Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 7, 2014

Original Article

ISOLATION AND CHARACTERIZATION OF A NOVEL CHEMICAL COMPOUND FROM *EUGENIA CARYOPHYLLUS* FLOWER BUD EXTRACT

ROMA GHAI[1]*, K. NAGARAJAN[2], NEESHU GUPTA[2]

[1]Department of Pharmacology, KIET School of Pharmacy, Ghaziabad (U.P.), India, [2]Department of Pharmaceutical Chemistry, KIET School of Pharmacy, Ghaziabad (U.P), India.
Email: romaghai30@gmail.com

Received: 23 May 2014 Revised and Accepted: 05 Jul 2014

ABSTRACT

Objective: The objective of this study was to isolate and characterize the bioactive principles from the aqueous ethanolic extract of flower buds of *Eugenia caryophyllus*.

Methods: The isolation was done using column chromatography using gradient elution with different mobile phases. The isolated compound was subjected to spectral analysis. Structure elucidation was carried out on basis of spectral analysis.

Results: The infra-red spectra showed specific absorption bands for flavanoids viz. 1245.86 to 1111.01 cm^{-1} for HCO stretch of ether; 1330 to 1050 cm^{-1} for CO stretch of lactone. In addition IR spectrum showed specific absorption bands for flavanoids viz. 3432.57cm^{-1} for OH stretch for alcohol and phenol. Mass spectra showed pseudomolecular ion (M^+ion) peak at m/z 464 which corresponds to molecular formula $C_{21}H_{20}O_{12}$.

Conclusion: From the spectral characteristics, the isolated compound from the extract was confirmed to be Gossypetin 7-O rhamnopyranoside (Rhodiolgin).

Keywords: *Eugenia caryophyllus*, Myrtaceae, Flower buds, Gossypetin, *Syzygium aromaticum*

INTRODUCTION

Natural products like plant extracts, either as pure compounds or as standardized extracts provide unlimited opportunities for new drug discoveries due to unmatched availability of chemical diversity [1]. According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [2]. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Due to various adverse effects of the available synthetic drugs, men have turned to ethnopharmacognosy. Since plant extracts usually occur as a combination of various bioactive compounds or phytochemicals with different polarities, isolating the pure compound becomes very necessary and it holds a big challenge. Column chromatography technique is the most widely used for separation, isolation & purification of chemical constituents from natural drugs.

Eugenia caryophyllus is a small ever-green tree that belongs to the botanical family *Myrtaceae* (subfamily: *Myrtoideae* and tribe: *Syzgieae*) and scientifically known as *Syzygium aromaticum* (L.) Merr. & L.M. Perry. Dried flower buds of *Eugenia caryophyllus* are used as a spice. The essential oil obtained from the buds of *Eugenia caryophyyllus* L. is widely known for its medicinal properties. Chemical analysis has identified the major constituents as eugenol, eugenyl acetate, beta-caryophyllene, 2-heptanone [3]. Its oil has anti-oxidant properties and in dentistry used as a topical application to relieve pain and to promote healing and also finds use in the fragrance and flavouring industries. The essential oil has shown to have anti-microbial activity [4,5] and anti-fungal properties against dermatophytes [6,7]. In addition, it is found to have antimutagenic [8], anti-inflammatory [9], antiulcerogenic [10,11], antithrombotic [12] and antiparasitic [13] and an anti-convulsant [14] activity. The anti-oxidant effect of eugenol has been reported on carbon tetrachloride induced erythrocyte damage in rats [15]. Pharmacological studies with *Eugenia caryophyllus* extract have also demonstrated anti-stress activity [16], analgesic activities [17,18] and found to be more potent than aspirin in inhibiting platelet aggregation [19]. Due to such wide therapeutic activities, it was decided to investigate bioactive compounds from aqueous ethanolic extract of *Eugenia carophyllus* flower buds.

MATERIALS AND METHODS

Plant materials

The flower buds of *Eugenia caryophyllus* was collected from an authorized vendor Global Herbs and authenticated by Professor Mohammed Ali, Department of Pharmacognosy, Faculty of Pharmacy, Jamia Hamdard, New Delhi. A voucher specimen coded PRL/JH/11/01 was deposited in the Jamia Hamdard.

Extraction procedure

The flower buds of *Eugenia caryophyllus* were dried at room temperature and reduced to coarse powder. The powdered material was subjected to qualitative tests [20,21] for the identification of various phytoconstituents like alkaloids, flavanoids, phenols, phytosterols etc. Then the powder was subjected to cold maceration with 70% ethanol for 7 days at room temperature. After 7 days, it was filtered and the filtrate was concentrated on water bath to obtain a dark brownish residue.

