ISOLATION AND SPECTRAL IDENTIFICATION OF QUERCETIN FROM THE ALCOHOLIC ROOT EXTRACT OF *Clerodendrum paniculatum* Linn.

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Abstract

Plant materials are used throughout developed and developing countries as home remedies, over the counter drug products and raw materials for the pharmaceutical industry and represent a substantial proportion of the global drug market. It is therefore essential to establish internationally recognized guidelines for assessing their quality. Some of quality control parameters of the root *Clerodendrum s*pecies belonging to Verbenceae family were analyzed. It includes root powder characters, moisture content determination by LOD method, FOM determination, R_f value detection by TLC, using different solvents, Ash values, extractive values, bitterness value, Haemolytic activity, detection of tannins, Foaming Index, Detection of Arsenic and heavy metals, determination of micro organism .The isolation of the compound from the extract by column chromatography by using different solvents ,purified ,analysed by various spectral studies . The study ensures that the quality control parameters do help in the proper standard of the crude drugs in drug development process for global acceptances. The current study may be useful to progress further investigation on the isolation of other flavonoids and their biological potential for the treatment of human ailments.

Keywords: *C. paniculatum*, UV, IR , NMR, MASS spectroscopy .

INTRODUCTION

Clerodendrum paniculatum Linn, (Family :Verbenaceae) [1] is a species found in India, It is reported as folk remedy for tumours , leprosy , fever , infection , inflammation .The roots have been reported to possess laxative ,diuretic ,analgesic ,anti inflammatory, anti tumour and antibacterial activities[1]. In the present study the root portions of *Clerodendrum paniculatum* Linn comprising,phytochemical and spectral analysis..The root was extracted with ethanol, extraction. The vaccum dried extracts were screened various phytoconstituent and TLC, HPTLC, spectral analysis analysis. The flavonoids of plant origin are versatile in biological activities .Their presence in plants may be due to one of the purpose such as defence such as microbial attack as precursor or as metabolic end product of plant metabolism .Isolation of flavonoids by solvent extraction supposed to be a very tedious process because of its magnitude of reactivity with other molecule of plants .

MATERIALS AND METHODS

EXPERIMENTAL SECTION

The plant *C.paniculatum* was collected from Pathanamthitta district of Kerala and identified by Thomas Mathew, HOD of Botany, Marthoma College Tiruvalla, Kerala .Voucher no. VSCI-15 was deposited in the Pharmacognosy department, Pushpagiri College of pharmacy, Tiruvalla.

PREPARATION OF EXTRACT

The root portion of the plant was washed with running water to remove soil and other matter and dried in shade for 20 days, powdered, extracted 500gm with ethanol (EECP) by cold extraction to yield the extract. The extract were reduced to molten mass by rotary vacuum evaporator and the yield was 21% w/w.

PHYTOCHEMICAL SCREENING

The root portion of the plant was washed with running water to remove soil and other matter and dried in shade for 20 days, powdered, extracted 500gm with ethanol by cold extraction to yield the extract. The extract were reduced to molten mass by rotary vacuum evaporator and the yield was 25%w/w. Preliminary phytochemical screening was performed as per standard procedure and various phytochemical constituents were identified[6,7]

such as carbohydrates ,starch, mucilage saponins ,flavonoids ,tannins , phenolic compounds in the different extract .

Isolation and characterisation of active constituents

Alcoholic extract of *Clerodendrum paniculatum* Linn was successively partitioned with hexane ,dichloromethane ,ethyl acetate and butanol .The combined organic layer of each fractions was evaporated to dryness to get molten mass. The ethyl acetate fraction 6.8gm was fractionated on silica gel column chromatography using subjected to column chromatography using $CHCL_3$ up to 100% followed by increasing gradient of MeOH up to 100% .The isolate were collected in 50 ml portions and monitored on TLC using solvent Dichloromethane MeOH(7.5:2.5).The fractions that showed similar R_f were mixed and concentrated .The isolate named as CiA (71.5mg) These isolate were subjected to HPTLC, Physico-Chemical and spectroscopic characterisation. From the result obtained it can be say that isolate is a Flavonoid and Quercetin

RESULT AND DISCUSSION

Phytochemical screening of the alcoholic extract of Cp were carried out for the determination of groups of organic compounds present in them .As a result they are carbohydrates ,starch, saponins, muscilge, flavonoids, tann ins and phenolic compounds The isolated compound were subjected to TLC ,HPTLC,Physico- Chemical and spectroscopic characterisation.

