Research Article

Comparative Evaluation of Total Flavonoid Content and Antioxidant Activity of Methanolic Root Extract of *Clerodendrum infortunatum* and Methanolic Whole Plant Extract of *Biophytum sensitivum*

Tapan Kumar Barman*, Pallab Kalita, Tapas Kumar Pal
*Professor & H.O.D.(Pharmacology), NSHM Knowledge campus Kolkata, Group of institution, 124- B.L. Saha road, Kolkata, West Bengal, India.
***Corresponding author's E-mail:** tapan.barman@nshm.com

Accepted on: 10-06-2013; **Finalized on:** 31-08-2013.

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. *Biophytum sensitivum* linn. (Oxalidaceae), is an important and widely used medicinal plant. *Biophytum sensitivum* linn. is used as a traditional folk medicine in ailments such as inflammation, arthritis, wounds, tumors and burns, gonorrhea, stomach ache, asthma, cough, degenerative joint disease, urinary calculi, diabetes, snake bite, amenorrhea and dysmenorrheal. The total flavonoid content of methanolic root extract of C. infortunatum (MECI) and methanolic whole plant extract of *Biophytum sensitivum* (MEBS) were determined by using aluminium chloride colorimetric method. Flavonoid compounds were found to be 5.5 and 9.49 µg of quercetin equivalent [QE] per mg of MECI and MEBS respectively. In this study phytochemical analysis of MECI and MEBS have 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.* and B. sensitivum 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 and 43.96 of MECI and MEBS at concentration of 110.46µg/ml, when compared with Quercetin. The reducing capabilities were found to be in dose dependent manner.

Keywords: Aluminium chloride, *Biophytum sensitivum*, *Clerodendrum infortunatum*, DPPH, Quercetin.

INTRODUCTION

For thousands of years mankind is using plant sources to alleviate or cure illness.[1] Novel chemical compounds synthesis from the plant active constituents, which are of potential use in medicine and other useful application. Herbal remedies are popular remedies for diseases used by a vast majority of the world's population.[2] 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, anthelminthic, hepatoprotective, anticonvulsant, wound healing, analgesic activities of their different parts and Biophytum sensitivum (family- Oxalidaceae) having different pharmacological activities such as dengue, anticancer, anti-inflammatory, chemo protective, antidiabetic and wound healing activities of their different parts. [4-13]

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 (O2·), hydroxyl (OH·), hydroperoxyl (OOH·), peroxyl (ROO·), alkoxyl (RO·) radicals non free radicals are hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3) singlet oxygen (1O2). RNS are mainly nitric oxide (NO·), peroxynitrite (ONOO·) 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 diseases.[14] 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 activity.[15] Therefore, the objective of our present study is to determine the antioxidant and total flavonoid content of root extract of *Clerodendrum infortunatum* and whole plant extract of *Biophytum sensitivum* 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.** *Biophytum sensitivum* were collected from the forest of Medinapur, WB. 30 kg of plant materials was identified taxonomically by expert taxonomist at the Botanical survey of India, Howrah, West Bengal, India. Voucher specimen of the plant at B.S.I. is 942 (dated 17-9-68) and the collected sample has been matched with the voucher specimen taxonomically and deposited in the institution herbarium for future reference.

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 both plant materials were taken in a soxhlet apparatus separately and 80% methanol was added up to 2 siphons that is up to 500ml. The temperature is set to 700C and the extraction was carried out up to 5 hours. Then the extract obtained is filtered and concentrated at 700C. Dried extracts were kept in refrigerator and used for further study.[16]

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, quarcetin is used as standard materials. Various concentrations of standard quarcetin solution were used to make a standard calibration curve.[15]

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 µg/ml. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm (λmax of quercetin) with a Shimadzu UV-1800 spectrophotometer. Aluminium chloride, 1% and potassium acetate, 1M solutions were prepared.[15,17,18]

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 aluminum 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 aluminum 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 activities of methanolic root extract of *Clerodendrum infortunatum* (MECI) and methanolic whole plant extract of B*iophytum sensitivum* (MEBS) were 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 stochiometrically depending on the number of electrons taken up.[14]

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.[14,19]

Percentage inhibition = (1– absorbance of test/absorbance of control) × 100

Reducing Ability

Principle

Like the antioxidant activity, the reducing power increased with increasing amount of the extract. When

potassium ferricyanide reacts 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 form.[19]

Procedure

1 ml of different concentrations (25 to 900 µg/ml) of the extract fractions was 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 power.[19-21]

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.