Isolation

A column tube 1ft X 2.5 cm was taken and dried. The lower end of the column was plugged with absorbent cotton. The column was clamped and fitted in vertical position on a stand. The column was then half filled with n-hexane & silica gel slurry poured in small portions and allowed to settle gently till a necessary length of the column was obtained. The extract which was adsorbed in silica gel, then poured onto the bed of silica, covered it again with a layer of cotton wool and more amount of solvent was poured over it. The column was then run by gradient elution technique. 70% ethanolic extract was subjected to column chromatography using different solvent systems (100% benzene, 75%benzene: 25% chloroform, 50% benzene: 50% chloroform, 25% benzene: 75% chloroform, 100% chloroform, 75% chloroform: 25% acetone, 50% chloroform: 50% acetone, 25% chloroform: 75% acetone, 100% acetone, 60% acetone: 40% ethyl acetate, 40% acetone: 60% ethyl acetate, 100% ethyl acetate, 80% ethyl acetate: 20% methanol, 60% ethyl acetate: 40% methanol, 40% ethyl acetate: 60% methanol, 20% ethyl acetate: 80% methanol, 100% methanol, 80% methanol: 20% water, 60% methanol: 40% water, 40% methanol: 60% water, 20%

methanol: 80% water, ethylacetate-methanol-water (10:1.35:1) and ethyl acetate: formic acid: glacial acetic acid: water (10:1.1:1.1:2.6) in a glass column.

All the fractions collected in the conical flasks were marked. The marked fractions were subjected to thin layer chromatography to check the homogeneity of various fractions.

Elution of drug in column using methanol: water (0.4:0.6) (fraction No. 20) designated as F_{20} yielded darkish orange coloured residue which was subjected to characterization.

Physiochemical characterization

One pure bioactive was isolated and the isolated pure fraction was tentatively identified using qualitative chemical analysis. Further identification & characterization was done using I.R, NMR & Mass spectroscopy. Infrared (I.R) Spectra analysis was recorded on FT-IR 6700 Thermoscientific (Mcleods) spectrophotometer.

The isolated test compound was subjected to IR using KBr/chloroform for the study of absorption of infra-red radiation with its corresponding functional groups clearly [22]. The ^{1}H-NMR & ^{13}C-NMR were recorded at room temperature using Bruker Avance III 500 NMR spectrophotometer and the solvent used was DMSO. The chemical shifts were given in δ (ppm) value relative to TMS as internal standard. The mass spectral analysis was performed on micro TOF QII. Electrospray ionization was used as a source.

The scan began from 50m/z and end at 1500m/z in low tune method. In wide tune method, scan began from 50m/z and end at 3000m/z.

RESULTS AND DISCUSSION

The IR spectra of the isolated compound is shown in Fig.1. The ^{1}H NMR spectra is shown in Fig. 2. ^{13}C NMR spectra of the isolated compound are shown at δ<200 ppm, δ<180 ppm, δ<65 ppm and δ<63 ppm respectively in Fig.3-6. The mass spectra of the isolated compound are expressed in Fig.7 (ranging from 370-505 m/z ratio) and in Fig. 8 ranging from 320-500m/z ratio. The interpretation of all the spectras are given in Tables 1 to 4. An IR spectra shows an absorption peak at 2864.02 cm^{-1} which indicates an ether linkage in the compound which is further confirmed by its ^{1}H NMR & ^{13}C NMR with singlet at δ 4.106 ppm and δ 153.16 ppm respectively. Presence of the alcohol/ phenol is confirmed by the presence of absorption peak at 3432.57 cm^{-1} in the IR spectra and a narrow long singlet is observed at δ 3.095 ppm in ^{1}H NMR. Further the presence of lactone in sugar, ketonic linkage, aromatic hydrocarbon and cycloalkane are confirmed and the values are given in the Tables 1, 2 and 3. Mass spectral analysis also indicates the evidence of alcohol, gossypetin methyl ester, terminal vinyl, some ethyl esters and butyl esters with the corresponding M^{+} ion peak at m/z 464 (Table:4). All these spectral data, suggests that the isolated compound eluted from the column chromatography was found to be Gossypetin 7-O-Rhamnopyranoside (Rhodiolgin) and have chemical formula $C_{21}H_{20}O_{12}$ (Fig.9).

