International Journal of Research and Development in Pharmacy and Life Sciences
Available online at http//www.ijrdpl.com
August - September, 2014, Vol. 3, No.5, pp 1180-1188
ISSN: 2278-0238

Research Article

PHYTOCHEMICAL EXAMINATION AND GC-MS STUDIES OF THE MEDICINAL PLANT - *Naravelia zeylanica*

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(Received: May 17, 2014; **Accepted:** July 12, 2014**)**

ABSTRACT

In this study the phytochemical analysis of leaves of Naravelia zeylanica (Ranunculaceae) plant in various ether extracts were taken up. The GC-MS analytical studies on the ethanol extract, revealed the presence of 17 compounds of different types like alkaloids, flavonoids, phenolics, terpenoids, etc. One of those phenolic type of compound was isolated by the chromatographic method and was characterized by chemical and spectral studies.
Keywords: GC-MS, phytochemicals, Naravelia zeylanica.

INTRODUCTION

Naravelia zeylanica is a small genus woody climber distributed in Himalayas (Wealth of India, 1998). Roots are tuberous, leaves with two opposite ovate, cordate leaflets and a terminal 3 branched tendril, flowers in pinacles, small with pleasant scent, achenes red with long feathery styles. The plants are propagated by seeds or cuttings. The stems can be twisted into strong ropes. They are also reported to be used as tooth sticks to cure toothaches. Roots when crushed emit a smell which is said to relieve headache(Gamble, 1915).It is used as an astringent,anti-inflammatory , anthelmintic , rheumatic pain ,wounds ,ulcers, intestinal worms leprosy and skin diseases (Khare, 2007). The micro propagation of the plant, isolation and characterization of berberine from leaves of Naravelia zeylanica was carried out (Raja Naika and Krishna, 2008). Antiulcer activity of the leaf extract of Naravelia zeylanica was reported(Shenoy et al., 2009). The quantitative estimation, antioxidant studies on aerial parts of Naravelia zeylanica were investigated(Suther Singh et al., 2011). The anti-inflammatory activity of Naravelia zeylanica leaves extract was reported (Moses et al., 2012). The present study focus to characterize and analyse the phytochemicals by GC-MS, which will throw more insight into identifying the formula of bimolecular therapy in drug studies. A phenolic compound (C9H10O2) was identified based on various chemical and spectral analysis.

Experimental

The fresh plant (Naravelia zeylanica) samples were obtained locally from the Kolli Hills,Trichy. The plant species was verified with authentic specimen at Rapinat Herbarium, Trichy, Tamilnadu,India. The leaves were washed in tap water; shade dried, crushed and was taken for various phytochemical analysis.

The shade – dried plant material was cut into pieces and packed in a wide-mouthed bottle (2 lit). The moisture free

ethanol was poured into the bottle just to soak the plant material completely. The bottle was closed air-tight and allowed to stand for 72 hours, undisturbed. After 72 hours, ethanol was collected in a pure dry bottle (2 lit). The ethanolic extract was subjected flash-evaporation to get the concentrated extract (Harborne, 1973).

The concentrated ethanolic extract was subjected to various qualitative tests for the presence of the different kinds of metabolites. Thus, the phytochemical screening was carried out. The ethanolic extract was divided into two parts. One part was treated with Sodium hydroxide and then with ether. The top organic layer was taken as Ether Layer I. The bottom aqueous layer was neutralized with hydrochloric acid and further extracted with ether to get Ether Layer II (Di Stefano, 2011). The second part was treated with hydrochloric acid and then with ether. The top organic layer was taken as Ether Layer III. The bottom aqueous layer was neutralized with Sodium hydroxide and further extracted with ether to get Ether Layer IV. The extraction scheme is given in (Figure 1).

The ether layer II was taken for study as it was screened positively for the phenolic type of compound. The extract was chromatographed (Table 1) by preparative-TLC using Benzene : Ethyl Acetate (8:2) as the eluant and silica gel (100 m mesh size) as stationary phase. The compound NZ2 was isolated. It was recrystallized from acetone and its taken for melting point measurement. Then, the compound NZ2 was subjected to the routine chemical and spectroscopic analyses. The structural characterization was done with UV-VIS,IR, H-NMR,C-13-NMR and Mass spectral studies .

