The Scientific Temper
Vol. 2; No.1&2 : Jan.-July, 2011 : pp77-80
Email: letmepublish@rediffmail.com

ISSN 0976 8653
e-ISSN 2231 6396

EVALUATION OF ANTIOXIDANT ACTIVITY OF THE POLYOXYGENATED XANTHONES FROM *SWERTIA CHIRATA* BUCH., HAM.

SUVRA MANDAL[A*], PRIYABRATA DAS[C], ASHES DAS[A], DHIRENDRA NATH MONDAL[A], ANINDITA GHOSH[B], DEBARATI MUKHERJEE[C], RAGHWENDRA MISHRA[C] ATANU BHATACHARYYA[D] AND MANOJ KAR[C]

a. Department of Chemistry, National Research Institute for Ayurvedic Drug Development, Bidhannagar, Kolkata-700091, INDIA
b. Department of Chemistry, University of Calcutta, 92 A.P.C.Road, Kolkata-700009, INDIA
c. Department of Biochemistry, N.R.S.Medical College and Hospital, Kolkata, INDIA
d. Department of Zoology, Vidyasagar College for Women, 39, Sankar Ghosh Lane, Kolkata, INDIA
* Corresponding author, E-mail address: mandal.suvra@gmail.com

ABSTRACT

Swertia chireta is an indigenous medicinal plant which are used for variety of ailments.Moreover, several phytochemicals are present in this plants.The major bioactive compound within the plant is xanthones.This plants are the mojor source of secondary metabolites, like flavonoides and triterpenoid etc . Naturally, the plant *Swertia* chireta posses a multiple source of different biological component which are solely responsible for different physiological activities.

Naturally, bioassay of this plants were investigated with the hexane.Hexan extract of aerial parts of the Himalayan plant, *Swertia chirata* Buch.Ham. ex Wall.(Gentianaceae) resulted in the isolation of seven polyoxygenated xanthones, fraction Sc-1 – Sc-7. Out of seven compounds extracted from this plant , five components (Sc - 3 - Sc-7) exhibited antioxidant activity at different magnitude of potency.The paper deals with these facts.

KEYWORDS *Swertia chirata*; aerial parts; antioxidant activity; seven polyoxygenated xanthones.

INTRODUCTION

Considerable attention has been focused on plants which are source of natural antioxidant compounds and medicinal value, because most of them have modulatory role on physiological functions and biotransformation reactions involved in the detoxification process(Ahsan, *et al*., 2009, Kumar ,*et al*., 2011).

Excessive free radical production has been implicated in the process of diabetes mellitus, arteriosclerosis,ischemia-reperfusion injury, liverdisease, inflammation, renal failure, neurodegenerative disorders, aging, carcinogenesis and various other diseases in human [Bulkley, 1983, Dormandy, 1983,]. The increased oxidative stress can be reduced by various phytochemicals obtained from plants having antioxidant property. The compounds of *Swertia chirata* Buch., Ham. (Gentianaceae), reduce free radical activity and thereby protect the cells from oxidative damage and thus may possess therapeutic activities [Tiwari,2004,Selvamathy *et al*.2010].

The Himalayan plant *Swertia chirata* Buch., Ham. (Gentianaceae), commonly known as 'Chirayita' or Kirata-tikta' in Sanskrit, is well known for its multifarious therapeutic value since the era of 'Atharva Veda' (Charak Samhita) and is widely used in the Indian System of Medicine as crude drug. It is used as an antimalarial, a bitter stomachic, febrifuge, anthelmintic, as remedy for scanty urine, epilepsy, ulcer, diabetes, bronchial asthma, melancholia and certain type of mental disorder. [Chatterjee and Pakrashi, 1995, Mandal, *et al.*, 1997.]