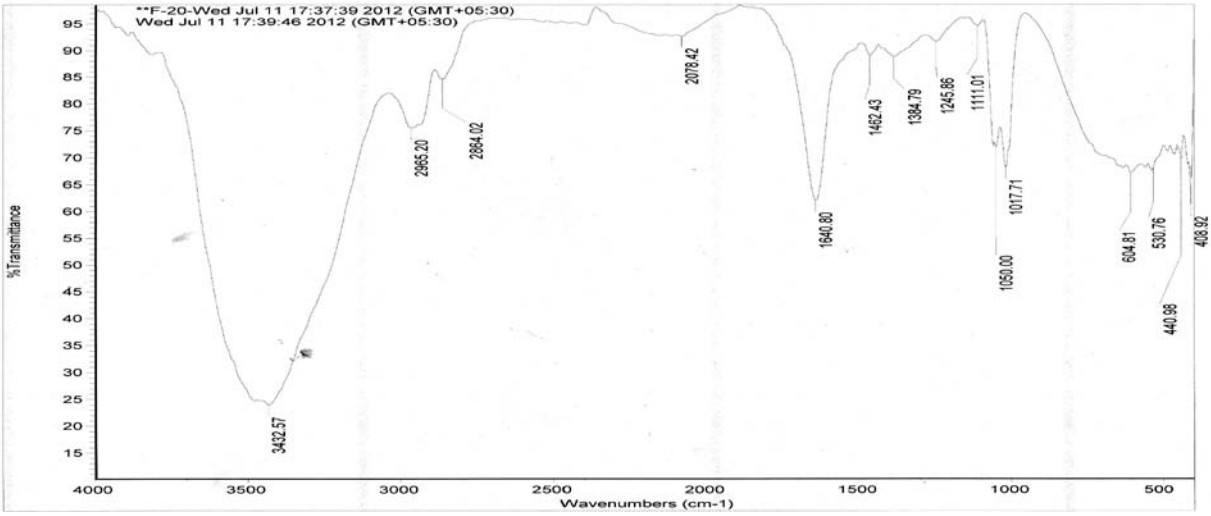


Fig. 1: IR spectra of the isolated lead compound

Table 1: Interpretation of IR spectra of isolated compound

S. No	Wave Number cm^{-1}	Characterstic functional group	Compound type/Expected group
1.	3432.57	-O-H st	Alcohols/Phenols
2.	2864.02	H-C(-O-)st.	Ether
3.	1640.80	C=Ost.	Ketone
4.	1450.00	Ar-C-C st	Benzene ring
5.	1245.86	C-O st.	Lactone in sugar
6.	1017.71	C-O(H)st	Alcohols/Phenols
7.	604.81	=C-H δ bend	Alkene

Table 2: Interpretation of ^{1}H-NMR spectra of the isolated lead

S. No.	Assignment	Range (delta)	Nature of peak	Comments/significance
1.	CH_2-O	4.106 ppm	Broad elongated singlet	Presence of Ether
2.	-CH-OH	3.095 ppm	Narrow long singlet	Presence of alcohol & phenol of sugar
3.	CH-(C)	2.859	Narrow low singlet	Presence of aromatic hydrocarbon
4.	CH_2-(C)	1.844	Narrow singlet	Presence of aromatic hydrocarbon
5.	CH_2-(C=C)	1.837 (scale)		Presence of cycloalkane
6.	CH2	1.044	Triplet	Presence of alkanes

