International Journal of Pharmaceutical Archive-3(1), 2014, 273-284
Available online through www.ijpaonline.info ISSN 2319-7226

RESEARCH ARTICLE

EXTRACTION AND PHARMACOLOGICAL SCREENING OF CARVONE AND IT'S DERIVATIVES

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(Received on: 21-12-13; Revised & Accepted on: 30-01-14)

ABSTRACT

P*hytoconstituents are responsible for many therapeutic activities. The aim of this research work is to separate the phytoconstituents, derivatization of phytoconstituents and its characterisation. And lastly compounds were evaluated for antioxidant and anticancer activity. The present work explains the antioxidant property of N-[(1 E)-2-methyl-5-(prop-1-en-2-yl) cyclohex-2-en-1-ylidene] methanamine obtained from caraway seeds (Carum carvi).*

The (1E)-1-[2-methyl-5-(prop-1-en-2-yl) cyclohex-2-en-1-ylidene]-2-phenyl hydrazine obtained from caraway seeds possess anticancer activity on MCF7 (breast), HeLa (cervix) and SK-OV3 (ovary) cell lines. This can prove as a new drug candidate for anticancer activity which can be explored further.

Keywords: *Carum carvi, phytoconstituents, antioxidant, anticancer.*

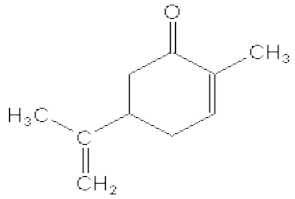
INTRODUCTION

Natural products contain a variety of chemical components belonging to different classes of compounds which provide a potent source for drug discovery and thus eventually lead optimization. Rationalizing the use of various traditionally used herbs is needed. Keeping this vision we have aimed our studies in separating the phytoconstituent from the herb that are traditionally used and evaluate their biological activity.

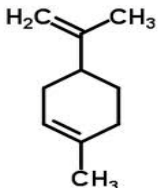
Carum carvi Linn. (Apiaceae) herbal medicine widely explored for biological activities. Traditionally the whole plants has been used for antimicrobial, anti-inflammatory, analgesic, antipyretic, diuretic, antiseptic, cytotoxic activity.

Caraway
It consist of seeds of *Carum carvi* (Linn.) belonging to family Apiaceae. The plant is similar in appearance to other members of the carrot family, with finely divided, feathery leaves with thread-like divisions, growing on 20–30 cm stems. The main flower stem is 40–60 cm tall, with small white or pink flowers in umbels. Strongly aromatic caraway is a member of parsley/Apiaceae family, the family that also includes commonly known herbs and spices such as dill, anise, funnel and cumin etc. In addition to its use as medicinal values, caraway indeed has many health benefiting nutrients, minerals, vitamins and anti-oxidants.

Chemical constituents- The chemical ingradients in caraway plant, Principle volatile compounds are (45-65%) *carvone, limonene,and dihydro-carvone.*[2] It also contain traces of *carveol, pinen, cumuninic aldehyde, carotene, crypto-xanthin* and *zea-xanthin.* Minerals like iron, copper, calcium, potassium, manganese, selenium, zinc and magnesium.



carvone



Limonene

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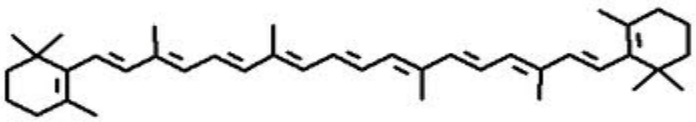
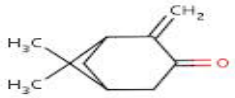
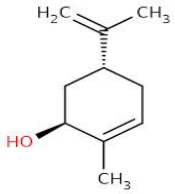


beta-carotene



Pinen



Carveol

The present research work is undertaken for the separation of phytoconstituents and screening of pharmacological activity of traditionally used herb such as caraway.

The present works aims at extraction, separation, derivatization and characterisation of separated and derived compounds by IR, NMR and Mass spectroscopy. Followed by pharmacological evaluation of antioxidant and anticancer activity.

MATERIAL AND METHODS

PART-1: EXTRACTION, SEPARATION AND DERIVATIZATION OF PHYTOCONSTITUENTS

General information for the extraction, separation and derivatization of phytoconstituents.
This point explains procedure followed for the extraction, separation and derivatization of phytoconstituents and characterization. Starting materials used for each step and the products obtained were assessed for purity by physical constant determination and Thin Layer Chromatography (TLC). The structures of the final compounds were characterized by Infrared (IR) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy, and Elemental Analysis. IR spectroscopy was carried out using KBr pellet method on the SHIMADZU IR Affinity-1. NMR spectra were recorded on a Bruker Avance 500 spectrophotometer operating at 500.13 MHz (^{1}H). Samples were dissolved (0.1 mmol/ml) in deuterated chloroform (CDCl3), and spectra were recorded at 303 K.

Caraway seeds was obtained from Yucca enterprices, Mumbai and shatavari roots was obtained from MET college of pharmacy, Nashik. Standard sample of carvone was obtained from Altavista phytochemicals, Hydrabad and standard sample of shatavarin IV was obtained from Natural remedy, Banglore. All chemicals obtained from SD Fine chemicals, Mumbai.

