Original Research Article

Isolation of active molecules from the stems of passion vine

ABSTRACT

Plants contain large number of phytochemical components which are bioactive and useful in the treatment of various disease conditions. But there is a need to investigate the constituents present in medicinal plants responsible for their pharmacological action. Preliminary phytochemical analysis revealed the presence of flavonoids, glycosides and steroids in the stems of *Passiflora foetida L* commonly known as Passion fruit. The present work aims at extraction, isolation & characterization of active constituents present in the stems of *Passiflora foetida L*.The ethanolic extract of stems was subjected to preliminary phytochemical tests.
Extraction was carried by a Soxhlet extractor and the following compounds were isolated from different solvents.
Three constituents were isolated, Kaempferol is a polyphenol isolated from from n- butanol extract. A flavonoid, Apigenin from ethyl acetate extract and Beta- Sitosterol a phytosterol was isolated from petroleum ether extract.
All the components isolated were characterized by IR, 1HNMR, 13CNMR and mass spectroscopical data.

Keywords: Passion vine, Keampferol,Apigenin,Beta sitosterol,spectral analysis

1. INTRODUCTION

Passion vine known as Passiflora foetida. This is also known as stinking passion flower, bush passion fruit. Since it grows widely it is also known as wild maracuja, wild water lemon, stone flower, love-in-a-mist, or running pop. It is a perennial climber [1,2]. Passion vine has number of medicinal uses, it is useful to treat worm infestation in children where the fresh, whole plant is boiled and used. The dried plant in the form of decoction is used to treat cold and cough and it is reported to have antitubercular and antispasmodic properties. It is said to improve fertility in women by utilizing the fluid obtained from pressing the leaves and stem. Fresh leaves from the plant are used as antidote for snake bite and leaves also contain antimicrobial properties [3,4]. The important steps to elicit the biologically active compound from plant resources are extraction, isolation and characterization of bioactive compound, pharmacological screening, toxicological evaluation and clinical evaluation [5-7]. Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. The basic operation includes pre-washing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system. Proper actions must be taken to assure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples [8-10]. The isolated compounds have anticancer,anti inflammatory and cholesterol lowering properties [11,12]
As plant extracts occur as a combination of various type of bioactive compounds with different polarities, their separation still remains a task for the process of identification and characterization. In isolation of these bioactive compounds,

number of different separation techniques such as TLC, column chromatography and other versatile chromatographic techniques to obtain pure compounds, structural characterization and pharmacological activity [13,14].

2. MATERIAL AND METHODS

For the present investigation the plant stem were collected from local area of Mangaluru, The plant stem was corroborated by Dr. Noeline J. Pinto, Professor and Head, Dept. of Botany, St.Agnes College, Mangaluru,Karnataka State.
Toshniwal apparatus was used for recording of melting points. Perkin – Elmer model 700 IR spectrophotometer for obtaining of IR spectra. Bruker AM 400 (400 MHz) NMR spectrometer using DMSO for 1H NMR spectra, 13C NMR was taken on Bruker AM (400-100 MHz) using DMSO as solvent. Mass spectra were taken on EI-MS.

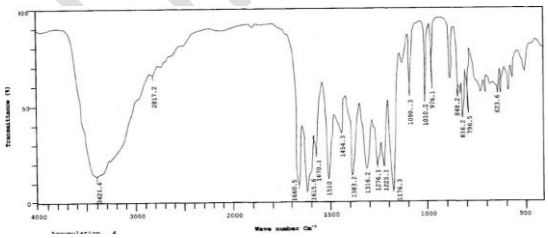
Experimental procedure for preparation of ethanolic extract
The stems of *Passiflora foetida L* (3 kg) were cleaned, shade dried and broken down into pieces, with help of a mechanical grinder coarse powder. The powder was then passed through sieve no. 40. 750 gms of powder was extracted with ethanol in soxhlet extractor for 72 hrs. . The ethanol extract was thus fractioned into n-butanol soluble extract (60 g) petroleum ether soluble fraction (30 g) and ethyl acetate soluble fraction (20 g).