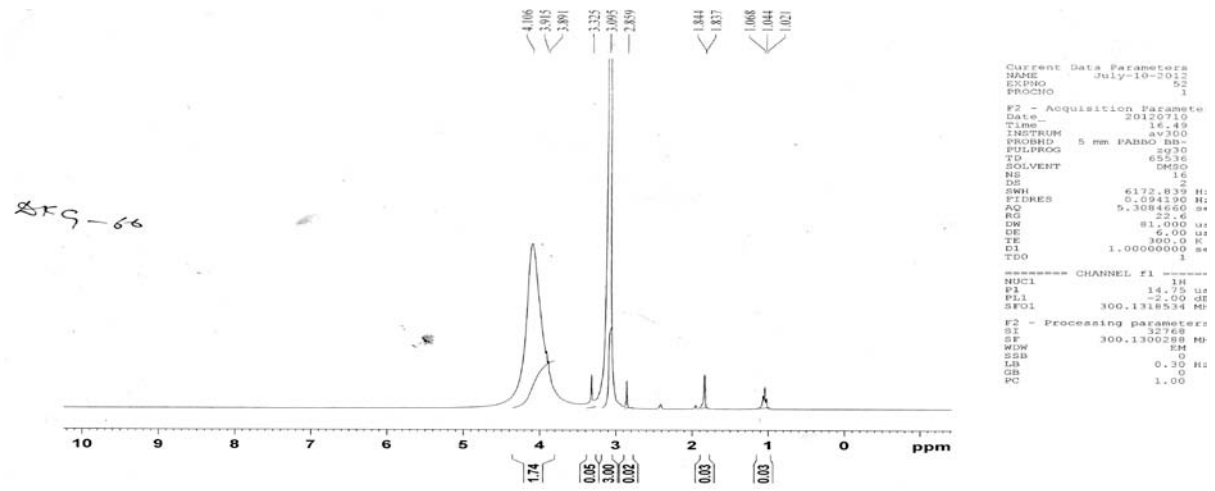


Fig. 2: ^{1}H-NMR spectra of the isolated lead compound

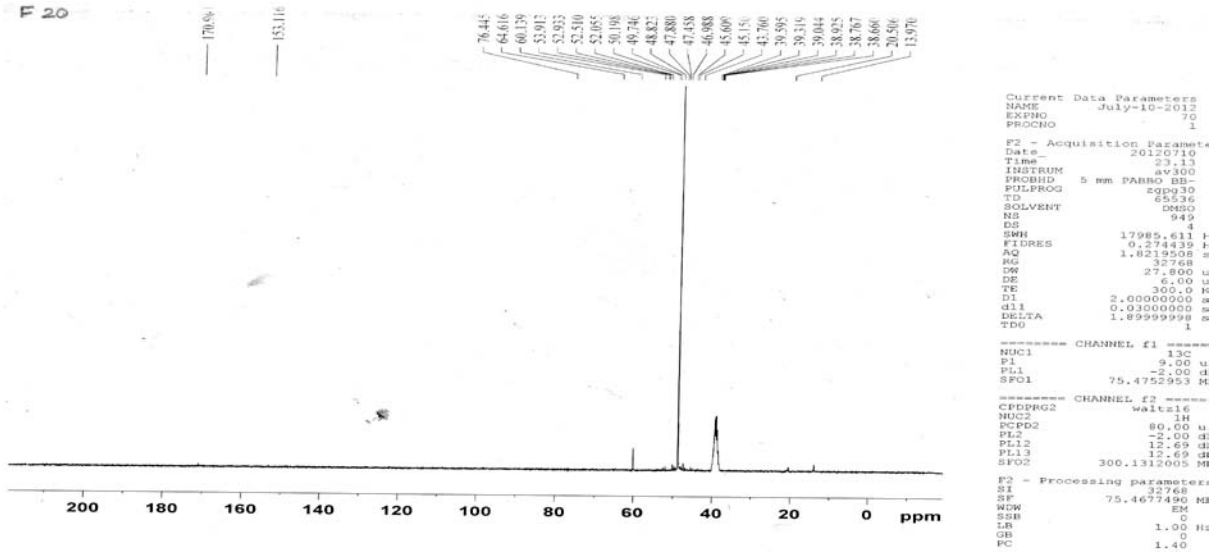


Fig. 3: ^{13}C- NMR spectra of the isolated lead compound (δ <200 ppm)

