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Research Article

ISOLATION, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF AN AROMATIC ESTER FROM *IPOMOEA CARNEA* STEM BARK

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ABSTRACT

Medicinally important plant species *Ipomoea* has been reported in Indian system of medicine since ancient times. *Ipomoea carnea* is one of those reported as used in folk medicine. *Ipomoea carnea* belongs to the family convolvulaceae and fistulosa sub-family. It is a wild herb, largely available in all states of India and a native of South America. Many Ipomoea species having antimicrobial activities were reported in literature. Taking this into consideration, steam distillation of fresh stem bark of *Ipomoea carnea* was carried out to isolate potentially bioactive compound. Antibacterial activity of steam volatile mixture was performed and it showed significant activity against *Klebseilla pneumonia* (ATCC33495), *Proteus mirabilis* (ATCC12453) and *Pseudomonas aeruginosa* (ATCC10662). Therefore, bioguided separation and isolation of compound **A** was performed. Compound A, a bioactive secondary metabolite; (Z)-4- hydroxyphenyl-4', 5'-dimethylcyclooct- 4'-ene-carboxylate was isolated for the first time from stem bark of this plant source. The structure of the isolated compound was elucidated by IR, MS, ^{1}H-NMR, ^{13}CNMR, DEPT, etc. Isolated compound; A exhibited promising activity against above mentioned bacteria. MIC study of the isolated compound was also carried out. *Pseudomonas aeruginosa* was found most susceptible as the compound showed the highest activity against this strain. The antibacterial activity was determined by using disc diffusion method. Streptomycin was used as a standard.

Keywords: *Ipomoea carnea,* Steam distillation, Antibacterial activity.

1. INTRODUCTION

Steam distillation is the most common method of essential oil production. Volatile organic matters and essential oils of plant material can be extracted using steam distillation method. Many organic compounds tend to decompose at high sustained temperatures. Separation by normal distillation would then not be an option. This method is specially offered for steam volatile compounds. It also offers the advantage of selectivity, since some water insoluble substances are steam volatile and some volatilize so slowly that sharp separation is possible. The technique is useful in processing natural oils and resins, which can be separated into steam volatile and non steam volatile. Some well known examples are the steam volatile components of lemongrass oil such as citral, farnesol, nerol, citronellal and myrcene and the steam volatile components of citronella oil such as citronellal, citronellol etc [1]. The steam distillate of *Ipomoea cairica* leaves was toxic against mosquito species *Culex triaeniorhynchus, Aedes aegypti, Anopheles stephensi* [2].

Ipomoea carnea is a native of South America and available in plenty in all the states of India due to its adaptation to the Indian climatic conditions [3]. It belongs to convolvulaceae family and fistulosa subfamily [4]. Several reports are available on the biological activities of *Ipomoea carnea*. It is reported for wound healing activity [5]. Extract of the whole plant was widely used as antirheumatic remedy in Bolivia [6]. Immunomodulatory activity of aqueous extract of *Ipomoea carnea* was tested on peritoneal cells of rat [7]. There are reports on synergistic effect of insecticides with plant extracts of *Ipomoea carnea* against malarial vector, *Anopheles stephensi* [8]. Aqueous and petroleum ether extracts of *Ipomoea carnea* leaves have the potential to be used as an ideal eco-friendly approach for the control of the major lymphatic filariasis vector, *Culex quinquefasciatus* [9]. A saponin had been isolated from *Ipomoea carnea* with anticarcinogenic and oxytoxic properties [10]. Isolation and characterization of secondary metabolites, their bioactivity study along with antioxidant capacity, antimicrobial activity

and carbohydrate contents were reported in authors' earlier studies [11-15]. In line with the objectives of the current research work to isolate secondary metabolites having potential biological activities, an aromatic ester (compound A) was isolated from *Ipomoea carnea* stem by steam distillation and antibacterial activities were investigated. Considering the significance of medicinal and industrial importance of steam volatile material, efforts were made towards steam distillation of fresh stem bark of *Ipomoea carnea*. The steam volatile mixture was tested against some bacterial strains *Klebseilla pneumonia* (ATCC33495), *Proteus mirabilis* (ATCC12453) and *Pseudomonas aeruginosa* (ATCC10662) and these were found active against the above bacterial strains. Therefore, bioguided separation and isolation of compound A was performed. The compound showed significant activity against the tested bacterial strains. As per the authors' information, isolation and antimicrobial activity of compound A from *Ipomoea carnea* stem bark is reported for the first time.

