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Research Article

ISOLATION, CHARACTERIZATION AND QUANTIFICATION OF BERGENIN FROM *SYZYGIUM CUMINI* STEM BARK

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ABSTRACT

Diabetes is one of the major health problems, the world is facing today and its rising rate necessitates alternative treatment methods. An anti-diabetic chemical constituent of *Syzygium cumini* (L.) Skeels, bergenin was chosen for the study. The present study aims to isolate, characterize and quantify bergenin from methanol extract of stem bark of *S. cumini*. This compound was extracted, characterized by spectroscopic methods and estimated quantitatively by HPTLC.

Keywords: Diabetes mellitus, bergenin, HPTLC, HPLC

INTRODUCTION

Diabetes mellitus is a metabolic disorder, which is characterized by chronic hyperglycemia (elevation of blood glucose) with complications in carbohydrate, protein and fat metabolism resulting from the defects in insulin secretion and action. In the past two decades, there has been an explosive increase in the number of diabetic patients. Predominant changes in the human environment and human behavior coupled with sedentary life style have resulted in escalating rates of diabetes. The long term effects of diabetes mellitus leads to various complications which substantially increase the rate of morbidity and mortality and reduce the quality of life of the diabetic individual[1]. There are many therapeutic approaches for the treatment of diabetes, one among is herbal drugs. They are more effective than other drugs because of their quick positive response and being safe on the body without side effects. Therefore, investigation of these herbal plants for anti-diabetic activity and isolation of active principle responsible for anti-diabetic property is an important area of research. *Syzygium cumini* is a medicinal plant with an illustrious medical history. It is widely distributed throughout India and Indian systems of medicine mention its use for the treatment of diabetes mellitus. Different anatomical parts of the plant were also reported for antioxidant, anti-inflammatory, neuropsycho-pharmacological, anti-microbial, anti-HIV, anti-leishmanial, anti-diarrheal, anti fertility, anorexigenic, gastro-protective, anti-ulcerogenic and radio-protective activities[2,3]. The stem bark is reported with betulinic acid, friedelin, epi-friedelanol, β-sitosterol, eugenin and fatty acid ester of epi-friedelanol[4], β-sitosterol, quercetin kaempferol, myricetin, gallic acid and ellagic acid[5], bergenin[6], flavonoids and tannins[7]. Among these chemical constituents, bergenin possess the antidiabetic property and hence its isolation, characterization, quantification by HPTLC and HPLC finger print profiling is aimed in the present study.

MATERIALS AND METHODS

Plant Material

The stem bark was collected from the Anna Hospital campus, Arumbakkam, Chennai, Tamil Nadu, India. The collected stem bark was shade dried, coarsely powdered and used for the study.

Extraction

The powdered drug (500 g) was soaked in methanol (2 liters) for 48 hours. The extract was filtered and the solvent was distilled out. The process was repeated again for complete extraction of the chemical constituents. The extract was triturated with water and partitioned with ethyl acetate. The ethyl acetate soluble portion was collected and concentrated.

Isolation and characterization

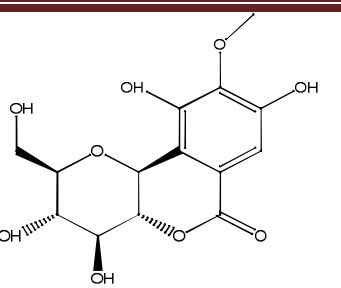
This extract was subjected to column chromatography over silica gel (Acme, 60-120 mesh) as adsorbent. The column was eluted with chloroform. All the chloroform fractions were distilled, compared by TLC and combined. Elution was continued with chloroform : methanol mixture with varying proportions. The fractions eluted with mixture of 8.5:1.5 ratios answered for phenol. Hence these fractions (6 nos.) were combined and rechromatographed over silica gel (Acme, 100-200 mesh) which afforded colourless compound. The colourless compound was confirmed as bergenin by chemical tests, melting point and spectral data.

Instrumental

IR spectra were taken in KBr disc on a Perkin – Elmer grating FT-IR instrument. UV spectrum was taken in a Shimadzu spectrometer. ^{1}H NMR was taken in DMSO and ^{13}C NMR was taken in $CDCl_3$ in a Bruker instrument (400 and 100 MHz respectively). Linomat IV (CAMAG, Muttenz, Switzerland) applicator, CAMAG TLC twin trough chamber (20 x 10 cm), CAMAG TLC scanner 030618 attached with WINCATS software, CAMAG visualizer were used for High Performance Thin Layer Chromatographic estimation and photo documentation. For High Pressure Liquid Chromatographic finger print profile development, Shimadzu LC 10 AD instrument with Gemin C18 column was utilized.

Estimation of Bergenin by HPTLC

TLC plate precoated with silica gel 60 F_{254} (E. Merck) of 0.2 mm thickness. Toluene : Ethyl acetate : Acetic acid (6:6:1.0, v/v/v) was finalized as the mobile phase. Extracted accurately weighed 1 g of powdered dried drug with 50 ml of methanol in a Soxhlet apparatus consecutively three times. Filtered, concentrated the combined extracts under vacuum and made up the volume to 10 ml with methanol. Dissolved 10 mg of bergenin in methanol in a 10 ml volumetric flask and made up to the mark. 4, 6, 8, 10 and 12 µl of standard solution corresponding to 4, 6, 8, 10 and 12 µg of bergenin were applied on the precoated TLC plate. 10 µl of the sample solution was also applied on six tracks on the same plate. Developed the plate in the solvent system in a chamber (previously saturated with solvent vapor for 30 min) till the solvent rises to a distance of 8 cm; dried the plate in air and scanned densitometrically at 273 nm; recorded the peak area of each spot and linear regression calibration curve; calculated the amount of bergenin present in the sample from the calibration curve. The TLC plate was detected under UV 254 nm and UV 366 nm and the chromatograms were recorded.