I) CARAWAY

A) Extraction of carvone from carum carvi

1) The dried seeds of caraway were cracked in mortal-pestle. Set up assembly for distillation apparatus using 500 ml round bottom flask. Taken 50 gms cracked caraway seeds in flask, 300 ml of distilled water and 3-4 porcelian pieces were added. We used the main arm of claisen head to add water during the procedure. Placed 250 ml separating funnel with joint in the main arm of the claisen adapter, inserting it through the ring clamp. It was assured that stopcock was closed and poured in about 200 ml distilled water. Turned on cold water to flow briskly through the condenser. Distilled the mixture, making sure that it boils rapidly but that foam does not get into the condenser. The distillate was opaque. Continued distilling until no more oily material was seen in condenser (50-60 min), collected 150-200 ml of distillate.
2) 1gm of sodium chloride was added in 10 ml of distillate and stirred the solution to dissolve the salt. Transferred this mixture in separating funnel and 30 ml of DCM was added. Separating funnel vigorously shaken for 4-5 min. Separate the organic layer in dry flask and remaining aqueous layer once again washed with 30 ml chloroform. 10 gm of sodium sulfate was added in flask to dry the organic solution and the solution was swirled for 5 min. and separate the organic layer from sodium sulfate.
3) Set up the assembly for distillation. Simply distilled the DCM from above mixture and light yellow colour oil obtained as product.[10]

Optimization:
Since the method in the literature has reported less yield. The optimization is done at the second stage. DCM is replaced by $CHCl_3$, the extraction was done in the same way.

Sr. No.	Caraway seeds	Solvent used at second stage	Carvone oil obtained	Yield
1	50 gms	DCM	1.1 ml	1.69 %
2	50 gms	$CHCl_3$	1.65 ml	2.5 %

Table: 1

B) Derivatisation of carvone

a) Reaction of carvone with potassium benzoate (C1)

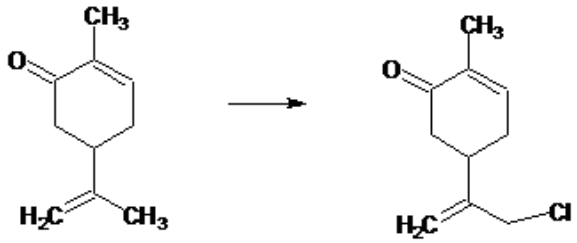
Reagents; sodium hypochlorite, n-hexane, potassium benzoate, potassium carbonate, N, N-dimethyl formamide, chloroform, anhydrus sodium sulphate.

Procedure (Step I)
5ml carvone was dissolve in 10 ml mixture of chloroform: water (1:1) and acetic acid was added. The mixture was vigourously stirred.

Added approximately 1 ml of dilute sodium hypochlorite solution drop wise for 5 min. and stirred. The reaction mixture was kept at room temperature for 30 min.. After 30 min., mixture extracted with chloroform. The organic layer was dried over anhydrous sodium sulphate [7].

Removal of solvent in vacuo afforded the corresponding chlorine. This procedure give a intermediate (I) with chlorination.

Reaction



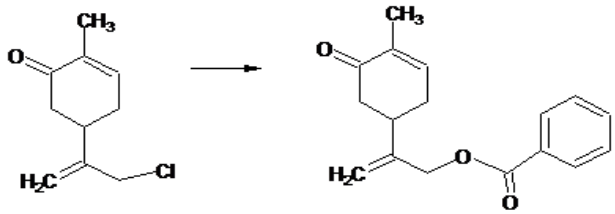
(C) (I)

Procedure (Step II)
This intermediate reacted with a potassium benzoate in presence of 0.5gm potassium carbonate in 5 ml N, N-dimethyl formamide[8].

This gives ester containing compound. In that reaction leave the chlorine with potassium from potassium benzoate and forms ester linkage with methyl group of carvone.

Pale yellow coloured liquid obtained as a product. The reaction was monitored by thin layer chromatography with benzen: ethyl acetate (7:3) as a mobile phase.

Reaction



(I) (C1)
2-(4-methyl-5-oxocyclohex-3-en-1-yl) prop-2-en-1-yl benzoate

Characterization of C1.

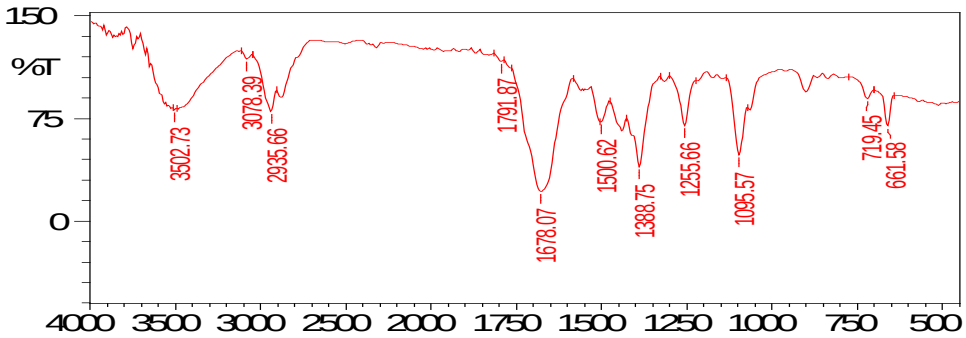
Product	Physical constant (B.P.)	Solubility	R_f	Yield
C1	110 ^{0}C	Soluble in DCM, $CHCl_3$, alcohol	0.56	50%

