



ESTIMATION OF TOTAL FLAVONOIDS CONTENT (TFC) AND ANTI OXIDANT ACTIVITIES OF METHANOLIC ROOT EXTRACT OF CLERODENDRUM INFORTUNATUM LINN

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Abstract:

Clerodendrum infortunatum(C.I.) Linn. (Lamiaceae), commonly known as *Bhant* in Hindi, is a small shrub occurring throughout the plains of India. This plant has been used in Indian folk medicine in the treatment of bronchitis, asthma, fever, burning sensation, diseases of the blood, inflammation, and epilepsy. The total flavonoid content of methanolic root extract of *C. infortunatum* (MECI) was determined by using aluminium chloride colorimetric method. It was found that *Clerodendrum infortunatum* root extracts contain total flavonoids in 0.0055% gm quercetin equivalent per gram of extract. In this study phytochemical analysis of methanolic extract of *C. infortunatum* L. has indicated the presence flavonoid. Since these compounds are of pharmacological interest, coupled with the use of this plant in traditional medicine, prompted us to check *C. infortunatum* L. for possible antioxidant activity by DPPH scavenging activity and reducing power ability. The maximum percentage inhibition by DPPH method was found about 92.99 at concentration of 110.46µg/ml, when compared with Quercetin. The reducing capabilities were found to be in dose dependent manner.

Key words: *Clerodendrum infortunatum*, aluminium chloride, quercetin, DPPH

Introduction:

For thousands of years mankind is using plant sources to alleviate or cure illness¹. Novel chemical compounds synthesis from the plant active constituents, which are of potential use in medicine and other usefull application. Herbal

remedies are popular remedies for diseases used by a vast vast majority of the world's population². Herbal plants having many pharmacologically active compounds like flavonoids, alkaloids, tannin, steroids, glycosides, phenols, fixed oils, which is stored in their specific parts of leaves, bark, flowers, seed, fruits, root etc.³. *Clerodendrum infortunatum* (family- Lamiaceae) having different pharmacological activities such as antimicrobial, anthelmintic, hepatoprotective, anticonvulsant, wound healing, analgesic activities of there different parts⁴⁻⁸.

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There is an increased evidence for the participation of free radicals in the etiology of various diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases, aging etc. A free radical is defined as any atom or molecule possessing unpaired electrons. Antioxidants are agents which scavenge the free radicals and prevent the damage caused by reactive oxygen species (ROS), reactive nitrogen species (RNS). ROS is composed of superoxide anion ($O_2\cdot^-$), hydroxyl ($OH\cdot$), hydroperoxyl ($OOH\cdot$), peroxy ($ROO\cdot$), alkoxy ($RO\cdot$) radicals non free radicals are hydrogen peroxide (H_2O_2), hypochlorous acid ($HOCl$), ozone (O_3) singlet oxygen (1O_2). RNS are mainly nitric oxide ($NO\cdot$), peroxy nitrite ($ONOO\cdot$) nitrogen dioxide (NO_2). Antioxidants can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells prevent damage to lipids, proteins, enzymes, carbohydrates DNA. A wide range of antioxidants from both natural and synthetic origin has been proposed for use in the treatment of various human diseases⁹. Flavonoids are potent antioxidants and have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity¹⁰. Therefore, the objective of our present study is to determine the antioxidant and total flavonoid content of root extract of *Clerodendrum infortunatum* using quercetin, Aluminium Chloride colorimetric method. In the study quercetin taking as a standard flavonoids.

Materials and Methods:

Plant material: The root of *Clerodendrum infortunatum* was collected from Ischadagharia village of Kamrup, Assam. The authentication of plant material was done by a botanist at Botanical survey of India, Howrah, W. Bengal and the Voucher no is **CNH/24/2013/Tech.II/1005**.

Chemicals:

Quercetin, aluminium chloride, Diphenylpicryl hydrazine (DPPH), Trichloroacetic acid (TCA) and $FeCl_3$.

