www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol. 3, No.2, pp 1107-1112, April-June 2011

Screening of some Medicinal Plants for their Antityrosinase and Antioxidant activities

Nithya Narayanaswamy, Arun Duraisamy and K .P. Balakrishnan*

ITC R&D Centre, Peenya Industrial Area Phase I, Bangalore 560 058, India

Corres. Author: kp.balakrishnan@itc.in

Abstract: The present study was designed to appraise the depigmenting and antioxidant abilities of five medicinal plants. The various solvent extracts of medicinal plants were prepared by using decoction method. Mushroom tyrosinase was used as the model system for evaluating the antityrosinase activity of plant extracts. The results of the study show that the aqueous extract of *Asparagus racemosa* (43.29%) exhibited the maximum inhibiting potential of the tyrosinase enzyme. The antioxidant ability of the plant extract was determined by using the 2, 2-diphenyl -1-picryl hydrazyl (DPPH) radical scavenging assay. The aqueous and ethanolic extract of *Holarrhena antidysentrica* was found to have the highest inhibition of DPPH radical amongst the plant extracts tested. The plant extracts were also quantified for their total phenolic content. The aqueous and ethanolic extracts of *Pachygone ovata* were found to possess the greater phenolic content. From our findings it was concluded that the aqueous extract of *Asparagus racemosa* can be used as an active to inhibit tyrosinase and protect the skin from free radical toxicity.

Key words:I Depigmenting, antioxidant, Mushroom tyrosinase, *Asparagus racemosa*, 2, 2-diphenyl -1-picryl hydrazyl, *Holarrhena antidysentrica, Pachygone ovata.*

1. Introduction

The skin is the primary defense against invasion by bacteria, viruses, and other toxic elements and is the largest organ exposed to various oxidative insults. Reactive oxygen species (ROS) generated exogenously reacts with various biomolecules present in the skin and they play an important role in the skin disorders [1]. The ultraviolet radiation from the sunlight is the most common exogenous factor and pernicious to the skin. It leads to the alterations in the composition of the skin including the accumulation of elastic fibers [2], collagen reduction and degeneration [3] and deposition of glycosaminoglycans[4]. Such damage to the skin causes reduction in the elasticity of skin and the linearity of dermal elastic fibers, inducing wrinkling and sagging, resulting in aging appearance of the skin [5].

The exposure of the skin to ultraviolet radiation also induces the secretion of melanin due to rapid proliferation of melanocytes [6]. The abnormal secretion of melanin leads to hyperpigmentation of the skin. Melanin in the epidermal layers of the skin is produced by a pathway called melanogenesis in which tyrosinase is the important rate limiting enzyme. It catalyses three steps of melanin biosynthesis including the hydroxylation of tyrosine to 3, 4 – dihydroxyphenylalanine (DOPA), Oxidation of DOPA to DOPA quinone and oxidation of 5, 6-dihydroxyindole to indolequinone. So, the enzyme tyrosinase is the key target for finding out the skin lightening agents either from natural or synthetic origin [7]. Many tyrosinase inhibitors are used as skin-whitening agents including licorice extract, arbutin, and kojic acid. In recent times, there has been much attention focused on the application of natural plant extracts as skin lightening agents in cosmetic industry.

Reactive oxygen species are also implicated in various other disease conditions namely cardiovascular diseases, diabetes mellitus, cancer and

neurodegenerative diseases [8]. Medicinal plants exhibit a wide range of pharmacological activities, and have been shown to have anticancer, anti-inflammatory and anti-aging properties [9, 10]. So, the present investigation was performed to explore the potential skin whitening and antioxidant agents from natural sources because of their effectiveness, lower cost and less adverse effects when compared to synthetic ingredients.

2. Materials and Methods

2.1. Plant material

The various parts of plant materials were procured from commercial vendors in Coimbatore district, Tamilnadu and were authenticated by botany department of Mount Carmel College, Bangalore.

2.2. Plant extracts preparation

One gram of herbal powder was dissolved in 10ml of water/ethanol/Petroleum ether. The solution was heated in a boiling water bath at 60 degree centigrade for 60 minutes. The mixture was cooled to room temperature and centrifuged at 6000 rpm for 10 minutes. The supernatant was filtered and the filtrate was collected and used for the analysis.

2.3. Antioxidant assays

The antioxidant activity of the various solvent extracts of the plant was determined by employing the following methods.