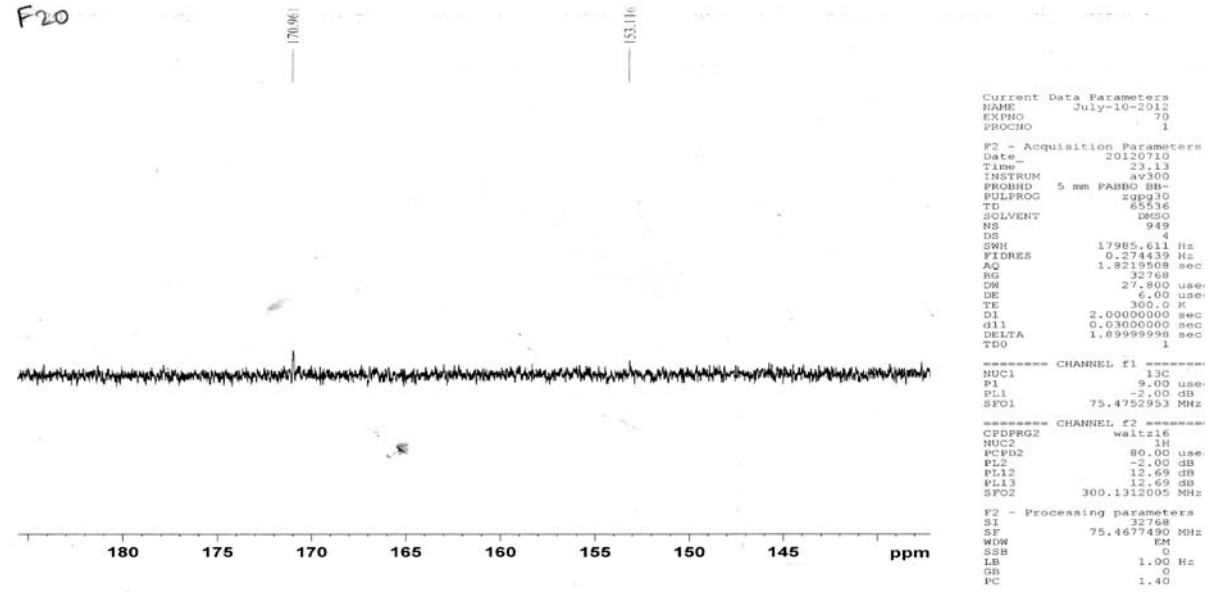


Fig. 4: ^{13}C- NMR spectra of the isolated lead compound (δ<180 ppm)

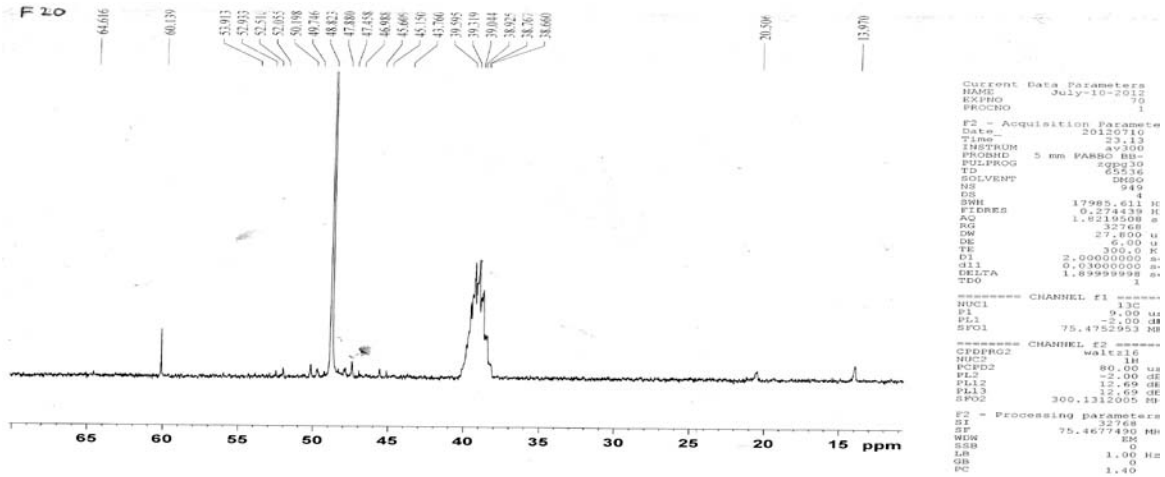


Fig. 5: ^{13}C- NMR spectra of the isolated lead compound (δ<65 ppm)