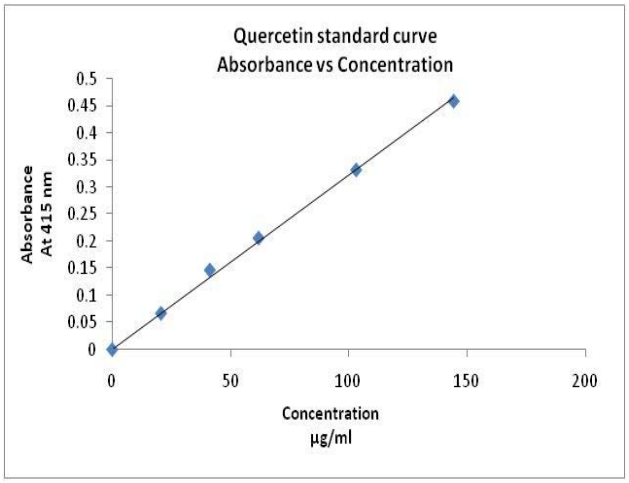

Figure 1: Quercetin standard curve

Table 1: Results of calibration curve

Concentration (µg/ml)	Absorbance at 415 nm	
	MECI	MEBS
1218	0.169	0.429
1827	0.272	0.659
2436	0.357	0.855

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

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

Where,

R - Result obtained from the standard curve

D.F - Dilution factor

V - Volume of stock Solution

100 - For 100 g dried plant

W - Weight of plant used in the experiment

Table 2: % yield and Total flavonoid content of extract

Sr. no.	Yield (% w/w)	TFC (µg of QE/mg of extract)
Methanolic root extract of C. infortunatum	5	5.5
Methanolic whole plant extract of B. sensitivum	7	9.49

The % total flavonoid content of the extracts is given in table 2. The soxhlet method gave the yield of crude extract 5% and 7% w/w, respectively.

Table 3: Dpph Radical Scavenging Activity

Concentration (µg/ml)	Absorbance at 517 nm		
	MECI	MEBS	Quercetin
22.08	3.285	3.64	2.968
44.16	2.871	3.54	2.392
55.20	2.382	3.29	1.911
77.28	1.214	2.88	1.252
110.40	0.267	2.135	0.681

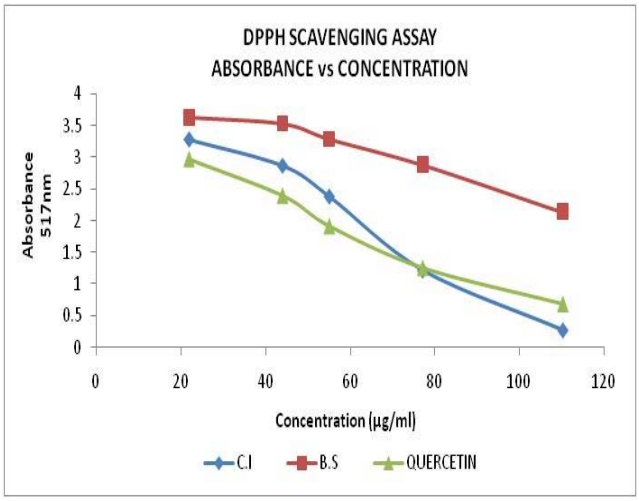

Figure 2: DPPH scavenging assay MECI and MEBS with respect to standard Quercetin

Table 4: Evaluation of DPPH free-radical scavenging activity of MECI and MEBS With respect of standard quercetin

Concentration(µg/ml)	% of inhibition		
	MECI	MEBS	Quercetin
22.08	13.78	4.51	22.10
44.16	24.65	7.17	37.22
55.20	37.48	13.54	49.84
77.28	68.14	24.33	67.14
110.46	92.99	43.96	82.13

