Annals of Phytomedicine 2(2): 96-104, 2013

ANNALS OF PHYTOMEDICINE
An International Journal

ISSN 2278-9839

Isolation and identification of quercetin and emodin from *Cassia tora* L.

Durgesh Nandani, Roop Narayan Verma and Amla Batra

Plant Biotechnology Laboratory, Department of Botany, University of Rajasthan, Jaipur-302055, *Rajasthan, India*

Received May 26, 2013: Revised June 30, 2013: Accepted July 25, 2013: Published online December 30, 2013

Abstract

During the present research work, a flavonoid (quercetin) and an anthraquinone (emodin) were isolated from the *in vivo* leaves and *in vitro* callus of *Cassia tora* L. Their extraction and isolation was done by using their standard protocols, respectively. However, their identifications were carried out through thin layer chromatography, NMR and IR spectroscopy.

Key words: *Cassia tora* L., TLC, IR, NMR, quercetin, emodin

Introduction

Plants are an important part of our everyday diet, their constituents and nutritional value has been intensively studied for decades. In addition to essential primary metabolites (*e.g.*, carbohydrate, lipid, protein and amino acids), higher plants are also able to synthesize a wide variety of low molecular weight compounds,the secondary metabolites. Plant secondary metabolites can be defined as compounds, that have no recognized role in the maintenance of fundamental life processes in the plants but they do have an important role in the interaction of the plants with its environment (Sirikantaramas *et al*., 2008). The production of these compounds is often low (less than 1% dry weight) and depends mainly on the physiological and developmental stages of plants. Many secondary metabolites have complex and unique structures and their production is often enhanced by both biotic and abiotic stress conditions. They are stored in specific cells and organs of the plant and are often accumulated in vacuoles (Chaudhuri *et al*., 2009).

Almost 1,00,000 secondary metabolites have been discovered from the plant kingdom, but only with half of the structures fully elucidated (Hartmann *et al*., 2005). Secondary metabolites are characterized by enormous chemical diversity and every plant has its own characteristic and specific secondary metabolite (Pichersky and Gang, 2000). Secondary metabolites can be classified on the basis of chemical structure, *e.g.*, having rings, containing a sugar, composition containing nitrogen or not, their solubility in various solvents or the pathway by which they are synthesized, *e.g.*, phenylpropanoid, which produces tannins.

Many higher plants are major sources of natural products, which are used as pharmaceuticals, agrochemicals, flavour and fragrance ingredients, food additives and pesticides. The search for new plant derived chemicals should, thus, be a priority in current and future efforts towards sustainable conservation and rational utilization of biodiversity. In the search of alternatives for the production of desirable medicinal compounds from plants, biotechnological approaches specifically, through plant tissue culture, having great potential as a supplement to traditional agriculture, in the industrial product and by-product production of bioactive plant metabolites (Rao and Ravishankar, 2002; Srivastav and Srivastav, 2007; Jesse *et al*., 2009). Cell suspension culture systems could be used for large scale culturing of plant cells from which secondary metabolites could be extracted. The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products (Vanisree *et al*., 2004; Rai *et al*., 2009).

Present studies deal with the isolation and identification of flavonoid (quercetin) and an anthraquinone (emodin) from *in vivo* (leaves) and *in vitro* (callus) tissues of *C. tora* L.

Author for correspondence: Dr. Roop Narayan Verma
Plant Biotechnology Lab., Department of Botany, University of Rajasthan, Jaipur-302055, Rajasthan, India
E-mail: dr.rnv26may@gmail.com
Tel.: +91-09214524163

Cassia tora L., which is chosen for the present research endeavour, is a medicinally and economically important annual plant. It dies in late winter and exists for several months of the year, only as a seed bank within the soil. The plant is generally propagated by seeds. Seeds lose their viability within one month of ripening and, hence, storage of seeds is major problem for propagation. It shows wide genetic variability in terms of plant size, morphology, fruit size and fruit production. However, the type and amount of chemical constituents also vary from plant to plant. It is critically important to produce plants having same genotypes, suited for the local climate and soil conditions and for the production of desirable chemicals.

Flavonoids are a group of polyphenolic compounds, possessing 15 carbon atoms, in which two benzene rings joined by a linear three carbon chain. It constitutes one of the most characteristic classes of compounds in higher plants. Many flavonoids are easily recognised as flower pigments in most angiosperm families (flowering plants). However, their occurrence is not restricted to flowers but includes all parts of the plant.

Anthraquinones is one of the largest groups of natural quinines, including more than 170 natural compounds (Thomson, 1997; Harborne *et al*., 1999). Among them, half of the natural anthraquinones are found in lower fungi, particularly in *Penicillium* and *Aspergillus* species and in Lichens. Others are found in higher plants and in some instances, show their presence in insects (Evans, 1996). Further, certain families like Rubiaceae, Rhamnaceae, Fabaceae, Polygonaceae, Bignoniaceae, Verbenaceae, Scrophulariaceae and Liliaceae are particularly rich in anthraquinones (Van and Labadie, 1989).

Material and Methods

Plant material

Fresh green leaves were collected from mature plant of *C. tora* L., growing in the campus of University of Rajasthan, Jaipur. Callus was obtained from leaves, cultured on MS medium, supplemented with 2,4-D (1.0 mg/l). Further, 8 week old maintained stock callus cultures, developed from leaf explants, were also used for the extraction experiments. Leaves and calli were dried under shade at room temperature for 7 days and then powdered using grinder. Total 20 gm of powdered samples were taken for, further experimental work.

Extraction procedure

The dried samples were soxhlet extracted in 80% ethanol (100 ml/gdw) on a water bath for 24 h (Subramanian and Nagrajan, 1969). The extracts were concentrated and re-extracted in petroleum ether (40^0-60^0C, Fraction first), ethyl ether (Fraction second) and ethyl acetate (Fraction third) in succession. Each step was repeated thrice to ensure complete extraction in each case. Fraction first and second were rejected and fraction third was analyzed for flavonoids. Fraction third was hydrolyzed by refluxing with 7% sulphuric acid for two hours. The mixture was filtered and the filterate was extracted with ethyl acetate in separating funnel. The ethyl acetate layer (upper layer) was washed with distilled water to neutralize and dried in vacuum for, further, analysis of flavonoids.

