Int J Pharm Bio Sci 2016 April; 7(2): (P) 98 - 100

Original Research Article Pharmacognosy

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

ISOLATION OF KAEMPFEROL AND QUERCETIN FROM PODOPHYLLUM HEXANDRUM RHIZOME.

SNEHAL B. BHANDARE* AND KIRTI S. LADDHA

Medicinal and Natural Product Research Laboratory, Department of Pharmaceutical Sciences and Technology,Institute of Chemical Technology, Matunga, Mumbai, India-400 019.

ABSTRACT

Podphyllum hexandrum Royle (Berberidaceae) has gained much attention in phytochemical and pharmacological research as it shows excellent anticancer activity and has been used in the treatment of skin diseases, sunburns and radioprotection. Chemically it contains lignans and flavonoids such as kaempferol, quercetin and their glycosides. The objective of this study is to develop simple method for isolation of Kaempferol and Quercetin from Podophyllum rhizome. The isolated compounds were characterized by TLC, UV, IR, MS and NMR spectral analysis and their purity was confirmed by HPLC analysis.

KEYWORDS: flavonoids, kaempferol, quercetin, podophyllum rhizome

SNEHAL B. BHANDARE

Medicinal and Natural Product Research Laboratory, Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Matunga, Mumbai, India-400 019.

INTRODUCTION

Podophyllum hexandrum family Berberidaceae, is a perennial herb native to an inner range of Himalayas from Kashmir to Sikkim[1]. It is known as devine drug as it is used in Indian and Chinese traditional system of medicine and now it is also used in an allopathic systems for curing leukaemia, lymphoma, viral/ bacterial infections, cancers[2]. The dried roots and rhizomes of *P. hexandrum* contain around 8% of resin; known as podophyllin which is prepared by precipitating its alcoholic extract in slightly acidic water. It is commonly used as cholagogue, purgative, alterative, emetic, bitter tonic, as a cathartic in veterinary medicine and recently used in controlling some forms of cancers. Podophyllin contains podophyllotoxin (32-54%), quercetin (8%), kaempferol, astragalin and essential oil (3.7%), wax (8.6%) and mineral salts[1]. As flavonoids have shown beneficial effects on human health, it has gained increased interest in the last years[3]. Especially flavonols such as kaempferol and quercetin which has been reported to show decreased risk of coronary heart diseases and cancer[4]. Since these flavonols are present in very good amount; an attempt was made to isolate kaempferol from podophyllum rhizome. It has been reported previously that kaempferol was isolated from podophyllum rhizome[5]. In the present research paper, isolation and characterization of kaempferol and quercetin from podophyllin, with good yield has been reported.

Experimental

Plant Material

The rhizomes of *P. hexandrum* were collected from the local market of Mumbai, India. The plant was identified and its voucher specimen was deposited at Medicinal Natural Product Research Laboratory, Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Mumbai.

Chemicals

Kaempferol and quercetin reference standards were purchased from Total Herb Solutions, Mumbai. All chemicals and solvents used for extraction and isolation were of laboratory grade. Solvents used for HPLC analysis were of analytical grade obtained from Merck, India.

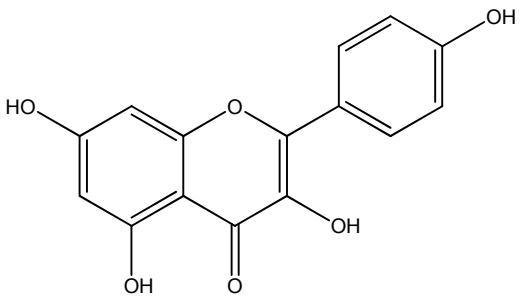
Instrumentation

UV/Vis Spectrum was recorded in methanol on a Jasco V-530 Spectrophotometer. HPLC analysis was performed with Jasco (Hachioji, Tokyo, Japan) system with a manual sample injection valve equipped with 20 µl loop and UV-visible detector (UV-1575), RP-18 endcapped column (250 mm × 4.5 mm i.d., 5 µm particle, Hibar Purospher Star, Germany). IR spectra were recorded on Shimadzu instrument. Mass spectrum was recorded on Micromass Q-TOF MS Mass Spectrometer. 1H NMR spectra was recorded on a JOEL 400-MHz instrument with an internal standard of tetramethylsilane (TMS).