n-Butanol extract: The residue (20 g) was dissolved in a small volume of methanol (5 ml) and was made into slurry with silica gel G. This was then loaded onto a silica gel column (150 g) prepared in ethyl acetate. The column was eluted with 100% ethyl acetate followed by graded mixture of 1%, 5% and 10% methanol in ethyl acetate. Elutes of the different fraction were continuously monitored by TLC [Silica gel G; ethyl acetate: methanol and visualized by UV/NH_3] .The 100% ethyl acetate and 99:1 (ethyl acetate: methanol) elutes showed similar spot. On concentration this was deposited as a yellow colored compound. It was recrystallized from methanol obtained as yellow amorphous powder and was designated as compound I (27 mg). It gave orange colour with shinoda's test for flavonoids and a yellowish green colour with NH_3, and melting point was found 3480C. The ethyl acetate soluble fraction was chromatographed over silica gel column using a solvent of CHCl3: EtOAc: MeOH; 3:2:1 to give compound I. (Eluates of the different fractions were continuously monitored by TLC and visualized by UV/NH3).and subjected to spectroscopic analysis and the spectrographs are shown in figure I(a-d) and the compound was identified as Keampferol.

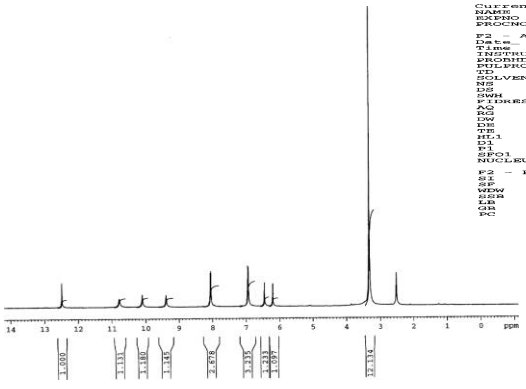
Spectral Data of I:
IR (KBr cm-1): 3421.4(O-H str), 2817(C -H str),1660(C=Ostr),1570.2(C=C str),1225.1(C-O str)
1HNMR(dmso-d6): d12.48(1H.s,5-0H),d10.70(1H.s,3-0H), d10.1(1H.s.3-OH), d9.4(1Hs.5'-OH), d8.04-d8.06(2H d. 8 Hz, H-2', H-6'), d6.8(2H, d 8 Hz , H-3', H-5'), d6.79(1H, d 2Hz, H-8), d6.16(1H, s 3Hz), d6.9(1H.s H-7), d6.45- 6.44(1H 2Hz H-6).
13C NMR(dmso-d6): d 146.75 (C-2), 135.52 (C-3), 175.79(C-4),160.61(C-5), 98.09 (C-6),163.78(C-7), 93.36 (C8), 156.09 (C-9), 103 (C-10) 121.57(C1'), 129.36(C2',6'), 115.32(C-3',5'), 159.08(C-4')
Mass Spectra (EI-MS):Molecular Formula : $C_{15}H_{10}O_6$,Molecular weight : 286
EIMS (m/z) : 286 (M+, $C_{15}H_{10}O_6$, 100%);268 (33%), 258 (15%);153(9.8%), 123 (26%); 93(29.52%), 77(8.8%), 69 (17.9%)

Fig 1:Spectral data of compound I

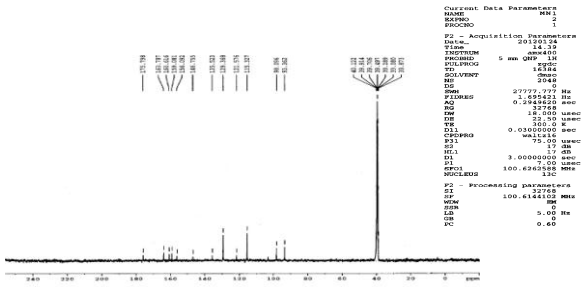
I-a (IR spectra)



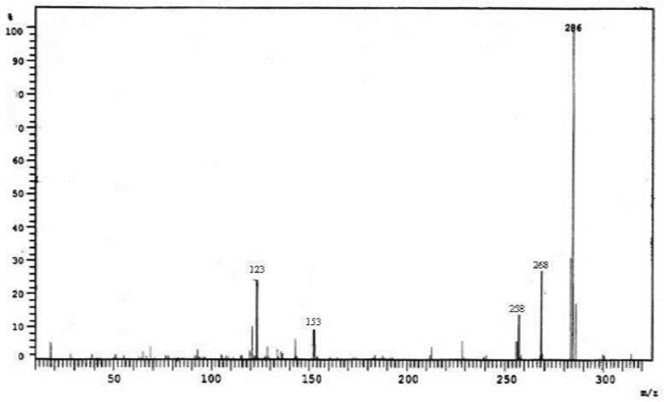
I-b (HNMR spectra)