Table: 2

Sepectroscopic data

Infrared spectroscopy

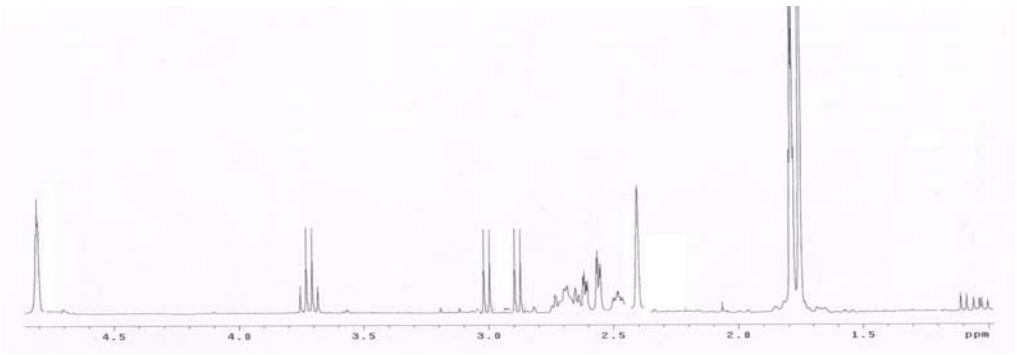
FTIR (KBr, cm^{-1}): 3502.73 (O-H stretch), 3078.39, 2935.66 (C-H stretch), 1791.8 (C-O stretch), 1678, 1500, 1388.7, 1095.57, 719.45, 661.



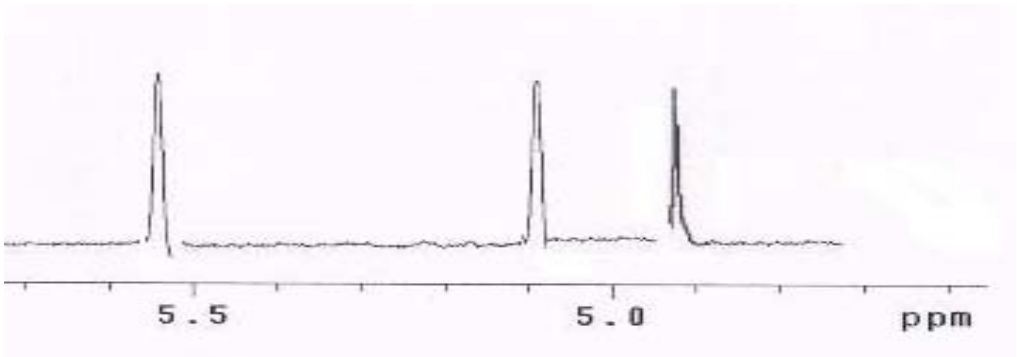
IR- 1

^{1}H NMR Spectroscopy

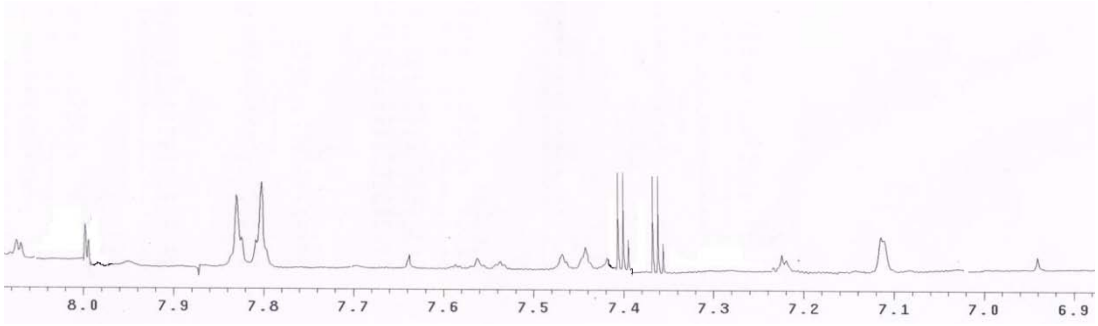
CDCl3 δ 8.07(2H,d), 7.4 (1H,t), 7.3 (1H,t), 5.1 (1H,s), 5.56 (1H,s), 4.8(2H,s), 2.81(2H,t), 2.43(3H,s), 2.45(1H,m).



NMR- 1a



NMR-1b



NMR-1c

b) Reaction of carvone with amines (benzylamine, methylamine and phenyl hydrazine)

Reagents: benzylamine,methylamine and phenyl hydrazine, n-hexane, montmorillonite k-10.

PROCEDURE

48.07ml n-hexane, 5ml carvone, 3.85ml amine (benzylamine BA, methylamine MA and phenylhydrazine PH) and 0.96gm montmorillonite k-10 was added in 250ml round bottom flask with a water separator and reflux condenser.