Chemical investigations on *Swertia chirata* by the present authors include the first isolated dimeric xanthone, 'chiratanin' from higher plant, a large number of polyoxygenated monomeric xanthones, the bitterest secoiridoid glycoside, amarogentin and triterpenes[Mandal and Chatterjee,1987, Mandal, *et al.*, 1997.]. Previously, we have biologically studied amarogentin and the crude aqueous extract of *Swertia chirata* which exhibited significant cancer chemopreventive potential as well as anticarcinogenic activity on DMBA induced mouse skin carcinogenesis model[Saha,*et al*., 2004, Saha, *et al*., 2006]. We also examined the mixture of six monomeric xanthone derivatives for antiinflammatory property on animal models and got significant activity [Mandal, *et al*., 1992]. Based on the above details , the present study was directed the efficacy of *Swertia chirayita*, against ethanol

(EtOH), extract of aerial parts of *Swertia chirayita* Buch. Ham. (Gentianaceae).

MATERIALS AND METHODS.

Experimental

Plant material

Swertia chirata (aerial parts), collected from a plant supplier of Kolkata, was authenticated by Dr. M.N.Das, Ex-Research Officer (Pharmacognosy), National Research Institute for Ayurvedic Drug Development (NRIADD), Kolkata. The plant specimen has been preserved in the Herbarium of NRIADD, Kolkata.

Extraction and Isolation

The bioassay guided fraction of the hexane extract of aerial parts of *Swertia chirata* led to the isolation of seven oxygenated xanthones from Sc-1 to Sc-7 shown in the table-1 in accending order of the phenolic character of the xanthones. All the compounds have been isolated by column chromatography on silica gel and purified by crystallization from appropriate solvents. The xanthones (Sc-1 - Sc-7) have been characterised by spectral analysis (UV, IR, PMR,CMR, MS) and by comparative study with the available literature data [Mandal, *et al.*, 1992.] .

Biochemical Estimation.

Total antioxidant status (TAS)

TAS was estimated by the method [13] based on the inhibition of radical cation, $ABTS^{+}$[2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] which has a characteristic long wavelength absorbance maxima at 734 nm. $ABTS^{+}$ is formed by interaction with potassium per sulphate.

Reagent required

i) ABTS

ii) Trolox

iii) Potassium per sulphate

iv) PBS (Phosphate Buffer Saline)- 5mM

Method

ABTS radical cation ($ABTS^{+}$) was formed from 7mM ABTS in PBS (pH 7.4) solution by reacting with 2.45 mM potassium per sulphate (final concentration) keeping at room temperature for 16hr. As ABTS and Potassium per sulphate react stoichiometrically at a ratio 1:0.5, this resulted incomplete oxidation of the ABTS. Oxidation of the ABTS commenced immediately, but the absorbance was not maximal and stable until more than 6 hr elapsed. ABTS solution was diluted with PBS to an absorbance of 0.7 (±0.02) at 734 nm and equilibrated at 30ÚC.

After 16hr of formation of $ABTS^{+}$, 10ìl solution of active compound (1mM) was incubated with 1 ml diluted ABTS solution and the change in colour intensity was measured at 734 nm after 6 minutes exactly. Similarly the inhibition of colour against the solvent blank was estimated by the absorbance at 734 nm and its reading was substracted from that of active compound. Here trolox was used as a standard antioxidant and the result was expressed as mM Trolox equivalent.

RESULT

The present report furnishes results of antioxidative study of seven tetraoxygenated xanthones presented in Table-1.The total antioxidant status(TAS) was estimated against the standard antioxidant, Trolox. All the compounds have been isolated by column chromatography on silica gel and purified by crystallization from appropriate solvents. The xanthones (Sc-1 - Sc-7) have been characterised by spectral analysis (UV, IR, PMR,CMR, MS) and by comparative study with the available literature data as we have discussed earlier. [Mandal, *et al.*, 1992.] (Table-2). Moreover, total antioxidant status –TEAC, is the millimolar concentration of a Trolox solution having the antioxidant capacity equivalent to a 1.0mM solution of the substances under investigation and described in the table-3.