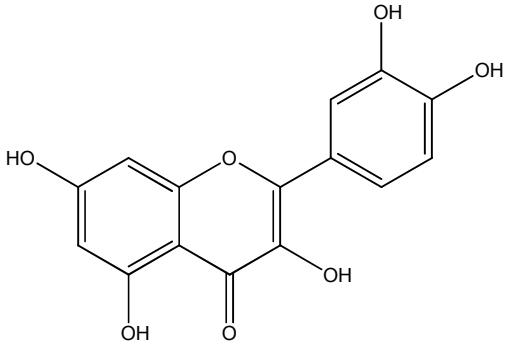
Extraction and isolation

The powdered rhizome of *P. hexandrum* (1kg) was subjected to Soxhlet extraction with methanol (5 l) as solvent for 42 hrs. This methanolic extract obtained was concentrated under reduced pressure to obtain 1/20th of its original volume. This methanolic extract was further added to 1 l of acidified water with hydrochloric acid to obtain podophyllin precipitate. This precipitate obtained was then filtered under Buchner funnel and dried to obtain podophyllin. Podophyllin was then extracted with chloroform and ethyl acetate using a Soxhlet apparatus. Ethyl acetate extract obtained was concentrated to form dry mass (19 gm) which is the mixture of flavonoids. The dried residue was subjected to column chromatography using petroleum ether and ethyl acetate solvent system. The purified kaempferol (600 mg) was first isolated at petroleum ether: ethyl acetate (100: 20). On further increasing the ethyl acetate concentration to (100: 22), purified quercetin (300 mg) was isolated. TLC and HPLC studies were then carried to determine the purity of the isolated compounds and structure was elucidated and confirmed by UV IR, MS and ^{1}HNMR spectral analysis.

Structures



Kaempferol



Quercetin

RESULTS AND DISCUSSION

The main aim of the study was to isolate kaempferol and quercetin simultaneously from *P. hexandrum* which gives better yield. Literature survey revealed that an attempt has been made previously to isolate kaempferol from *P. hexandrum* rhizomes which involved column chromatography[5] however, the yield obtained was very low. The advantage of this method is to isolate kaempferol and quercetin with comparatively better yield and purity. Podophyllin obtained represents the mixture of flavonoids and lignans. Since lignans

have high solubility in chloroform, it was removed by extracting it with chloroform. Further extraction was carried out with various solvents such as methanol, acetone and ethyl acetate. But podophyllin was totally soluble in methanol and acetone hence ethyl acetate was used for further extraction of flavonoids. Both IR spectra exhibited prominent absorption bands for free phenolic OH at 3277 and 3296.2 cm^{-1} and for conjugated C=O at 1597 and 1659.7 cm-1 respectively. The Molecular ion peak of kaempferol and quercetin were found to be at m/z 287 and 303.09 respectively. The UV/Vis maximum in methanol was found to be at 259, 360 nm and 260, 370 nm which are identical with standard kaempferol and quercetin respectively. 1H NMR analysis of both isolated compounds exhibited typical four-peak pattern of two doublets at ð 6.82 and ð 7.9 which was assigned to H-3',5' and H-2',6' respectively. These were found similar with reported values in literature[6,7]. The purity of the compound was confirmed by TLC and HPLC. Kaempferol and Quercetin showed 98.1% and 97% purity by HPLC using isocratic elution program of 15 min with the mobile phase methanol: water containing 0.05 % phosphoric acid (60:40) at UV 370 nm[8]. Based on the chemical and spectral studies, the isolated compounds were identified as kaempferol and quercetin. The results showed that method is suitable for isolation of kaempferol and quercetin from *P. hexandrum* rhizomes.

CONCLUSION

Easy and simple method for isolation of Kaempferol and Quercetin was developed. Various spectral data obtained showed the purity of the compounds. The reported method of isolation has shown good reproducibility in terms of purity and yield**.**

ACKNOWLEDGEMENT

The authors thanks to University Grant Commission, New Delhi, India, for providing financial assistance.

REFERENCES

1. Wealth of India, A dictionary of Indian raw materials and industrial products, raw materials. B. N. Sastri (Ed), Council of Scientific and Industrial Research, New Delhi, 2005, vol. VIII: Ph-Re, p.170-174.
2. Sultan P, Shawl AS, Abdellah AA and Ramteke PW. Isolation, Characterization and Comparative Study on Podophyllotoxin and Related Glycosides of *Podophyllum heaxandrum.* Current Research Journal of Biological Sciences. 2010; 2(5): 345-51.
3. Padmapriya N, Poonguzhali T. Evaluation of anticancer activity of flavonoid isolated from the acetone extract of the aerial parts of phyllanthus *niruri against* human lung cancer cell line (a549). Int J Pharm Bio Sci. 2015; 6(3): 296 – 304.
4. Francisco PV, Juan D. Flavonols and cardiovascular disease. Molecular Aspects of Medicine. 2010; 31:478–94.
5. Chaudhary A, Kaur P, Katiyar A, and Singh B. HPTLC–Densitometry Method for Simultaneous Determination of Major Lignans and Flavonoids in *Podophyllum hexandrum.* Journal of Planar Chromatography. 2012; 25(4): 314–19
6. Farzin H, Naaman K, Hossein H,, Randa K-A. Kaempferol from Saffron Petals. Iranian Journal of Pharmaceutical Research. 2003; 251-252.
7. Maria S, Irena M. Quercetin and its glycosides in the flowers of *Asclepias syriaca* L. Acta polonie pharmaceutica- drug research. 2000; 57 (4): 321-24.
8. Magdalena T, Validation of the analytical method for the determination of flavonoids in broccoli. Journal of Horticultural Research. 2014; 22(1): 131-40.