GC-MS studies

The GC-MS analysis of ethanolic extract of leaves of Naravelia zeylanica was performed on GC Clarus 500 Perkin Elmer interfaced to a mass spectrometer (GC-MS). The conditions were as follows:

- Column Elite -5ms fused silica capillary column (30× 0.25 mm ID x 0.25µm film thickness, composed of 5% phenyl 95% Dimethyl polysiloxane
- Carrier gas Helium (99.999% purity) was at a constant flow of 1 ml /min temperature 270 ºC
- Ion-source temperature was at 200ºC.
- The oven temperature was programmed from 50ºC, with an increase of 8ºC/min, to 250ºC hold for 5 min.
- Mass spectra were taken at 70eV (electron ionization technique) at a scan interval of 0.2 seconds and fragments were scanned from 40 to 600 Da.

The database of National Institute of Standard and Technology (NIST 2005) having more than 62,000 patterns was referred for the Interpretation on mass spectrum of each component that got separated by the Gas-chromatograph. The spectrum of the separated components was compared with the spectrum of NIST library database for about 95% matching to predict the compound. The details of the characterization studies are given in Table 2.

The given chemical conversions in (Figure 2) indicated position of side groups in the phenolic compound. Treatment of the phenolic compound with CH2I2 indicated the presence of -OH at 1and 2 position (Finar, 2005). When the phenolic compound was treated with alkaline KMnO4 to give a 3, 4-dihydroxy benzoic acid that indicated the presence of alkyl substituent 4th position to that of the phenolic functionality (Vogel, 2009). Using mild oxidizing agent, the phenolic compound yielded a diol type of product. It was on further treatment with K2Cr2O7 and H2SO4 formed ketone (Fieser,1981).

RESULTS AND DISCUSSION

The results of the GC-MS analysis of the ethanolic extract of the leaves of Naravelia zeylanica are listed in Figure 2. The list of constituents are given in Table 3. The major components were Benzaldehyde, 3-hydroxy-4-methoxy-(11.89); Dihydroactinidiolide (15.38), 3,4-Pyridinedimethanol, 6-methyl-(16.48); 4-Hydroxy-á-ionone (17.07); 5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol(19.30); Hexadecanoic acid, methyl ester(20.86) and phytol (22.86).

The GC-MS study of the ethanolic extract of the leaves of Naravelia zeylanica had shown the presence of many phytochemicals which might contribute to the medicinal activity of that plant.

The UV-VIS spectral data of phenolic was found to show a strong band (K band) at 213 nm (12640) represent the π-π* transition. It was due to the presence of –C=C-double bonds of the aromatic ring. A weak band (B band) at 276 nm (2580) due to the forbidden transition of pi-electrons in aromatic -C=C- bond. The strong absorption K band at 248.1 nm (14583) and weak band at 287.4nm (653) were

Table 1: The summary of chromatographic separation of some compounds

Plant Name	Solvent Ratio	Ether layer I & III	Ether layer II	Ether layer IV
Naravelia zeylanica	Benzene: EtOAc 8:2		NZ 2 (Phenolic)	
	EtOH:EtOAc 8.5:1.5		NZ 3 (Flavanoid)	
	$CHCl_3$: C_6H_6 6:4	NZ 1 (Terpenoid)		
	Benzene: EtOAc 8:2			NZ 4 (Alkaloids)

Table 2: Experimental Data of the Compound NZ2

S.No.	Compound Label	NZ2
1	Molecular Formula	$C_9H_{10}O_2$
2	Molecular Mass	150.18g/mol
3	Optical Activity	Inactive
4	Solubility	Polar solvents like chloroform, methanol, acetone
5	**Chemical Tests**	
	i. Elemental Analysis % Composition	%C 74.95 , %H 10.78, %O 14.26
	ii. Test for unsaturation	To the extract added 1ml bromine water.
	iii. Test for aromatic nature	The extract was taken in a Ni spatula and kept in flame
	iv. Test for phenol	To the small portion of the extract added 1ml of alcohol and neutral $FeCl_3$.
6	**Spectral Data**	
	UV-VIS Shimadzu UV-2100 $CHCl_3$, λ_{max} nm, (λ_{max})	213.8(12640), 248(14580), 276.5(2580), 287(653)
	FT-IR JASCO KBr Pellet ν (cm^{-1})	2968m, 2885s,3085s,1640s, 991s, 1350m, 3010s, 1604-1499m, 816-746s, 546m, 3561m, 3369s, 1380m, 693s,1234s, 3067 b,m.
	^{1}H-NMR Bruker 400MHz $CDCl_3$, TMS $d_{(ppm)}$	9.412s, 6.561d, 6.764d, 6.56d,2.016s, 4.892d, 5.337d
	^{13}C-NMR Bruker 400MHz $CDCl_3$, TMS , $d_{(ppm)}$	114.21, 139.91, 142.14, 115.43, 142.92 120.83, 26.51, 155.9, 107.55
	Micro Mass ESI-TOF *m/z*	M$^+$ **150.175** ,58.036,92.138, 51.067, 148.159, 107.087, 121.157, 108.138,135.140, 109.103, 135.140, 15.035, 41.072, 110.111, 40.064