2.3.1. DPPH radical scavenging assay [19]

DPPH (2, 2-diphenyl -1-picryl hydrazyl) is the most commonly used stable free radical, which is purple in colour, antioxidant molecules when incubated, reacts with DPPH and converts it into 2,2 – diphenyl-1-picryl hydrazine, which is yellow in colour. The degree of discoloration of purple to yellow was measured at 520 nm, which is a measure of scavenging potential of plant extracts. 5 µl of plant extract was added to 195 µl of DPPH solution (0.1mM DPPH in methanol) in a microtitre plate. The reaction mixture was incubated at 25 ^{0} C for 5 minutes, after that the absorbance was measured at 520 nm. The DPPH with corresponding solvents (without plant material) serves as control. The methanol with respective plant extracts serves as blank. The DPPH radical scavenging activity of the plant extract was calculated as the percentage inhibition.

% inhibition of DPPH radical =

$$\frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

2.3.2. Determination of total phenolics [20]

Phenols react with phosphomolybdic acid in Folin-ciocalteau reagent in alkaline medium and produce a blue colored complex (molybdenum blue) that can be estimated colorimetrically at 650 nm. Pipette out different aliquots (0.2 to 2 ml) into test tubes and made up the volume in each tube to 3.0 ml with water. Added 0.5 ml of Folin-Ciocalteau reagent. After 3 minutes, added 2.0 ml of 20% sodium carbonate solution to each tube. Mixed thoroughly, placed the tubes in a boiling water bath for exactly 1 minute, cooled and measured the absorbance at 650nm against reagent blank.

Sr.No	Botanical name	Part used	Family	Traditional uses
1	*Asparagus racemosa* (AR)	Rhizome	Asparagaceae	Phytoestrogenic therapy [11], galactogogue [12], diarrhoea and dysentery [13].
2	*Curcuma zedoaria* (CZ)	Rhizome	Zingiberaceae	Digestive and gall bladder disorders, cough, hepatic disorders, anti-inflammatory and antimicrobial activities [14].
3	*Lippia nodiflora* (LN)	Leaves	Verbenaceae	Diuretic, pain in knee joints and in lithiasis [15].
4	*Holarrhenna antidysentrica* (HA)	Leaves	Apocynaceae	Dysentery, diarrhea, intestinal worms [16, 17], antidiabetic drug [18].
5	*Pachygone ovata* (PO)	Leaves	Menispermaceae.	Central nervous system stimulant, analgesic properties

2.4. Skin whitening assay

2.4.1. Tyrosinase inhibition assay [21]

Tyrosinase (Phenoloxidase activity) which catalyses the transformation of L-tyrosine into L-DOPA by hydroxylation and further into *O*-dopaquinone by oxidation. Then, through a series of non-enzymatic reactions, *O*-dopaquinone is rapidly transformed into melanin, which is measured at 492 nm in a spectrophotometer. Each plant extract was assayed for tyrosinase inhibition by measuring its effect on tyrosinase activity using a 96-well reader. The reaction was carried out in a 50 mM potassium phosphate buffer (pH 6.8) containing 20 mM L-tyrosine and 312.5 U/ml mushroom tyrosinase at 30°C. The reaction mixture was pre-incubated for 10 min before adding the enzyme. The reaction mixture without the enzyme serves as blank. The reaction mixture with the corresponding solvents (without plant material) serves as control. The change of the absorbance at 492 nm was measured. The percent inhibition of tyrosinase was calculated as follows:

$$\% \text{ inhibition of tyrosinase} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

2.5. Statistical analysis:

Samples were analyzed in triplicate and the results were given as Mean ± S.D.

3. Results and Discussion:

3.1. Tyrosinase inhibitory activity of certain medicinal plants.

Melanogenesis is an important biological phenomena occurring in the melanocytes to protect the skin from free radical attacks which causes potential cellular injury to the skin. However, the excess secretion of melanin from melanocytes results in hyperpigmentation disorders namely skin darkening. Therefore, the discovery of tyrosinase inhibitors is becoming an urgent concern.

The results of tyrosinase inhibiting potential of various solvent extracts of five medicinal plants were illustrated in figure 1. Among the aqueous extract of five medicinal plants tested for their tyrosinase inhibiting potential, *Asparagus racemosa* possessed the maximum inhibition (43.29 %). The rest of the plants exhibited the antityrosinase activity which ranged from 30-38%. In the case of ethanolic extract of medicinal plants examined, *Holarrhena antidysentrica* (15.54%) ranked first in its tyrosinase inhibiting activity followed by *Curcuma zedoaria* (13.03%). Inhibition of tyrosinase activity by the remaining herbs was below 10 %.

