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QUANTITATIVE DETERMINATION OF SWERTIAMARIN, MANGIFERIN AND AMAROGENTIN IN CALLUS CULTURE OF *SWERTIA CHIRATA* BY HPLC ANALYSIS

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ABSTRACT

The paper deals with the quantitative determination of swertiamarin, mangiferin and amarogentin contents in Swertia chirata by HPLC analysis. The extracted plant material (20mg) was transferred in to 15ml borosil test tubes. 20ml of methanol was added and the tubes were capped securely. The content was heated in a water bath at 80°c in order to extract metabolites of interest. The extract was filtered and the residue was re-extracted twice in 20ml of methanol. The final volume was made up to 10ml by evaporation. The methanol extracts were combined and filtered through 0.22µ filter membrane. The solvents, namely water and acetonitrile used in the study were filtered and degassed. This was essential since storage of the solvent cause gas bubbles to accumulate. The results showed that the callus culture contained 0.67% swertiamarin, 0.09% mangiferin and 0.05% amarogentin and in contrast, tissue cultured plants contained 0.71% swertiamarin, 1.04% mangiferin and 0.08% amarogentin. On the other hand 2.91% swertiamarin, 0.07% mangiferin and 0.08% amarogentin was found in field grown plants of *Swertia chirata.*

KEYWORD: *Swertia chirata***,** swertiamarin, HPLC, mangiferin, amarogentin.

INTRODUCTION

India has a rich culture of medicinal herbs and spices with more than 2000 species and has a broad geographical area catering to Ayurvedic, Unani, Siddha and other conventional medicines. So far only few plants have been systematically studied chemically and pharmacologically for their potential medicinal value.[1,2] Man has used plants for the

treatment of diverse ailments for thousands of years.[3,4] According to the World Health Organization, most communities still depend upon the traditional medicines for their psychological and physical health requirements,[5] as they have no access to the products of Western pharmaceutical industries,[6] and lack healthcare facilities.[7] Rural areas of many developing countries still rely on traditional medicine for their primary health care needs.

Herbal medicines are in significant demand in both developed and developing countries as a source of primary health care owing to their properties having wide biological and medicinal activities and lesser costs. Herbal molecules are safe to use and would overcome the resistance produced by pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell.[8,9] Similarly with the occurrence of modern or allopathic medicine, a number of important modern drugs have been derived from plants used by indigenous people.[10] Traditional use of herbal medicine is recognized as a way to learn more about potential future medicines.

Researchers have identified number of compounds used in main stream medicine which are derived from "ethno medical" plant sources.[11] Plants are used medicinally in different countries and are a source of many vigorous and strong drugs.[12,13]

Swertia chirata is chemically characterized by the existence of taxonomically important groups of secondary metabolites like xanthones, iridoids, mangiferin and C-glucoflavones.[14] Between the different seco-iridoids present in *Swertia chirata*, swertiamarin is the one of the major and have well-established, important therapeutic applications. For example[15] reported the CNS depressant effects of swertiamarin isolated from *Swertia chirata*. Similarly, methanol extract of *Swertia japonica* was found to be useful anti-cholinergic[16] and this anti-cholinergic activity was recognized to swertiamarin, which was originate in amounts of about 30% on fractionation and purification of extract.

The present study was undertaken to analyze the Swertiamarin, mangiferin and amarogentin content in *in vitro* growing callus culture derived from leaf explants. Different reports on quantitative analysis of Swerchirin, swertiamarin and other secondary metabolites are presented for different genera or species of family Gentianaceae.[17,18] described the production of gentiopicroside and swertiamarin, the two pharmacologically significant seco-iridoids, from *in vitro* cultures of *Gentiana davidii* var. *formosana* by using HPLC. Also,[19] engaged capillary electrophoresis to break up and establish the quantity of swertiamarin in

Swertia herb.[20,21] conducted a relative biochemical analysis of *Swertia* species by Thin layer chromatography and High performance liquid chromatography, using isolated xanthones and seco-iridoids like amarogentin and amaroswarin. However, similar analysis of swertiamarin content in *Swertia chirata* and its related species is lacking in the literature. The present study, therefore attempted the HPLC profiling of *Swertia chirata* extracts for swertiamarin, mangiferin and amarogentin content.

MATERIALS AND METHODS

Source of plant material

The authentic plants of *Swertia chirata* were procured from Jaypee University of Information Technology, Waknaghat, Solan. The plants were maintained in the herbal garden of Shoolini University for further studies.