Table 3. Phytoconstituents of the ethanolic extract of the leaves of *Naravelia zeylanica* (GC-MS studies)

S.No	Peak Name	Retention Time	Activity	% Peak area
1	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.70	Antimicrobial, antiinflammatory	0.8957
2	(S)-(+)-2',3'-Dideoxyribonolactone	7.31	Antimicrobial	12.0056
3	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	7.93	Antimicrobial	0.2284
4	Butane, 1,1-dibutoxy-	8.01	Antioxidant,Antibacterial	0.5059
5	2-Methoxy-4-vinylphenol	9.54	Antibacterial	0.7323
6	Benzaldehyde, 3-hydroxy-4-methoxy-	11.89	Antibacterial	0.2325
7	Dihydroactinidiolide	15.38	Insecticide	1.3884
8	3,4-Pyridinedimethanol, 6-methyl-	16.48	Antimicrobial	0.4891
9	4-Hydroxy-á-ionone	17.07	No activity reported	0.4316
10	p-Menthane, 1,2:8,9-diepoxy-	17.33	No activity reported	0.5900
11	1-{2-[3-(2-Acetyloxiran-2-yl)-1,1-dimethylpropyl]cycloprop-2-enyl}ethanone	17.94	No activity reported	1.3284
12	5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol	19.30	Antimicrobial	4.4293
13	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	19.36	No activity reported	2.0849
14	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	19.80	Antimicrobial, antiinflammatory	58.1199
15	Farnesyl acetone	20.77	No activity reported	0.9425
16	Hexadecanoic acid, methyl ester	20.86	Antioxidant,hypocholesterolenic, antiandrogenic,flavor,nematicide hemolytic5-alpha reductase inhibitor	4.4721
17	Phytol	22.86	Antimicrobial, anti-inflammatory,diuretic ,anticancer	11.1225

