28]. Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The good antioxidant activity of HEWNN and HEPNN might be attributed due to the presence of phytochemical, such as Flavonoids and biflavones.

Hence, both HEWNN and HEPNN flowers were found to have the potential to be used as nutraceutical supplement due to its antioxidant activity as well as to exert potent supplement in prevention of degenerative diseases. However, in comparison with HEPNN flower, HEWNN flower extract exhibited significant antioxidant activity which might be due to the structural variation of antioxidant compounds such as number of phenolic hydroxyl or methoxyl groups, flavones hydroxyl, keto groups free carboxylic groups and other structural features. Therefore if a systematic investigation is initiated the traditional medicinal systems practiced in India can offer promising leads for the discovery of potent antioxidants that can have therapeutic and dietary use globally.

IV. CONCLUSION

The results of the study suggest that white flower of *N.nucifera* extract exert strong antibacterial and potent antioxidant activity when compared to pink flower of *N.nucifera* which might be due to the presence of rich phytochemical constituents. Future work is therefore under progress to identify and elucidate the bioactive principles

that are responsible for free radical scavenging activity and to establish its potential in animal models.

REFERENCES

[1] J. Parekh, S. Chanda, "Antibacterial activity of the crude methanol extract of Flower (Lythraceae)," Brazilian Journal Microbiology, vol. 38, 2007, pp. 204-207.

[2] M.V.Kumaraswamy, H.U.Kavitha and S.Sathish, "Antibacterial evaluation and phytochemical analysis of Betula utilis against some human pathogenic bacteria," Word journal of agricultural science, vol.4(5), 2008, pp. 661-664.

[3] D. Pitchai, R. Manikkam, S.R. Rajendran, G. Pichai, "Database on pharmacophore analysis of active principles from medicinal plants," Bioinformation, vol.5, 2010, pp. 43-45

[4] M. Valko, C.J. Rhodes, J. Moncol, M. Izakovic and M. Mazur, "Free radical metals and antioxidants in oxidative stress-induced cancer," Chemico. Biol. Interaction, vol. 160, 2006, pp.1–40.

[5] C. Saengkhae, W.Arunnopparat, P. Sungkhajorn, "Antioxidant activity of on oxidative stress-induced Erythrocyte hemolysis in hypertensive and normotensive rats," Journal of physiological sciences, vol. 20, 2008, pp.70-78.

[6] K.R. Sridhar, B. Rajeev, "Lotus- A potential nutraceutical source," J Agri Tech, vol. 3, 2007, pp. 143-155.

[7] Y. Kashiwada, A. Asoshima, Y. Ikeshiro, "Anti-HIV benzylisoquinoline alkaloids from leaves of and structure activity correlations with related alkaloids," vol.13, 2005, pp.443-448.

[8] C.W. Saengkhae, Arunnopparat and P. Sungkhajorn, "Antioxidant activity of Nelumbo nucifera Gaertn on oxidative stress-induced Erythrocyte hemolysis in hypertensive and normotensive rats," J. Physiol. Sci., vol. 20, 2008, pp. 70-78.

[9] P.N. Tongtorsak, N. Chaichit and J. Panyajirawut, "Acute and chronic hypotensive effects of crude leaf extract of Nelumbo nucifera Gaertn. TJPS, vol.17, 2004, pp.68-69.

[10] J.Parekh and S.Chanda, "Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity," Indian journal of Pharmacology, vol.37, 2005, pp. 408-409.

[11] Eucast definitive Document E. Def. 3.1. European Committee for antimicrobial susceptibility testing: "Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution", Clin. Microbial & Infec, vol. 6, 2000, pp 509-515.

[12] I.F.F. Benzie and J. J. Strain, "Ferric reducing antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration," Meth. Enzymol., vol. 29, 1999, pp. 15–27.

[13] D.K. Pal and S. Dutta, "Evaluation of the Antioxidant activity of the roots and rhizomes of Cyperus rotundus L.," Indian J. Pharmaceut. Sci., vol.68, 2006, pp. 256-258.

[14] M. Oyaizu, "Studies on product of browning reaction prepared from glucose amine," Jap. J. Nutr., vol.44, 1986, pp.307-315.

[15] P.M. Prieto Pineda and M. Aguilar, "Spectrometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex:specific application to the determination of vitamin E," Anal.biochem.,vol.269, 1999, pp 337-341.

[16] A. Bose, S. Mondal, J.K. Gupta, T. Ghosh, "A study on antimicrobial activity of Cleome rutidosperma DC," Journal of Natural remedies, vol.7, 2007, pp.132-134.

[17] Y. Rogger, L. Stanier, L. Mark, "Assessment of antimicrobial and antifungal activity of common medicinal plants," International Journal of Pharmacology, vol.3, Ed.6, 2000, pp.499-504

[18] T. Ushimaru, G. Silva, "Antibacterial activity of common plants against different bacterial strains," Food Chem., vol. 29, 2007, pp. 275-283.

[19] V.G. Kamatha, A. Chandrashekarb and P. S. Rajini, "Antiradical properties of sorghum (Sorghum bicolor L. Moench) flour extracts," J. Cereal. Sci., vol. 40, 2004, pp. 283–288.

[20] Y. Sumazian, A. Syahida, M. Hakiman, Maziah M, "Antioxidant activities, flavonoids, ascorbic acid and phenolic contents of Malaysian vegetables," J. Med. Plant Res., vol. 4, 2010, pp. 881-890

[21] T. Sun and C. T. Ho, "Antioxidant activities of buckwheat extracts," Food Chem., vol.90, 2005, pp. 743–749.

[22] C.R. Suresh, V. Annam, K. Pratibha K and M. Prasad, "Total antioxidant capacity-a novel early biochemical marker of oxidative stress in HIV infected individuals," J. Biomed. Sci., vol. 1, 2009, pp. 61-65.

[23] T.K. Maria, A. Szentmihalyi, A. Sarkozi and S. Varga, "Estimation on antioxidant activity in the greater Celandine (Chelidonium Majus L.) extracts by FRAP method, Acta. Biol. Szegediensis, vol.47, 2003, pp.115-117.

[24] R.J. Koeing, C. M. Peterson, R. L. Jones and C. Saddek, "Correlation of glucose regulation and HbA1c in diabetes," N. Engl. J. Med., vol. 295, 1976, pp. 417-419.

[25] S. Meir, J. Kanner, B. Akiri and P. Hadas, "Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves," J. Agri. Food Chem., vol. 43, 1995, pp. 1813-1817.

[26] P. D. Duh, Y. Y. Tu and G. C. Yen, "Antioxidant activity of water extract of Harng Jyur (Chrysanthemum moifolium Ramat)," Lebensm–Wiss Technol, vol. 32, 1999, pp. 269-277.

[27] C.Nagarajan, D.Alam, G.Subhan and P.Nyeen, "Antioxidant and hepatoprotectant action of crude alhoholic extract of Wrightia tomentosa," Oriental Pharmacy and Experimental medicine, vol.81, 2008, pp. 241-248.

[28] Y.Lu and L.Y.Foo, "Antioxidant activities of polyphenols from sage (Salvia officinalis)," Food chemistry, vol.75, 2000, pp. 197-202.