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In vitro antioxidant potential of methanol flower extracts of *Cassia auriculata Linn.*

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ABSTRACT

The phytochemical screening of methanolic extract C. *auriculata* showed the presence of secondary metabolites such as of flavonoids, saponin, steroids, glycosides, while phenols and tannins were absence. The plant has significant antioxidant activity which

is attributed to its high vitamin C content. Therefore *C. auriculatais* regarded as excellent source for bioactive compound that can be further developed into drug to combat oxidative stress.

Keyword: *C. auriculatais,* flavonoids, antioxidant, cervical cancer

1. INTRODUCTION

The medicinal plants are used traditionally to prevent or cure disease. The medicinal values of the plants are due to presence of phytochemical constituents, which produce definite physiological action on the human body. According to World Health Organization more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance (Diallo *et al.,* 1993). Plants are exploited as medicinal source since ancient age. The traditional and folk medicinal system uses the plant products for the treatment of various infectious diseases.

The *C. auriculata* belongs to family-Caesalpiniaceae which has been claimed to possess the wound healing and antioxidant activities**.** Traditional background of Indian medicine shows widespread use of plant product in cancer. Fresh flower of *C. auriculata* is widely used in traditional system of medicine as a cure for rheumatism. The plant has been reported to possess antipyretic, hepatoprotective, antidiabetic and antiperoxidative, antihyperglycemic and microbial activity. *C. auriculata* plant contains preliminary phytochemical constituents such as alkaloids, phenols, glycoside, flavonoids, tannin, saponins, protein, carbohydrate and anthraquinine derivatives are responsible for the pharmacological activities. The MTT assay of ethanolic extract of flowers of *C. auriculata* shows that all concentration is having anticancer activity (Murugananthan *et al.,* 2015).

2. MATERIALS AND METHODS

Collection of plant materials and identification

The leaves of *C. auriculata* (Fig: 1) were collected from in and around Erode district, Tamilnadu, India. The collected leaves were identified by Dr. Nagarajan Department of Botany kongunadu Arts and Science College Coimbatore, The collected plant leaves *C. auriculata* were washed twice with tap water and rinsed with distilled water to remove or dust particles attached with leaves and the plant leaves subjected to dry in shade. Followed by this step, the dried plant leaves were then subjected to cold percolation method to obtain *C. auriculata* leaves powder.

Fig: 1 Study the plant

PREPARATION OF PLANT EXTRACT

About 10 g of air dried powder was taken in 100 mL of methanol. Plugged with cotton wool and then kept on a rotary shaker at 220 rpm for 24 h. Then the supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume and stored at 4 ^{o}C in air tight container.

PHYTOCHEMICAL ANALYSIS

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Phytochemical components of the extracts of *C. auriculata* were screened by using standard methods. The components analyzed were Alkaloids, Flavonoids, Saponins, Tannins, Triterpinoids and Glycosides (Raaman, 2006).

DPPH RADICAL SCAVENGING ACTIVITY

DPPH radical scavenging activity The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H. The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method.9 Briefly, an 0.1mM solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 mL of the solution of all extracts at different concentration (100, 200, 300, 400 & 500µg/mL).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula. DPPH scavenging effect (% inhibition) = {(A0 –A1)/A0)*100} Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

ANTIOXIDANT ACTIVITY BY RADICAL CATION (ABTS +)

Antioxidant activity by radical cation (ABTS +) ABTS assay was based on the slightly modified method of (Huang *et al.,*2011) ABTS radical cation (ABTS+) was produced by reacting 7mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS + Solution were diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm. After addition of sample or trolox standard to 3.9 mL of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC). The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

HYDROXYL RADICAL SCAVENGING ACTIVITY

Hydroxyl radical scavenging activity the scavenging capacity of hydroxyl radical was measured according to the modified method of (Halliwell *et al.,* 1987) 10 Stock solutions of EDTA (1mM), FeCl3 (10 mM), Ascorbic Acid (1mM), H2O2 (10mM) and Deoxyribose (10 mM) were prepared in distilled deionized water. The assay was performed by adding 0.1mL EDTA , 0.01mL of FeCl3, 0.1mL H2O2, 0.36mL of deoxyribose, 1.0mL of the extract of different concentration (100, 200, 400 & 500µg/mL) dissolved in distilled water, 0.33mL of phosphate buffer (50mM, pH 7.9), 0.1mL of ascorbic acid in sequence . The mixture was then incubated at 37°C for 1 hour. 1.0mL portion of the incubated mixture was mixed with 1.0mL of 10%TCA and 1.0mL of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