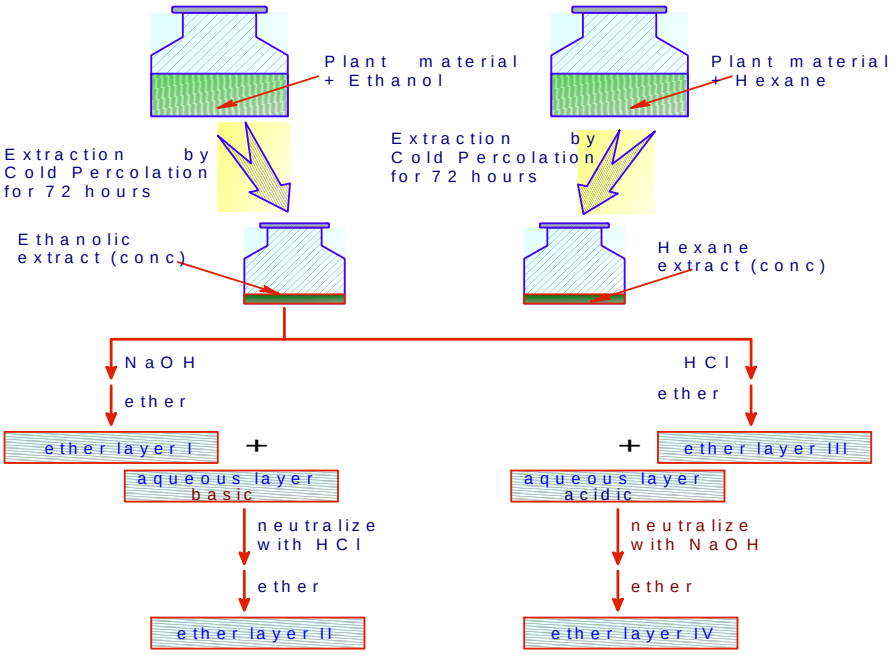


Figure 1. Extraction process of the plant - *Naravelia zeylanica*

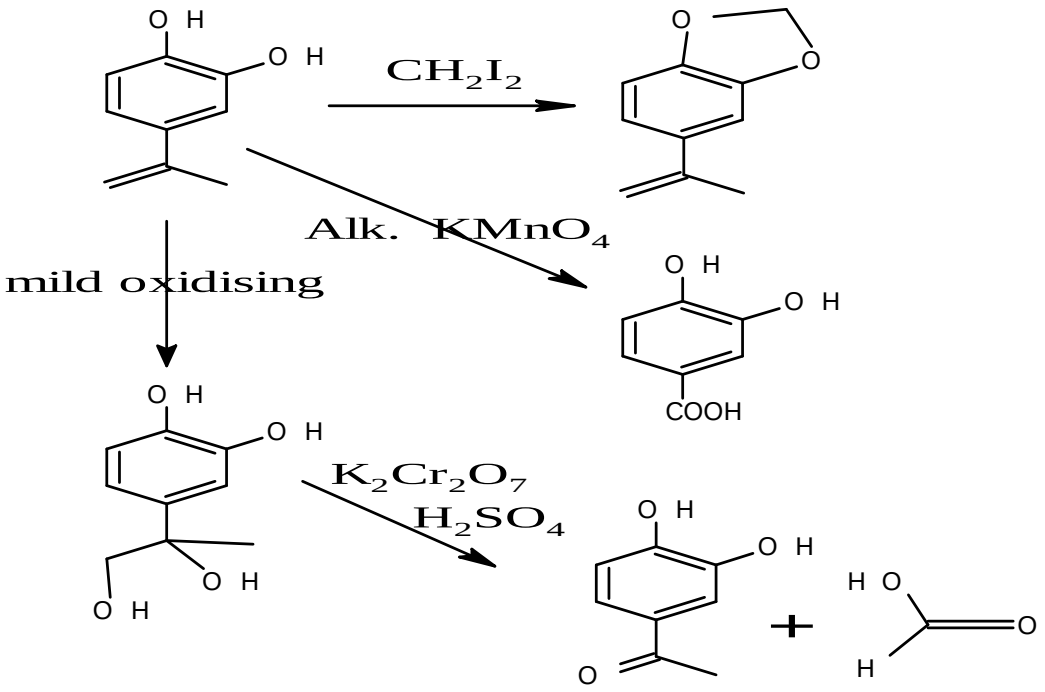


Figure 2. Chemical conversion of NZ 2