For the extraction of the emodin, each of the dried and powdered test samples were soxhlet extracted in chloroform (50mg/l) for 30 minutes. The chloroform extracts were filtered and the filterate evaporated to dryness, which were then taken up with 10 ml of 5% NaOH in 2% NH_3 solution (v/v) and analyzed chromatographically.

Thin layer chromatography

Thin glass plates (20 ´ 10 cm) coated (0.2-0.3 mm thick) with silica gel 'G' (30 gm/60 ml) were dried at room temperature. The dried plates were activated at 100^0 C for 30 minutes in an oven and cooled at room temperature. Ethyl acetate fraction was separately applied 1 cm above the edge of the plates along with the standard reference compound (Figure 1A).

The glass plates were developed in an air tight chromatography chamber, containing about 200 ml of solvent mixture of n-butanol, acetic acid and water (4:1:5) for quercetin. Some other solvent mixtures such as 1-Propanol: Ethyl acetate: water in the ration of 4:4:3 were tried for TLC of anthraquinone. The developed plates were air dried and visualized under UV light, ammonia vapours and I_2 vapours to observe the colour of the spots (Figure 1B). Further, the developed plates were sprayed with 5% ethanolic ferric chloride solution, to observe the colour of the spots. R_f values were calculated for isolated samples and compared with coinciding standards. The fluorescent spot coinciding with standard reference compound of quercetin was marked. The marked spots were scrapped and collected along with the silica gel 'G' and eluted with ethanol. Elute was crystallized with chloroform. The purified crystals were subjected to its IR and NMR spectral analysis.

Quantification of quercetin

Identified quercetin was quantitatively estimated by spectrophotometric methods, developed by Mabry *et al*. (1970) and Kariyone *et al*. (1953), respectively. Stock solutions of quercetin were prepared in methanol (1mg/l), out of which varied concentrations (20-100 µg) were separately spotted on TLC plates and developed as above. Plates were air dried and visualized under UV light and in I_2 vapours. Spots marked on the basis of fluorescence were collected along with the adsorbent in separate test tubes. Later, to each 5 ml of spectroscopic methanol was added and shaken vigorously and centrifuged. The supernatants were collected separately.

The volume of each was raised to 10 ml by methanol to which, 3 ml of 0.1 M $AlCl_3$ solution was added and kept at room temperature for 20 minutes. The OD of each of the samples was taken on spectrophotometer set at 440 nm for quercetin against the blank. A regression curve for each of the authentic compounds was plotted in between the various concentrations of standard quercetin and their respective OD, which followed the Beer's law. Amount of quercetin present in experimental samples, was calculated by using regression curve.

Results

During the present research endeavor, a quinone named anthraquinone (emodin) was isolated from the leaves and callus tissues of *C.tora*. L. When the developed plates were sprayed with 5% ethanolic ferric chloride solution, it showed bluish grey spots, which coincided with that of the reference quercetin (Figure 1A). When plates were placed in a chamber, saturated with ammonia vapours, it also showed deep yellow colour of quercetin (not shown in the form of figure), R_f value (0.82) of quercetin isolated from the sample, coincided with the R_f value of standard quercetin. The plates developed under UV light, showed fluorescent blue spot in the sample, coinciding with the standard sample of quercetin (not shown in the form of figure). Isolated compound was subjected to its IR spectral analysis. Its IR spectra showed following peaks in particular visiation:

1670 cm^{-1}	C=O stretching in ester group
1200cm^{-1}-1400 cm^{-1}	A broad peak at this region is due to -OH group
1070 cm^{-1}	C-O-C stretching
690cm^{-1} and 800cm^{-1}	Streching shows meta position of -OH group
710cm^{-1} and 830cm^{-1}	Streching shows ortho position of -OH group

When the developed plates were sprayed with 5% KOH solution, they showed reddish brown spots, which coincided with that of the reference emodin (Figure 1B). When plates were placed in a chamber, saturated with ammonia vapours, they also showed deep yellow colour of emodin (not shown in the form of figure). R_f value (0.96) of emodin isolated from the samples coincided with the R_f value of the standard emodin, respectively. The plates developed under UV light showed fluorescent spot in the sample coinciding with the standard sample of emodin (yellowish brown, not shown in the form of figure). Its IR spectra showed following peaks in particular visiation:

3055 cm^{-1}	Ar-H Stretching
2910 cm^{-1}	-C-H Stretching in $-CH_3$
1675 cm^{-1}	Strong peak for >C=O group
1640 cm^{-1}	Medium peak for -C=C- group
1300 cm^{-1}	Strong and broad peak due to –OH group
700 cm^{-1} and 810 cm^{-1}	Stretching shows meta position of –OH group

These spectral data are comparable to the literature data for quercetin (Yamamoto *et al*., 1986) and emodin (Yamaguchi, 1970). Its identification was confirmed by co-TLC and matched m.p. with an authentic sample.

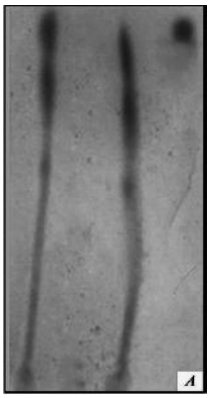

Figure 1A: Developed TLC plate of quercetin

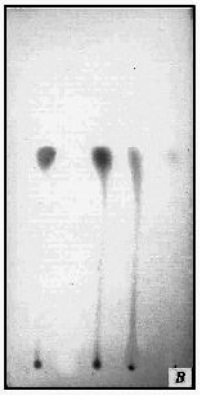

Figure 1B: Developed TLC plate of emodin

Discussion

Primary metabolites are the compounds, which are present almost everywhere in nature and are essential for all life forms. These compounds include the common carbohydrates, fats, proteins and nucleic acids that are needed to create and maintain the life. Typically they are involved in the energy regulation of organisms and with the growth and development of tissues, in short, they are the building blocks of organisms.