NITRIC OXIDE SCAVENGING ASSAY

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which were measured at 540 nm. The reaction mixture (6.0 ml) containing sodium nitroprusside (4.0 ml), phosphate buffer saline (PBS, 1.0 ml) and different concentrations (100 - 500 µg / ml) of a leaf extract (1.0 ml) in DMSO was incubated at 25°C for 15 minutes after incubation, 0.5 ml of the reaction mixture containing nitrite was removed, 1.0 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 minutes for completion of diazotization and 1.0 ml of naphthyl ethylene diamine dihydrochloride was added, mixed well and allowed to stand for 30 minutes in diffused light. A pink coloured chromophore was formed. The absorbance of these solutions was measured at 540 nm against corresponding blank solutions. Rutin was used as a standard. (Madan *et al*., 2005)

The inhibition was calculated according to the equation,

$$I = Ao - A1/Ao \times 100,$$

Where, Ao is absorbance of control reaction, A1 is absorbance of test compound.

3. RESULTS AND DISSUSION

The phytochemical analysis

The phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each works differently. Many plants and their extracts used against microbial infections due to the presence of secondary metabolites such as phenols (Kazmi *et al.,* 1994) Phytochemical analysis of methanolic extracts and acetone of flower sample of *C. auriculata* showed the presence of flavonoids, saponin, steroids, glycosides, while phenols and tannins were absence. Likewise in the acetone extracts of flower sample of *C. auriculata* shown the presence of saponin, steroids, glycosides, while flavonoids, phenols, tannins were absence (Table 1).

Table 1 Phytochemical analysis of methanolic and acetone flower extract of *C .auriculata*

Phytochemical compounds	Methanol	Acetone
Flavanoids	+	-
Phenols	-	-
Saponin	+	+
Tannin	-	-
Steroids	+	+
Glycosides	+	+

Present/Absent

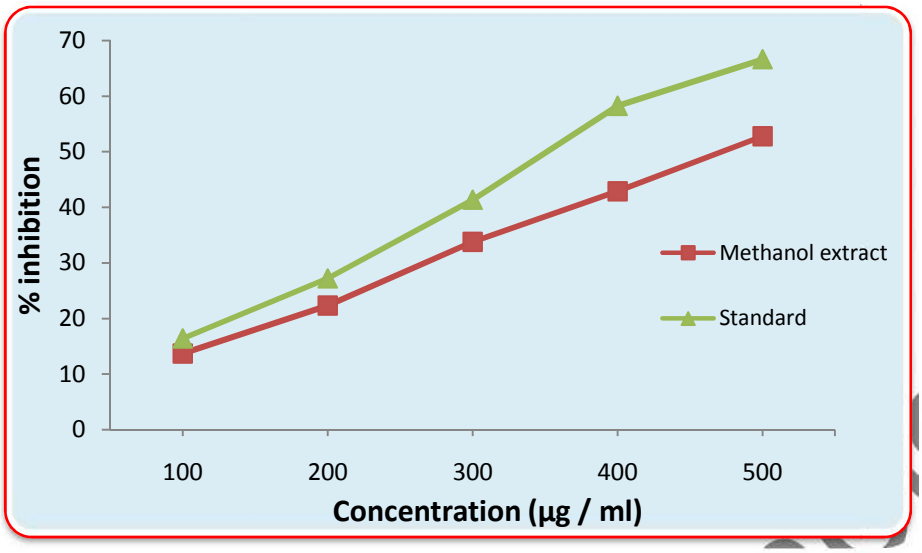
DPPH FREE RADICAL SCAVENGING ACTIVITY

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of the plant extract, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action.(Raquibul Hasan *et al.,*2009) the DPPH assay has been largely used as a quick, reliable and reproducible parameter to search the *In-vitro* general antioxidant activity of pure compounds as well as plant extracts (Koleva *et al.,* 2002). The reducing capacity of compounds could serve as indicator of potential antioxidant property. In the present study, the percentage of scavenging effect on the DPPH radical was concomitantly increased with the increase in the concentration of both methanol flower extracts of *C. auriculata* from 100 to 500µg/mL and 100 to500µg/mL. The percentage of inhibition existed from 423.73µg/Ml for methanol they were 473.19µg/ML (Table-2 fig-2). From the results it is known that the species, *C. auriculata* Linn. Possess hydrogen donating capabilities and does scavenging free radicals. Furthermore, it was noticed that the methanol extracts has more pronounced scavenging activity than that of the acetone extracts of *C. auriculata*.