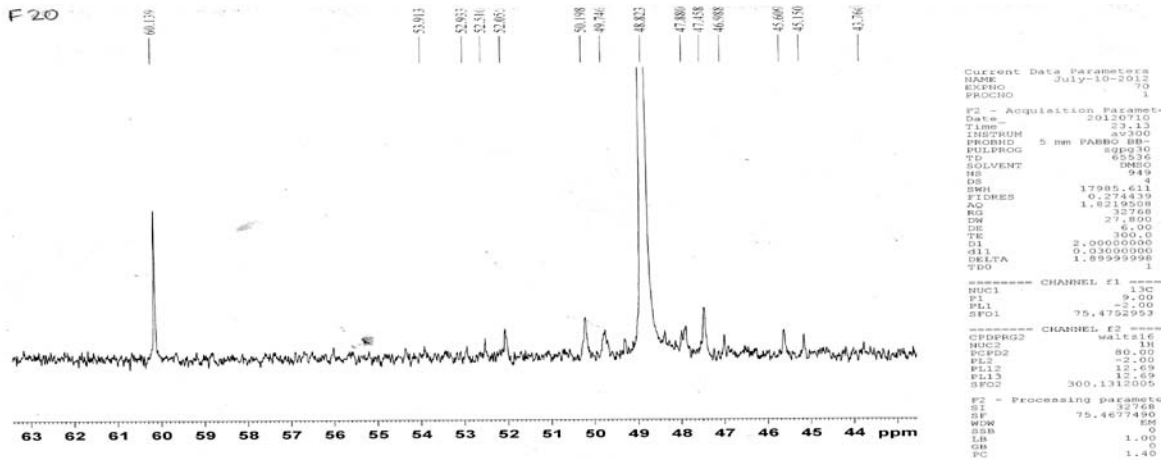
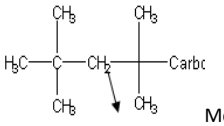
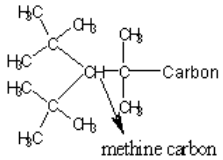
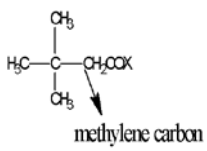


Fig. 6: ^{13}C- NMR spectra of the isolated lead compound (δ<63 ppm)

Table 3: Interpretation of ^{13}C NMR spectra of the isolated lead compound

S. No.	Assignment	Range observed	Nature of peak	Comments/Expected group
1.	COOR group	170.961	Singlet	Carboxylic ester and lactone (sugar)
2.	Ar-C-X or Ar-C-OH or Ar-C-O	153.16	Singlet	Heteroaromatic compound Alcohol and Phenol Ether
3.	 Methylene Carbon	60.139	Singlet	methylene Carbon
4.	  X=C,O,N	48.823	Singlet	Methine Carbon Methylene Carbon
5.	CH-Carbon	20.506	Singlet	alkane or cycloalkane
6.	CH2 carbon	13.970	Singlet	methylene Carbon
7	d_6-DMSO	39.319	Triplet	Solvent

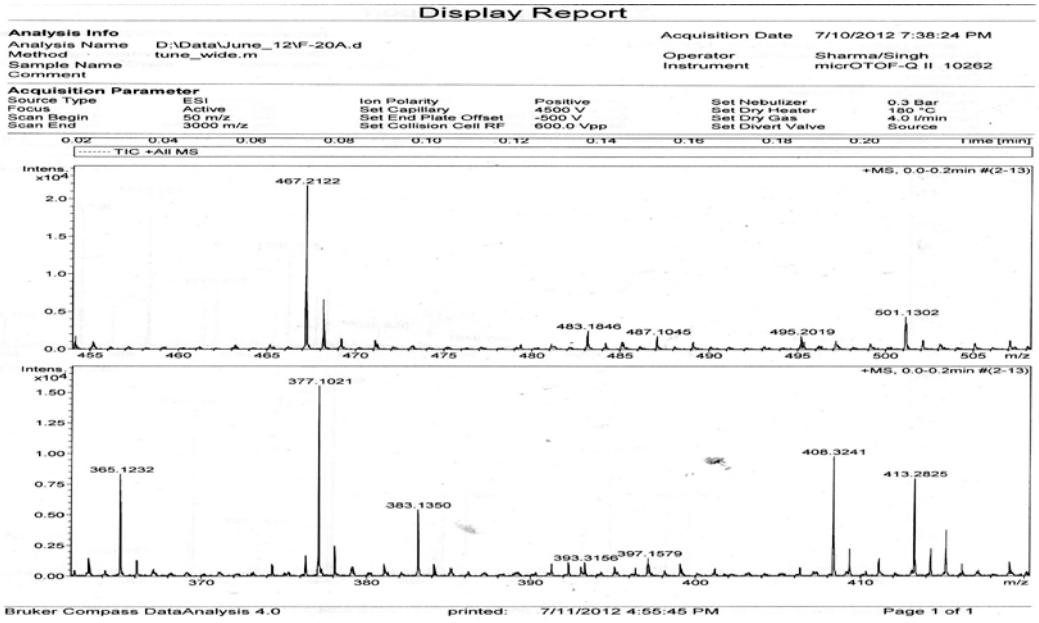


Fig. 7: Mass spectra of the isolated lead compound (370-505 m/z ratio)