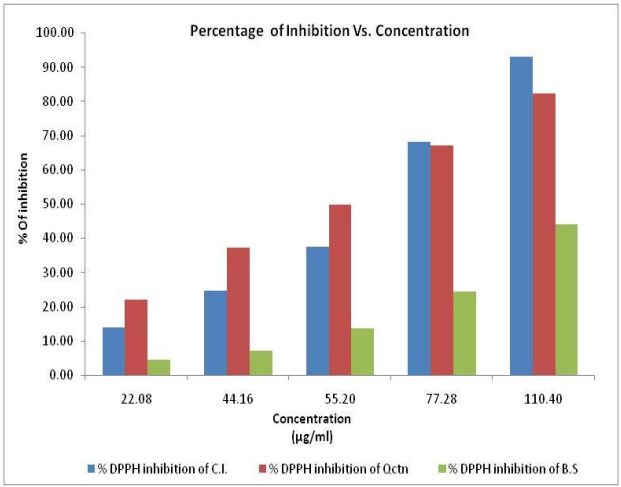

Figure 3: Evaluation of DPPH free-radical scavenging activity of MECI and MEBS With respect of standard Quercetin

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* and methanolic whole plant extract of *B. sensitivum 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 and 43.96 at a concentration of 110.46µg/ml.

Table 5: Reducing ability of MECI and MEBS With respect to standard quercetin at 700 nm

Concentration (µg/ml)	Absorbance at 700 nm		
	C.I.	B.S.	Quercetin
400	0.833	0.317	2.421
600	1.059	0.538	2.548
1000	1.662	0.695	2.855
2000	2.089	1.171	2.917

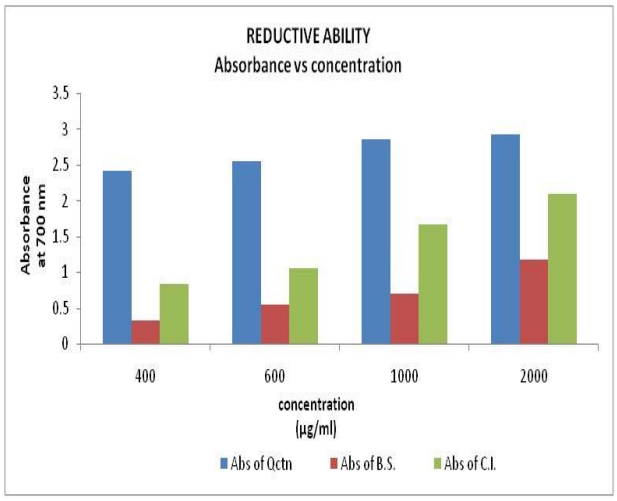

Figure 4: Reducing ability of methanolic root extract of C.I. With respect to standard Quercetin

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-5). Increase in optical density indicates higher reductive ability. The reducing capabilities of the root extract of *C. infortunatum L.* and B. sensitivum were found to be in dose dependent manner when compared with Quercetin.

CONCLUSION

The methanolic root extract of *C. infortunatum L.* and methanolic whole plant extract of *B. sensitivum 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.

Acknowledgement: The authors gratefully acknowledge Mr. Lalmohon Masanta and Mr. Subhas Kumar Manna for providing the laboratory oriented research facilities.

