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Studies on the chemical constituents of leaves of *Phyllanthus emblica* (L.)

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ABSTRACT

A Phytochemical study was carried out on the leaves of *Phyllanthus emblica.* By using different chromatographic techniques the separation of the chemical compounds were done and structure of the compounds were elucidated by spectroscopic methods including nuclear magnetic resonance as well as mass spectrometry. Two compounds were isolated and identified; that are quercetin and β-sitosterol.

Key words: Euphorbiaceae, *Phyllanthus emblica*, quercetin, β-sitosterol, NMR.

INTRODUCTION

Phyllanthus emblica Linn., belongs to the family Euphorbiaceae, commonly known as Indian gooseberry and amla. It is distributed in tropical and subtropical regions of India. It is excellent source of vitamin C, easily assimilated by human body[1].It is found all over India, along the sea-coast districts, in deciduous forest of Madhya Pradesh. It is helpful in lowering cholesterol level[2] and protects from heart disease[3-4], strengthens senses[5], strengthens liver[6]. It is useful in diabetes[7-8], gonorrhea, diuretic fevers, diarrhoea[9], mouth ulcers, inflammations, hair growth, headache, colic, asthma, respiratory problems. It is used as antioxidant[10], aphrodisiac, antifungal, antiviral, anticancer, antigenotoxic[11], antimutagenic[12], chelating agent.

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance 400 MHz spectrometer. The EI-mass was recorded on Shimadju QP 2000 mass spectrometer. The leaves of *Phyllanthus emblica* was collected from Agra College, Agra. The leaves were air dried under shade for ten days. Then the leaves were powdered with the help of warming blender. The air dried powdered leaves (500gm) were subjected to successive hot extraction in a Soxhlet apparatus with solvents petroleum ether, ethyl alcohol and ethyl acetate. The average time period for extraction was 72 hours. The individual extracts were filtered twice and then concentrated by distillation on vaccum. The ethanolic extract (5gm) was subjected to silica gel chromatography using isopropanol-

formic acid-water (2:5:5), to give compound 1 quercetin.

A portion of the ethyl acetate extract (4 gm) was subjected to silica gel thin layer chromatography using n-hexane: acetone (80:20) solvent system to give compound 2 that is identified β-sitosterol. Quercetin and β-sitosterol were identified by comparison with data from previous NMR and mass spectra [13-15].

Quercetin

Compound (1) Slightly yellow powder; m.p 316 °C; ^{1}H NMR (400 MHz, Me OD): δ (ppm) = 6.20 (1H, *d, J* = 2.0 Hz, H-6), 6.42 (1H, *d, J* = 2.0 Hz, H-8), 6.90 (1H,*d, J* = 8.2 Hz, H-5'), 7.64 (1H, *dd*, *J* = 8.3; 2.1 Hz, H-6'), 7.76 (1H, *d*, *J* = 2.1 Hz, H-2'),^{13}C NMR (100MHz, Me OD): δ (ppm) = 148.4 (C-2), 137.1 (C-3), 177.4 (C-4),162.4(C-5), 99.2 (C-6), 165.6 (C-7), 94.8 (C-8), 158.2 (C-9), 104.6 (C-10), 124.5 (C-1'), 116.2 (C-2', C-5'), 146.4 (C-3'), 150.2 (C-4'), 121.7 (C-6').

β-Sitosterol

Compound (2) White powder, m.p136^{0}C, ^{1}HNMR ($CDCl_3$ 400MHz): 5.38(1H,dd,J=5.2Hz,H-6),3.56(1H,tt,J=11.3;5.3Hz,H-3), 2.34 (1H,ddd, J=13.0;5.0;2.0Hz,H-4a) 0.74,0.87,0.88, 0.89,0.93,1.06 (each3H,s,MeX6). ^{13}CNMR($CDCl_3$, 100MHz): δ37.1(C-1), 31.5 (C-2),71.7(C-3),42.2(C-4),140.6(C-5),121.6(C-6), 31.8 (C-7),31.7(C-8),50.2(C-9),36.4(C-10), 21.2(C-11),39.7(C-12),42.2(C-13),56.7(C-14),24.2(C-15),28.1(C-16),56.0(C-17), 11.7(C-18), 19.3(C-19), 36.3 (C-20), 18.9(C-21), 33.8(C-22),26.1(C-23), 45.7(C-24), 29.2(C-25),19.7(C-26),19.1(C27),23.0 (C-28), 12.1(C-29).