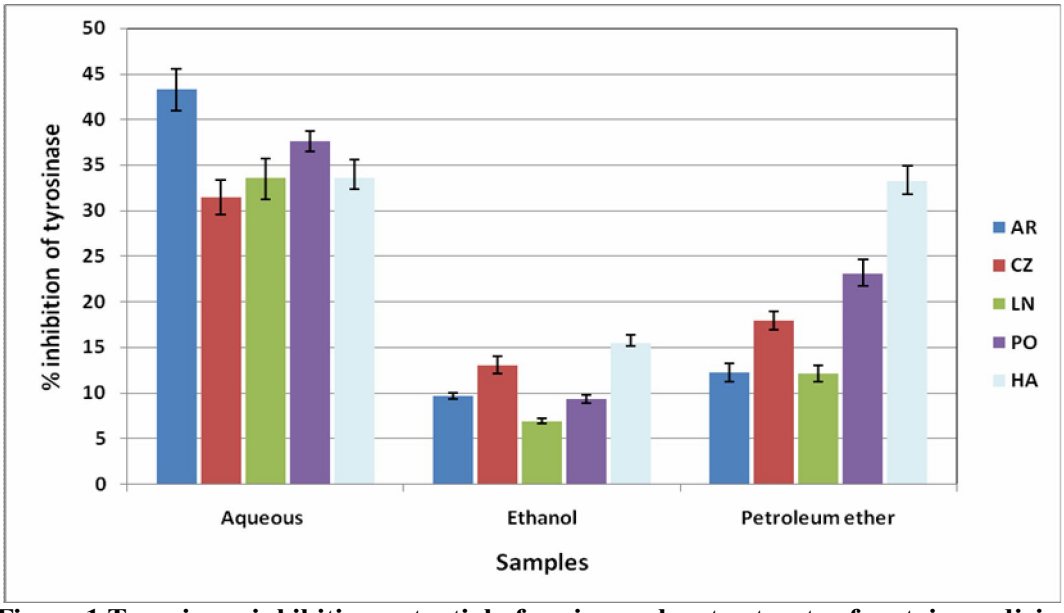

Figure 1.Tyrosinase inhibiting potential of various solvent extracts of certain medicinal plants

If the petroleum ether extract of five medicinal plants was taken into consideration for their antityrosinase activity, *Holarrhena antidysentrica* was greater in its inhibiting potential (33.3%). The order of antityrosinase activity of the remaining herbs was found to be *Pachygone ovata*>*Curcuma zedoaria*> *Asparagus racemosa*>*Lippia nodiflora*. *Asparagus racemosa* (12.27) and *Lippia nodiflora* (12.22) exhibited the similar inhibiting potential of tyrosinase enzyme.

Among the various solvent extracts of five medicinal plants studied for their antityrosinase activity, the aqueous extract of herbs showed good inhibiting property. Particularly, the aqueous extract of *Asparagus racemosa* had maximum inhibiting efficacy of tyrosinase enzyme. The highly water soluble compounds in the plant extract might be responsible for its superior inhibiting potential.

3.2. DPPH radical scavenging ability of various solvent extracts of medicinal plants.

There are different methods by which the antioxidant potential of the plant extracts can be determined. One such method is DPPH radical scavenging assay. DPPH is a relatively stable nitrogen centered free radical that easily accepts an electron by reacting with suitable reducing agents. As a result, the electrons become paired off and the DPPH solution losses its violet color depending on the number of electrons taken up [22]. The decrease in the absorbance of DPPH radical after the addition of plant extract was measured at 520 nm. The DPPH scavenging efficacy of five herbs in different solvents was examined and the results were shown in figure 2. The aqueous extract of *Holarrhena antidysentrica* (95.10%) exhibited the maximum quenching ability of DPPH radical when compared to the aqueous extract of other medicinal plants. The aqueous extract of *Asparagus racemosa* (88.40) ranked second in its inhibiting effect on DPPH radical. This is followed by the aqueous extract of *Lippia nodiflora* and *Pachygone ovata*. The inhibiting effect of DPPH radical was least in *Curcuma zedoaria*.