REFERENCES

1. Kamboj VP, Herbal medicine, Current science, 78, 2000, 35-51.
2. Pal S, Shukla Y, Herbal medicine: current status and the future, Asian pacific journal of cancer prevention, 4, 2003, 281-288.
3. Sharma A, Shankar C, Tyagi L, Singh M, Rrao C, Herbal Medicine for Market Potential in India: An Overview, Academic journal of plant sciences, 2, 2008, 26-36.
4. Modi AJ, Khadbadi SS, Farooqui IA, Ghorpade, Studies on antimicrobial activity of Clerodendrum infortunatum, Argyreia nervosa and Vitex negundo: A comparison, Scholars Research Library, 2(1), 2010, 102-105.
5. Modi A, Khadabadi SS, Deore SL, In vitro Anthelmintic Activity of Clerodendrum infortunatum, International Journal of PharmTech Research, 2(1), 2010, 375-377.
6. Sannigrahi S, Mazumder UK, Pal D, Mishra, Hepatoprotective potential of methanol extract of Clerodendrum infortunatum Linn. against CCl4 induced hepatotoxicity in rats, Indian Journal of Experimental Biology, 5(20), 2009, 394-399.
7. Pal D, Sannigrahi S, Mazumder U, Analgesic and anticonvulsant effects of saponin isolated from the leaves of Clerodendrum infortunatum Linn. in mice, Indian Journal of Experimental Biology, 47, 2009, 743-747.
8. Kuluvar G, Mahmood R, Mohamed R, Ahamed K, Babu P, Venkatarangaiah K, Wound healing activity of Clerodendrum infortunatum Linn. Root extracts, International Journal of Biomedical and Pharmaceutical Sciences, 3(1), 2009, 21-25.
9. Ananda Prabu K, Kumarappan CT, Sunil Christudas, Kalaichelvan VK, Effect of Biophytum sensitivum on streptozotocin and nicotinamide induced diabetic rats, Asian Pacific Journal of Tropical Biomedicine, 2012, 31-35.

10. V. H. Bhaskar, V. Rajalakshmi, Anti-tumor activity of aqueous extract of *Biophytum sensitivum* Linn, Annals of Biological Research, 1(3), 2010, 76-80.
11. D. Natarajan, MS Shivakumar, Srinivasan, Antibacterial activity of leaf extract of Biophytum sensitivum, Journal of pharmaceutical sciences and research, 2(11), 2010, 717-720.
12. C Guruvayoorappan, Biophytum sensitivum: Ancient medicine, modern targets, JAPTR, 3(2), 2012, 83-91.
13. Shivakumar MS, Srinivasan R, Natarajan D, Bioefficacy of *Biophytum sensitivum* (L.) leaf extracts against Dengue Mosquito Vector *Aedes Aegypti* (L.), RJPBCS, 3(3), 2012, 885-892.
14. Naskar S, Mazumder U, Pramanik G, Bala A, Haldar P, Islam A, Gupta M, 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, 3(3), 2011, 104-107.
15. Kiranmai M, Kumar M, Mohammed I, 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, 2, 2011, 254-261.
16. Gupta A, Naraniwal M, Kothari V, Modern extraction methods for preparation of bioactive plant extracts, International Journal of Applied and Natural Sciences (IJANS), 1(1), 2012, 8-26.
17. Siddique NA, Mohd M, Kalam A, Mohd A, Evaluation of antioxidant activity, quantitative estimation of phenols and flavonoids in different parts of Aegle marmelos, African Journal of Plant Science, 4(1), 2010, 001-005.
18. Fernandes A, Assunc M, Ferreira A, Randau KP, DeSouza T, Total Flavonoids Content in the RawMaterial and Aqueous Extractives fromBauhinia monandra Kurz (Caesalpiniaceae), The ScientificWorld Journal, 2012, 1-7.
19. Lavanya R, Maheshwari S, Harish G, Raj B, Kamali S, Hemamalani D, Varma J, Reddy C, In-vitro Antioxidant Activity of Methanolic Extract in Leaves of *Anisomeles malabarica Linn,* Research Journal of Pharmaceutical, Biological and Chemical Sciences, 1(4), 2010, 737-744.
20. Kanchan. Chauhan PK, Jaryal M, Kumari K, Singh M, Phytochemical and invitro anti oxidant potential ofaquous leaf extracts of *Brassica juncea* and *Coriandrum sativum,* IJPS, 3(8), 2012, 2862-2865.
21. Aghdam M, Dehghan G, Kafshboran HR, Comparative study of ABTS radical scavenging activity and flavonoid contents in several populations of *Teucrium polium,* International Conference on Life Science and Technology, 3, 2011, 55-58.

Source of Support: Nil, **Conflict of Interest:** None.