Table 1: Pharmacological and biological activities of quercetin

Activity	References
Mutagenic	Bjeldanes and Chang, 1977
Antioxidant	Murota and Terao, 2003; Lee *et al*., 2003; Sakanashi 2008
Antidepressant	Anjaneyulu *et al*., 2003
DNA binding	Tan *et al*., 2003
Anticancer activity	Richter *et al*., 1999; Hung, 2007; Siegelin *et al*., 2009
Improve blood circulation	Zhao *et al*., 1999; Lan and Zhang, 2006
Erythrocyte deformability	Begum and Terao, 2002
Anti-inflammatory	Guardia *et al*., 2001; Boots, 2008
Antitumor	Wang and Mazza, 2002; Indep *et al*., 2006
HIV Inhibition	Nair *et al*., 2009
Hypolipidemia	Kamada *et al*., 2005; Park *et al*.,2008
Antihypertensive	Yukiko and Eriko, 2006.
Neuroprotection	Ossola, 2008

Table 2: Pharmacological and biological activities of emodin

Activity	References
Immuno-supperesive	Huang *et al*., 1992
Immunostimulant, Antiulcer	Boik, 1995
Mutagenicity and Genotoxicity	Westendorf *et al*., 1990; Van den Gorkom *et al*., 1999
Antioxidant	Yuan and Gao, 1997; Yen *et al*., 2000
Laxative	Rauwald, 1998; Van den Gorkom *et al*., 1999; Mueller *et al*., 1999
Diuretic	Zhou and Chen, 1988
Vaso relaxive	Huang *et al*., 1991
Cardiac stimulant	Dwivedi *et al*., 1988
Antitumor	Su *et al*., 1995; Sun *et al*., 2000
Induces muscle contraction	Cheng and Kang, 1998
Central nervous system depressant	Ram *et al*., 1994
Hypolipidemic	Boik, 1995
Improves liver functions	Yutao *et al*., 2000
Antibacterial	Wang and Chung, 1997
Antifungal	Singh *et al*., 1992
Antiparasitic	Wang, 1993
Antiviral	Barnard *et al*., 1992
Anti-inflammatory and Analgesic	Chang *et al*., 1996

The secondary metabolites are of limited occurrence. Some metabolites do not have a proven function in the organism, while others apparently play a vital role. Some secondary metabolites of plants are found only in certain species of families, showing the individuality of species. Their production may also vary with conditions, the environment of the organism or the available nutrition. They may have a role in providing defence against pest and pathogens, providing protection against UV radiation and stress, or acting as attractive volatile odour compounds or pigments (Miyagawa, 2009). Some of them are pharmacologically active and useful as medicines *i.e*., salicin isolated from salix (Wagner *et al*., 2008); taxol isolated from taxus (Wu *et al*., 1999); morphine isolated from opium (Minemasa *et al*., 2004), respectively.

During the present research endeavor, quercetin was isolated and quantified from leaf and callus tissues of *C. tora* L., respectively. Present studies showed that in leaves per mg dry weight around 0.74 µg of quercetin was present, while in callus, 0.69 µg quercetin per mg dry weight of tissues was observed.

In consonance to this, Meena and Patani (2008) have quantified quercetin from *in vivo* (leaves, stem, fruit and root) and *in vitro* (callus developed from nodal segments) plant parts of *Citrullus colocynthis* (Linn.) Schrad. and evaluated that amount of quercetin was lower in callus tissues developed from nodal segments. Besides this, quercetin has also been quantified from *in vivo* and *in vitro* plant parts of different sps. *i.e*., *Scutellaria baicalensis* (Yamamoto *et al*., 1986), *Acacia catechu* (L.f.) Willd (Jain *et al*., 2007) and *Ricinus communis* L. (Khafagi, 2007) *etc*.

In contrast to this, Arya *et al*. (2008) have studied the quercetin content in callus tissue of *Pluchea lanceolata* Oliver & Hienr. They revealed that maximum amount of quercetin was present in 6 week old callus tissues (0.23 mg/gdw), followed by 8 week callus tissue (0.20 mg/gdw), while 4 and 2 week callus tissues showed 0.17 mg/gdw and 0.12 mg/gdw quercetin, respectively.

Quercetin, has been reported to exhibit a wide range of biological activities. Various pharmacological activities of quercetin have been demonstrated in the last couple of decades, including antioxidation by scavenging free radicals, prevention of atherosclerosis, antitumor activities *etc*., as shown in Table 1.

Besides, the extraction of 'quercetin', another secondary metabolite, anthraquinone 'emodine' was isolated from the leaves and callus tissues of *C. tora* L. Present studies showed that high amount of emodin was present in callus tissues derived from leaves. In consonance to this, Tabata *et al*. (1975) have studied anthraquinone (emodin, physcion and chrysophenol) content in seedling and seedling derived callus of *C. tora*. L. Their studies showed that higher amount of anthraquinone was present in callus cultures, which showed that hormonal concentration of culture medium, influenced the concentration of anthraquinone. Besides this, some researchers have seen the effect of nutritional factors, on the formation of anthraquinones in callus cultures. The addition of increasing concentrations of vitamins promoted callus growth, but vitamin free medium suppressed anthraquinone formation (Sepehr and Ghorbanli, 2002). Work similar to our studies has also been reported in some other plants *viz*., *Rhamnus alaternus* L. (Charles *et al*., 1982), *Aloe vera* L. (Acurero, 2008), *Cassia podocarpa* (Rai, 1988) *etc*.

In contrast to this, Lin *et al*. (2003) have studied the quantification of emodin in *in vivo* and *in vitro* grown plants of *Polygonum multiflorum* THUNB and revealed that significantly higher amounts of emodin was observed in the *in vitro* propagated shoots than the *in vivo* grown plants. Further, Nazif *et al*. (2000), studied that anthraquinone production and quantification in suspension cultures of *C. acutifolia* and evaluated that anthraquinone production could be enhanced by salt stress.