If we consider the ethanolic extracts of medicinal plants, *Holarrhena antidysentrica* had the strongest inhibition on DPPH radical. The order of DPPH radical scavenging activity of ethanolic extracts of the remaining herbs is as follows. *Asparagus racemosa*>*Pachygoneovata*>*Lippia nodiflora*> *Curcuma zeodoaria.* Among the petroleum ether extracts of medicinal plants studied for their DPPH scavenging activity, *Holarrhena antidysentrica* (15.05%) possessed the high quenching of DPPH radical. The rest of the plants showed the minimum inhibition ranged from 1-7%.

Among the various solvent extracts of medicinal plants tested, aqueous and ethanolic extracts of the herbs were found to possess good antioxidant activity. Out of five medicinal plants investigated for their radical quenching activity, *Holarrhena antidysentrica* extract was found to have superior radical scavenging efficacy in all the solvent systems. The percentage inhibition of DPPH radical by aqueous and ethanolic extracts of *Holarrhena antidysentrica* was almost in similar range.

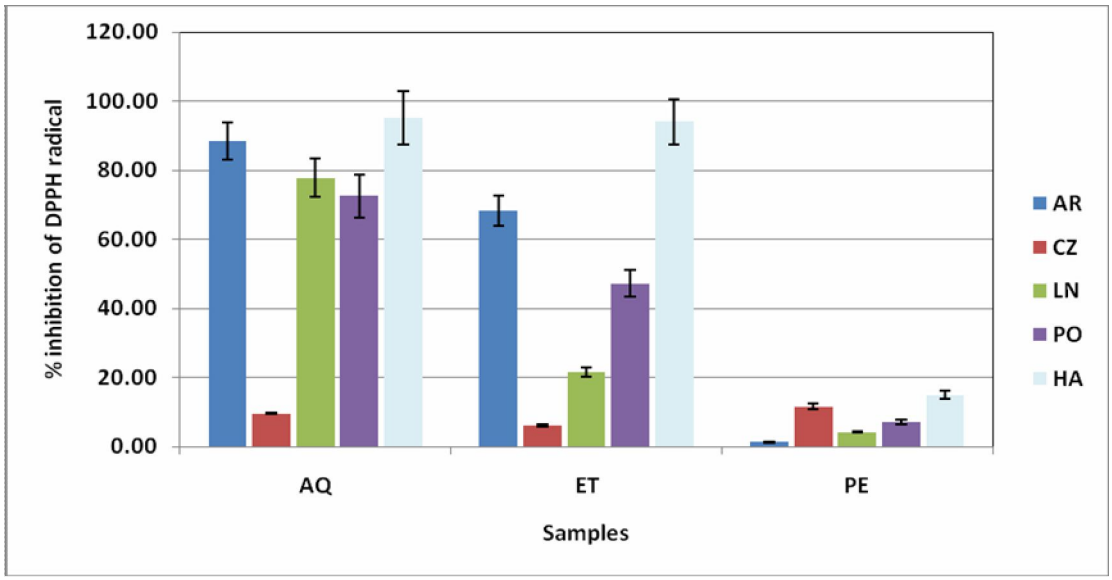

Figure 2 depicts the DPPH scavenging potential of different solvent extracts of medicinal plants.

3.3. Determination of total phenolics in various solvent extracts of medicinal plants

Plant phenolics constitute a major group of phytochemicals that acts as primary antioxidants [23]. They have high redox potentials which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [24]. The total phenolic content of plant extracts in terms of catechol equivalents were shown in figure 3.

If we consider the aqueous extract of medicinal plants, *Pachygone ovata* possessed the highest phenolic content (8.93 mg of catechol/g of plant tissue). *Asparagus racemosa, Lippia nodiflora* and *Holarrhena antidysentrica* had mariginal differences in their total phenol content. The least amount of phenolics was present in *Curcuma zedoaria* (3.21 mg of catechol/g of plant tissue).

Among the ethanolic extract of five medicinal plants studied for their phenolic content, large amount was detected in *Pachygone ovata* (10.18 mg of catechol/g of plant tissue). This was followed by *Holarrhena antidysentrica (*4.45mg of catechol/g of plant tissue), *Asparagus racemosa* (3.08 mg of catechol/ g of plant tissue) and *Lippia nodiflora* (1.27 mg of catechol/g of plant tissue). The least amount of phenolics was observed in *Curcuma zedoaria*.