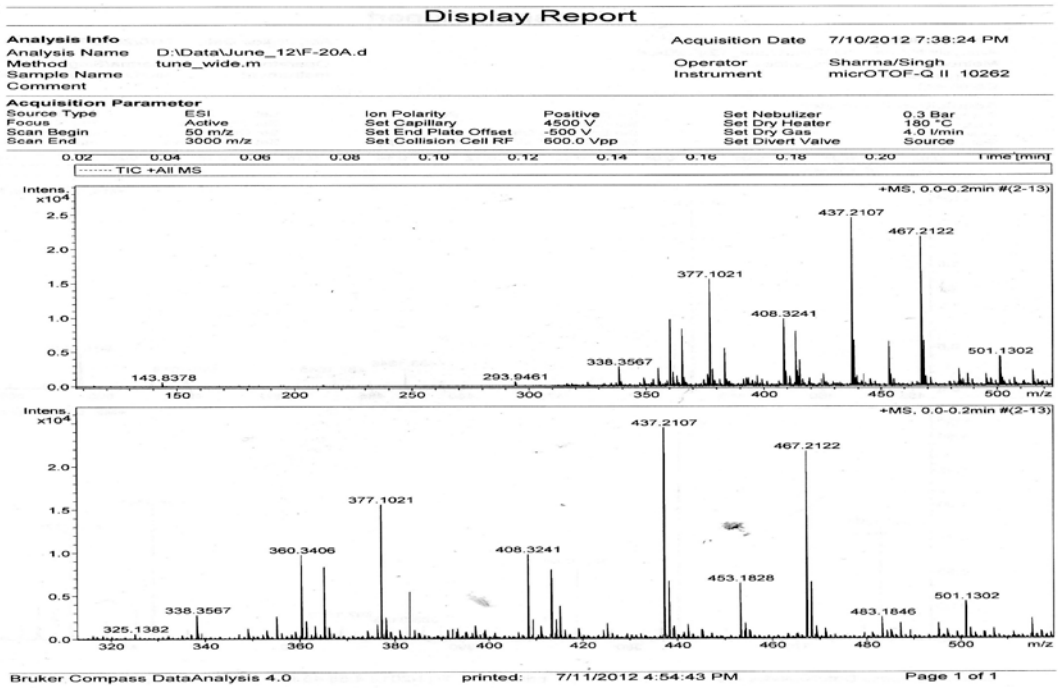


Fig. 8: Mass spectra of the isolated lead compound (320-500m/z ratio)

Table 4: Mass spectral interpretation of the isolated lead compound

S. No.	Mass	Ion	Product ion & composition of neutral particle lost/Adduct peak formation	Sub-structure or compound type	m/z ratio of the compound fragmented peak
1.	23	Na^+	$[M+23]^+$	Gossypetin methyl ester In presence of Na+	487
2.	27	$C_2H_3^+$	$[M-27]^+$ (C_2H_3)	Terminal vinyl, some ethyl esters and N-ethylamides	437
3.	56	$C_4H_8^+$, $C_3H_4O^+$	[M-56]+ (C_4H_8), $C_3H_4O^+$	Butyl esters Methyl cyclohexenone	408
4.	87	$C_5H_{11}O^+$, $C_4H_7O_2^+$	$[M-87]^+$ ($C_5H_{11}O$), ($C_4H_7O_2^+$)	Alcohol, ethylester Esters, acid	377
5	104	$C_8H_8^+$	$[M-104]^+ C_8H_8$	Phenylethyl derivative	360

CONCLUSION

From the interpretation of all the spectras, the novel compound was elucidated to have chemical formula $C_{21}H_{20}O_{12}$ and the structure (Fig.9) was confirmed to be flavanoid Gossypetin 7-O-Rhamnopyranoside (Rhodiolgin), a flavanoid class compound.

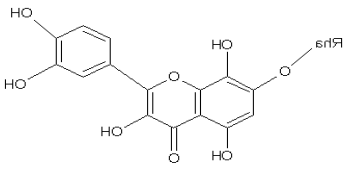


Fig. 9: Structure of Gossypetin 7-O-Rhamnopyranoside (Rhodiolgin)

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

ACKNOWLEDGEMENT

We remain highly grateful to Dr. Narendra Kumar, Director KIET Group of Institutions, Dr. UmaKant Bajaj, Principal KIET School of Pharmacy & Dr. Vinay Kumar (Head of Department,Pharmacology) for their co-operation and moral support with necessary facilities.