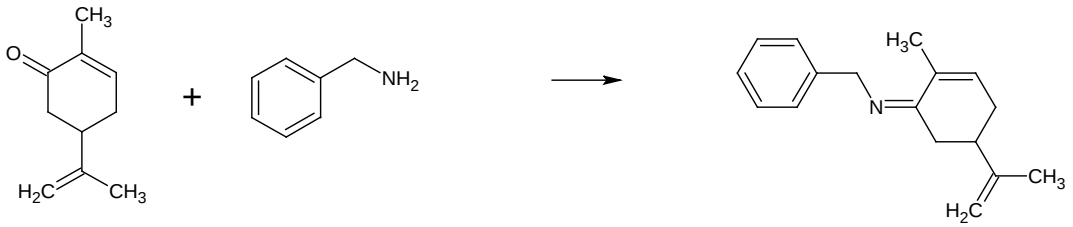
Under stirring with a magnetic stirrer the reaction mixture was heated to reflux until no more water was separated (3-4 hrs).

After cooling to room temperature the suspension was filtered through a fluted filter paper into a 250 ml round bottom flask and the residue was washed with 20ml n-hexane. The solvent was evaporated at rotary evaporator and reddish orange liquid was obtained and characterised. n-hexane was recycled.

The reaction was monitored by thin layer chromatography with benzene: ethyl acetate as a mobile phase.[11]

REACTIONS

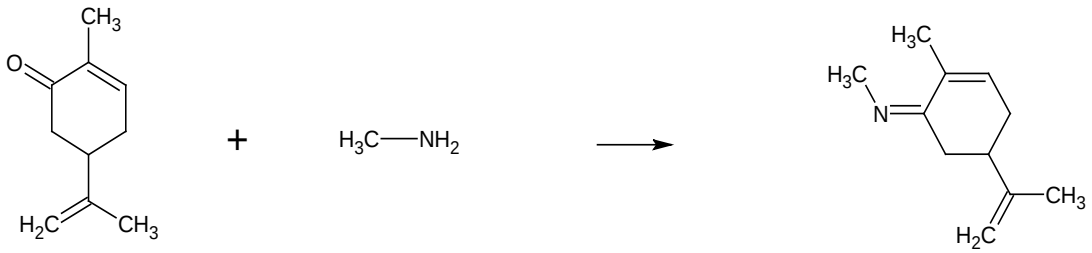
I)



(C) benzyl amine (C2)

N-[(1E)-2-methyl-5-(prop-1-en-2-yl) cyclohex-2-en-1-ylidene]-1-phenyl methanamine

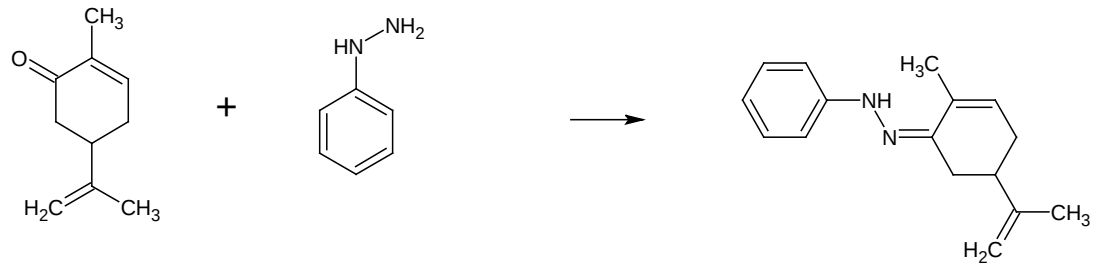
II)



(C) Methyl amine (C3)

N-[(1E)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-ylidene] methanamine

III)



(C) phenyl hydrazine (C4)

(1E)-1-[2-methyl-5-(prop-1-en-2-yl) cyclohex-2-en-1-ylidene]-2-phenyl hydrazine

Characterization of C2, C3 and C4:

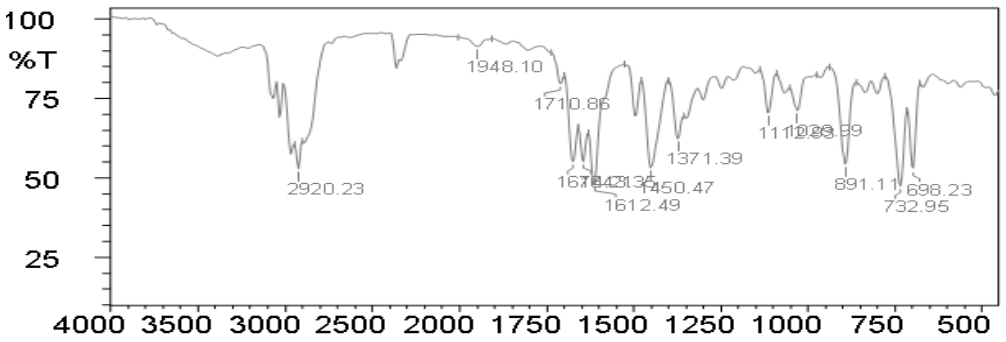
Product	Physical constant (B.P.)	Solubility	R_f	Yield
C2	144 ^{0}C	Soluble in $CHCl_3$, DCM, n-hexane	0.64	72.5 %
C3	156 ^{0}C	Soluble in DCM, n-hexane, $CHCl_3$	0.70	71.20 %
C4	220 ^{0}C	Soluble in $CHCl_3$, DCM, n-hexane	0.58	74.5 %