Holarrhena antidysentrica (7.01 mg of catechol/g of plant tissue) had the highest level of phenolics among the five medicinal plants tested in petroleum ether solvent. Petroleum ether extract of *Pachygone ovate* was found to be the rich in the total phenol content among the rest of the medicinal plants screened. The amount of phenolics in petroleum ether extract of medicinal plants clearly demonstrated that the extractability of phenolic compounds is too low in this particular solvent.

Among the various solvent extracts of medicinal plants examined for their total phenolic content, it was noticed that all the five herbs exhibited good phenolic content in aqueous extract. This clearly indicates that the extractability of phenolic compounds is good in aqueous medium. Eventhough the extractability of total phenols in the aqueous medium is comparatively better, the ethanolic extract of *Pachygone ovata* has shown to have the maximum content of phenolics expressed in terms of catechol equivalents.

4. Conclusion

Out of five medicinal plants selected for testing their antityrosinase activity, the aqueous extract of *Asparagus racemosa* possesses the strongest inhibition. In order to support the skin whitening potential of the aqueous extract of *Asparagus racemosa*, all the plant extracts were assessed for their *in-vitro* antioxidant potential. Among the plants screened, the aqueous and ethanolic extract of *Holarrhena antidysentrica,* was found to be superior and followed by the aqueous extract of *Asparagus racemosa.* The same plant extracts were also analyzed to quantify the total phenolic content. Here the ethanolic extract of *Pachygone ovata,* followed by the aqueous extract of *Asparagus racemosa* was found to be the richest among the screened herbs. Irrespective of the parameters used for determining the antioxidant potential, the aqueous extract of *Asparagus racemosa* was found to be the second potent extract among the screened plants. These findings support the use of aqueous extract of *Asparagus racemosa* in cosmetic formulations to deliver the skin lightening and antiaging benefits, once the dermal safety is ensured.

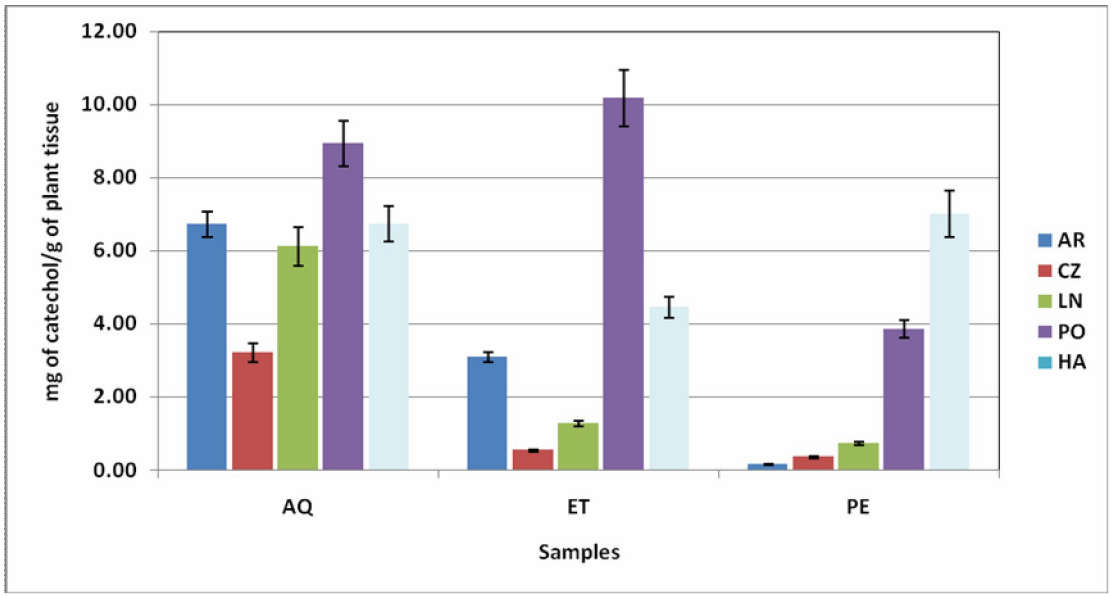

Figure 3 illustrates the total phenolic content of medicinal plants.