I-c (^{13}C NMR spectra)



I-d (Mass spectra)



Petroleum ether extract:
The extract obtained from petroleum ether (20 g) was made into solution with $CHCl_3$ (20 ml) and adsorbed onto alumina (20 g),after evaporation of the solvent it is loaded onto alumina column (150 g). The extract was prepared in petroleum ether (60–80°C). The column was eluted first with petroleum ether (60-80°C), petroleum ether: chloroform (95:5, 90:10, 80:20) The elution was monitored by TLC (Silica gel G; visualization: vanillin sulphuric acid reagent heated at 110°C). Each time 10 ml were collected in a test tube and identical eluates (TLC monitored) were combined and concentrated to 15 ml and kept in a dessicator. Elution carried out with petroleum ether (60- 80°C): chloroform mixture (90:10) resulted in a spot on TLC and was designated as compound II (55mg) and found to be beta sitosterol through spectral analysis and shown in figureII(a-d) .
Physical state : Pearl white crystals
Rf Value : 0.7 (solvent system;Pet ether:$CHCl_3$,80:20) Melting Point : 138-140°C

Spectral Characterization of compound II:

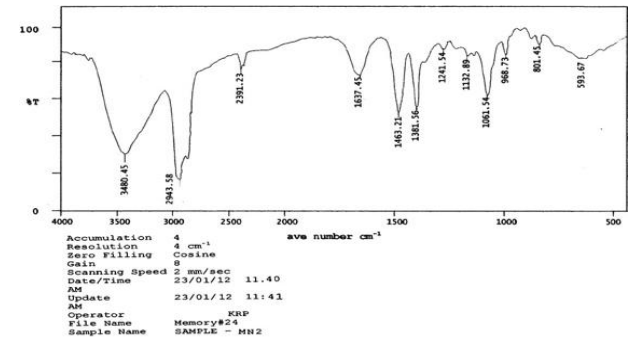
IR (KBr cm-1): 3480.1 (O-H), 2943.58, 2391.23 (C-H stretching in CH_2-CH_3)
1637.6 (C=C stretching) 1463.4(C-H deformation inCH_3),1381.56(C-H deformation in gem dimethyl)(C-O stretching),1061.5 (C-O str of secondary alcohol)
1HNMR(dmso-d6): δ 0.64 to 1.007, (18H, 6xCH_3), δ 1.03 to 1.235 (m, 22H,11xCH_2)
δ 1.44 to 2.26 (m, 8H, methane protons) δ 3.55 (br, 1H, OH),δ 5.35 (m, 1H,Vinylic proton at C-12)
13CNMR(dmso- d6):140.7(C-5), 121.67 (C-6), 56.02 (C-17),5.79 (C-13) 36.09
(C-22) 33.9 (C-1), 35.46 (C-8), 33.9 (C-23),36.0(C-10),32.36 (C-16), 31.81(C-7), 29.11(C-25), 30.23 (C-24),40.14(C-29.11(C-25),30.23(C-24),40.14(C-31.5(C-26),77.7(C-28)

Mass Spectra (EI-MS): Molecular formula $C_{29}H_{50}O$, Molecular wt:414
EIMS (m/z): 414 (M+, $C_{29}H_{50}O$, 54%), 397 (18%) 329 (12%), 303 (10%), 288 (4%),273 (10%), 255(M+)side chain H_2O, 6%), 231(10%), 199 (20%),161 (30%), 147 (34%), 133 (24%)105 (50%), 91 (76%), 71 (44%), 57 (100%).