Emodin forms the basis of a purgative anthraquinone derivatives and from ancient times has also been widely used as a laxative compound (Evans, 1996; Bruneton, 1999). It is believed that the presence of hydroxyl groups in position 1 and 8 of the aromatic ring system is essential for the purgative action of this compound (Paneitz and Westendorf, 1999). Recent studies have shown in Table 2 that emodin also exhibits numerous other biological activities which affect the immune system, vasomotor system and the metabolic processes.

Conclusion

The medicinal plant *Cassia tora* L. appears to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. The antimicrobial, antiviral, anti-inflammatory, antispasmodic, antianalgesic and antidiuretic *etc*., can be attributed to their high flavonoids, anthraquinones, steroids, tannins, terpenoids and saponins. Exploitation of these pharmacological properties involves, further investigation of these active ingredients by implementation techniques of extraction, purification, separation, crystallization and identification.

Acknowledgement

Authors are thankful to CSIR for providing financial assistance in the form of major emeritus project [No. 21(0822)/ 10/EMR-II] sanctioned to Professor Amla Batra, Department of Botany, University of Rajasthan, Jaipur.

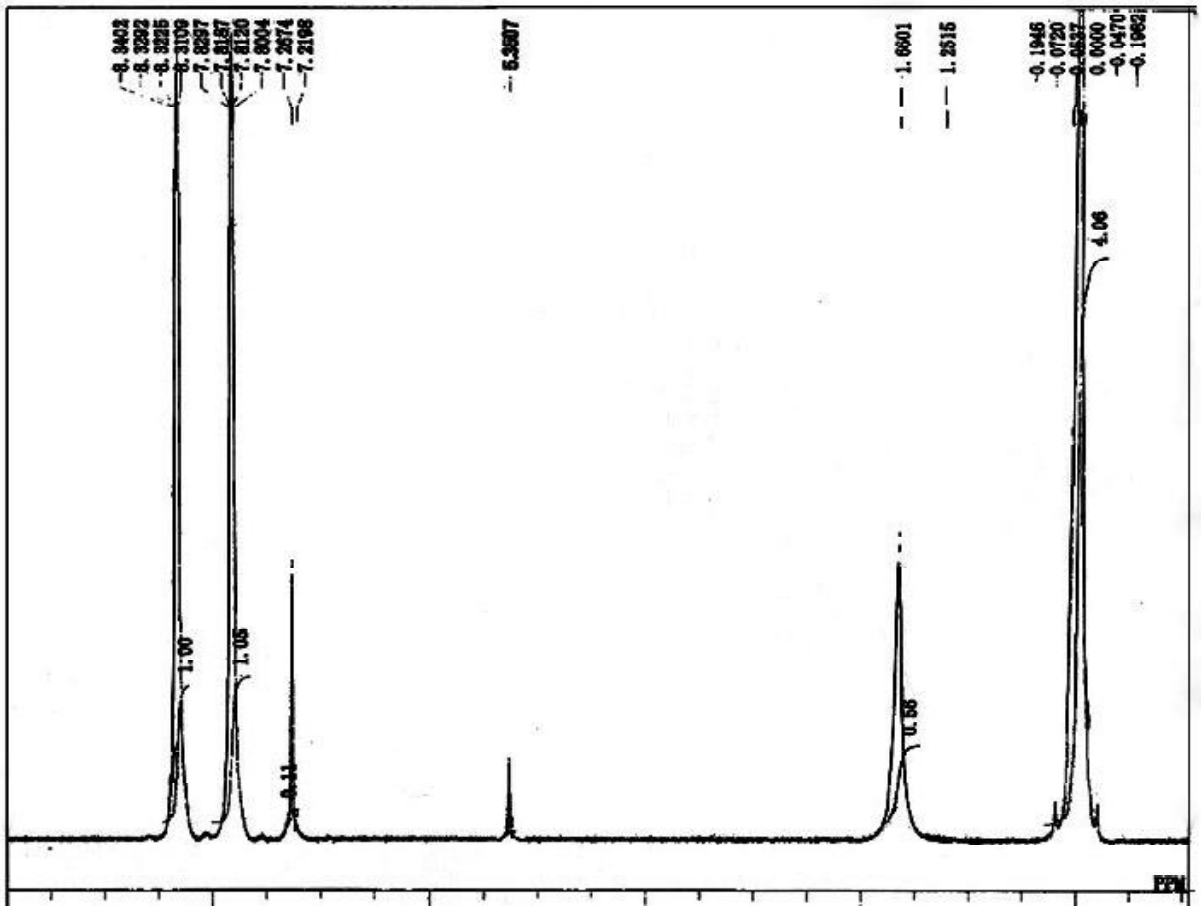

Figure: NMR spectra of isolated emodin

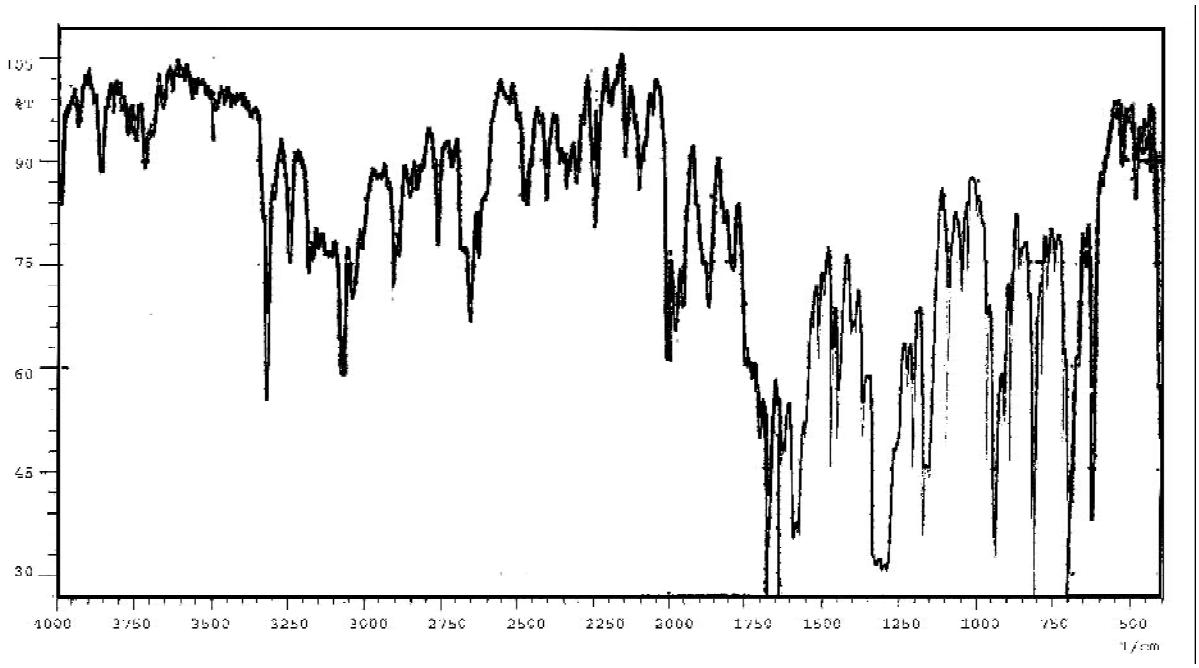

Figure: IR spectra of isolated emodin

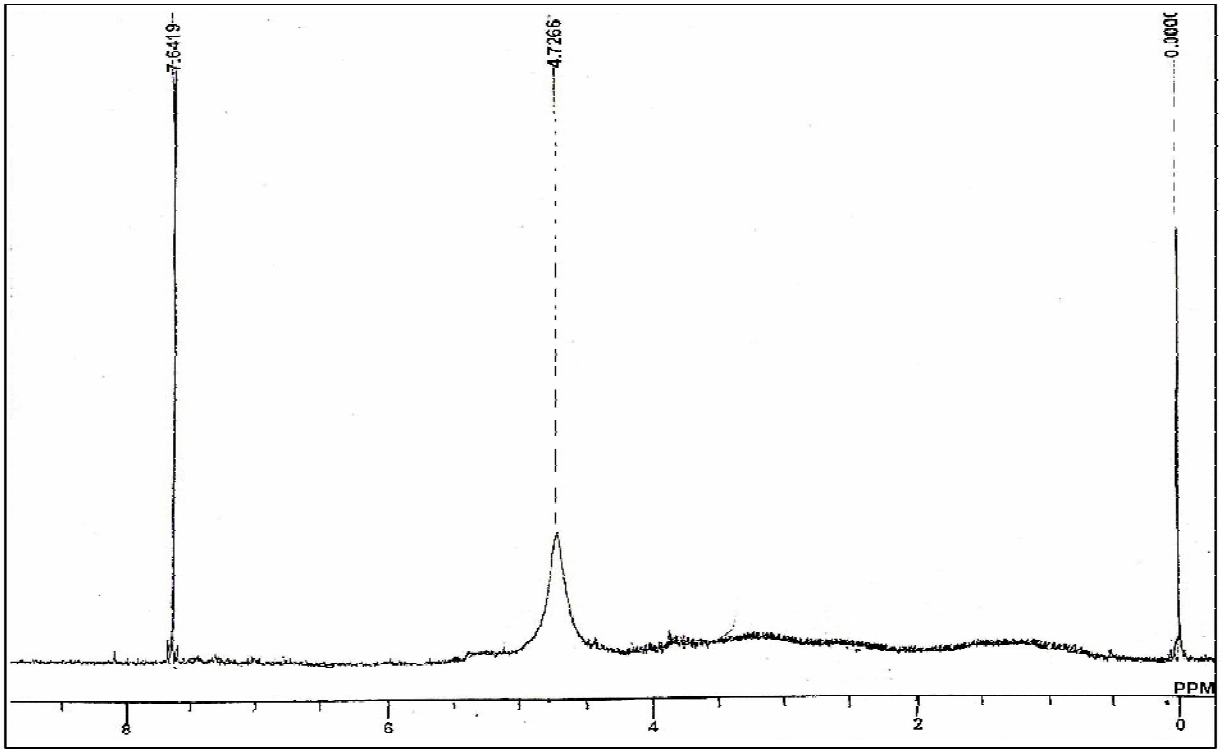

Figure: NMR spectra of isolated quereetin

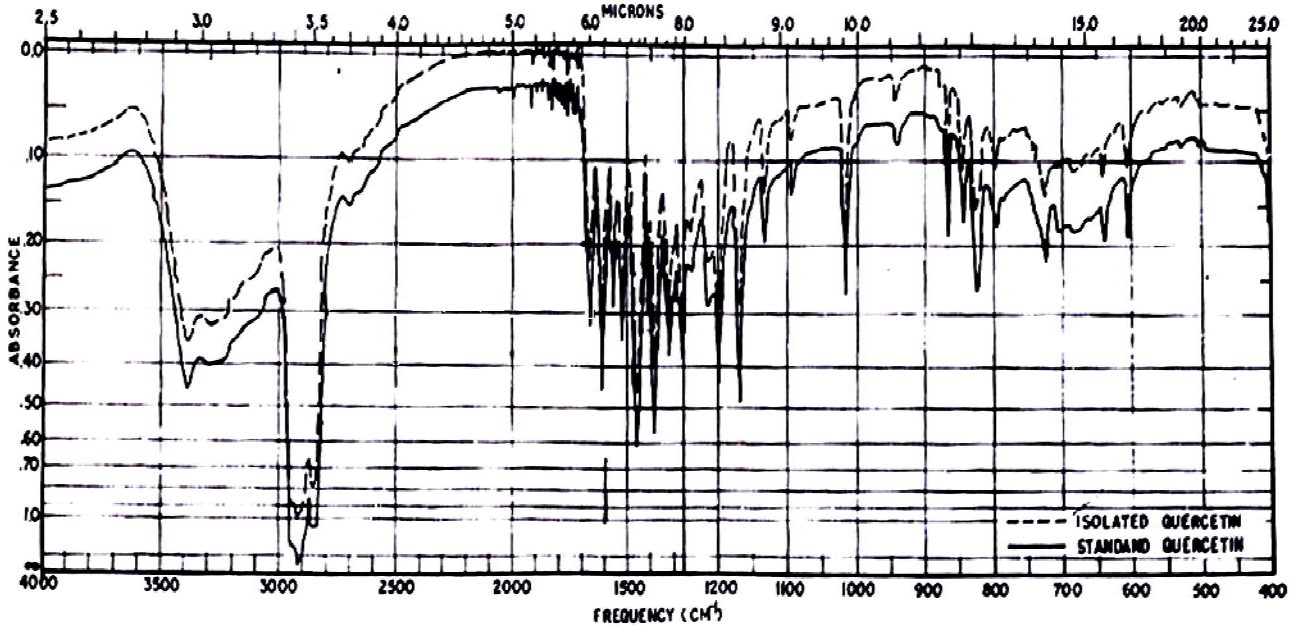

Figure: Superimposed IR spectra of isolated quereetin and standard quereetin

Conflict of interest

We declare that we have no conflict of interest.

Reference

Anjaneyulu, M., Chopra, K. and Kaur, I. (2003). Antidepressant activity of quercetin, a bioflavonoid, in streptozotocin-induced diabetic mice. *J. Medic. Food,* **6**(4):391-395.