DPPH was obtained from Hi media laboratories Pvt. Ltd. Mumbai. Aluminium chloride, TCA, $FeCl_3$ were obtained from Merck, Mumbai, India; Quercetin was obtained from Sisco research laboratories Pvt. Ltd. (SRL) Mumbai, India.

Preparation of extracts by using soxhlet extracting methods:

100g of root bark material was taken in a soxhlet and 80% methanol was added up to 2 siphons that is up to 500ml. The temperature is set to 70°C and the extraction was carried out up to 5 hours. Then the extract obtained is filtered and concentrated at 70°C. Dried extracts were kept in refrigerator and used for further study¹¹.

Estimation of total flavonoid content Aluminium Chloride Colorimetric Method:

Principle :

Formation of acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols in addition with aluminium chloride. Aluminium chloride also forms acid labile complexes with the ortho - dihydroxyl groups in the A- or B- ring of flavonoids. For building the calibration curve, quercetin is used as a standard materials. Various concentrations of standard quercetin solution were used to make a standard calibration curve¹⁰.

Procedure:

In this method, quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in methanol and then diluted to 6.25, 12.5, 25, 50, 80, and 100 $\mu\text{g/ml}$. A

calibration curve was made by measuring the absorbance of the dilutions at 415 nm (λ_{max} of quercetin) with a Shimadzu UV-1800 spectrophotometer. Aluminium chloride, 1% and potassium acetate, 1M solutions were prepared^{10,12,13}.

Stock Solution of Extracts:

100 mg of the each extract was accurately weighed and transferred to 10 ml volumetric flask and made up the volume with methanol.

Preparation of Test Solutions:

0.5ml of each extract stock solution, 1.5 ml methanol, 0.1 ml aluminium chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminium chloride with distilled water. Sample and sample blank of all four extracts were prepared and their absorbance was measured at 415 nm. All prepared solutions were filtered through whatmann filter paper before measuring.

Antioxidant Activity:

In this study free radical scavenging activity of methanolic root extract of *Clerodendrum infortunatum* was determined by in vitro assay models such as DPPH free radical, reducing ability. Quercetin was used as reference standard.

DPPH radical scavenging activity:

Principle:

DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant by their hydrogen donating ability. The electrons become paired off and solution loses colour stoichiometrically depending on the number of electrons taken up⁹.

Procedure:

DPPH radical scavenging activity was measured using the method of Kiranmai *et al.*; with some modifications. 2 ml of reaction mixture containing 1 ml of DPPH (100 μM in methanol) 1 ml of test solution, at various concentrations of the extract fractions was incubated at 37°C for 30 min absorbance of the resulting solution was measured at 517 nm using Beckman model DU-40 spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the following equation^{9,14} :

Percentage inhibition = $(1 - \text{absorbance of test} / \text{absorbance of control}) \times 100$

Reducing Ability:

Principle:

Like the antioxidant activity, the reducing power increased with increasing amount of the extract. when potassium ferricyanide react with ferric chloride in the present of anti oxidant, potassium ferrocyanide and ferrous chloride are found as a product. Presence of reducers causes the conversion of the Fe^{3+} /ferricyanide complex used in this method to the ferrous form¹⁴.

Procedure:

1 ml of different concentrations (25 to 900 $\mu\text{g/ml}$) of the extract fractions were mixed with potassium ferricyanide (2.5 ml, 1%) 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50°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 water and 0.5 ml FeCl_3 (0.1%) were added to it. The absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power¹⁴⁻¹⁶.

Results and Discussion:

Determination of Total flavonoid content:

To perform the calculations of total flavonoid content in the studied plant using Kiranmai *et al.*, method, a standard curve is

needed which is obtained from a series of different quercetin concentrations.