Table 2 DPPH free radical scavenging activity of methanolic flower extracts of *C. auriculata*

S. No.		% inhibition				
		100 (µg/ml)	**200 (µg/ml)**	**300 (µg/ml)**	**400 (µg/ml)**	**500 (µg/ml)**
1	Methanol extract	16.34 ± 0.29	24.39 ± 0.34	36.28 ± 0.72	47.28 ± 0.90	59.28 ± 0.29
2	Standard (Vitamin C)	20.00 ± 0.33	31.95 ± 0.20	43.50 ± 0.23	51.44 ± 0.27	65.40 ± 0.50

The experiment was conducted in triplicates (n=3)

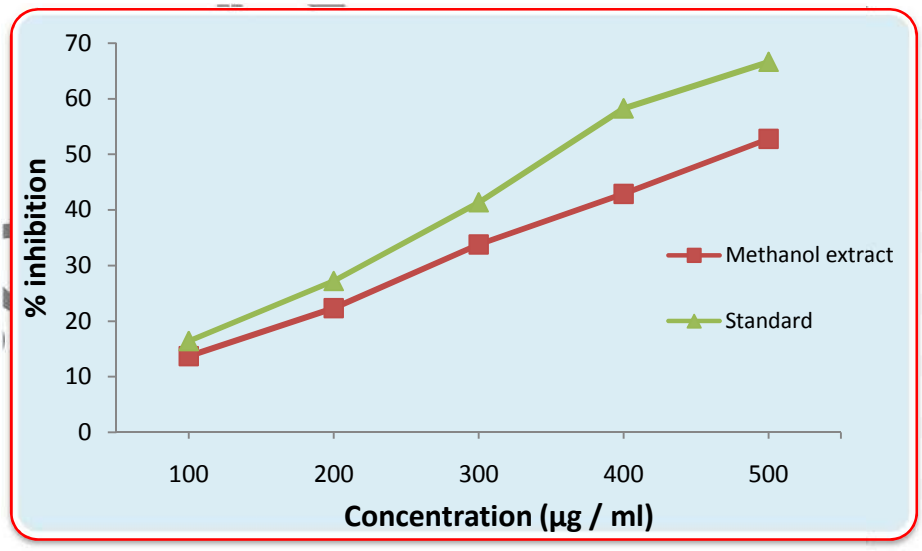


IC_{50} value of Methanol extract : 423.73 µg/ml
IC_{50} value of Vitamin C (Standard) : 384.77 µg /ml

Figure 2 DPPH free radical scavenging activity of methanolic flower extracts of *C. auriculata*

$ABTS^{\bullet+}$ RADICAL SCAVENGING ACTIVITY

Assay measures the relative antioxidant ability to scavenge the radical $ABTS^{\bullet+}$ and is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and chain breaking antioxidants. The methanol and acetone extracts of flower parts of *C. auriculata* are taken to examine the antioxidant activity. The methanol and acetone plant extracts were most active and fully scavenged by $ABTS^{\bullet+}$. The aerial extract showed high amount of $ABTS^{\bullet+}$ radical scavenging activity in 100 to 500 µg/mL concentration. The flower part extract exhibited moderate activity. Methanolic flower extracts exposed decreasing activity with increasing concentrations. The results were expressed as Trolox equivalent in µ mol/g extract (Table-3 & fig-3).



IC_{50} value of Methanol extract : 414.75 µg/ml
IC_{50} value of Vitamin C (standard) : 355.35 µg/ml

Figure 3 ABTS radical scavenging assay of metanolic flower extracts of *C. auriculata*

Table 3 ABTS radical scavenging assay of metanolic flower extracts of *C. auriculata*

S. No.		% inhibition				
		100 (µg/ml)	200 (µg/ml)	300 (µg/ml)	400 (µg/ml)	500 (µg/ml)
1	Methanol extract	17.27 ± 0.38	26.28 ± 0.38	37.10 ± 0.18	48.36 ± 0.19	59.37 ± 0.84
2	Standard (Vitamin C)	20.90 ± 0.23	31.22 ± 0.10	44.50 ± 0.20	54.30 ± 0.54	67.20 ± 0.10

The experiment was conducted in triplicates (n=3).