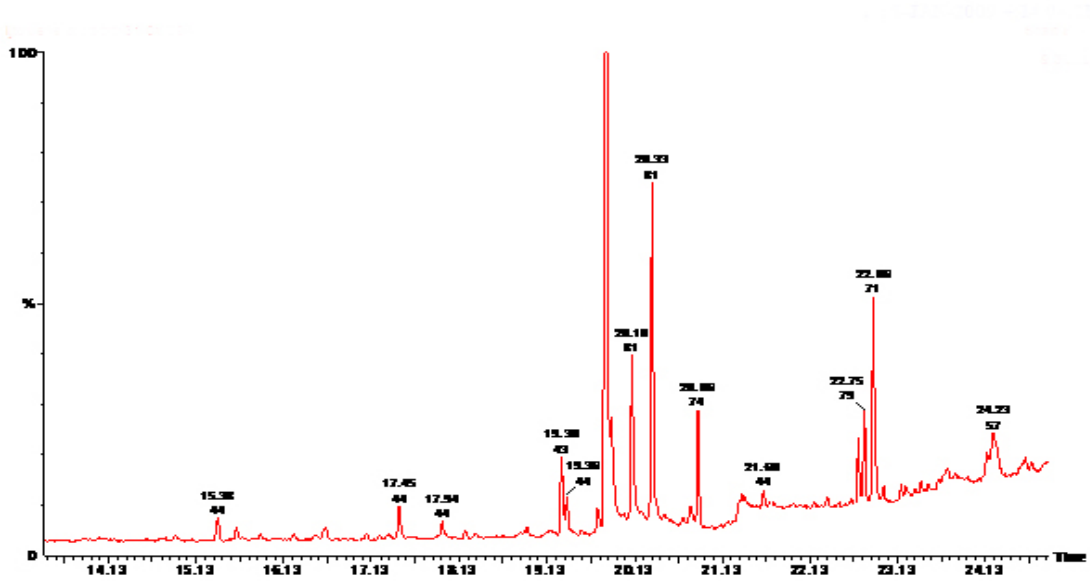


Figure 3. Chromatogram of *Naravelia zeylanica* leaves by GC-MS

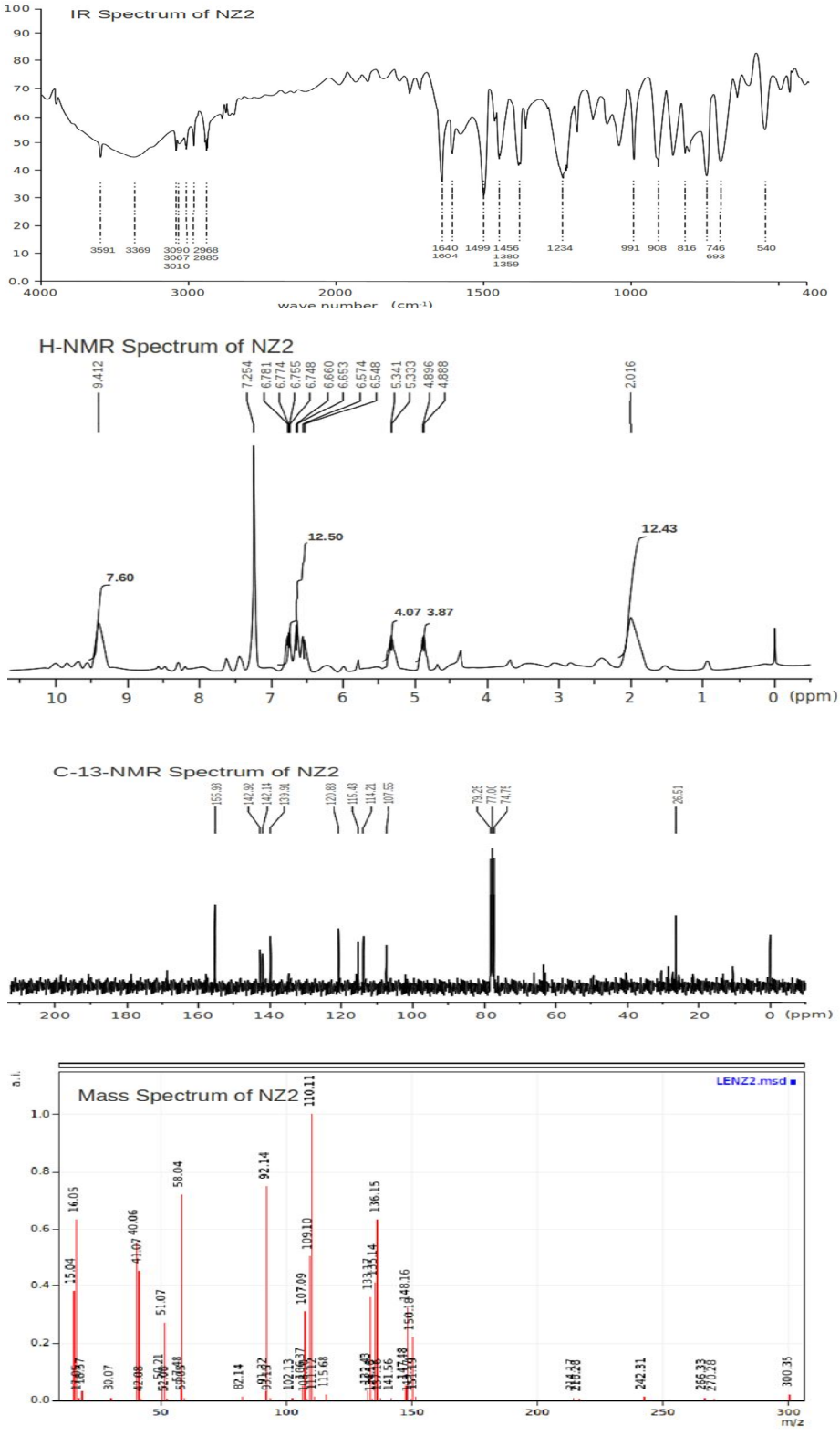


Figure 4. IR, NMR, C-13 and Mass spectra of NZ 2