FIGURE1: Quercetin standard curve

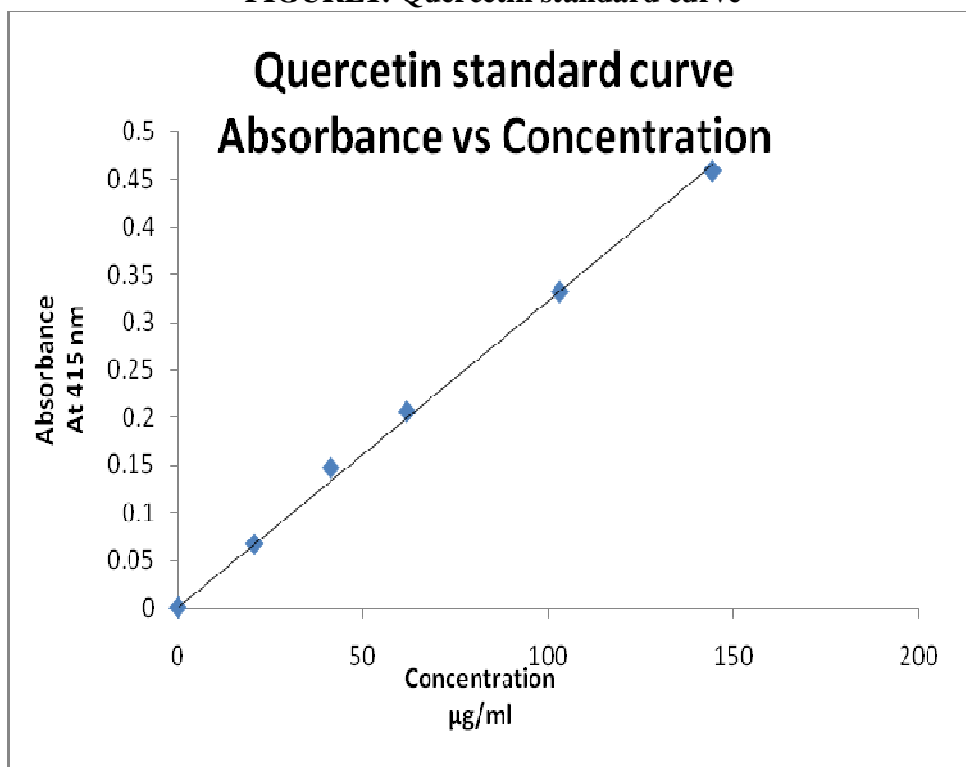


TABLE 1. Results of calibration curve

SR. NO	Concentration of plant Extract(µg/ml)	Absorbance at 415nm
1.	438	0.065
2.	1096	0.154
3.	2192	0.271

Concentration values of extracts were obtained from Quercetin standard curve, by interpolating to the X- axis. TFC was calculated by using the following formula [18]

$$TFC = \frac{R \times D.F \times V \times 100}{W}$$

Where,

W - Weight of plant used in the experiment

V - Volume of stock Solution

100 - For 100 g dried plant

R - Result obtained from the standard curve

D.F - Dilution factor

Table2. % yield and % total flavonoid content of extract

Extract	Yield (% w/w)	% TFC in gram
Methanolic root extract of <i>Clerodendrum infortunatum</i>	5	0.0055

The % total flavonoid content of the extracts are given in table 6. The soxhlet method gave the yield of crude extract 5% w/w.

Table3. DPPH Radical Scavenging Activity

Sr. no.	Concentration (µg/ml)	Absorbance at 517 nm	
		MECI	QUERCETIN
1.	22.08	3.285	2.968
2.	44.16	2.871	2.392
3.	55.20	2.382	1.911
4.	77.28	1.214	1.252
5.	110.40	0.267	0.681

Figure 2: DPPH scavenging assay of methanolic root extract of C.I. with respect to standard quercetin