The authors are very much thankful to Mr. Dharmendra Singh (Lab Instructor, Pharmaceutical Chemistry department) and Mr. Kapil Kr. Sharma (Lab Instructor, Research Pharmacology Lab) who helped in carrying out such challenging task of isolation.

REFERENCES

1. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. Extraction, isolation and Characterization of Bioactive compounds from Plant extracts. Afr J Tradit Complement Altern Med 2011;8(1):1-10.
2. WHO Report on Traditional Medicine WPR/RC 52/76th. Aug. 2001.
3. Chaieb K, Hajlaoui H, Zmantar T, Nakbi ABK, Rouabhia M, Mahdouani K, *et al*. The chemical composition and biological activity of essential oil, *Eugenia cryophyllata* (*Syzygium aromaticum* L. Myrtaceae):a short review. Phytother Res 2007;21(6):501-6.
4. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu India., BMC Complement Altern Med 2006;6:35.
5. Fu Y, Zu Y, Chen L, Shi X, Wang Z, Sun S, *et al*. Antimicrobial activity of clove and rosemary essential oils alone and in combination. Phytother Res 2007;21:989–94.
6. Park MJ, Gwak KS, Yang I, Choi WS, Jo HJ, Chang WJ, *et al*. Antifungal activities of the essential oils in *Syzygium aromaticum* (L.) Merr. Et Perry and *Leptospermum betersonni* Bailey and their constituents against various dermatophytes. J Microbiology 2007;45:460-65.
7. Cai L, Wu CD. Compounds from Syzygium aromaticum possessing growth inhibitory activity against oral pathogens. J Nat Prod 1996;59(10):987-90.
8. Miyazawa M, Hisama M, Antimutagenic activity of phenylpropanoides from clove (*Syzygium aromaticum*). J Agric Food Chem 2003;51(22):6413-22.
9. Magalhaes CB, Riva DR, DePaula LJ, Brando-Lima A, Koatz VL, Leal-Cardoso JH, *et al*. In vivo anti-inflammatory action of eugenol on lipopolysaccharide-induced lung injury. J Appl Physiol (1985);108(4):845-51.
10. Li Y, Xu C, Zhang Q, Liu JY, Tan RX. *In vitro* anti-Helicobacter pylori action of 30 Chinese herbal medicines used to treat ulcer diseases. J Ethnopharmacol 2005;98(3):329-33.
11. Bae EA, Han MJ, Kim NJ, Kim DH. Anti-Helicobacter pylori activity of herbal medicines. Biol Pharm Bull 1998;21(9):990-92.
12. Srivastava KC, Malhotra N. Acetyl Eugenol, a component of oil of cloves (*Syzygium aromaticum*) inhibits aggregation and alters arachidonic acid metabolism in human blood platelets. Prostaglandins Leukot Essent Fatty Acids 1991;42:73-81.
13. Cosa P, Vlientinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: How to develop a stronger *In vitro* 'proof-of-concept'. J Ethnopharmacol 2006;106:290-302.
14. Pourgholami MH, Kamalinejad M, Jayadi M, Majzoob S, Sayyah M. Evaluation of anti-convulsant activity of essential oil of *Eugenia caryophyllata* in male mice. J Ethnopharmacol 1999;64(2):167-71.
15. Kumaravelu P, Subramaniyam S, Dakshinamoorthy DP, Devaraj NS. The antioxidant effect of eugenol on CCl_4-induced erythrocyte damage in rats. J of Nut Biochem 1996;7(1):23-8.
16. Singh AK, Dhamanigi SS, Asad M. Anti-stress activity of hydro-alcoholic extract of *Eugenia caryophyllus* buds (clove). Indian J Pharmacol 2009;41:28-31.
17. Hossseini M, Kamakar M,Rakhshandeh H. Analgesic effect of clove essential oil in mice. Avicenna J Phytomed 2011;1(1):1-6.
18. Kamkar M, Nazaribourun A, Hosseini M. Analgesic effect of aqueous and ethanolic extracts of clove. Avicenna J Phytomed 2013;3 (2):186-92.
19. Srivastava KC. Antiplatelet principles from a food spice clove (*Syzgium aromaticum*). Prostaglandins, Leukotrienes Essent. Fatty Acids 1993;48:363-72.
20. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 41st ed. India:Nirali Prakashan;2008.
21. Harborne JB. Phytochemical Methods:A guide to modern techniques of plant analysis. 3rd ed. UK:Springer;1998.
22. Silverstein RM, Webster FX. Spectrometric identification of organic compounds. 6th ed. India:Wiley;2005