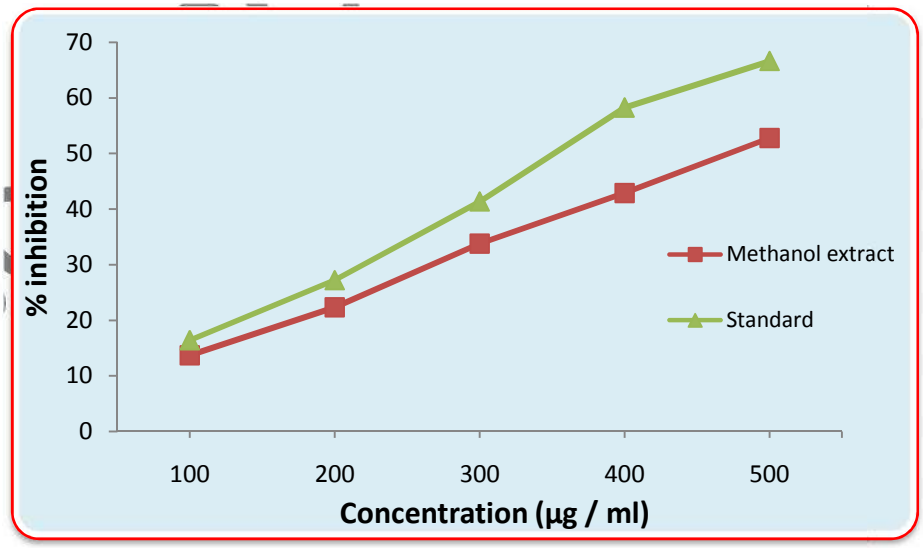
HYDROGEN PEROXIDE-SCAVENGING ASSAY

Hydrogen peroxide is generated In vivo by several oxidase enzymes. In this method, when an antioxidant is incubated with hydrogen peroxide, the decay or loss of hydrogen peroxide is measured spectrophotometrically.(Pellegrini et al., 1999) Hydrogen peroxide is a weak oxidizing agent which inactivates enzymes by oxidation of the essential thiol (SHe) groups. It rapidly transverses cell membranes and once inside the cell interior, interacts with Fe2þ and Cu2þ to form hydroxyl radicals, which is harmful to the cell.(Contreras-Guzma´n *et al.,* 1982). The extracts showed good scavenging effects with IC50 values 405.00µg/mL of methanol and 484.44µg/mL of acetone observed in (Table-4 and fig-4). The composition of hydrogen peroxide into water may occur according to the antioxidant compounds, as the antioxidant component present in the extract are good electron donors, they may accelerate the conversion of H2O2 to H2O.

Table 4 Hydroxyl radical scavenging assay of methanolic flower extracts of *C. auriculata*

S. No.		% inhibition				
		100 (µg/ml)	200 (µg/ml)	300 (µg/ml)	400 (µg/ml)	500 (µg/ml)
1	Methanol extract	14.12 ± 0.19	25.83 ± 0.56	36.38 ± 0.18	49.38 ± 0.20	60.48 ± 0.23
2	Standard (Vitamin C)	16.30 ± 0.20	32.32 ± 0.20	44.25 ± 0.50	56.34 ± 0.30	68.50 ± 0.40

The experiment was conducted in triplicates (n=3).



IC_{50} value of Methanol extract : 405.00 µg/ml

IC_{50} value of Vitamin C (standard) : 348.40 µg/ml

Figure 4 Hydroxyl radical scavenging assay of methanolic flower extracts of *C. auriculata*

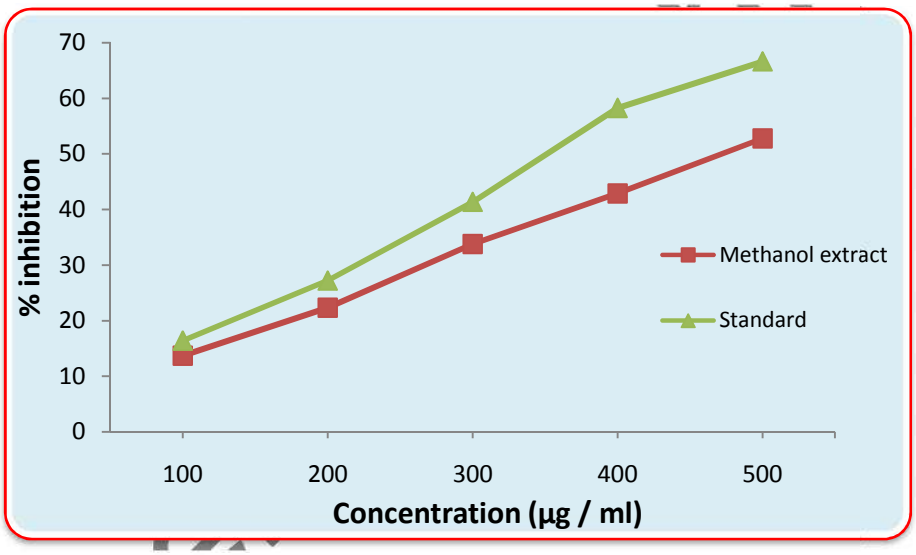
NITRIC OXIDE ASSAY

Nitric oxide generated from sodium nitroprusside is measured by the Greiss reagent reduction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Table-5 and fig-5).