Table 1: Xanthones isolated from *Swertia chirata*

Name of compound	Molecular formula (M^{+})	Mp.^{0}C	Yield (%)	Chemical name	Reference
Sc-1	$C_{16}H_{14}O_{6}$ (302)	140	0.045	1,3,7-Trimethoxy -8-hydroxy xanthone	11
Sc-2	$C_{16}H_{14}O_{6}$ (302)	203-204	0.005	1-Hydroxy 3,5,8-Trimethoxy xanthone	12
Sc-3	$C_{15}H_{12}O_{6}$ (288)	185	0.061	1,8-dihydroxy 3,5, -dimethoxy xanthone	5,12
Sc-4	$C_{14}H_{10}O_{6}$ (274)	224-225	0.152	1,7,8-Trihydroxy 3 -methoxy xanthone	12
Sc-5	$C_{14}H_{10}O_{6}$ (274)	274	0.238	1,5,8-Trihydroxy- 3 -methoxy xanthone	5,11
Sc-6	$C_{14}H_{10}O_{6}$ (274)	196 (Acetate)	0.004	1,5,6-Trihydroxy- 3 -methoxy xanthone	5,11
Sc-7	$C_{13}H_{8}O_{6}$ (274)	>360	0.006	1,3,7,8-Tetrahydroxy xanthone	5,11

DISCUSSION

A compound can exert antioxidant activity in various ways. It can directly scavenge various reactive oxygen species (ROS) such as super oxide radicals, hydrogen peroxide, lipid peroxides, hydroxyl radical etc. It can also inhibit the formation of such species by reacting with their promoter namely, iron and copper orgenerate the enzymes or it can enhance the activity of other antioxidants [Mathur, *et al.*, 2011, Abirami and Gomathinayagam, 2011].

Although ROS can help the immune system clean out extrusive microorganisms and excessive ROS can also react with biological molecules such as DNA, proteins and phospholipid and ultimately cause oxidative damage in tissue and free radical- related diseases such as inflammation, heart disease, diabetes, gout, cancer etc [Slater,1984]. For aerobic organisms,

Table-2
Spectroscopical data of Xanthones Sc-1 – Sc-7

Name of the compound	$UV_{ëmax}$,in ETOH(nm)	IR, $í_{max}$ in KBr(Cm^{-1})	1_{H-NMR}
Sc-1	250,268,330	2900,,1650, 1620,1600, 1570 1480	ä($CDCl_3$): 4.02, 3.93, 3.85, (C-1, C-3, C-7 0Me), 6.36, 6.39 (d, J=2H_Z each; C-2 and C-4-H), 6.71, 6.55, (d, J=10H_Z each, C-5 and C-6-H) 11.77 (C-8-OH)
Sc-2	230,254, 275,330	3100,1655, 16251610, 1600,1570 1480	ä($CDCl_3$): 3.81, 3.93, 3.93 (C-3, C-5, C-8-OMe), 6.28, 6.44 (d, J=2H_Z each, C-2 & C-4-H), 6.65, 6.85 (d,J= 9 H_Z each C-5, C-6-H), 13.08 (C-1-OH)
Sc-3	249, 273 & 325	3150, 1660, 1620, 1600, 1550 1420	ä(d_6-DMSO): 3.86, 3.92 (C-3, C-5-OMe), 6.29, 6.48 (d, J= 3H_Z each, C-2 & C-4-H), 6.65, 7.17 (d, J= 9H_Z each, C-6, C-7-H), 11.30, 11.88 (C-1 & C-8-OH)
Sc-4	254, 265, 327	3450, 3100, 1650, 1615, 1600, 1570 1480	ä(d_6-DMSO): 3.92 (C-3-OMe), 6.30,6.35 (d, J= 2H_Z each, C-2,C-4-H), 6.65, 6.72 (d, J= 10 H_Z each, C-5 & C-6-H), 11.77, 11.82 (C-1 & C-8-OH)
Sc-5	255, 279, 335 & 393	3500, 3150, 1655, 1620, 1600, 1560 1480	ä(d_6-DMSO):3.81 (C-3-OMe), 6.27, 6.49 (d, J=2.5 H_Z each, C-2 & C-4-H), 6.54, 7.15 (d, J= 9H_Z each, C-6 & C-7-H), 11.1- 12.0 (C-1 & C-8-OH)
Sc-6	249, 277 & 315	3460, 3100, 1650, 1625, 1610, 1600, 1570 1450	ä(d_6-DMSO): 3.90 (C-3-OMe), 6.35, 6.55 (d, J= 2H_Z each, C-2 & C-4-H), 7.06, 7.89 (d, J=10H_Z each, C-7 & C-8-H), 7.55, 10.04 (C-5 & C-6-OH), 12.76 (C-1-OH)
Sc-7	247, 266, 331 & 385	3300, 3150, 1667, 1613, 1590 1470	ä(d_6-DMSO): 6.20, 6.34 (d, J= 3H_Z each, C-2 & C-4-H) 6.88, 7.26 (d, J=10H_Z each, C-5 & C-6-H), 9.25 (br.s, C-3 & C-7-OH) , 11.53, 11.73 (br.s, C-1 & C-8-OH)