2. MATERIAL AND METHODS

2.1. Collection and Identification of Plant material

The plant material was collected from the river sides of Pune, Maharashtra, India. The plant was authenticated at Botanical Survey of India, Pune. The authentication no is ELICAI.,BSI/WC/Tech/2009/96.

2.2. Preparation of plant extract and isolation

Steam distillation of fresh stem bark of *Ipomoea carnea* was performed. The distillate was extracted using ether as a solvent. It was dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure to get impure crystalline matter. Repeated crystallization using ethanol resulted in white shining crystalline compound (Compound A), (Z) - 4- hydroxyphenyl- 4', 5'-dimethyl-cyclooct- 4'- ene- carboxylate which was characterized by modern spectral techniques. The compound was isolated for the first time from the stem bark of *Ipomoea carnea*.

2.3. Bacterial Strains

On the basis of pathogenic importance, three pathogenic bacterial strains, gram negative bacteria, *Klebseilla pneumonia* (ATCC33495), *Proteus mirabilis* (ATCC12453) and *Pseudomonas aeruginosa* (ATCC10662) were selected. All bacterial strains were maintained at 4°C on nutrient agar (Hi-Media) slants and cultured at 37°C using same agar medium.

2.4. Antibacterial activity assay

The paper disc diffusion method was used to determine the antibacterial activity. Sample of steam distillation product and isolated pure compound (10mg) were dissolved separately in respective solvent (1ml) to prepare stock solution. Sterile filter paper discs (5 mm) were impregnated with 40 µl of these solvent extracts. Adequate amount of Muller-Hinton Agar was dispensed into sterile plates and allowed to solidify under aseptic conditions. The count of the bacterial strains was adjusted to yield $1x10^7$ to $1x10^8$ ml^{-1}. The test organisms (0.1ml) were incubated with a sterile spreader on the surface of the solid medium in plates. The agar plates inoculated with test organism were incubated for one hour before placing the extract impregnated paper discs on the plates. The sterile discs impregnated with different extracts were placed on agar plates. The plates were incubated at 37°C for 24 hours. Acetone was used as solvent for dissolving the extract and pure compound and was used as control in the assay. The antibacterial activity results were calculated as a mean of three replicates. Streptomycin was used as standard. Anti-bacterial activity was assessed based on the measurement of the diameter (in mm) of the clear zones of growth of inhibition.

3. RESULTS AND DISCUSSION

Compound A (fig.1), (Z)-4-hydroxyphenyl-4', 5' dimethylcyclooct- 4'- ene-carboxylate, was isolated as white shining crystals. It was purified by repeated crystallization using ethanol. It showed sharp melting point at 143°C.

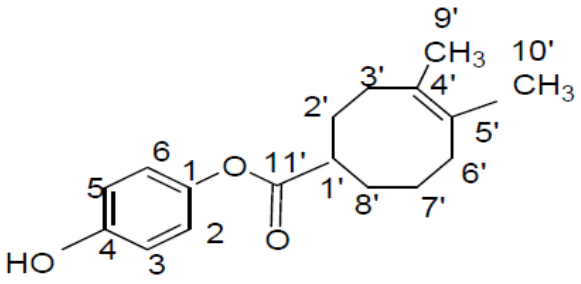

Fig. 1: Compound A: (Z) - 4- hydroxyphenyl- 4', 5'-dimethylcyclooct- 4'- ene- carboxylate

Mass spectrum (fig. 2) demonstrated molecular ion peak at *m/z* 275 $[M+1]^+$ suggesting the molecular formula to be C_{17} H_{22} O_3. IR spectrum (fig. 3) showed characteristic bands at 3356 (O-H stretching), 1745 (ester carbonyl), 1155 and 1094 cm^{-1} (C- O- stretching).