Table 5 Nitric oxide scavenging assay of methanolic flower extracts of *C. auriculata*

S. No.		% inhibition				
		100 (µg/ml)	200 (µg/ml)	300 (µg/ml)	400 (µg/ml)	500 (µg/ml)
1	Methanol extract	15.49 ± 0.27	25.39 ± 0.28	37.39 ± 0.87	49.88 ± 0.20	58.28 ± 0.26
2	Standard (Vitamin C)	16.40 ± 0.25	27.20 ± 0.30	41.35 ± 0.40	58.23 ± 0.34	66.60 ± 0.50

The experiment was conducted in triplicates (n=3).



IC_{50} value of Methanol extract : 401.14 µg/ml

IC_{50} value of Vitamin C (standard) : 348.51 µg/ml

Figure 5 Nitric oxide scavenging assay of methanolic flower extracts of *C. auriculata*

4. CONCLUSION

In brief summarizing the above results, it is well cleared that flowers extract of *Cassia auriculata (Linn.)* exhibited significant antioxidant potential. Antioxidant potential of plant was investigated with the help of various *in vitro* antioxidant assays like DPPH Scavenging assay, $ABTS^{\bullet+}$ radical scavenging activity, Hydrogen peroxide-scavenging assay and Nitric oxide scavenging assay. This medicinal plant by in vitro results appears as interesting and promising and may be effective as potential sources of novel antioxidant drugs.

REFERENCE

1. Contreras-Guzma´n ES, Strong FC. 1982. Determination of tocopherols (vitamin E) by reduction of cupric ion. Journal of Assoc Anal Chemistry. ; 65:1215-1221.
2. Diallo D, B Hveem, MA Mahmoud, G Berge, BS Paulsen and A Maiga, 1999.An ethnobotanical survey of herbal drugs of Gourma district, Mali. Pharmaceutical Biology. 37:80-91.
3. Giao, M.S., Gonzalez, S.M. L., Rivero, P.M. D., Pereira, C.I., Pintado, M.E and Malcata, F.X. (2007). Infusions of

Portuguese medicinal plants: Dependence of final antioxidant capacity and phenolic content on extraction features. *J Sci Food Agric*., 87: 2638-2647.

4. Halliwell B, Gutteridge JMC, Aruoma OI., The deoxyribose method: a simple test tobe assay for determination of rateconstants for reaction of hydroxylradicals. Ana. Biochem, 65: 215-219 (1987)
5. Huang MH, Huang SS, Wang BS, Sheu MJ, and Hou WC., Antioxidant and antiinflammatory
6. Kazmi, M., Malik, A., Hameed, S., Akhtar, N. and Ali, S. An anthraquinone derivative from Cassia italica. Phytochemistry., 1994; 36(8): 761-763.
7. Koleva II, Van Beek TA, Linssen JPH, de G root A, Evstatieva LN. 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemistry Anal. 13:8 -17.
8. Muruganantham N, Solomon S, Sentamnselvi MM. 2015. Anticancer activity of Cassia auriculata (flowers) against human liver cancer. 6(1):19-24.
9. Pellegrini N, Proteggente A, Pannala A, Yang M, Rice Evans C. 1999Antioxidant activity applying an improved ABTS radical cation decolorization. Free Radical Biological Medicinal. 26:1231e1237. properties of Cardiospermum halicacabum and its reference compounds ex vivo and in vivo. J. Ethnopharmacol, 133: 743-750 (2011).
10. Rahman MM, MR Habib, SMR Hasan, MA Sayeed and MS Rana, 2011. Antibacterial, Cytotoxic and Antioxidant potential of Methanolic extract of Phyllanthus Acidus L.
11. Rajeshwar, Y., Senthilkumar, G. P., Malay, A.G and Mazumder, U.K. (2005). Studies on in vitro antioxidant activities of methanol extract of Mucuma pruriens (Fabaceae) seeds. Eur Bull Drug Res., 13: 131-138.
12. Raquibul Hasan SM, Mokarram Hossain MD, Raushanara A, Mariam J, Ehsanul Hoque Mazumder MD, Rahman Shafiqur. 2009. DPPH free radical scavenging activity of some Bangladesh medicinal plants. Full Length Research Paper. 3:875e879.
13. Shimada, K., Fujikawa, K., Yahara, K and Nakamura, T. (1992). Antioxidative properties of xanthan on the antoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem., 40:945-8.