TLC-Spectrophotometric Determination of Alkaloid Berberine From Marketed Tablet Formulation of *Berberis Aristata* DC

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GALENİK PREPARATLARDAKİ ALKOLOİD BERBERİN DÜZEYLERİNİN İNCE TABAKA KROMATOGRAFİSİ VE SPEKTROFOTOMETRİ İLE BELİRTİMİ

Özet

Piyasada satılan bazı galenik preparatlarında terapötik etkiye sahip etkin madde düzeyleri belirtilmemektedir. Alkaloid özelliği olan Daruhalad *(Berberis aristata)* ekstraktı içeren böyle bir preparatın berberin düzeyleri incelendi. Bu araştırma, bu tür bir preparat içerisindeki etkin madde miktarının İTK - spektrofotometrik yöntemle tesbit edilebileceğini göstermektedir.

Yapılan literatür taramasında benzer bir çalışmaya rastlanmadı.

Summary

Ayurvedic tablet formulations sold in the market do not mention the amount of active ingredient having therapeutic effect. Such one tablet formulation containing extract of Daruhalad *(Berberis aristata),* was analysed for berberine content. The present work deals with finding out the actual amount of the active ingredient present there in, by TLC - Spectrophotometric method.

Literature survey does not reveal any work on such product.

Keywords : *Berberis aristata - Berberine content - TLC-spectrophotometric determination*

Literature Survey

Kimura and *Noro* (1) determined berberine (*5,6 - Dihydro - 9,10 - dimethoxybenzo [g] - 1,3 - benzodioxolo[5,6-a]quinolizinium ; 7,8,13 a - tetradehydro - 9,10 - dimethoxy - 2,3 - (methylenedioxy) berbinium*) from crude drugs, by applying a sample solution on a water saturated Sephadex G-25 column followed by the elusion with 0.05m NaCl and measurement of absorption at 421 nm.

Mura and *Tominga* (2) found out a 2-dimensional TLC method for the separation and quantitative determination of berberine from *Phelladandron* bark. BuOH-AcOH-H$_2$O (7:1:2) and cyclohexane - Et$_2$NH (9:1) were used as developing systems consecutively on Kieselgel G plate. It was eluted from the adsorbent and determined photometrically at 420 nm.

Tsesko and *Ladygina* (3) separated berberine from plant raw materials by extracting it from *Berberis vulgaris* and putting it on TLC using solvent system CHCl$_3$ - EtOH - 25% NH$_3$ Solution (3:3:1). The spot corresponding to berberine by its Rf value and UV fluorescence was eluted with 0.1 N H$_2$SO$_4$ and measured spectrophotometrically at 347 nm.

Naidovich et *al* (4) macerated barberry roots with 25% NaOH and extracted with ether. Subsequent extracted ether layers were combined and the whole was extracted with 2% H$_2$SO$_4$ and berberine content of the solution was determined spectrophotometrically at 420 nm.

Chen and *Liu* , extracted berberine from the dried root powder by methanol and after evaporation the residue was dissolved in ethanol and purified by TLC using CHCl$_3$ - MeOH - NH$_4$OH (15:4:0.5) solvent system. Scrapings were treated with H$_2$SO$_4$ and 10% chromotropic acid at 100°C for 30 min and then determined spectrometrically at 570 nm.

Experimental

The label claim on the tablet formulation
Each tablet contains,
Berberis aristata ....32 mg.

Preparation of Test Solution

22 tablets of the formulation were wedged and powdered. Powder equivalent to the weight of 20 tablets was taken. This was extracted with ammoniacal chloroform stirred for 20 min. The chloroform layers were combined and evaporated to dryness. The residue was dissolved in ethanol and diluted to 100 ml with ethanol.

Preparation of Standard Solution

8 mg of berberine was weighed accurately and dissolved in 70 mL of ethanol, and diluted to 100 mL with the same solvent.

Separation and Quantitation of Berberine Alkaloid

Chromoplates of 20 x 20 cm size were prepared with Silica gel G of thickness 500 μm and then activated at 105°C - 110°C for one hour. Each three activated chromoplates were taken and streaked

using 0.25, 0.50 and 0.75 ml of test and standard solutions. The plates were developed in a saturated developing chamber using methanol - benzene (10 : 40) as a mobile phase. The plates were run to 10 cm which took 35 min. Visualization was done under UV light and treating with iodine vapours. For the purpose of scrapping, a reference plate under the same conditions was prepared and then knowing Rf value, scrapping was done. The Rf value for berberine was 0.8. The corresponding band was scrapped out and analysed by Shimadzu UV 240/visible spectrophotometer at 267 nm in ethanol (5).

Recovery Experiment

To the powder equivalent to weight of 20 tab. 100 mg of pure berberine was added. From this admixture a quantity of powder equivalent to weight of 20 tablets, was analysed by the proposed method, the percentage recovery for the alkaloid was obtained. Further statistical evaluation indicated the precision of the proposed method.

Drug	A	B	C	D	E	F
Berberine	0.269	100	99.67	99.67 %	±0.039	15.64

A: Content per tablet by the proposed method (mg), B: Amount of drug added in mg,
C: Amount of drug recovered in mg, D: Percentage recovery, E: Standard deviation,
F: Coefficient of variation.

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