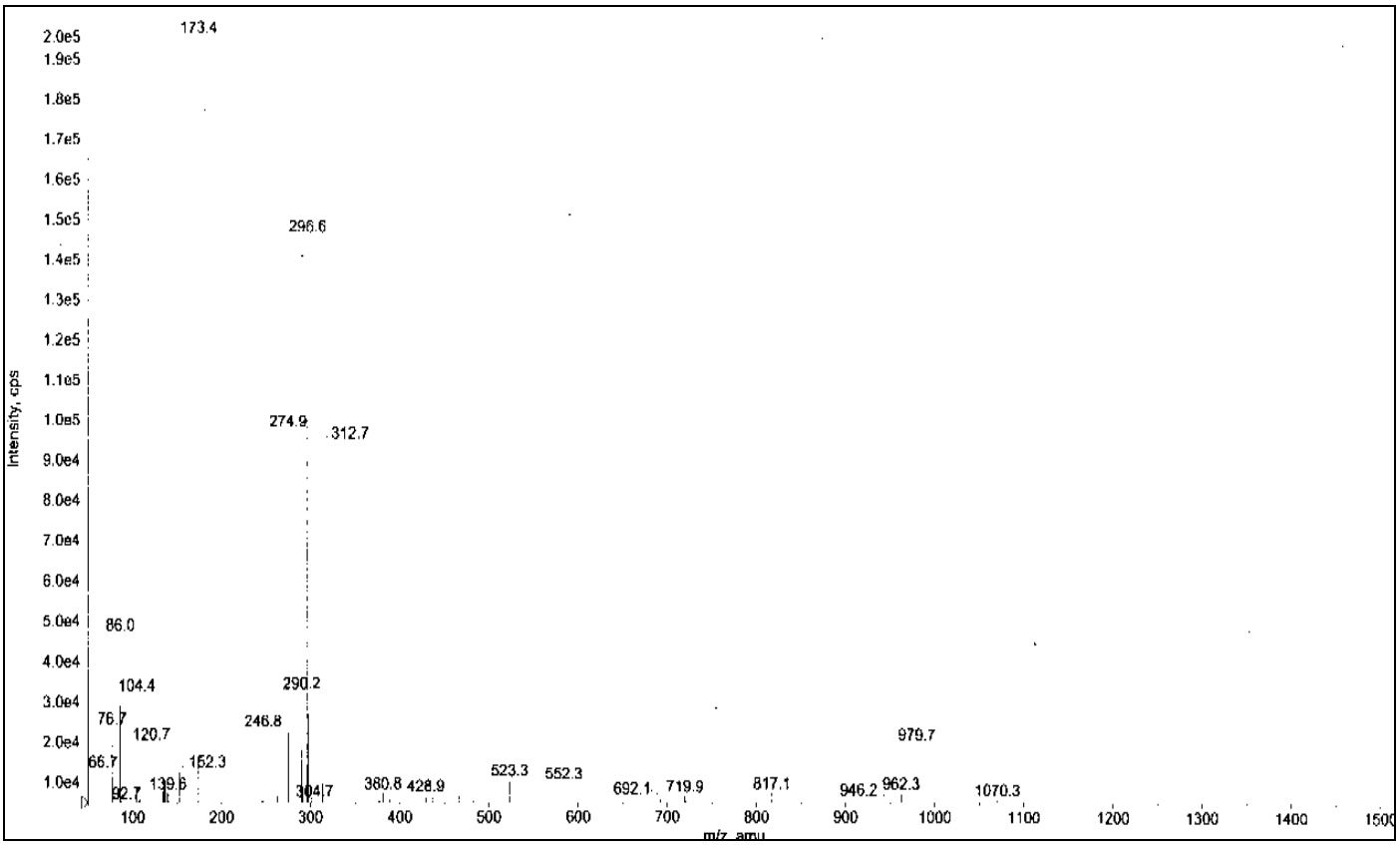

Fig. 2: Mass spectrum of compound A

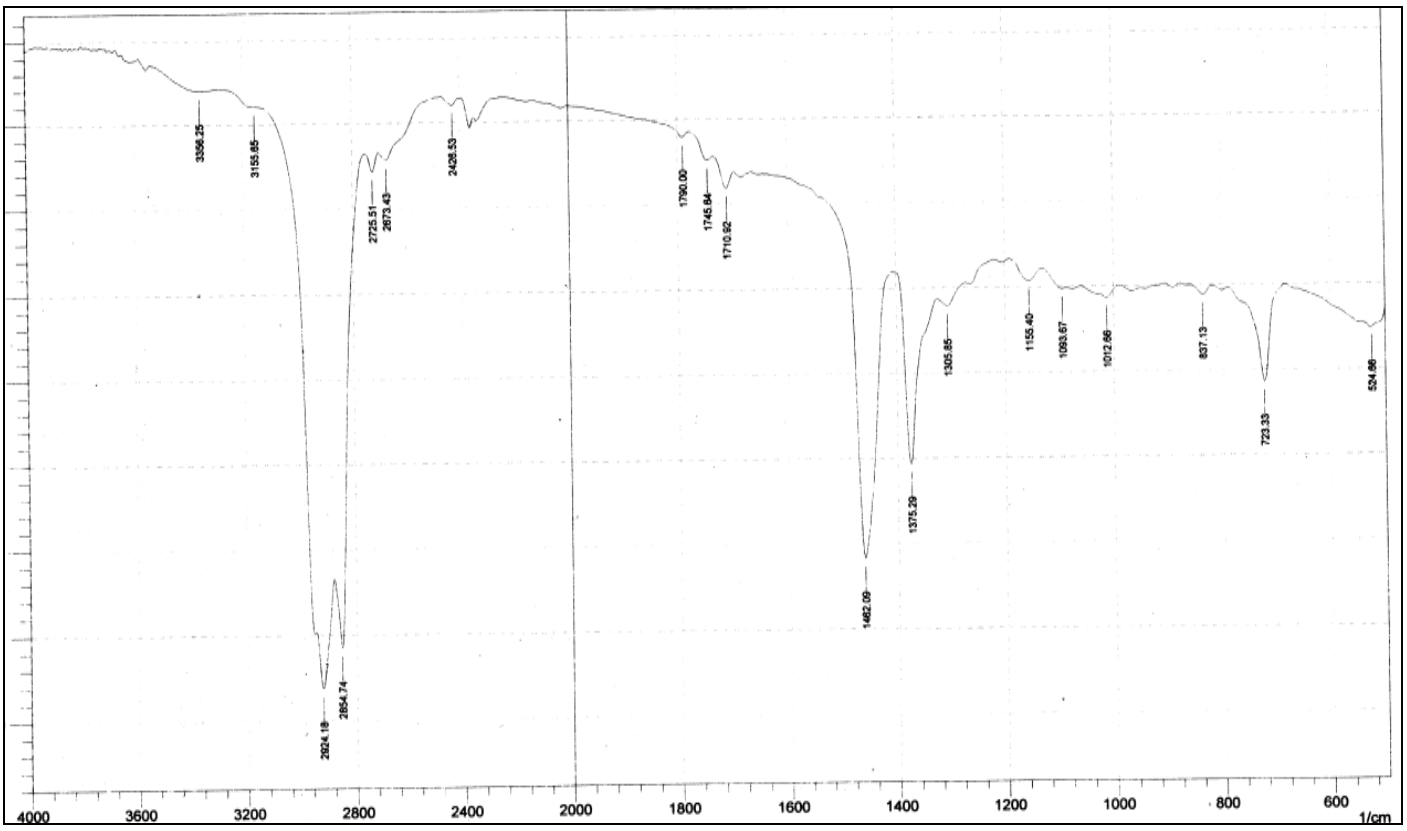

Fig. 3: IR spectrum of compound A

^{1}H NMR spectrum (fig. 4, table 1) displayed a broad singlet at δ 7.52 (br, s, 2H), for aromatic protons (H 2 & H 6). Another broad singlet at δ 6.99 (br, s, 2H) was noticed indicating (H 3 & H 5) aromatic protons. A broad and short peak indicated the presence of hydroxy proton at δ 3.5. A multiplet at δ 2.18 was observed that indicated (H 1', 1H) proton. A broad peak at δ 2.01 (br, s, 4 H) was depicted as (H3'& H6') methylene protons. Another merged multiplet at δ 1.55 indicated (H 2' & H 8') methylene protons. A singlet at δ 1.55 (s, 6 H) was observed for (H9' & H10') methyl protons. An upfield multiplet was present at δ 1.25 (m,2H) for H 7'.

The ^{13}C-NMR spectrum (fig.5, table 1) displayed seventeen carbon atoms. The spectrum exhibited downfield singlet for ester carbonyl carbon atom at δ 160. A strong singlet was noticed at δ148 and δ145 for (C 4 and C1) carbon atoms. Another strong singlet at δ 128 was emerged for (C 4' & C 5') carbon atoms. Doublets at δ 120 and δ118 can be assigned for (C 2& C 6) and (C 3 & C5) aromatic carbon atoms. A doublet at δ 45 was assigned for C 1' carbon atom. A triplet at δ 32 was well assigned for (C 8' & C 6'). Three triplets were observed at δ 26, δ24 and δ23.5 for C 2', C 7' and C 3' carbon atoms respectively.