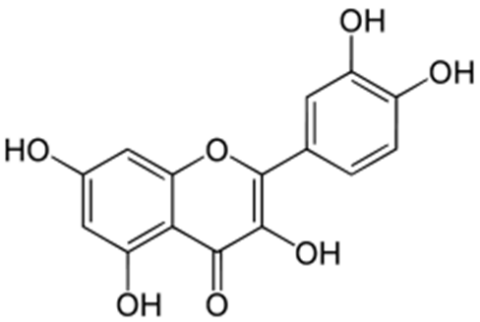

Fig. 1: Structure of Quercetin

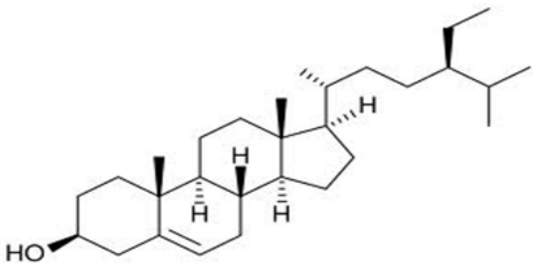

Fig. 2: Structure of β-Sitosterol

RESULTS AND DISCUSSION

Aim of this study was to identify and characterize the bioactive principles from the leaves of *Phyllanthus emblica*. Compound 1 is a slightly yellow powder, m.p 316 °C. The EI-mass spectrum of 1 showed the molecular ion at m/z 302 [M$^+$] corresponding to the formula $C_{15}H_{10}O_7$ and in agreement with other spectroscopic data. The ^{1}H NMR showed that the proton of H-6 and H-8 appeared as a duplet at δ 6.20 and 6.42 .The proton of H-5',H-6'and H-2' appears at ä 6.90,7.64 and 7.76 respectively.^{13}CNMR spectrum showed a carbonyl group at δ177.4 .The carbon bonded to hydroxyl group appeared at δ137.1, 146.4, 150.2, 162.4 and165.6.

Compound 2 was isolated as white powder, m.p 136^{0}C. The EI-mass spectrum of 2 showed the molecular ion at m/z 414[M$^+$] corresponding to the molecular formula $C_{29}H_{50}O$ and in agreement with other spectroscopic data. The ^{1}H NMR spectrum showed a broad triplet at δ 5.38 corresponding to H-6 olefenic proton and multiple at δ 3.56 corresponding to H-3 alpha proton six tertiary methyl singlets.^{13}C NMR of the compound showed 29 signals for steroid skeleton which was represented by six methyl groups. The carbon bonded to the hydroxyl group C-3 appeared at 71.7.The quercetin and β- sitosterol has been earlier reported in various plants [16-20].

CONCLUSION

From the physical, chemical and spectral characteristics, compound 1and 2 were concluded as Quercetin (Fig.1) and β- sitosterol (Fig.2). Quercetin is flavonoid, used in asthma, eczema, heyfever, and hives. It possess anti-inflammatory, anticancer, antiviral activity, inhibit inflammatory leukotriene production[21]. β- sitosterol is phytosterol, used as antioxidant and an anti- diabetic agent. Human liver microsome studies reveals that β-sitosterol inhibits the cholesterol absorption, reduces the symptoms of benign prostatic hyperplasia[22], anti-inflammatory[23] and anti-pyretic activity. So medicinal properties of *Phyllanthus emblica* is due to the quercetin and β- sitosterol, furthermore scientific evaluation are required to establish therapeutic efficacy.

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