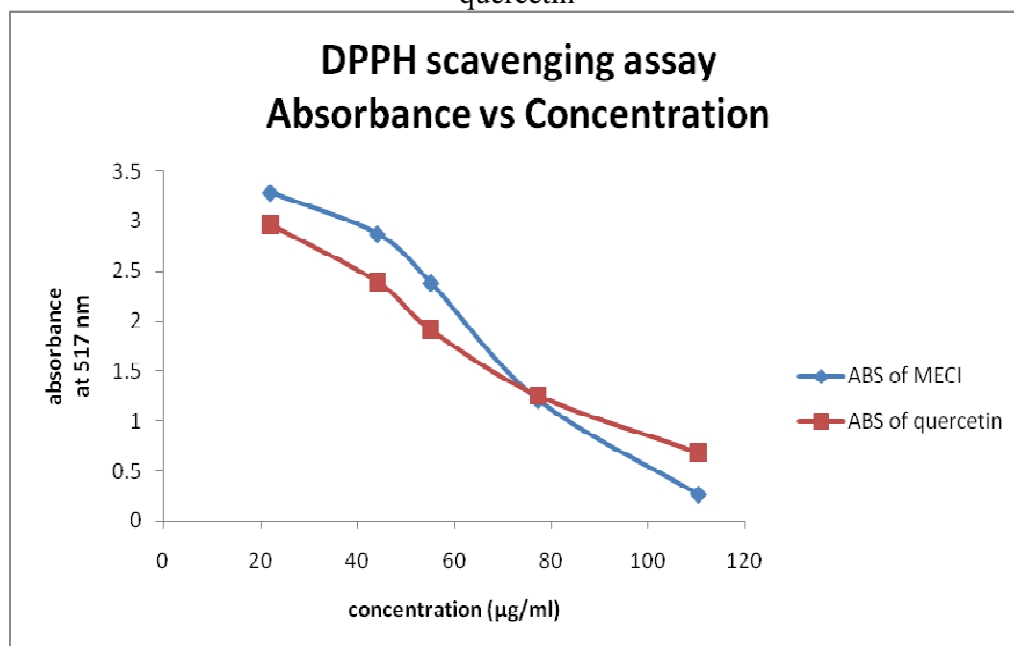
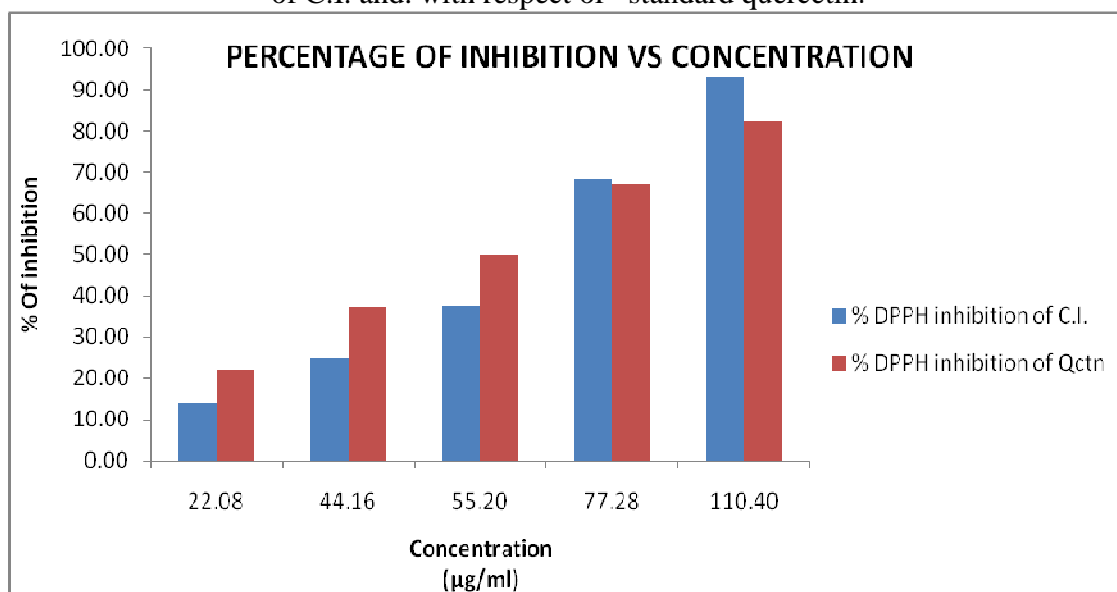


Table4. Evaluation of DPPH free-radical scavenging activity of methanolic root extract of C.I. and. With respect of standard quercetin

Sr. no	Concentration($\mu\text{g/ml}$)	% of inhibition	
		MECI	QUERCETIN
1.	22.08	13.78	22.10
2.	44.16	24.65	37.22
3.	55.20	37.48	49.84
4.	77.28	68.14	67.14
5.	110.46	92.99	82.13

Figure- 3 Evaluation of DPPH free-radical scavenging activity of methanolic root extract of C.I. and. with respect of standard quercetin.