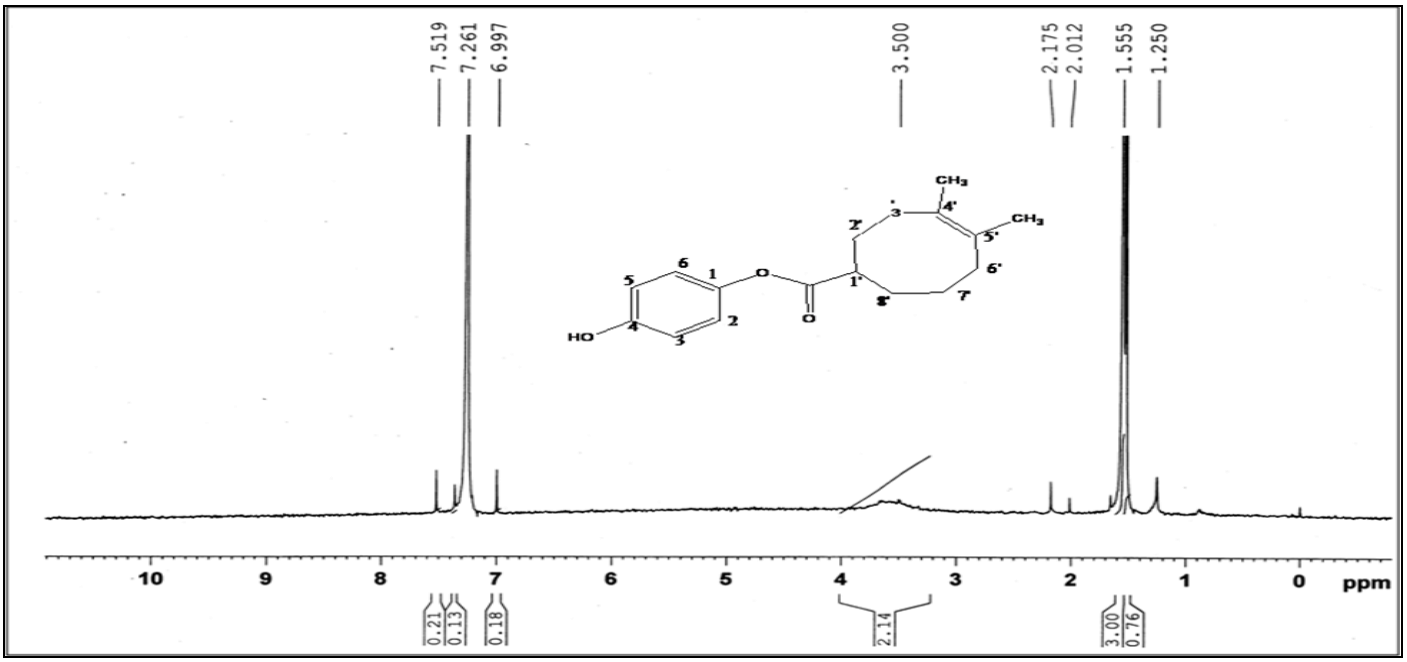

Fig. 4: ^{1}H- NMR of compound A

Table 1: ^{1}H NMR, ^{13} C-NMR data of Compound A

^{1}H-NMR [500M*Hz*, MeOD, δ (ppm)]		^{13} C-NMR [125 M*Hz*, MeOD, δ (ppm)]	
Atom No	**δ (ppm)**	**Atom No**	**δ (ppm)**
H 2 & H 6	7.52 (br, s, 2H)	Ester carbonyl carbon	160 (s)
H 3 & H 5	6.99 (br, s, 2 H)	C 4	148 (s)
Hydroxy proton	3.5 (br, s, 1 H)	C 4' & C 5'	128 (s)
H 1'	2.18 (d, 1H)	C 2 & C 6	120 (d)
H 2' & H 8'	1.55 (m, 4H)	C 3 & C 5	118 (d)
H 3' & H 6'	2.01 (br, s, 4 H)	C 1'	45(d)
H 9' & H 10'	1.55 (s, 6 H)	C 6' & C 8'	32 (t)
H 7'	1.25 (m, 2 H)	C 2'	26 (t)
		C 7'	24 (t)
		C3'	23.5 (t)
		C 9' & C 10'	16.9 (q)

The DEPT pulse sequence (fig. 6) demontrated the presence of five methine, five methylene, two methyl carbon atoms and five quarternary carbon atoms. In the NOSEY experiment (fig. 7a and 7b) H 8' specified corelations with H 6' & H 1' but not with H 7', H 2'. H 3' specified correlations with H 4' methyl protons and H 1' but not with H 2'. Similar types of correlations were observed with C 5' methyl protons. These correlations indicated that H 6', H 1' and H 3' are β oriented while H 7' & H 2' substituents are α oriented. The above spectroscopic data confirms the structure and stereochemistry of compound **A**.