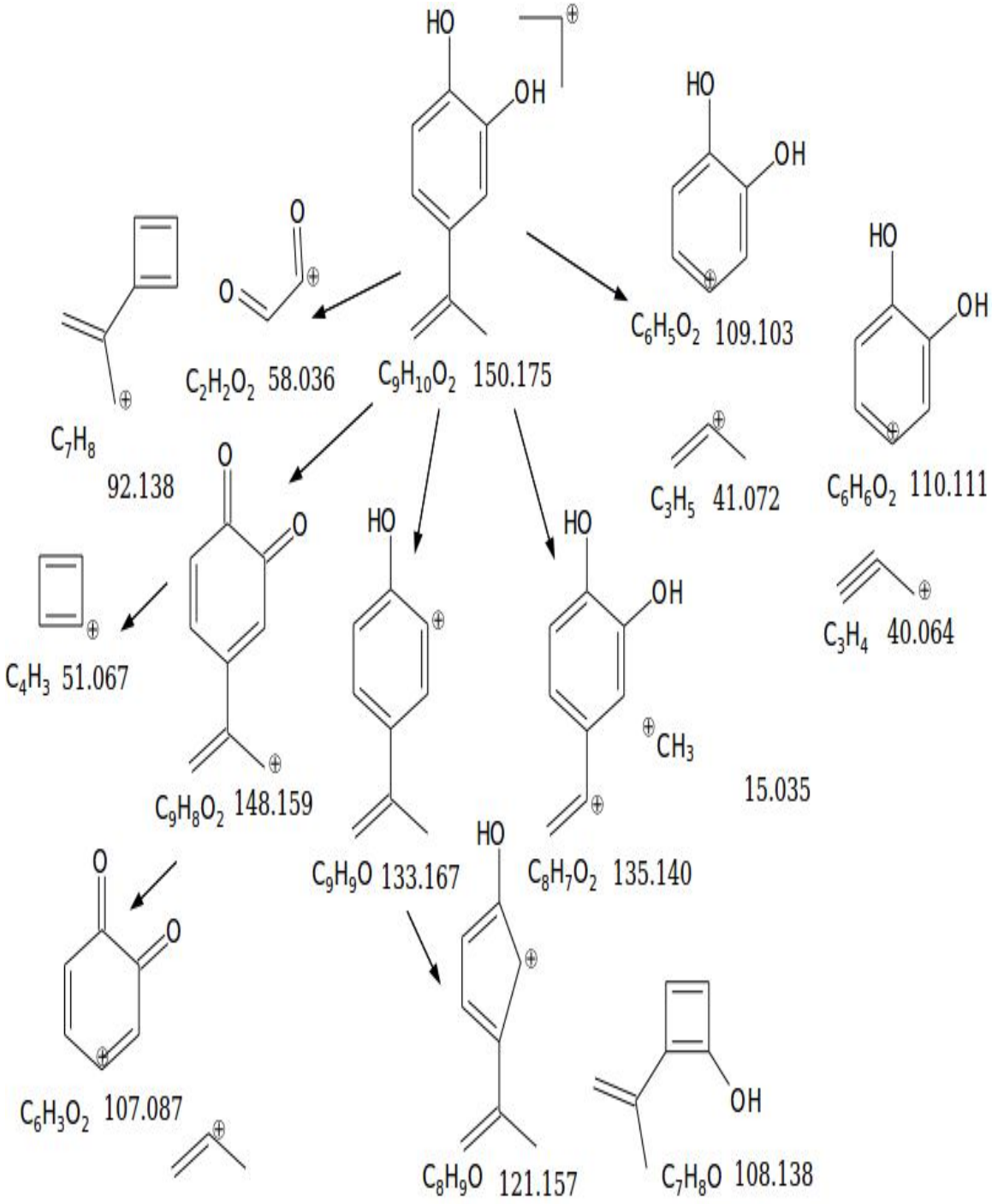


Figure 5. Mass fragmentation of NZ 2

significant of the π- π* transition of the –C=C-double bond in conjugation with the aromatic ring.