Table: 3

Spectroscopic data

Infrared spectroscopy (C2)

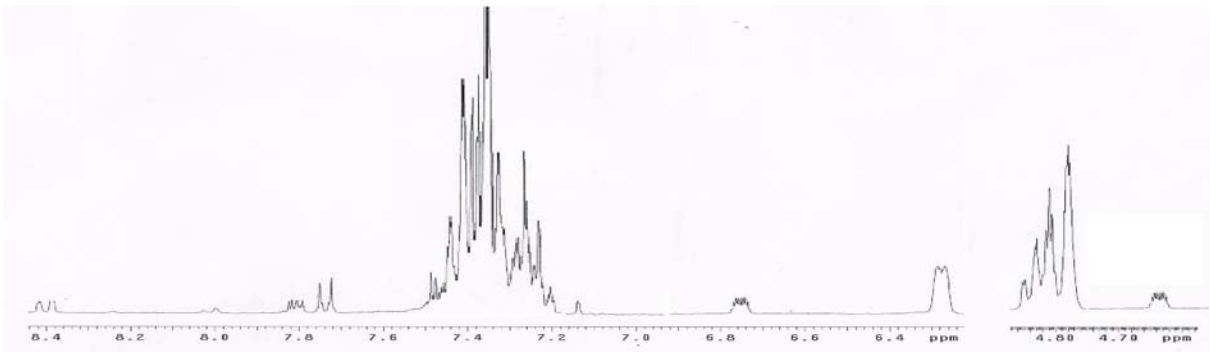
FTIR (KBr, cm^{-1}): 2920 (C-H stretch alkene), 1948.1, 1710.8, 1674.21, 1643.35 (N-H bend), 1450.47, 1371.39 (C-N vibration), 1112.93, 819,732.9, 698.28.



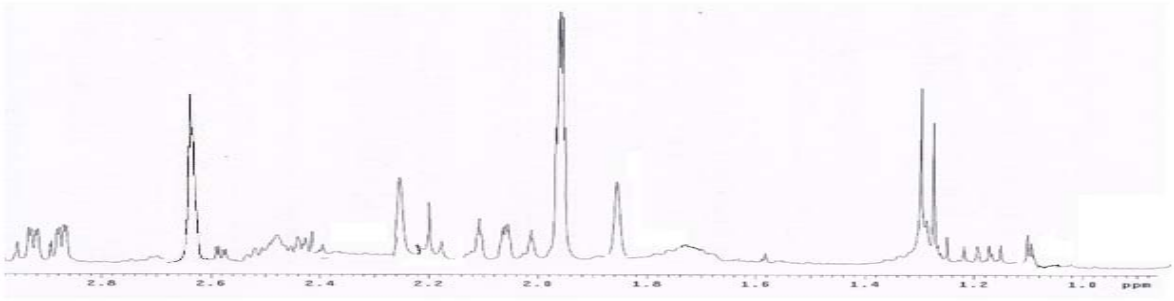
IR- 2

^{1}H NMR Spectroscopy (C2)

CDCl3 δ 7.36 (1H,d), 7.33 (1H,t), 7.26 (1H,t), 2.64(2H,s), 2.21 (3H,s), 2.20 (1H,m),1.82 (3H,s), 4.85 (2H,d), 1.33(2H,d).



NMR- 2a

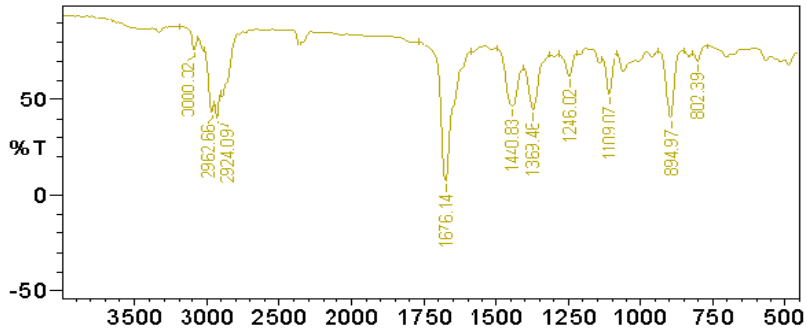


NMR- 2b

Spectroscopic data

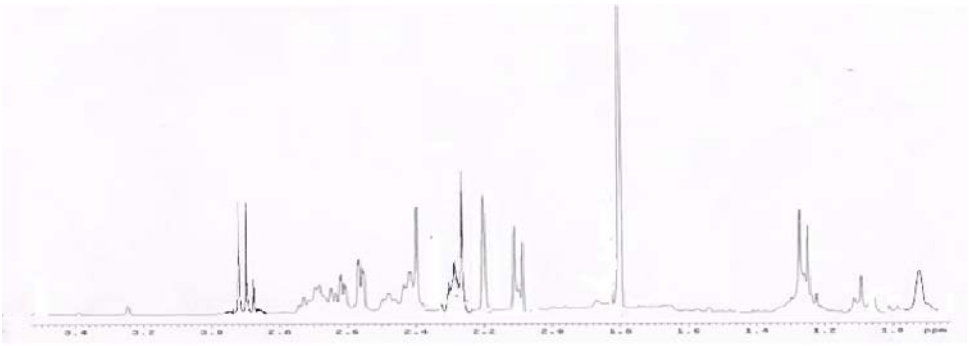
Infrared spectroscopy (C3)