Arya, D.; Patni, V. and Kant, U. (2008). *In vitro* propagation and quercetin quantification in callus cultures of Rasna (*Pluchea lanceolata* Oliver & Hienr). *Ind. J. Biotech*., **7**:383-387.

Barnard, D.L.; Huffman, J.H.; Morris, J.L.; Wood, S.G.; Hughes, B.G. and Sidwell, R.W. (1992). Evaluation of the antiviral activity of anthraquinones, anthrones and anthraquinone derivatives against human cytomegalovirus. *Antiviral Res.,* **17**:63-77.

Begum, A.N and Terao, J. (2002). Protective effect of quercetin against cigarette tar extract-induced impairment of erythrocyte deformability. *J. Nut. Biochem.,* **13**:265-272.

Bjeldanes, L.F. and Chang, G.W. (1977). Mutagenic activity of quercetin and related compounds. *Science,* **197**(4303):577-578.

Boik, J. (1995). Cancer and natural medicine. Princeton, MN, USA: Oregon Medical Press.

Boots, A.W. (2008). *In vitro* and *ex vivo* anti-inflammatory activity of quercetin in healthy volunteers. Nutrition, **24**:703-710.

Bruneton, J. (1999). Pharmacognosy, phytochemistry, medicinal plants, 2nd edn. Paris, France: Intercept Ltd. and Lavoisier Publishing.

Chang, C.H.; Lin, C.C.; Yang, J.J.; Namba, T. and Hattori, H. (1996). Antiinflammatory effects of emodin from *Ventiogo leiocarpa*. *Amer. J. Chin. Med.,* **24**:139-142.

Charles, I; Chaar, A. *and* Kabbara, R.A. (1982). A chromatographic study of the anthraquinones of *Rhamnus alaternus* L. II. The anthraquinones produced in callus culture. *Pharma. Bio.,* **20**(1):9-11.

Chaudhuri, K.; Das, S.; Bandyopadhyay, M.; Zalar, A.; Kollmann, A.; Jha, S. and Tepfer, D. (2009). Transgenic mimicry of pathogen attack stimulates growth and secondary metabolite accumulation. *Transgen. Res.,* **18**(1): 121-134.

Cheng, Y.W. and Kang, J.J. (1998). Emodin-induced muscle contraction of mouse diaphragm and the involvement of Ca2+ influx and Ca2+ release from sarcoplasmic reticulum. *Brit. J. Pharmacol.,* **123**:815-820.