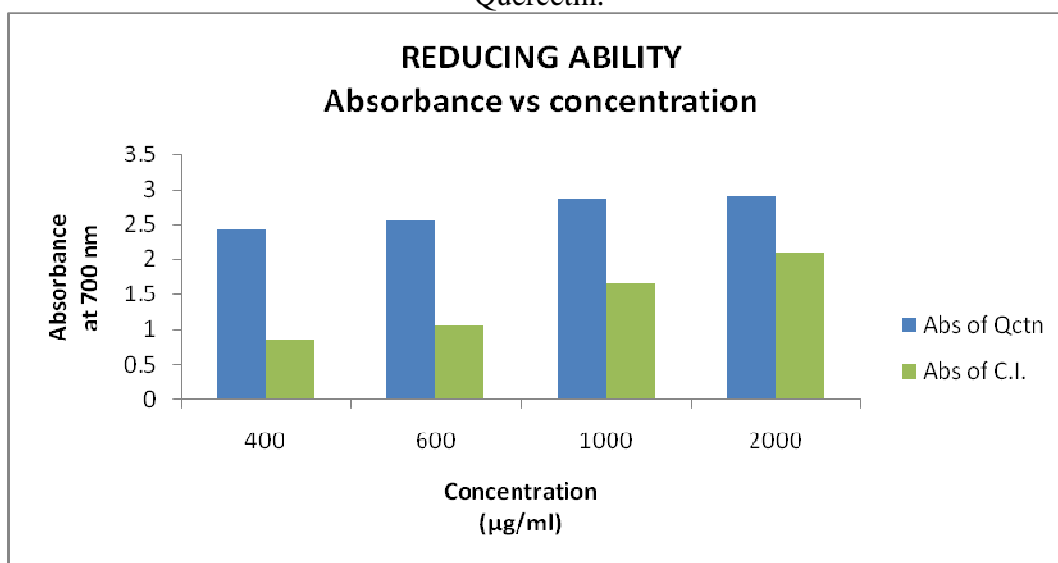
This assay is being used widely as a preliminary test which provides information on the reactivity of test compound with a stable free radical since odd electron of DPPH gives strong absorption band at 517nm(violet colour) and when it is quenched by the extract , there

is a decrease in absorbance. Methanolic extract of *C.infortunatum L* showed a very good anti-radical activity in scavenging DPPH radical (comparable to the standard, Quercetin) with a maximum inhibition of about 92.99 at a concentration of 110.46 $\mu\text{g/ml}$.

Table5.:Reducing ability of methanolic root extract of C.I. With respect to standard quercetin at 700 nm

SL. NO	CONCENTRATION (µg/ml)	ABSORBANCE AT 700 n.m.	
		C.I.	QUERCETIN
1.	400	0.833	2.421
2.	600	1.059	2.548
3.	1000	1.662	2.855
4.	2000	2.089	2.917

Figure 4: Reducing ability of methanolic root extract of C.I. With respect to standard Quercetin.



The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity . For the estimation of the reductive ability we investigated the Fe³⁺ to Fe²⁺ transformation using the method of Oyaizu, where the change in the optical density of the final mixture is measured at 700nm (Table-2). Increase in optical density

indicates higher reductive ability^{12, 13}. The reducing capabilities of the root extract of *C. infortunatum L.* was found to be in dose dependent manner when compared with Quercetin.

Conclusion:

The methanolic extract of the root of *C.infortunatum L.* contains flavonoids, which

possess antioxidant property. Hence further investigation and proper isolation of more active principles might help in the findings of new lead compounds which will be effective against free radical mediated diseases.