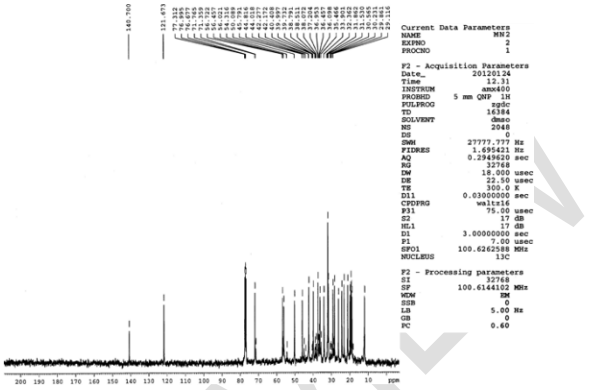
Acetylation of Compound II: 5 mg of the extract in dry pyridine (0.2 ml) and 1.0 ml of freshly distilled Ac_2O, kept at room temperature overnight, then added to crushed ice, stirred kept for 2 h, filtered and dried. The solid obtained was crystallized from benzene as white flakes, m.p. 126-128 °C

Fig II:Spectral data of compound II

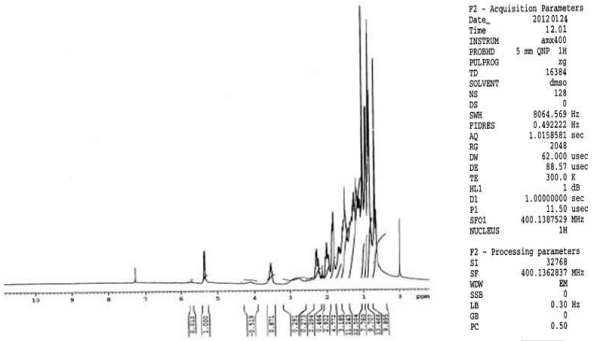
II-a (IR spectra)



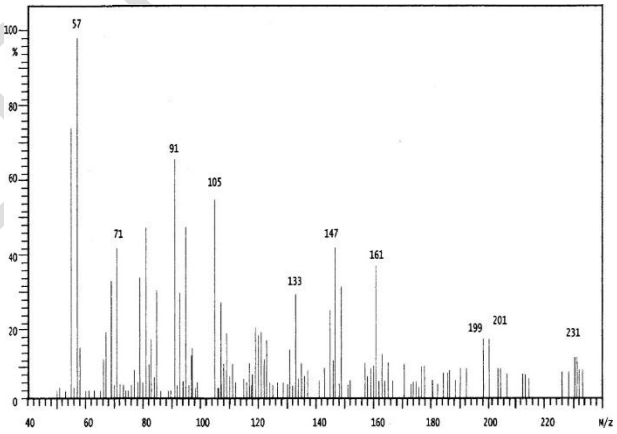
II-b (HNMR spectra)



II-c (^{13}C NMR spectra)



II-d (Mass spectra)



Ethyl Acetate Extract:

The residue (30 g) was dissolved in a small volume of methanol (5 ml) and was made into slurry with silica gel G. This was then loaded onto a silica gel column (150 g) prepared in ethyl acetate. . The column was eluted with 100% ethyl acetate followed by graded mixture of 1%, 5% and 10% methanol in ethyl acetate. Elutes of the different fraction were continuously monitored by TLC [Silica gel G; ethyl acetate: methanol and visualized by UV/NH3] .The 100% ethyl acetate and 99:1 (ethyl acetate: methanol) elutes showed similar spot. On concentration this was deposited as a yellow colored compound. It was recrystallized from methanol obtained as yellow amorphous powder and was designated as compound III. It gave orange color with Shinoda's test for flavonoids and a yellowish green color with NH_3 and melting point was found 348 0C. compound III was identified and found to be Apigenin through spectral analysis and shown in figures III(a-d).

Analysis of compound III:

Physical state : Yellow Crystals

Rf Value : 0.52 (Solvent system: EtOAc: MeOH (99:1)) Melting Point : 345 °C

Spectral Characterization of compound III :

IR (KBr cm-1): 3312.36 (br. O-H str),3093.43 (Ar. C-H str),1603.0 (C=Cstr)1667.12 (C=Ostr)

1HNMR(dmso-d6): δ 10.80 (s, 1H,3- OH),δ 10.33 (s, 1H.4'-OH),δ 10.33 (s, 1H.4'-OH)
δ 12.94 (s. 1H.7-OH),δ 7.92 (d.2H.H-2', H-6'),δ 6.92 (d,2H, H-3',H-5') δ 6.77 (d,1H,H-8)
δ 6.47 (d,1H,H-6), δ 6.18 (s,1H,H-3)

13C NMR(dmso-d6): δ 164.0 (C-2), 103.6 (C-3), 181.6 (C- 4), 161.34 (C-5),98.71 (C-6), 163.64 (C-7), 93.83 (C-8), 157.18 (C-9),102.75 (C-10), 121.08 (C-1'),115.84 (C-3',5'), 128.32 (C-6'-2'), 161.03 (C-4')