Cleaning of glassware and instruments

All the glassware were cleaned by washing with detergent (Tween-20) with the help of brush and rinsed thoroughly in tap water and rinsed with distilled water. The glassware was kept for drying in hot air oven at 80-100ºC for 1 hour.

All dissecting instruments (forceps, scalpel, blade) and dissection and washing glassware (petriplates, beakers) were either wrapped with paper or aluminum foil either singly or were put in closed aluminum cans for sterilization by autoclaving. Dissection and transfer of explants were carried out in these petriplates under aseptic conditions. Micro tips used for aseptic condition by micropipettes were wrapped with paper and autoclaved. Sterilization of glassware as well as instruments were carried out by autoclaving at 121°C for 1 hour at 15 psi pressure. The used glassware with contaminated cultures were first autoclaved to kill contaminating microorganisms and molten media was disposed off and later on culture vessels were cleaned as mentioned above for unused glassware.

Aseptic manipulation and culture conditions

All aseptic operations were carried out in laminar air flow chamber fixed with ultra violet (UV) light. The laminar air flow chamber was thoroughly wiped with rectified spirit (90% ethanol) and then the culture vessel containing medium or autoclaved petri-plates, autoclaved culture equipment, spirit and spirit lamp were kept inside the chamber. UV light was switched on for 15-20 minutes. The flow was allowed to run at least 10 minutes after putting off the UV light. Before starting the aseptic manipulations, hands were thoroughly washed

with soap and water. Hands were wiped with 90% ethanol which was allowed to evaporate. All surgical tools like scalpel, forceps and blades were autoclaved before use and flame sterilized at the time of use.

The rims of tubes and flasks were quickly flame sterilized before and after inoculations. Subsequently, in almost all experiments, the culture were inoculated in culture room maintained at a temperature of 25±2°C with 16h photoperiod at 32 µE m^{-2} s^{-1} light intensity provided by cool florescent tubes.

Production of Swertiamarin, mangiferin and amarogentin in indigenous bioreactor

The accretion status of metabolites was determined in plants by subjecting samples from different experiments to chemical analysis.

Sample preparation

The presence of metabolites was determined in plants by subjecting samples from different experiments to chemical analysis. The quantification of swertiamarin, mangiferin and amarogentin was carried out by reverse phase High Performance Liquid Chromatography (HPLC Waters Spheri-sorb 5µm ODS2 515). The plant material was ground in liquid nitrogen, suspended in 80% methanol and sonicated for 10-15 min. The samples were centrifuged at 10,000 rpm for 15 minutes and supernatant was filtered by using 0.22µ syringe filter. A novel HPLC method was developed optimized for immediate quantification of major three metabolites such as swertiamarin, mangiferin and amarogentin. The mobile phase was composed of a mixture of acetonitrile: water (70:30). The linear gradient at a flow rate of 1.0 ml/min was started. The compounds were identified on the basis of their retention time and comparison of UV spectra with the standards procured from ChromaDex (Bangalore, India). The quantification was repeated twice for each sample and the data were interpreted in terms of amount of swertiamarin, mangiferin and amarogentin present.

HPLC analysis (Chromatographic system)

Waters HPLC system consisting of M-600 E quaternary gradient pump, 717 auto-sampler, Novapak Reverse phase C-18 (3.9 X 150 mm) column.

Assay

The standard solutions were injected into the HPLC system via auto-sampler. The different plant samples were run in isocratic mode. The peak area was used to calculate the amount of

metabolites of interest present in different samples. The calibration curve was developed through Empower software which also calculated the amount of Swertiamarin, mangiferin and amarogentin content in the prepared samples.

RESULTS

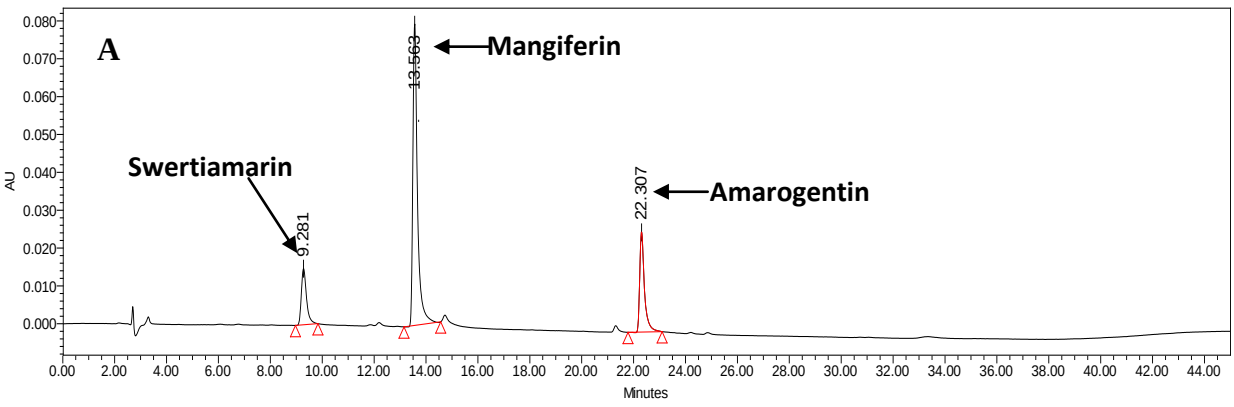
Production of swertiamarin, mangiferin and amarogentin in callus culture, tissue cultured plants and field grown plants of *Swertia chirata.*