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Reference:

1. Kamboj, V.P. 2000. *Herbal medicine*. Current science. Vol.78.35-51
2. Pal, S., Shukla, Y. 2003. *Herbal medicine: current status and the future*. Asian pacific journal of cancer prevention. Vol 4. 281-288.
3. Sharma,A., Shankar.C., Tyagi, L., Singh, M., Rrao,C.2008. *Herbal Medicine for Market Potential in India: An Overview*. Academic journal of plant sciences. Vol 2. 26-36.
4. Modi A. J., Khadbadi S.S., Farooqui I.A., Ghorpade, D.S. 2010. *Studies on antimicrobial activity of Clerodendrum infortunatum, Argyreia nervosa and Vitex negundo: A comparison*. Scholars Research Library. Vol 2(1). 102-105
5. Modi,A., Khadabadi S. S., Deore S. L. 2010. *In vitro Anthelmintic Activity of Clerodendrum infortunatum*, *International Journal of PharmTech Research*.Vol.2(1). 375-377.
6. Sannigrahi, S., Mazumder, U. K., Pal, D., Mishra. 2009. *Hepatoprotective potential of methanol extract of Clerodendrum infortunatum Linn. against CCl4 induced hepatotoxicity in rats*. *Indian Journal of Experimental Biology*. Vol 5(20).394-399.
7. Pal, D., Sannigrahi, S., Mazumder, U. 2009. *Analgesic and anticonvulsant effects of saponin isolated from the leaves of Clerodendrum infortunatum Linn. in mice*. *Indian Journal of Experimental Biology*. Vol.47.743-747
8. Kuluvar,G., Mahmood,R., Mohamed,R., Ahamed,K., Babu,P., Venkatarangaiah, K. 2009 .*Wound healing activity of Clerodendrum infortunatum Linn. Root extracts*. *International Journal of Biomedical and Pharmaceutical Sciences*.Vol. 3(1).21-25
9. Naskar, S., Mazumder,U., Pramanik,G., Bala, A., Halder,P., Islam, A., Gupta,M. 2011. *Comparative in vitro anti oxidant activity of different parts of Cocos nucifera linn. on reactive oxyzen and nitrogen species*. *International Journal of Pharmacy and Pharmaceutical Sciences*. Vol 3, Suppl 3. 104-107.
10. Kiranmai,M., Kumar,M., Mohammed, I.2011. *Comparison of total flavanoid content of Azadirachta indica root bark extracts prepared by different methods of extraction*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. Volume 2. 254-261
11. Gupta,A., Naraniwal,M., Kothari,V.2012.*Modern extraction methods for preparation of bioactive plant extracts*. *International Journal of Applied and Natural Sciences (IJANS)* .Vol.1, Issue 1. 8-26.
12. Siddique,N.A., Mohd, M., Kalam, A., Mohd, A.2010. *Evaluation of antioxidant activity, quantitative estimation of phenols and flavonoids in different parts of Aegle marmelos*. *African Journal of Plant Science* Vol. 4 (1). 001-005,
13. Fernandes,A.,Assunc,M.,Ferreira,A.,Rand au,K.P.,DeSouza,T.2012. *Total Flavonoids Content in the RawMaterial and Aqueous Extractives fromBauhinia monandra Kurz (Caesalpinaceae)*. *The ScientificWorld Journal* .Volume 2012.1-7.
14. Lavanya,R., Maheshwari,S., Harish,G., Raj,B., Kamali,S., Hemamalani,D., Varma,J., Reddy,C.2010.*In-vitro Antioxidant Activity of Methanolic Extract in Leaves of Anisomeles malabarica Linn*. *Research Journal of Pharmaceutical,*

- Biological and Chemical Sciences.vol-1(4).737-744.
15. Kanchan. Chauhan,P.K.,Jaryal,M., Kumari,K., Singh,M. 2012. *Phytochemical and invitro anti oxidant potential of aqueous leaf extracts of Brassica juncea and Coriandrum sativum* . IJPS.Vol. 3(8). 2862-2865.
16. Aghdam,M.,Dehghan,G., Kafshboran,H.R. 2011. *Comparative study of ABTS radical scavenging activity and flavonoid contents in several populations of Teucrium polium*. International Conference on Life Science and Technology. vol.3.55-58.