Mass Spectra (EI-MS)

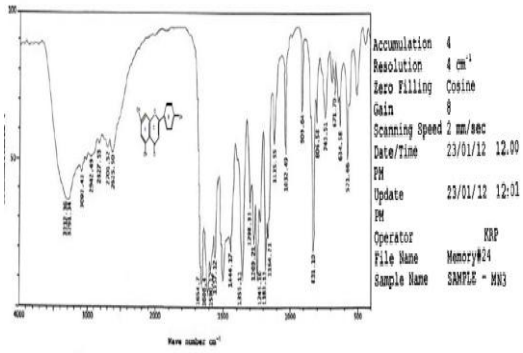
Molecular formula $C_{15}H_{10}O_5$

Molecular weight 270

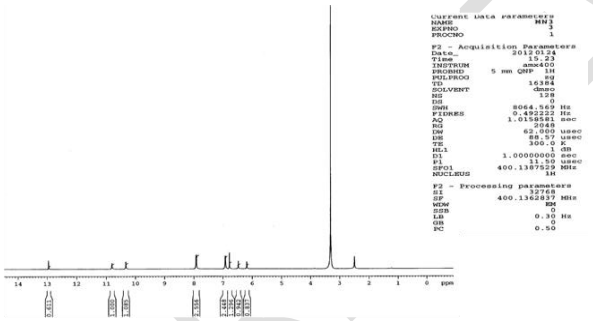
EIMS (m/z) 270 (M+, $C_{15}H_{10}O_5$, 100%), 242 (18%), 152 (22%),121(14%), 96 (8%) 69(9%).

Fig III:Spectral data of compound III

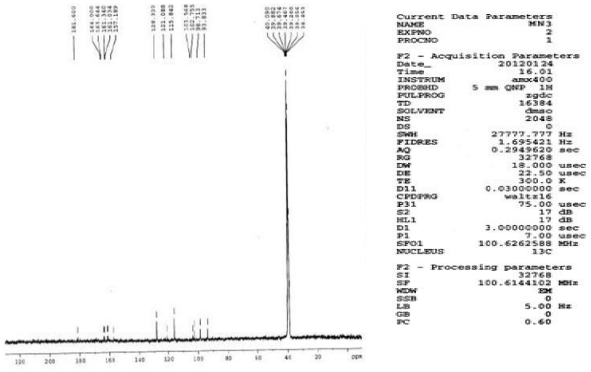
III-a (IR spectra)



III-b (HNMR spectra)



III-c (^{13}C NMR spectra)



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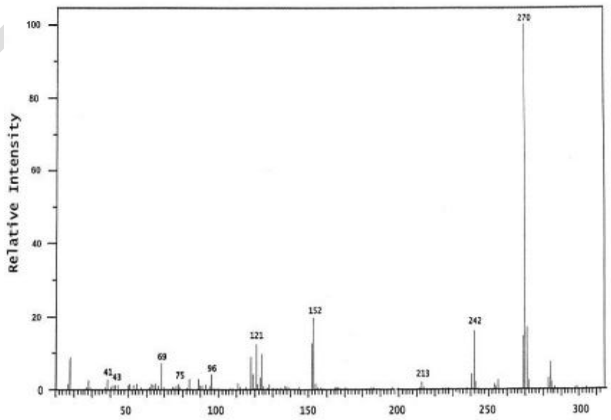


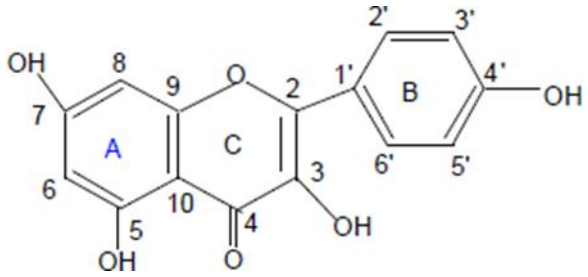
3.RESULTS AND DISCUSSION

The preliminary chemical investigation of the stems of Passiflora foetida L led to the isolation of Kaempferol from n-Butanol extract, β sitosterol from petroleum ether extract and Apigenin from ethyl acetate extract.