The swertiamarin is present in all the plant part, but comparatively maximum amount is present in the roots. The amount of swertiamarin, mangiferin and amarogentin produced in the callus culture was compared with tissue cultured plants and field grown plants, by HPLC analysis (Table 1).

Table 1: HPLC analysis for swertiamarin and its related metabolites production in callus culture, tissue cultured and field grown plants of *Swertia chirata*.

	Area			Percentagec (%)		
Swertia chirata	**Swertiamarin**	**Mangiferin**	**Amarogentin**	**Swertiamarin**	**Mangiferin**	**Amarogentin**
callus	279235	177495	36304	0.67	0.09	0.05
Tissue Culture	293249	1844517	59727	0.71	1.04	0.08
Field grown	1119530	119203	55196	2.91	0.07	0.08

In this study, it was found that the callus culture contained 0.67% swertiamarin, 0.09% mangiferin and 0.05% amarogentin and in contrast, tissue cultured plants contained 0.71% swertiamarin, 1.04% mangiferin and 0.08% amarogentin. On the other hand 2.91% swertiamarin, 0.07% mangiferin and 0.08% amarogentin was found in field grown plants of *Swertia chirata* (Figure 1). Since, we could detect all the three major metabolites (swertiamarin, mangiferin and amarogentin) in the callus culture of *swertia chirata*.