Dwivedi, S.P.D.; Pandey, V.B.; Shah, A.H. and Rao, Y.B. (1988). Chemical constituents of *Rhamnus procumbens* and pharmacological actions of emodin. *Phytother. Res.,* **2**:51-53.

Evans, W.C. (1996). Treas and evans pharmacognosy, 4th edn. London, UK: W.B. Saunders Company.

Guardia, T.; Rotelli, A.E.; Juarez, A.O. and Pelzer, L.E. (2001). Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. *Farmaco.,* **56**(9):683-687.

Harborne, J.B.; Baxter, H. and Moss, G.P. (1999). Phytochemical dictionary. A handbook of bioactive compounds from plants, 2nd edn. London, UK: Taylor & Francis.

Hartmann, T.; Kutchan, T.M. and Strack, D. (2005). Evolution of metabolic diversity. *Phytochem*., **66**:1198-1199.

Huang, H.C.; Chang, J.H.; Tung, S.F.; Wu, R.T.; Foegh, M.L. and Chu, S.H. 1992. Immunosuppression effect of emodin, a free radical generator. *Europ. J. Pharmacol.,* **211**:359-364.

Huang, H.C.; Chu, S.H. and Chao, L.P.D. (1991). Vasorelaxants from Chinese herbs, emodin and scoparone, possess immunosuppressive properties. *Europ. J. Pharmacol.,* **198**:211-213.

Hung, H. (2007). Dietary quercetin inhibits proliferation of lung carcinoma cells. *Tai, E.S. and Gillies, P.J. (Eds): Nutrigenomics opportunities in Asia. Forum. Nutr. Basel. Karger.,* **60**:146-157.

Indep, M.A.; Bhosle, S.C.; Shinde, A.D.; Barkume, M.S. and Ingle, A.D. (2006). Tumour response to quercetin, a bioflavonoid with some promises in therapies. *Ind. J. pharma. Res*., **68**(5):570-574.

Jain, R.; Patni, V. and Arora, D.K. (2007). Isolation and identification of flavonoid "quercetin" from *Acacia catechu* (L.f.) Willd-A Katha yielding plant. *J. Phytol. Res.,* **20**:43-45.

Jesse, W.; Li, H. and Vederas, J.C. (2009). Drug discovery and natural products: End of an era or an endless frontier? *Science,* **325**(5937): 161-165.