Kaempferol: The compound on crystallization (MeOH) yielded yellow needles with m.p 279- 278^{o}C. It gave orange colour with Mg/HCl. The molecular ion peak was observed at m/z 286. The 1HNMR spectrum showed the AB system at d 6.79 and d 8.04 and also showed two meta- coupled doublets at 6.44 and 6.45. The 13CNMR spectrum exhibited 15 carbon signals consisting of fourteen olefinic signals at d 93.36- 163.78 and a carbonyl carbon signal at d 175.79. The characteristic absorption peaks at 3427 cm -1 and 3317 cm -1 is due to phenolic O-H stretching, 2954 cm -1 and 2850 cm -1 due to C-H stretching, and at 1613 cm -1 due to C=O stretching.

These spectral data suggested that the compound I is a flavonol derivative.

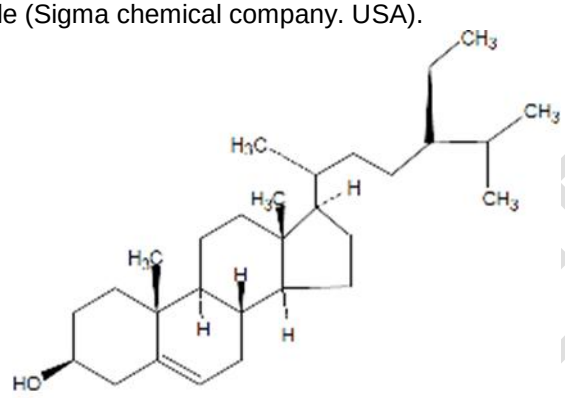
.Based on the above mentioned data and the reported chemical structures of flavonoid the structure of compound I-Keampferol was determined to be 3,4,5,7 tetra hydroxyl flavones of corresponding molecular formula $C_{15}H_{10}O_6$.



ß – Sitosterol: It gave a characteristic colour reaction for a sterol. The yellow colour obtained With tetranitromethane confirmed unsaturation in the molecule. Its acetate matched at 126-128°C . 13CNMR spectral data matched with that of s-sitosterol. The most downfield signals at δ140 was accommodated for sp2 (olefinic) carbon at C-5and the next downfield signal at δ121 to C-6 carbon the oxygenated carbon at C-3 gave a downfield signal at δ77 ppm. The next downfield at δ 56 was accommodated for C-17 other carbon atoms of the steroidal skeleton except that in the side chain appeared in the range δ45-δ30 ppm. The angular methyl groups and the side chain methyl carbon gave signal in the region δ19.8 - δ8.4 ppm.

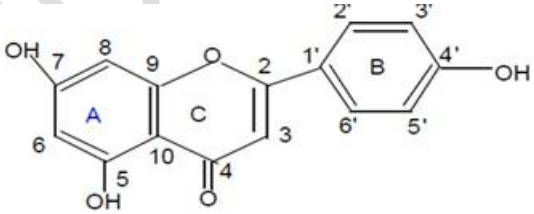
The absorption peak at 965 and 802cm -1 is due to C=C-H group. The absorption peak at 1242cm -1 is due to C-C stretching and peak at 725cm -1 indicated mono substitution in aromatic ring.

Its identity as ß – sitosterol was further confirmed by IR, 1HNMR, and mass spectral data and chromatography with an authentic sample (Sigma chemical company. USA).



β - Sitosterol

Apigenin: m.p.345 °C showed positive responses to shinoda test and a yellow green colour with NH_3. 13CNMR spectral data matched with that of apigenin. The IR spectra of apigenin showed a broad intermolecular OH stretch vibrations band at 3333 cm−1, an aromatic C–H stretch at 3040 cm−1, a vibration band at 1646 cm−1 characteristic for flavone of conjugation between the C=O and double bonded of C2–C3, and 1801 cm−1 for lactone ring. Its identity as Apigenin was further supported by the mass spectral data and confirmed by co- chromatography with an authentic sample of apigenin.



4. CONCLUSION

The three constituents isolated from the stems of Passiflora foetida L are kaempferol, beta-sitosterol and apigenin, they belong to class of flavanol, phytosterol and flavone respectively. The components isolated have various pharmacological activities. Kaempferol has anticancer and anti-inflammatory properties, beta-sitosterol has cholesterol lowering properties and reduces the risk of certain cancers and apigenin also has anti cancer properties These compounds were characterized by IR, 1HNMR, 13CNMR and Mass Spectroscopical data.

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