The solvent systems used and their corresponding R_f values are shown below

Sl No.	Mobile phase	No. of spots	R_f Values
1.	Ethyl acetate: formic acid: glacial acetic acid: water (100 : 11 : 11 : 27)	Single	0.63
2.	n- Butanol : glacial acetic acid : water (40 : 10 : 50)	Single	0.62
3.	n- Butanol : ethyl acetate : water (4 : 1 : 2.2)	Single	0.48

The compound-1 exhibited a positive test for flavonoid and phenols. It gave an olive green colour with ferric chloride, a reddish pink colour in Shinoda's test and a yellow colour with ammonia, It gave positive colour reaction in Molish's test indicating the presence of a qlycoside.

The melting point of isolated compound- was found to be in between 216-218°C (uncorrected) The isolated compound I was subjected to UV, IR, Proton NMR, ^{13}C NMR, and Mass Spectroscopic analysis to find out the structure.

Spectral studies of compound1

UV λ_{max} MeOH (nm): 253, 368 (Fig.1.

IR γ_{max} cm^{-1}: 3392, 3369,1654,1609,1558,1508,1458,1429 (Fig, 2).

^{1}**HNMR**: δ 7.15 (CH), 6.93(CH), 6.72(CH), 6.25(CH), 5.94(CH), 11.85(OH), 10.68(OH), 10.29(OH), 9.48(OH) (Fig.3).

^{13}**CNMR**: δ 136.5(C), 146.9(C) 146.5(C). 145.9(C), 158.8 (C) 161.8(C) 166.4(C), 122.8(C) 115.3 (CH), 104.5 (C) 117.2 (CH), 176.1 (C), 98.3(CH), 94.0(CH), 121.8(CH) (Fig 4).

FAB-MS: pso. lons 303 [M - H] (Fig. 5).

The spectral data matched with that of the quercetin, thus confirming the structure of quercetin.

Quercetin (3,3',4',5,7- pentahydroxy flavone)

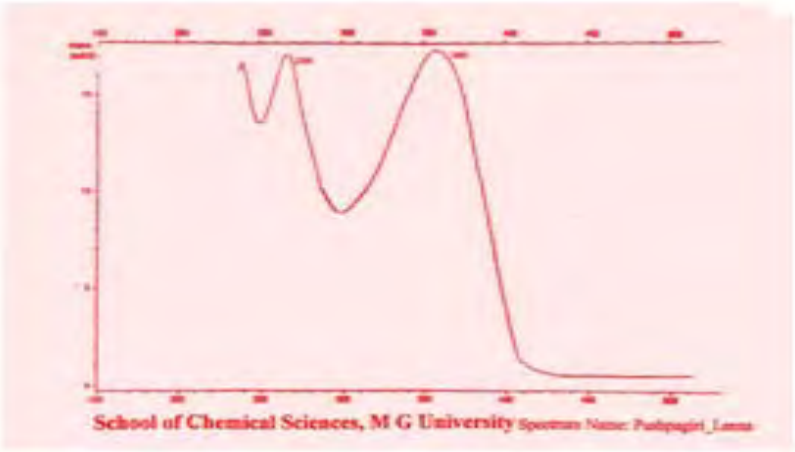


Fig 1-

Fig 2 :