FTIR (KBr, cm^{-1}): 3080.32 (C-H stretch), 2962.66, 2924.09, 1676.14 (C-C al kene), 1369.46 (C-N stretch), 1246.02, 1109, 894.97, 802.39

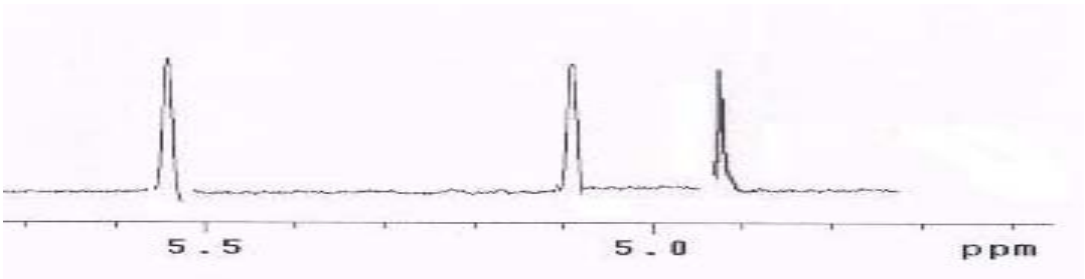


IR- 3

^{1}H NMR Spectroscopy (C3)



NMR- 3a

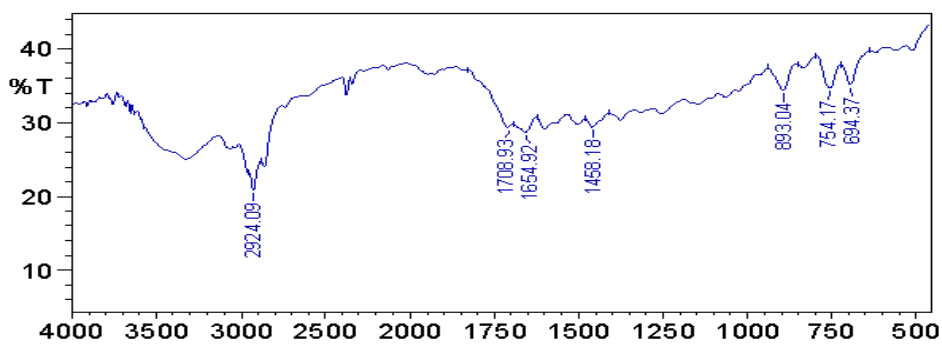


NMR-3b

Spectroscopic data

Infrared spectroscopy (C4)

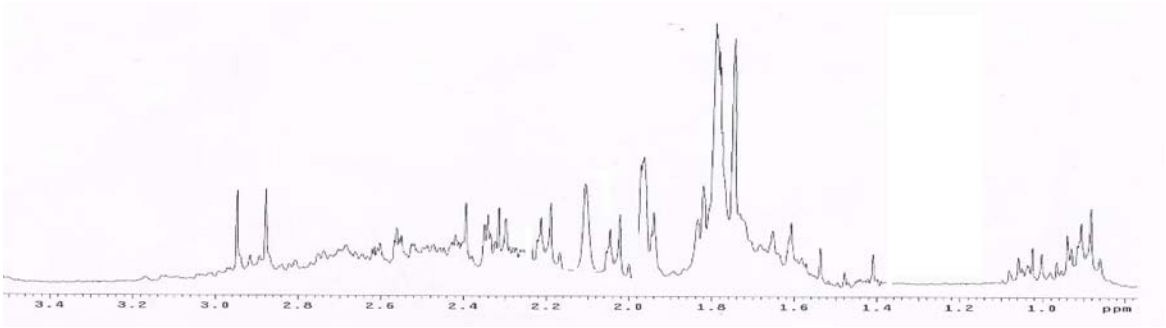
FTIR (KBr, cm^{-1}): 2924.09, 1708.93, 1654.92 (>C=N stretch), 1458.18, 893.04, 754.17, 694.



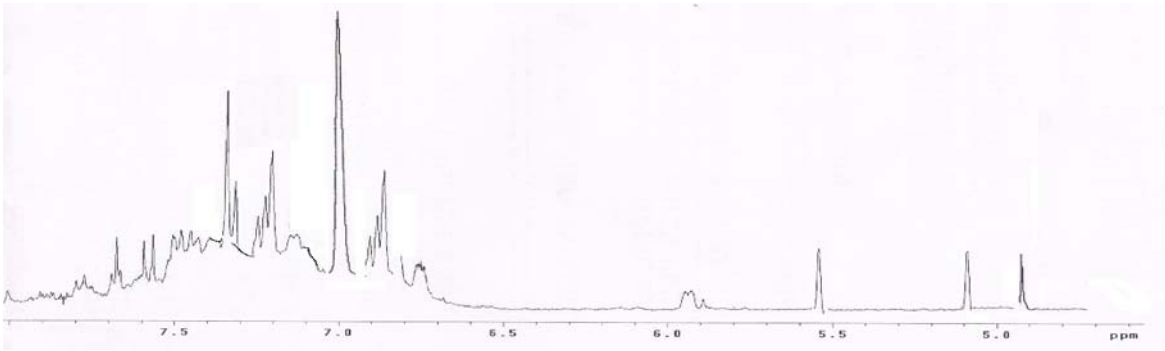
IR-4

^{1}H NMR Spectroscopy (C4)

CDCl3 δ 7.35 (1H,d),7.20 (1H,t),7.02(1H,t),6.9 (1H,s),5.09(1H,s),4.8(1H,s),5.55 (1H,s), 2.1(3H,s), 1.04(1H,d), 1.78(3H,s), 2.09(2H,t).