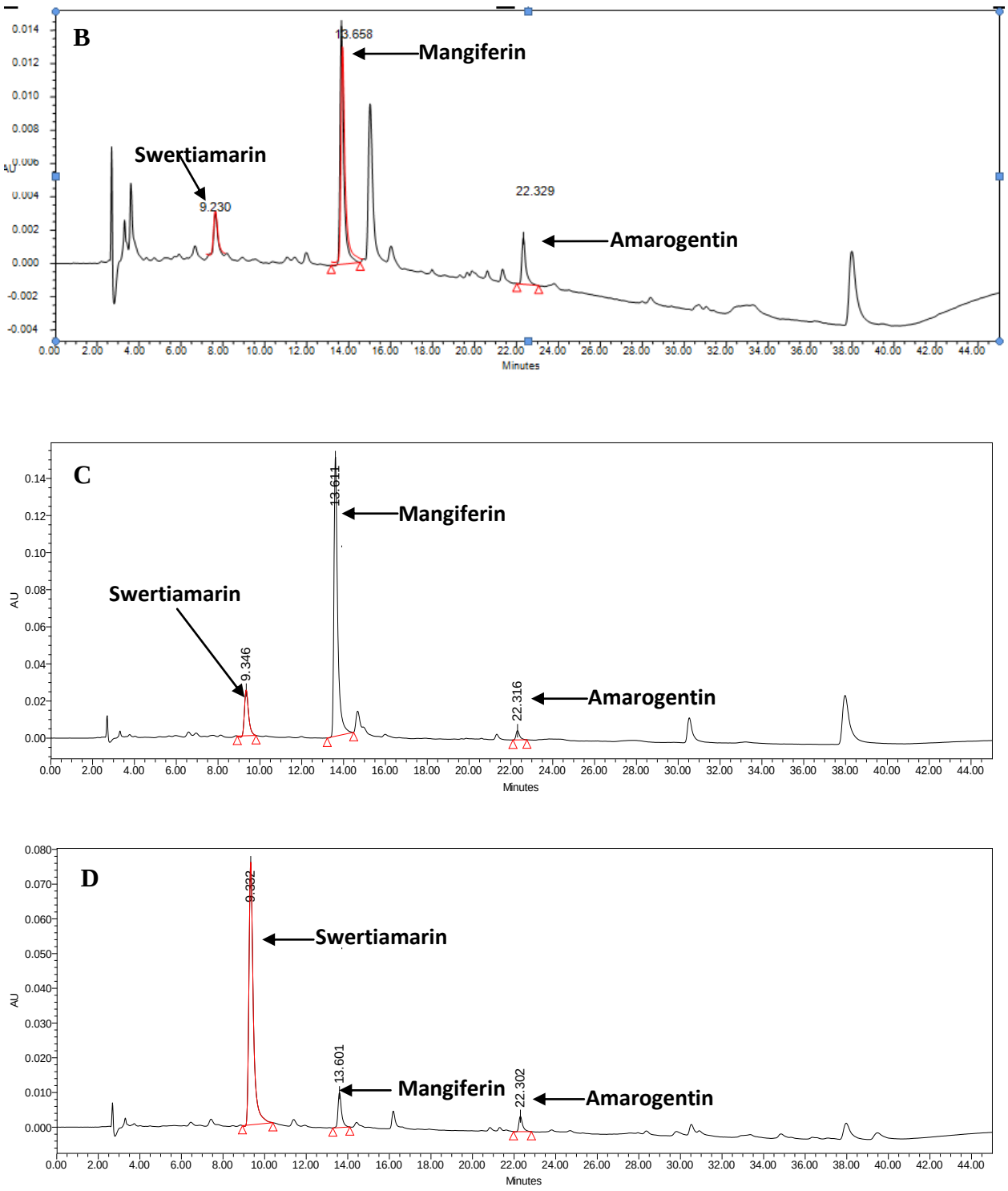


Figure 1: HPLC chromatogram showing the peaks of standard (A), callus culture of swertia (B), tissue cultured plants (C), and field grown plants of *Swertia chirata* (D).

DISCUSSION

HPLC analysis is a broadly employed technique in biotechnological, biochemical, biomedical, and pharmaceutical research areas. HPLC enables quantification of the secondary metabolites with a great accuracy, as it generates peaks for compound being quantified in

association to its concentration in each sample, which are quantified on the basis of standard curve generated from the known concentrations of the reference compound. With these advantages, HPLC was employed for the quantification of swertiamarin and its related metabolites.

S. chirata is reported as a potent root drug in the literature[22] and the whole plant is uprooted whenever harvesting the plant. The present study was carried out with an objective to production of Swertiamarin and related metabolites in indigenous bioreactor. The results of our study help us to conclude that the contents of these metabolites varied considerably in *Swertia chirata*. The age of the plant is known to influence the growth of secondary metabolite in plant.[23,24,25] The variation in these metabolites content, with the age of the plant as noticed in the present study has been described in different species of family Gentianaceae.[26]

In vitro callus and cell suspension studies have been reported in different species of Gentianaceae family for the production of secondary metabolites, especially the secoiridoid glucosides such as, gentiopicroside and swertiamarin.[18] developed a protocol for the *in vitro* propagation of *Gentiana davidii* var. *formosana* and reported a higher content of the secoiridoids as compared to marketed crude drug. The cell suspension cultures of callus derived from stem explants of *Gentiana davidii* at optimal conditions produced maximum swertiamarin (0.224% on dry weight basis) after twelve days of the culture period.[17] In the present study the friable callus obtained from the leaf explants and analyzed for these three metabolites content.[27] conducted a biochemical analysis of callus cultures and tissue cultured *swertia pseudochinensis* plantlets. 4% swertiamarin content was quantified in five-month-old regenerated plants, which decreased to 2.4% after eight months of growth. They detected swertiamarin in regenerated plantlets of *Swertia pusedochinensis*, but could not detect swertiamarin in callus cultures.

CONCLUSION

Based on the results of the present HPLC analysis of *S. chirata*, it can be concluded that in the present study callus culture was also a good source for the production of swertiamarin, mangiferin and amarogentin.

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Conflict of Interest

No potential conflict of interest to disclose.