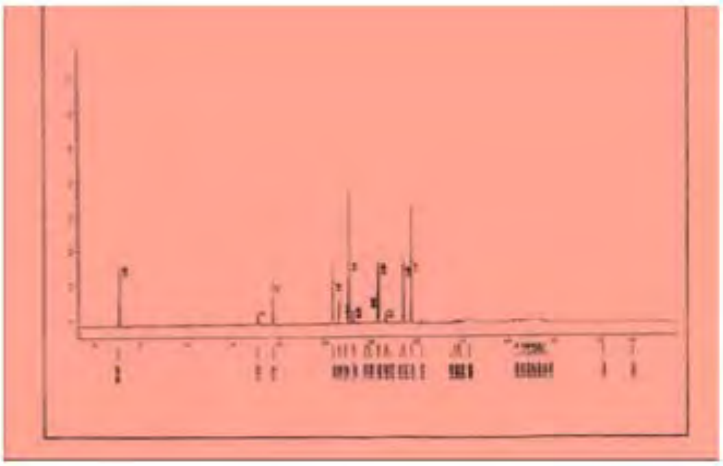
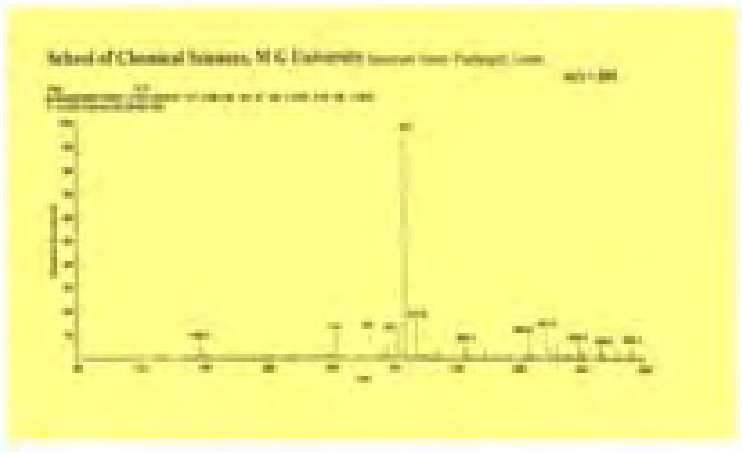
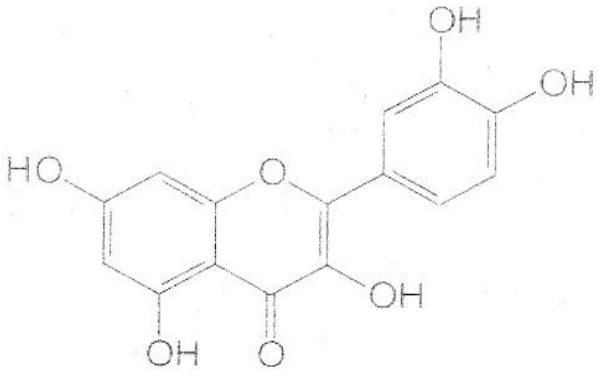


Fig 3 :



CONCLUSION

The U.V spectra of compound, CiA in different reagent showed the presence of 5,7,3'4' tetrahydroxy flavonol aglycones . IR spectra reveals the presence of hydroxyl, carbonyl, aromatic and ether group .The 1H NMR shows the presence of two meta coupled aromatic protons at H-6,H-8, position confirms the 5,7 di-substituted ring A. The C-13NMR spectrum indicate the presence carbon atoms .All the spectral data of compound CiA were found to be that of Quercetin .



REFERENCES

[1] Vaidyaratnam P.S Varier: Indian Medicinal plants Volume2, Arya Vaidyasala 124-125.
[2] KM, Nandkarni AK and Chopra RN: Indian Materia Medica, Popular Prakasan, Bombay, 3rd Ed, 1954, 31
[3] The Indian Pharmacopoeia, The Controller of publications, Government of India, New Delhi, Appendix 9, 1996, A100-A108
[4] Kokate CK, Purohith AP and Gokhale SB: Text book of Pharmacognosy, Nirali Prakasan, Pune, 29th Ed, 2004, 108-109.
[5] Kokate CK: Practical Pharmacognosy, Vallabh Prakashan, New Delhi, 4th Ed, 2005, 107-109.
[6] WHO: Quality Control Methods for medicinal plant materials Published by WHO Geneva 1998.
[7] J .B Harborne Phytochemical Methods ,A Guide to modern techniques of plant analysis third edition 45 ,78-79.
[8] Dr .Rajpal Standardization of Botanicals Vol.1 115,117,118,119.