the major system of defense against oxidative damage is the use of 'antioxidant' enzymes to convert excessive ROS into non toxic compounds. An imbalance between the amount of ROS and antioxidant enzymes is a problem for our health. So, daily intake of foods containing antioxidants is necessary [Re,*et al*., 1999 , Halliwell and Gutteridge , 1984].

S.chirata is a Himalayan medicinal plant traditionally used in India. The use of this plant is also found in China and Nepal (Phoboo *et al*., 2010 **)**. It is used to treat the different ailments for a long time. The reason and progress of some of these ailments, in some ways, are related to damage caused by ROS. For example, oxidants have been considered to be involved in the early stages of carcinogenesis,[Hochstein and Attallah,1988] whereas oxygen free radicals have played an important role in the development of the long-term complications of diabetes.[Boynes,991, Krishnamurthy, *et al*., 2006] Although the intake of antioxidantsmay not completely cure the disorders, it can reduce the oxidative stress in our body and consequently reduce the progress of diseases[Fasalu Rahiman , *et al*., 2011]

In this study, seven tetraoxygenated xanthones have been isolated from *S.chirata* in pure form. All the seven compounds were evaluated for their antioxidant potential along which three compounds Sc-5, Sc-6 and Sc-7 exhibited a very significant antioxidant property compared to the standardantioxidant, Trolox, a vitamin-E derivative. A promising antioxidant activity has been reflected in Sc-5 and Sc-6 as their activities are more than double as compared to Trolox.

We have previously reported that, the extract and the pure compounds of *S.chirata* are effective in inflammation and cancer in animal models [Mandal, 1992, Mandal (Sarkar) , 1980, Ghosal, *et al*., 1973]. Oxidative stress is common in both the above mention diseases. Our present finding is possibly partially related to that effectiveness. The study clearly indicates that the extract of *Swertia chirata* possesses antioxidant property. Further research is necessary for elucidating the active potent component of *Swertia chirata* out of the seven components of this plant . Moreover, studies are in progress to know the specific gene functions in relation to antioxident activity of the *Swertia chirata* in the several physiological processes.

ACKNOWLEDGEMENT

The authors (S.M, AD, and DNM) thank the Director General, CCRAS, New Delhi forfinancial assistance. The administrative support of the Director of NRIADD, Bidhannagar, Kolkata is also thankfully acknowledged.

REFERENCES

Ahsan,R., Islam, K.M., Musaddik,A. and Haqu, E. 2009. Hepatoprotective Activity of Methanol Extract of Some Medicinal PlantsAgainst Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats. Global Journal of Pharmacology, 3 (3) : 116-122.

Bulkley, G.B.1983. Role of oxygen derived free radicals in digestive tract disease .Surger. **94** : 407 – 411.

Dormandy, T .L. , 1983. An approach to free radicals. Lancet . **11** : 1010 – 14.

Kumar ,R.V., Kumar,S., Shashidhara ,S. , Anitha, S., Manjula, M ..2011 . Comparison of the Antioxidant Capacity of an Important Hepatoprotective Plants. International Journal of Pharmaceutical Sciences and Drug Research . 3(1) : 48-51.

Selvamathy, S.M.K.N. , Arumugam Geetha, A. and Saranya, P. 2010. GASTROPROTECTIVE EFFECT OF *SWERTIA CHIRAYITA* –A STUDY WITH ULCER INDUCED RATS. *Pharmacologyonline* 1: 332-355.