Kamada, C.; Da Silva, E.; Kameyama, M.O.; Moon, J.H. and Terao, J. (2005). Attenuation of lipid peroxidation and hyperlipidemia by quercetin glucoside in the aorta of high cholesterol-fed rabbit. Free Radical Res.

Kariyone, T.; Hashimoto, Y. and Kimura, M. (1953). Microbial studies on plant components IX, Distribution of flavonoids in plants by paper chromatography. *J. Pharm. Soc*., **73**:253-256.

Khafagi, I.K. (2007). Variation of callus induction and active metabolite accumulation in callus cultures of two varieties of *Ricinus communis* L. *Biotech*., **6**(2):193-201.

Lan, W.J. and Zheng, X.X. (2006). Activity of *Ginkgo biloba* extract and quercetin on thrombomodulin expression and tissue-type plasminogen activator secretion by human umbilical vein endothelial cells. *Biomed. Environ. Sci.,* **19**:249-253.

Lee, J.C.; Kim, J.; Park, J.K.; Chung, G.H. and Jang, Y.S. (2003). The antioxidant, rather than prooxidant, activities of quercetin on normal cells: quercetin protects mouse thymocytes from glucose oxidase-mediated apoptosis. *Exp. Cell Res.,* **291**(2):386-397.

Lin, L.C.; Nalawade, S.M.; Mulabagal, V.; Yeh, M.S. and Tsay, H.S. (2003). Micropropagation of *Polygonum multiflorum* THUNB and quantitative analysis of the anthraquinones emodin and physcion formed in *in vitro* propagated shoots and plants. *Biol. Pharm. Bull.,* **26**(10):1467-1471.

Mabry, T.J.; Markham, K.R. and Thomas, M.B. (1970). The systematic identification of flavonoids. Springer-Verlag Publication, New York. pp.261-266.

Meena, M.C. and Patni, V. (2008). Isolation and identification of flavonoid "quercetin" from *Citrullus colocynthis* (Linn.) Schrad. *Asian J. Exp. Sci.,* **22**(1):137-142.

Minemasa, H.; Toshiyuki, M.; Shin, T. and Hajime, O. (2004). Rapid and sensitive determination of morphine in street opium samples by thermal desorption gas chromatography using a microfurnace pyrolyzer. *J. Separ. Sci*., **27**(12):1030-1032.

Miyagawa, M. (2009). Studies on nitrogen-containing secondary metabolites playing a defensive role in plants. *J. Pesticide Sci.,* **34**(2): 110-112.

Mueller, S.O.; Schmitt, M.; Dekant, W.; Stopper, H.; Schlatter, J.; Schreier, P. and Lutz, W.K. (1999). Occurrence of emodin, chrysophanol and physcion in vegetables, herbs and liquors. Genotoxicity and anti-genotoxicity of the anthraquinones and of the whole plants. *Food Chem. Toxicol.,* **37**(5):481-491.

Murota, K. and Terao, J. (2003). Antioxidative flavonoid quercetin: implications of its intenstinal absorption and metabolism. *Arch. Biochem. Biophy.,* **417**:12-17.

Nair, M.P.N.; Saiyed, Z.M.; Gandhi, N.H. and Ramchand, C.N. (2009). The flavonoid, quercetin, Inhibits HIV-1 Infection in normal peripheral blood mononuclear cells. *Amer. J. Infect. Diseas.,* **5**(2):142-148.

Nazif, N.M.; Rady, M.R. and Seif El-Nasr, M.M. (2000). Stimulation of anthraquinone production in suspension cultures of *Cassia acutifolia* by salt stress. *Fitoterapia,* **71**(1):34-40.

Ossola, B. (2008). Paradoxical co-existence of protective and toxic effects of quercetin in the same *in vitro* neurodegeneration model. *Europ. J. Pharmaceut. Sci.,* **34**(1):33.

Paneitz, A. and Westendorf, J. (1999). Anthranoid contents of rhubarb (*Rheum undulatum* L.) and other Rheum species and their toxicological relevance. *Europ. Food Res. Technol*., **210**:97-101.

Park, J.; Ahan, J.; Kim, S. and Ha, T.Y. (2008). Quercetin suppresses the hyperlipidemia induced by an atherogenic diet in mice. FASEB J. **22**:707

Pichersky, E. and Gang, D.R. (2000). Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. *Trends Plant Sci.,* **5**(10):439-445.

Rai, M.; Deshmukh, P.; Gade, A.; Ingle, A.; Kovics, G.J. And Irinyi, L. (2009). *Phoma saccardo*: Distribution, secondary metabolite production and biotechnological applications. *Crit. Rev. Microbio.,* **35**(3):182-96.

Rai, P.P. (1988). Anthraquinone formation in callus cultures of *Cassia podocarpa*. *J. Nat. Prod.,* **51**(3):492-495.

Ram, S.N.; Pandey, V.B.; Dwivedi, S.P.D. and Goel, R.K. (1994). Further constituents of *Rhamnus triquetra* and CNS activity of emodin. *Fitoterapia,* **LXV**: 275-278.

Rao, R.S. and Ravishankar, G.A. (2002). Plant cell cultures: Chemical factories of secondary metabolites. *Biotech. Adv*., **20**(2):101-153.

Rauwald, H.W. (1998). Herbal laxatives: influence of anthrones anthraquinones on energy metabolism and ion transport in a model system. In: Lawson, L. D. and Bauer, R. (Eds). Phytomedicines of Europe, chemistry and biological activity. Washington, DC, USA: American chemical society., pp.97-116.

Richter, M.; Ebermann, R. and Marian, B. (1999). Quercetin induced apoptosis in colorectal tumor cells: possible role of EGF receptor signaling. *Nutr. Cancer,* **34**:88-99.

Sakanashi, Y (2008) Possible use of quercetin, an antioxidant, for protection of cells suffering from overload of intracellular Ca^{2+}: a model experiment. Life Sciences, **83**:164-169.