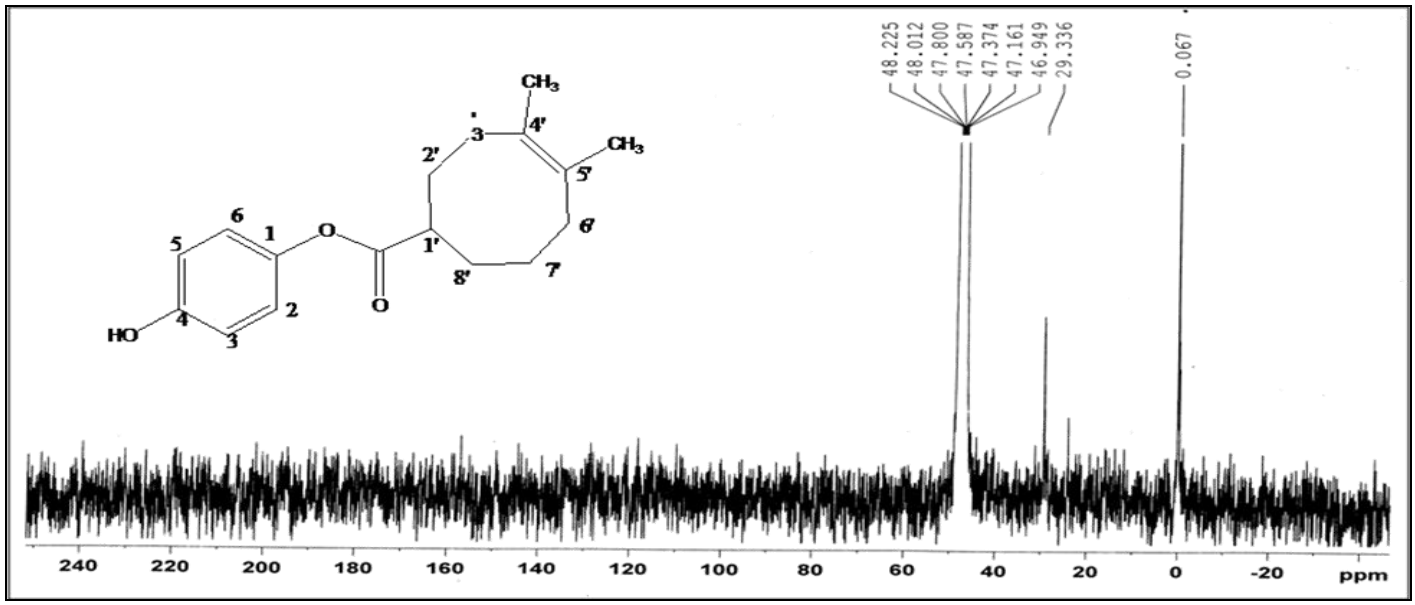

Fig. 5: ^{13}C- NMR of compound A

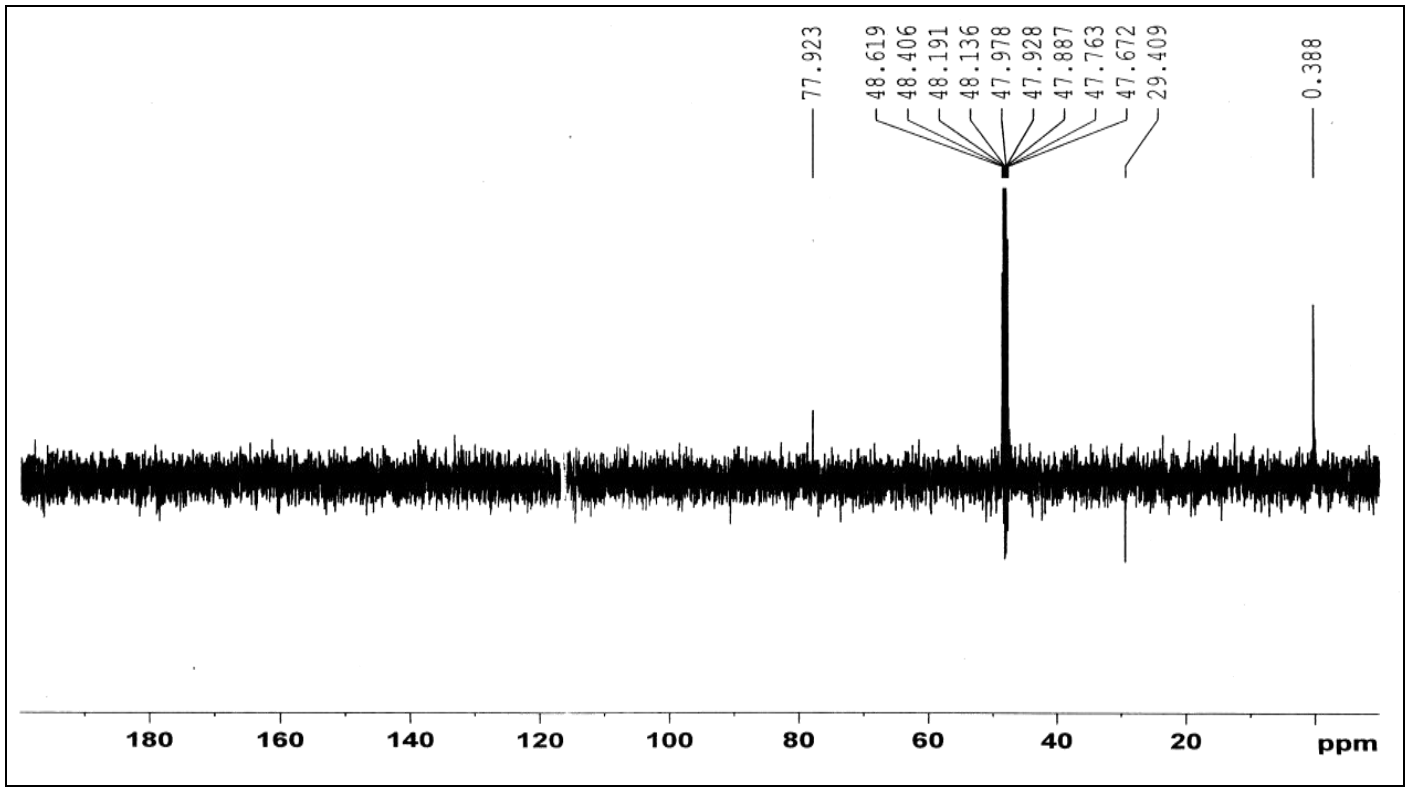

Fig. 6: DEPT of compound A

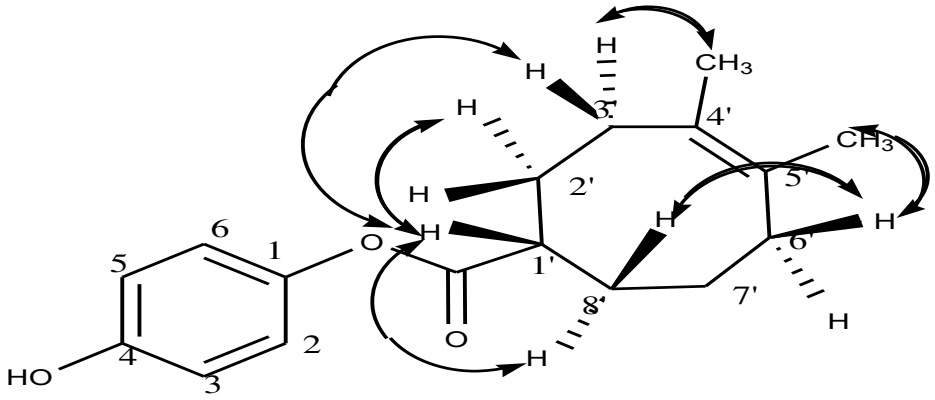

Fig. 7a: NOESY of compound A

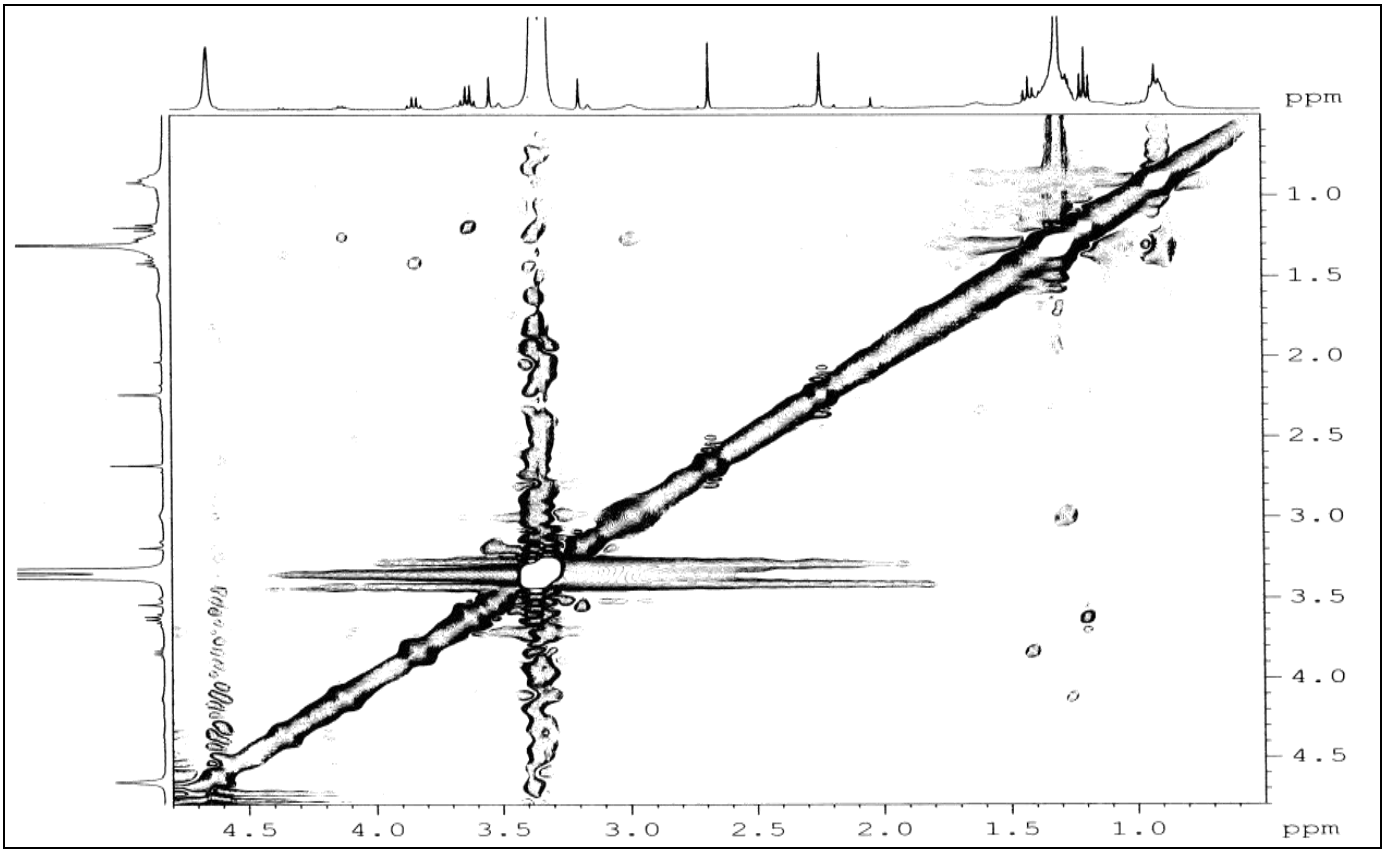

Fig. 7b: NOESY of compound A

The antibacterial activities of steam volatile mixture and isolated compound A were assayed *in vitro* by Agar disc diffusion method against three different bacterial strains. The results are presented in table 2.

Agar disc diffusion assay of activities of steam volatile mixture and isolated compound A showed a variable clear zone for different bacteria. Both the extract and pure compound exhibited good antibacterial activity against gram negative bacterial *Klebseilla pneumonia, Proteus mirabilis* and *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* was found the most susceptible bacteria as both extract and isolated pure compound showed the highest activity against this strain. Isolated compound A exhibited better activity than steam distillate mixture. The effect of concentration of compound A on anti-bacterial activity was analyzed further. MIC study of compound A against *Pseudomonas aeruginosa* was carried out and results are presented in table 3. For compound A the MIC was found 20 µg/ml and activity was increased with increasing concentration.

Table 2: Antibacterial activities of extract and compound A

Extract/Compound	Zone of inhibition		
	Klebseilla pneumonia	*Proteus mirabilis*	*Pseudomonas aeruginosa*
Steam volatile mixture	11	10	13
Compound A	13	11	15
Sreptomycin	17	15	20

**Zone of inhibition including the diameter of filter paper disc (5 mm)*

Table 3: MIC of compound A

Concentration (µg/ml)	Zone of inhibition (mm)
400	15
200	13
100	12
50	10
25	8
20	6
10	-

4. CONCLUSION

The bioactivity data from the present study offers a scientific proof for the traditional use of the plant *Ipomoea carnea*. It can be considered as a potential source of antibacterial compounds. The *in vitro* antibacterial evaluation of the isolated compound, (Z)-4- hydroxyl-phenyl- 4', 5'-dimethylcyclooct-4'- ene- carboxylate forms a primary platform for further phytochemical and pharmacological studies on this largely available plant.

5. ACKNOWLEDGEMENT

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Conflict of interest

The authors declare no conflict of interest.

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