Structure of bergenin

HPLC finger print profiling

1 ml of the sample solution used for HPTLC was diluted to 2.5 ml with methanol and used for HPLC finger print profiling.

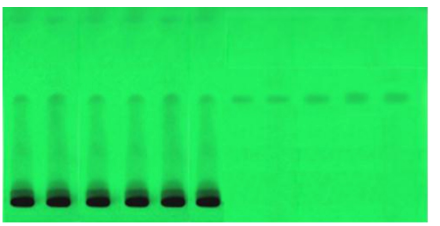


Figure 1. HPTLC chromatogram at UV 254 nm

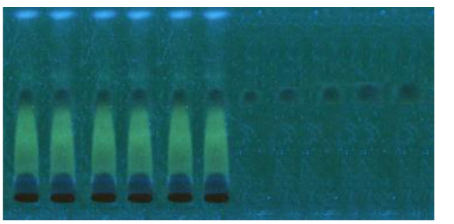


Figure 2. HPTLC chromatogram at UV 366 nm

Track 1-6: Ethanol extracts of *S. cumini* bark 10 µl;
Track 7-11: Standard Bergenin (4, 6, 8, 10 &12 µl respectively)

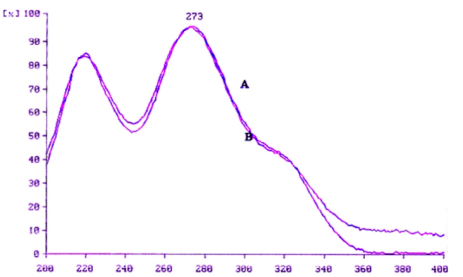


Figure 3. UV Super imposable spectra. A. Bergenin; B. Spot of *S. cumini* extract corresponding to R_f of bergenin

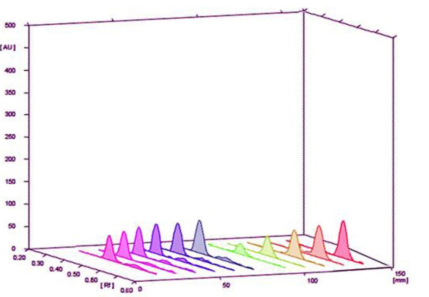


Figure 4. 3D Chromatogram of ethanol extract of *S. cumini* & bergenin at UV 273 nm

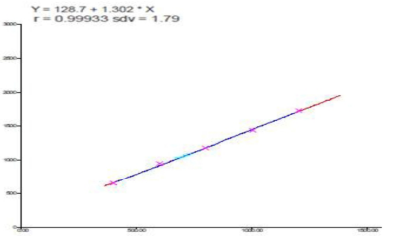


Figure 5. Linear regression curve of bergenin

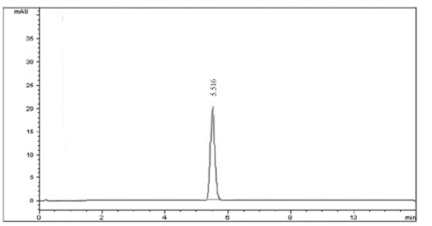


Figure 6. HPLC chromatogram of bergenin

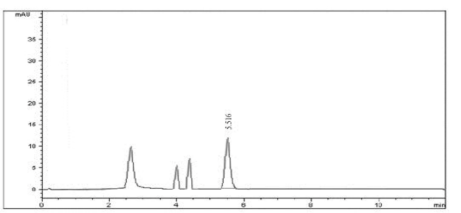


Figure 7. HPLC chromatogram of methanol extract of *S. cumini* stem bark

RESULTS

The compound was recrystallized as colourless needle from methanol (yield 27 mg, m. p. 239-240°C, lit.mp 237-240°C). It gave blue colour showing the presence of phenols (hydroxyl groups) and a single spot on TLC over silica gel (R_f = 0.52) with Toluene: Ethyl acetate: Acetic acid (6:6:1.0, v/v) as the developing system. The HPTLC chromatogram photo documented at UV 254 nm is shown in Figure 1 and that of UV 366 nm is shown in Figure 2. As the UV absorption maximum of bergenin was 273 nm, the estimation was done at UV 273 nm. Figure 3 shows the UV superimposable spectrum of bergenin with the corresponding spot of similar R_f of sample solution. The HPTLC 3D chromatogram of all 11 tracks is shown in Figure 4 and the linear regression curve obtained is shown in Figure 5. The HPLC chromatogram of bergenin showed sharp peak at R_t 5.516 (Figure 6). In the HPLC chromatogram of methanolic extract of *S. cumini* stem bark also, there is a peak in the same retention time. In addition, there are three more peaks in the sample solution at R_t 3.248, 4.083 and 4.462 (Figure 7).

DISCUSSION

The IR, UV spectrum, 1H NMR and 13C NMR of the isolated compound were compared with literature and the structure of the compound was elucidated as bergenin[8-10]. The standard deviation of the standard graph was 1.79 and the coefficient of variance was 4.136 % which show the precision and accuracy of the results. The regression coefficient (r) 0.99933 shows the similarity of the bergenin in standard and sample solutions which can also be confirmed by its UV superimposable spectrum. The compound bergenin was estimated quantitatively using HPTLC and the calculated content of bergenin from the methanol extract of S. cumini stem bark was 0.06 %.

CONCLUSION

The content of bergenin, an anti-diabetic chemical from the plant S. cumini stem bark was estimated by using HPTLC and its presence is ascertained, leading to the hope of its anti-diabetic activity. The TLC photo documentations at UV 254/366 nm and finger print profiles will also be useful in the identification of the drug.

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