References

1. Yamakoshi J, Otsuka F, Sano A, Tokutake S, Saito M, Kikuchi M and Kubota Y (2003).Lightening effect on ultraviolet-induced pigmentation of guinea pig skin by oral administration of a Proanthocyanidin rich extract from grape seeds.Pigment Cell Res.,16:629-638.
2. Braverman IM, Fonferko E (1982) Studies in cutaneous aging: I, the elastic fiber network. The Journal of Investigative Dermatology 78, 434-443.
3. Olikarnin A, Kallionen M (1989). A biochemical and immunohistochemical study of collagen in sunexposed and protected skin. Photo-dermatology 6, 24-31.
4. Smith JG, Davidson EA, Tindall JP, Sams WM (1962) Alterations in human dermal connective tissue with age and chroic sun damage. The Journal of Investigative Dermatology 39, 347-350.
5. Kambayashi H, Yamashita M, Odake Y, Takada K, Funasaka Y, Ichihashi M (2001) Epidermal changes caused by chronic low-dose UV irradiation induce wrinkle formation in hairless mouse. Journal of Dermatological Science 27, Supl. 1, 19-25.
6. Yasui H and Sakurai H (2003).Age-dependant generation of reactive oxygen species in the skin of live hairless rats exposed to UVA light.Exp.Dermatol, 12:655-661.
7. Salano F, Briganti S, Picardo M, Ghanem G (2006).Hypopigmenting agents: An updated review on biological, chemical and clinical aspects.Pigment Cell Res, 19, 550-71.
8. Gyamfi MA, Yonamine M, Aniya Y (2002). Free radical scavenging action of medicinal herbs from Ghana Thonningia sanguine on experimentally induced liver injuries.Gen. Pharmacol. 32: 661-667.
9. Pinnell SR (2003). Cutaneous photodamage, oxidative stress, and topical antioxidant protection. *J. Am.Acad. Dermatol. 48*, 1-19.
10. Noguchi N, Niki E (2000). Phenolic antioxidants: A rationale for design and evaluation of novel antioxidant drug for atherosclerosis. *Free Radic. Biol. Med.* 28, 1538-1546.
11. Mayo JL (1998). Black cohosh and chasteberry: herbs valued by women for centuries. Clinical Nutrition Insights 6, 1–4.
12. Pandey SK, Sahay A, PandeyRS, Tripathi YB (2005). Effect of *Asparagus racemosus* (Shatavari) rhizome on mammary gland and genital organs of pregnant rat. Phytotherapy Research 19, 721–724.
13. Nanal BP, Sharma BN, Ranade SS, Nande CV (1974). Clinical study of Shatavari (*Asparagus racemosus*). Journal of Research in Indian Medicine 9, 23–29.
14. Nicoletti MA, Bugno A, Orsine EMA, Zenebon O (2003). Estudo da atividade antimicrobiana do extrato fluido da *Curcuma zedoaria* (Christm) Roscoe – Determinação da concentração minima inibitória. *Rev. Bras. Farm,* 84(2): 39-41.
15. Kirtikar and Basu.Indian Medicinal Plants.2nd ed.Vol III.Dehradun, India: International Book distributors, 1935.
16. Sing KP (1983). Traditional research potentials of kutaja (*H. antidysenterica*). J of Research in Ayurved and Siddha IV: 6-16.
17. Kavitha D, Shilpa PN, Devaraj SN (2004). Antibacterial and antidiarrhoel effects of alkaloids of *Holarrhena antidysenterica* Wall. Indian J Exp Biol 42: 589-594.
18. Wahab MA, Yousuf M (2004). Ethno-medical uses of some medicinally active plants for treating diabetes in Chittagong Hill Tracts, Bangladesh. Hamdard Medicus 47: 36-38.
19. Mensor LL, Meneze FS, Leitao et al., (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother.Res.15, 127-130.
20. Mallick CP and Singh MB (1980). Plant enzymology and Histoenzymology (eds), Kalyani Publishers, New Delhi, pp.286.
21. Kang Tae Lee, Kwang Sik Lee, Ji Hean Jeong, Byoung Kee Jo, Moon Young Heo, and Hyun Pyo Kim (2003). Inhibitory effects of *Iamulus mori* extracts on melanogenesis. *J. Cosmet. sci.,* 54, 133-142.
22. Blois MS (2001). Antioxidant activity of grape seed extracts on peroxidation models *in vitro*. J. Agric. Food Chem. 55: 1018.
23. Hatano T, Edmatsu R, Mori A, Fujita Y and Yasuhara E (1989). Effects of tannins and related polyphenols on superoxide anion radical and on 1, 1-diphenyl-2-picrylhydrazyl radical. Chem. Pharm Bull., 37:2016-2023.
24. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kulaja TS and Heinonen M (1999). Antioxidant activity of plants extracts containing phenolic compounds. J.Agric Food Chem., 47:3954-3962.

*****