REFERENCES

1. Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V, Watal G. Hypoglycemic and antidiabetic effect of ethanolic extracts of leaves of *Annona squamosa* L. in experimental animals. *Journal of Ethnophamacology*, 2005; 99(1): 75-81.
2. Sandhu DS, Heinrich M. The use of health foods, spices and other botanicals in the Sikh community in London. *Phytotherapy Research*, 2005; 19(7): 633-642.
3. Sofowara A. Medicinal plants and antimicrobial activity. *Journal of Ethanopharmacology*, 1982; 100: 80-84.
4. Hill RJ, Implications of Monin-Obukhov similarity theory for scalar quantities. *Journal of the Atmospheric Sciences*, 1989; 46(14): 2236-2244.
5. Rabe T, Van Staden J, Isolation of an antibacterial sesquiterpenoid from *Warburgia salutaris*. *Journal of Ethnopharmacology*, 2000; 73: 171-174.
6. Salie F, Eagles PFK, Leng HMJ. Preliminary antimicrobial screening of four South African Asteraceae species. *Journal of ethnopharmacology*, 1996; 52(1): 27-33.
7. Griggs J, Metcalfe JC, Hesketh R. Targeting tumour vasculature: the development of combretastatin A4. *The lancet oncology*, 2001; 2(2): 82-87.
8. Lai PK, Roy J. Antimicrobial and chemopreventive properties of herbs and spices. *Current medicinal chemistry*, 2004; 11(11): 1451-1460.
9. Tapsell LC, Hemphill I, Cobiac L. Health benefits of herbs and spices: The past, the present, the future. *Medical Journal of Australia*, 2006; 185(4): 4-24.
10. Balick MJ, Cox PA. *Plants, people, and culture: the science of ethnobotany*. Scientific American Library, 1996.
11. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. *Environmental health perspectives*, 2001; 109(1): 69.

12. Srivastava J, Lambert J, Vietmeyer N. Medicinal plants: An expanding role in development. World Bank Technical Paper. No. 320. 1996.
13. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World journal of agricultural sciences*, 2008; 4(5): 839-843.
14. Jensen SR, Schripsema J. Chemotaxonomy and pharmacology of Gentianaceae. *Gentianaceae-Systematics and Natural History*, 2002; 5: 574-631.
15. Bhattacharya SK, Reddy PKSP, Ghosal S, Singh AK, Sharma PV. Chemical constituents of gentianaceae XIX: CNS-depressant effects of swertiamarin. *Journal of pharmaceutical sciences*, 1976; 65(10): 1547-1549.
16. Yamahara J, Kobayashi M, Matsuda H, Aoki S. Anticholinergic action of *Swertia japonica* and an active constituent. *Journal of ethnopharmacology*, 1991; 33(1): 31-35.
17. Chueh FS, Chen CC, Tsay HS. Studies on factors affecting the establishment of *Gentiana davidii* var. formosana (Hayata) TN Ho cell suspension cultures. *Journal of Food and Drug Analysis*, 2000; 8(4): 297-303.
18. Chueh FS, Chen CC, Sagare AP, Tsay HS. Quantitative determination of secoiridoid glucosides in *in vitro* propagated plants of *Gentiana davidii* var. formosana by high performance liquid chromatography. *Planta medica*, 2001; 67(01): 70-73.
19. Takei K, Sakakibara H, Sugiyama T. Identification of genes encoding adenylate isopentenyltransferase, a cytokinin biosynthesis enzyme, in Arabidopsis thaliana. *Journal of Biological Chemistry*, 2001; 276(28): 26405-26410.
20. Karan M, Vasisht K, Handa SS. Morphological and chromatographic comparison of certain Indian species of Swertia. *Journal of Medicinal and Aromatic Plant Sciences*, 1997; 19: 995-963.
21. Bhatia A, Karan M, Vasisht K. Morphological and chemotypic comparison of certain Indian species of Swertia. *Journal of Medicinal and Aromatic* Plant Sciences, 2003; 25: 336-343.
22. Kirtikar KR, Basu BD. Indian medicinal plants. (Lalit Mohan Basu, 49, Leader Road, Alahabad 3), 1984; 1664–1666.
23. Rossetti V, Lombard A, Sancin P, Buffa M, Borgarello E. Seasonal variations in components of dried *Gentiana lutea* roots from Western alpine region. *Quarterly Journal of Crude Drug research*, 1981; 19: 37-46.
24. Franz C, Franz G, Fritz D, Schultze J. Seasonal variations in the content of bitter substances and soluble sugars in *Gentiana lutea* L. *Scientia Pharmacia*, 1985; 53: 31-38.

25. Carnat A, Carnat AP, Fraisse D, Ricoux L, Lamaison JL. The aromatic and polyphenolic composition of Roman camomile tea. *Fitoterapia*, 2004; 75(1): 32-38.
26. Nishikawa K, Nishioka M, Shimomura K, Nakanishi F, Ishimaru K. Tissue cultures and secondary metabolites. *Japanese Journal of Food Chemistry*, 1998; 5: 111-115.
27. Kitamura Y, Dono M, Miura H, Sugii M. Production of swertiamarin in cultured tissue of *Swertia pseudochinensis. Chemical Pharmaceutical Bulletin*, 1988; 36: 1575-1576.