NMR-4a



NMR-4b

PART - 2: PHARMACOLOGICAL ACTIVITY

2.1 ANTIOXIDANT ACTIVITY EVALUATION

Evaluation of in-vitro antioxidant activity

The present work involved the in-vitro antioxidant study of methanolic (80%) solution of compounds by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging activity

METHODOLOGY

The reaction mixture consisted of 1 ml of 0.1 mM DPPH in methanol, 1 ml of methanol and 0.5 ml of methanolic (80%) solution of experimental compounds at various concentrations (0-300 µg/ml). Carvone was used as positive control. The absorbance of the mixture at 517 nm was measured 30 min later. The reaction solution without DPPH was used as a blank test. Measurements were performed in duplicate. The percentage of scavenging activity was determined by comparing the result of test material with those of standard antioxidant carvone. The radical scavenging activity was expressed as the inhibition percentage and monitored as per the equation:

Inhibition (%) = [(control – test) / control]*100

The result was expressed as IC_{50} value that is the concentration of solution required for 50% inhibition

RESULT

Compound	25	50	100	200	300
Standard Carvone	37.97	42.11	48.29	56.40	66.04

Table: 4- % inhibition by standard compounds at different concentrations

Concentrations in µg/ml						
Compound	**2**	**5**	**10**	**15**	**20**	**25**
C1	41.28	42.96	55.77	56.17	66.21	70.21
C2	68.59	73.04	76.37	78.02	81.44	88.64
C3	54.86	59.65	75.23	87.42	93.76	94.33
C4	46.02	47.81	61.39	67.24	75.93	89.41

Table: 5- % inhibition by compounds at different concentrations

Compounds	%inhibition (25 µg/ml)
Standard Carvone	37.97
C1	70.21
C2	88.64
C3	94.33
C4	89.41

Table: 6- % inhibition values at 25 µg/ml of experimental compounds by DPPH free radical scavenging activity

From the above table it has been observed that compound C3 possess more antioxidant activity when it is compared to Standard carvone and compound C1, C2 and C4.

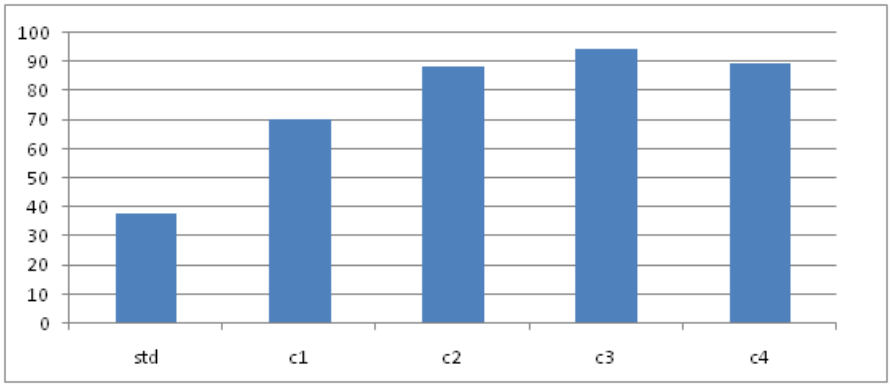


Fig. 3: % inhibition values (at 25 µg/ml) of experimental compounds by DPPH free radical scavenging activity

2.2 ANTI-CANCER ACTIVITY EVALUATION

Experimental Procedure for Sulforhodamine B Assay[15,16,17]**:-** Anticancer activity of the compounds was determined by sulforhodamine B (SRB) assay. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For screening experiment, cells were inoculated into 96 well microtiter plates in 100 µl. After cell inoculation, the microtiter plates were incubated at 37°C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 hrs prior to the addition of experimental drugs. After 24 hrs, one 96 well plate containing $5x10^3$ cells/well was fixed *in situ* with trichloroacetic acid (TCA) to represent a measurement of the cell population (Tz) at the time of drug addition. Experimental drugs were initially solubilized in ethanol at 100 mg/ml and diluted to 1 mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate (1 mg/ml) was thawed and diluted to 10^{-6} µg/ml, 10^{-5} µg/ml, 10^{-4} µg/ml, and 10^{-3} µg/ml with complete medium. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e. 10, 20, 40 and 80 µg/ml.

End Point Measurement:-After addition of compounds, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30 % w/v TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. SRB solution (50 µl) of 0.4 % w/v in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM TRIS base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100.