Tiwari , A.K. 2004. New generation therapeutic base for treatment of polygenic disorders,Curr Sci . **86** : 1092 – 1102

Chatterjee , A. and Pakrashi, S.C . 1995. The Treatise on Indian Medicinal Plants, publications and Information Directorate, CSIR, New Delhi . 4 : 92-94.

Mandal , S. and Chatterjee , A. 1987.Chiratanin, structure of a new bixanthone, Tetrahedron Lett. 28 : 1309 – 10

Mandal S, Joshi , P.C. and Das ,P.C. 1997. Drug value of Swertia chirata and its phytoconstitutents , Bull Medico Ethno Botany Res .**18**: 82 – 88

Saha , P., Mandal , S., Das, A. , Das , P.C. and Das , S . 2004. Evaluation of anti carcinogenicactivity of Swertia chirata Buch.Ham., an Indian Mdicinal plant, on DNBA induced mouse skin carcinogenesis model Phytother Res . 1**8** : 373– 377.

Saha , P., Mandal, S., Das , A. and Das , S. 2006. Amarogentin can reduce hyperproliferation by downregulation of cox-II and upregulation of apoptosis in mouse skin carcinogenesis model, Cancer Letters . **244**: 252 - 259.

Mandal, S., Das, P.C. and Joshi, P.C.1992. Antiinflammatory action of Swertia chirata Buch. Ham. . Fitoterapia . **63** : 122-128.

Mathur, A., Verma , S.K., Singh, S.K.,Mathur,D., Prasad, G.B.K.S.and Dua, V.K. 2011.. INVESTIGATION OF ANTI-INFLAMMATORY PROPERTIES OF *SWERTIA CHIRAYTA* AND *GLORIOSA SUPERBA*. *Recent Research in Science and Technology . 3(3): 40-43.*

Abirami, P. and Gomathinayagam, M. 2011. A REVIEW ON *ENICOSTEMMA LITTORALE*. *Pharmacologyonline* 1: 75-83.

Slater, T. F.1984.Free radical mechanism in tissue injury, Biochem. J. **222** : 1-15.

Re , R., Pellegrini , N. and Proteggente, A . 1999. Antioxidant activity applying and improved ABTS radical cation decolorisation assay, Free Radical Biology and Medicine . **26** : 1231 – 37

Halliwell, B. and Gutteridge ,J. M. C. 1984. Cell damage and antioxidant therapy, Lancet .**23** : 1396-1397.

Phoboo, S., Pinto,M.D.S., Bhowmik, P.C., Jha , P.K.and Shetty, K. 2010 .QUANTIFICATION OF MAJOR PHYTOCHEMICALS OF *SWERTIA CHIRAYITA,* A MEDICINAL PLANT FROM NEPAL. ECOPRINT 17: 59-68,

Hochstein, P. and Attallah, A. S.1988. The nature of oxidants and antioxidants systems in inhibition of mutation and cancer, Mutation Res. **202** :363-375.

Boynes, J. W.1991.Role of oxidative stress of complications in diabetes .Diabetes. **40** : 405-411

Krishnamurthy, P., Vaithinathan, S. and Bhuvaneswari, A.2006. Protective effect of Syzygium cuminii (Linn.) Skeels seed extract on lipid peroxidation in alloxan induced diabetic rats, Nat. Prod. Rad. **5** : 103-107.

Fasalu Rahiman , O.M. , Rupesh Kumar ,M. , Tamizh Mani , T. and Mohamed Niyas, K. 2011. A REVIEW OF HEPATOPROTECTIVE NATURAL PRODUCTS. International Journal of Pharmaceutical Sciences Review and Research. 8, (1) : 80-84.

Mandal , S., Das, P.C. and Joshi, P.C. 1992. Review on Naturally occurring xanthones from Terrestrial flora, J Indian Chem. Soc . **69**: 611 – 636.

Mandal (Sarkar) S. 1980. Ph.DThesis on 'Studies on phytochemicals and their transformation reactions, Calcutta University, India.

Ghosal , S., harma, P.V. and Choudhuri , R.K. 1973. J Pharm Sci. **62** : 926 – 29.

❑❑❑