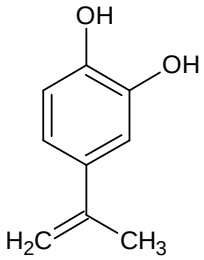
The IR spectrum of NZ2 was found to have a weakly sharp at 3561cm-1 was indicative of the -O-H stretching of the free hydroxyl group. The -O-H stretching of the hydroxyl group that was involved in hydrogen bonding gave a very broad band at 3369cm-1. The -O-H stretching of the intramolecularly hydrogen bonded -OH group showed a mildly broader weak band at 3067cm-1. The bending vibrations of the -O-H bond and the -C-O bond of the hydroxyl group attached to the carbon, were indicated clearly through the bands 1380 and 1234cm-1, respectively. The out-of-plane bending of the -O-H bond showed a strong band at 693cm-1.The methyl group showed a mediocre band and a strong band at 2968 and 2885cm-1, respectively. These were attributed to the C-H stretching vibrations. The bending vibration of the C-H bond of this methyl group was observed through a weaker band at 1350cm-1.The C-H stretching vibrations of the -C=C-H group was found as band at 3085cm-1. The bending vibrations of the same -C-H bond were indicated through the bands at 991 and 908cm-1. The- C=C- stretching of the alkenic part was identified as a strong band at 1640 cm-1 . The aromatic ring showed a strong band at 3010cm-1 for the stretching vibrations of the C-H bond. The same bond showed bending vibrations at 816 and 746 cm-1. The stretching vibrations of the -C=C- of the aromatic ring was known through the triplet bands at 1604, 1499 and 1456cm-1. The same bond showed a bending vibration through a strong band at 546cm-1.

In the H- NMR spectrum of NZ2, the deshielded methyl protons gave a singlet signal at 2.016ppm (3H) indicating that they had no neighbouring proton and the methyl group got attached to pi-bonded carbon. A set of two highly deshielded alkenic protons had given two doublet signals at 4.892ppm (J 2.9, 1H) and 5.337 (J 2.9, 1H) revealing that they are neighbours and attached to the same alkenic carbon. They had no neighbouring proton. The doublet signal of a deshielded proton with a chemical shift value 6.561ppm (J 10.3, 1H) was indicative of the aromatic proton with one neighbouring proton. Another doublet signal of a deshielded proton with a chemical shift value 6.656ppm (J 2.8, 1H) was indicative of the aromatic proton with one neighbouring proton at meta-position. The doublet-doublet of

another deshielded aromatic protons was shown at 6.764ppm (J 10.3, 2.8, 1H) signified the presence of one aromatic proton with two neighbouring protons – one at ortho-position and the other at meta-position.The highly deshielded hydroxyl proton showing a singlet signal at 9.412ppm (2H) indicated the presence of two -OH and no they had no neighbouring proton.

In the Off-Resonance and fully decoupled C-13 NMR spectrum of NZ2, the methyl carbons showed a signal at 26.51ppm. It was indicative of a deshielded environment of a pi-bonded carbon, to which the methyl was present. There were two alkenic carbons which gave the signals at 107.55 and 155.90 ppm. Of these two carbons, one was more deshielded as it got attached to the aromatic ring. A set of six aromatic carbons indicated signals at 114.21, 115.43, 120.83, 139.91, 142.14 and 142.92ppm. Of the six aromatic carbons three were slightly more deshielded than the other three as they got attached to an alkenic part and two hydroxyl groups.

The molecular ion peak at 150.175 showed that the NZ2 compound was in conformity with the molecular formula, C9H10O2. It was confirmed to be a phenolic type of compound by the chemical and spectral studies studies. The mass spectral fragmentation pattern was in agreement with the spectral data. The base peak at 110.11 was indicative of the formation of C6H6O2. Based on the chemical studies, spectral data and GC-MS studies, the structure of the compound was elucidated as phenolic as given below:



CONCLUSION

The GC-MS study on the leaf extract of Naravelia zeylanica revealed the compounds such as antioxidant, phenolics, flavonoids, alkaloids and terpenoids were present. It was further confirmed by the chemical methods and phytochemical screening. The ethanolic extract was further fractioned using alkali ,acid and ether to get ether layer I, II, III and IV. The ether layer II was taken for chromatographic separation. The compound NZ2 was isolated and it was taken for characterization studies. Thus, the compound NZ2 was deciphered to be a phenolic type of a compound.

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How to cite your article:
Easwaran L., Ramani V. L., "Phytochemical examination and gc-ms studies of the medicinal plant - Naraveliazeylanica", Int. J. Res. Dev.Pharm. L. Sci., 2014, 3(5), pp. 1180-1188.