Sepehr, M.F. and Ghorbanli, M. (2002). Effects of nutritional factors on the formation of anthraquinones in callus cultures of *Rheum ribes*. *Plant Cell. Tiss. Org. Cult.,* **68**:171-175.

Siegelin, M.D.; Reuss, D.E.; Habel, A.; Rami, A. and Deimling, A.V. (2009). Quercetin promotes degradation of survivin and thereby enhances death-receptor-mediated apoptosis in glioma cells. *Neuro. Oncol.* **11**(2):122-131.

Singh, U.P.; Singh, K.P.; Singh, S.P. and Ram, V.B. (1992). Effect of emodin isolated from *Rhamnus triquetra* on spore germination of some fungi. *Fitopatol. Brasil.,* **17**:420-422.

Sirikantaramas, S.; Yamazaki, M. and Saito, K. (2008). Mechanisms of resistance to self-produced toxic secondary metabolites in plants. *Phytochem. Rev.* **7**:467-477.

Srivastava, S. and Srivastava, A.K. (2007). Hairy root culture for mass-production of high-value secondary metabolites. *Crit. Rev. Biotech.,* **27**(1):29-43.

Su, H.Y.; Cheng, S.H.; Chen, C.C. and Lee, H. (1995). Emodin inhibits the mutagenicity and DNA adducts induced by 1-nitropyrene. *Mut. Res.,* **329**:205-212.

Subramanian, S.S. and Nagarajan, S. (1969). Flavonoids of the seeds of *Crotolaria retusa* and *C. striata*. *Curr. Sci*., **38**:65.

Sun, M.; Sakakibara, H.; Ashida, H.; Danno, G. and Kanazawa, K. (2000). Cytochrom P4501A1-inhibitory action of antimutagenic anthraquinones in medicinal plants and the structure-activity relationship. *Biosci. Biotech. Biochem.,* **64**:1373-1378.

Tabata, M.; Hiraoka, N.; Ikenoue, M.; Sano, Y. and Konoshima, M. (1975). The production of anthraquinones in callus cultures of *Cassia tora*. Lloydia.

Tan, J.; Zhu, L. and Wang, B. (2003). DNA binding and cleavage activity of quercetin nickel (ii) complex. *Dalton Trans.,* **24**:4722-4728.

Thomson, R.H. (1997). Naturally occurring quinines. IV. Recent advances. London, UK: Blackie Academic & Professional, Chapman & Hall.

Van den Gorkom, B.A.P.; De Vries E.G.E.; Karrenbeld, A. and Kleibeuker, J.H. (1999). Review article: anthranoid laxatives and their potential carcinogenic effects. *Ailment. Pharmacol. Therap.,* **13**: 443-452.

Van, D.B.A.J.J. and Labadie, R.P. (1989). Quinones. In: Dey, P.M. and Harborne, J. B. (Eds). Methods in plant biochemistry. Vol. 1 plant phenolics. London, UK: Academic press., pp.451-491.

Wagner, S.; Urena, A.; Reich, E. And Merfort, I. (2008). Validated HPTLC methods for the determination of salicin in *Salix sp*. and of harpagoside in *Harpogophytum procumbens*. *J. Pharma. Biomed. Anal.,* **48**(3):587-591.

Wang, H.H. (1993). Antitrichomonal action of emodin in mice. *J. Ethnopharmacol,* **40**:111-116.

Wang, J. and Mazza, G. (2002). Effects of anthocyanins and other phenolic compounds on the production of tumour necrosis factor alpha in LPS/IFN gamma-activated RAW 264.7 macrophages. *J. Agric. Food Chem.,* **50**:4183-4189.

Wang, W.H. and Chung, J.G. (1997). Emodin-induced inhibition of growth and DNA damage in the *Helicobacter pylori*. *Curr. Microbio.,* **35**: 262-266.

Westendorf, J.; Marqdt, H.; Poginsky, B.; Domnik, M.; Schmidt, J. And Marqdt H. (1990). Genotoxicity of naturally occurring hydroxyanthraquinones. *Mut. Res*. **240**:1-12.

Wu, Z.L.; Yuan, Y.J.; Liu, J.X.; Xuan, H.Y. and Hu, Z.D. (1999). Study on enhanced production of taxol from *Taxus chinensis* var. mairei in biphasic-liquid culture. *Acta Bot. Sin.* **42**(10):1108-1113.

Yamaguchi, K. (1970). Spectral data of natural products. Elsevier Pub. Co. Amsterdam and New York.

Yamamoto, H.; Chatani, C.; Kitayama, A. and Tomimori, T. (1986). Flavonoid production in *Scutellaria baicalensis* callus cultures. *Plant Cell. Tiss. Org. Cult.,* **5**(3):219-222.

Yen, G.C.; Duh, P.D. and Chuang, D.Y. (2000). Antioxidant activity of anthraquinones and anthrone. *Food Chem.,* **70**:437-441.

Yuan, Z. and Gao, R. (1997). Antioxidant actions of anthraquinonlines contained in *Rheum*. *Pharma. Pharmacol. Lett.,* **7**:9-12.

Yukiko, Y. and Eriko, O. (2006). Antihypertensive effect of quercetin in rats fed with a high-fat high-sucrose diet. *Biosci. Biotech. Biochem.,* **70**(4):933-939.

Yutao, Z.; Dingguo, L.; Hongshan, W.; Zhirong, W.; Xin, H.; Qinfang, X. and Hanming, L. (2000). Emodin on hepatic fibrosis in rats. *Chin. Med. J. Eng. Ed.,* **113**:599-601.

Zhao, X.; Gu, Z. and Attele, A. S. (1999). Effects of quercetin on the release of endothelin, prostacyclin and tissue plasminogen activator from human endothelial cells in culture. *J. Ethnopharmacol.,* **67**:279-285.

Zhou X.M. and Chen Q.H. (1988). Biochemical study of Chinese rhubarb XXII. Inhibitory effect of anthraquinone derivatives on sodium-potassium-ATPase of a rabbit renal medulla and their diuretic action. *Acta Pharm. Sin.,* **23**:17-20.