Six absorbance measurements [at time zero (T_z), control growth (C), and test growth in the presence of drug at the four concentration levels (T_i)] were used to calculate the percentage growth inhibition. Percentage growth inhibition at each of the drug concentration was calculated as:

$[(T_i–T_z)/(C–T_z)]$ x 100 for concentrations for which $T_i \geq T_z$ $(T_i–T_z)$ positive or zero

$[(T_i–T_z)/T_z]$ x 100 for concentrations for which $T_i < T_z$. $(T_i–T_z)$ negative

Human breast cancer cell line MCF-7

Table 7: % Growth compared to control

Drug concentration				
Compounds	10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml
C1	100.0	100.0	100.0	100.0
C2	96.6	94.6	91.0	81.0
C3	98.2	98.0	94.4	86.9
C4	73.8	69.5	45.7	35.0
ADR	10.9	10.3	6.8	-20.3

Cells were treated with at least four different concentrations levels ranging from 10 µg/ml – 80 µg/ml (as shown in table 7) and the sensitivity of MCF7 cell lines to each drug was assessed by TGI, LC50 and GI50.

Table 8: Dose response parameters

Compounds	LC50	TGI	GI50
C1	>80	>80	>80
C2	>80	>80	>80
C3	>80	>80	>80
C4	>80	>80	50.9
ADR	>80	51.2	<10

Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested as shown in table 8.

Human cervix cell line HeLa

Table 9:% growth inhibition compared to control

Drug concentration				
Compounds	10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml
C1	67.2	63.2	56.7	51.3
C2	61.1	57.2	50.0	40.0
C3	60.7	56.1	51.9	31.3
C4	32.6	28.2	9.5	-20.9
ADR	-47.9	-62.3	-63.9	-59.6

Cells were treated with at least four different concentrations levels ranging from 10 µg/ml – 80 µg/ml (as shown in table 9) and the sensitivity of HeLa cell lines to each drug was assessed by TGI, LC50 and GI50.

Table 10: Dose response parameters

Compunds	LC50	TGI	GI50
C1	>80	>80	69.4
C2	>80	>80	51.3
C3	>80	>80	45.9
C4	>80	55.5	14.5
ADR	49.4	<10	<10

Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested as shown in table 10.

Human ovarian cancer cell line SK-OV-3

Table 11: %growth inhibition compared to control

Drug concentration				
Compounds	10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml
C1	100.0	99.0	97.3	88.0
C2	100.0	94.1	87.8	78.9
C3	99.4	97.4	93.8	90.8
C4	98.5	75.7	43.4	6.8
ADR	-27.5	-39.3	-40.0	-48.9

Cells were treated with at least four different concentrations levels ranging from 10 µg/ml – 80 µg/ml (as shown in table 11) and the sensitivity of SK-OV-3 cell lines to each drug was assessed by TGI, LC50 and GI50.

Table 12: Dose response parameters

Compunds	LC50	TGI	GI50
C1	>80	>80	>80
C2	>80	>80	>80
C3	>80	>80	>80
C4	>80	55.5	43.0
ADR	62.7	21.9	<10

Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested as shown in table 12.

RESULT AND DISCUSSION

Caraway (*Carum carvi* L.) plant reported for the most therapeutic used in traditional system. We separated the active constituent; carvone from crude caraway seeds by method which are reported in literature.

Optimizes the extraction techniques using various solvents and found better yield of carvone when DCM was replaced by $CHCl_3$.

Four different derivatives of carvone were prepared in order to evaluate the antioxidant and anticancer potential of carvone oil and its derivatives. All the four derivatives were preparaed by condensation reaction and yield obtained was in range of 70-80 %. The reaction were carried out replacing by cyclohexane by n-hexane which gave better yield. And at the end n-hexane was recycled. The yield was 20-25 ml of recycled n-hexane. All the derivatives were characterized. All the derivatives of carvone were evaluated for antioxidant activity and have show good antioxidant activity as compared to standard carvone.

All the carvone derivatives then evaluated for anticancer activity on MCF7 (breast), HeLa (cervix) and SK-OV3 (ovary) cell lines at ACTREC, Mumbai. It was found that GI 50 of (1E)-1-[2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-ylidene]-2-phenyl hydrazine has anticancer potential. Other derivatives were shown to have poor GI 50 values.

ACKNOWLEDGEMENTS

The authors are thankful to H(S)NCB's Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar for availing all laboratory and library facilities for the completion of this investigation. We also extend our greetings to H(S)NC Board. At this moment of accomplishment I pay homage to my guide, Mrs. Anita Yadav, Research guide of pharmaceutical chemistry. Under her guidance I successfully overcame many difficulties and learned a lot.

REFERENCES

1. Semih otles, Eren Akcicek . Herbs as a Food Source in Turkey Dursun Esiyok. *Asian Pacific Journal of Cancer Prevention*, **2004**, 5.
2. Kokate C. K., Purohit A. P. et al. Book of pharmacognosy. *Nirali prakashan*, **2008**, ed.42, 8.56, 11.29.
3. Gomase V.S. and Sherkhane A.S.. Isolation, structure elucidation and biotransformation studies on secondary metabolites from *Asparagus racemosus. International Journal of Microbiology Research*. **2010**, Volume 2, 1, 07-09.
4. Arjun Kafle, Surya K. Kalauni, Mangala Devi Manandhar. Phytochemical studies and in vitro activity of *Asparagus racemosus. JNPA.* **2012,** XXVI (1), 48-53.
5. J. Sedlakova, B. Kocourkova, L. Lojkova, V. Kuban. Determination of essential oil content in caraway (*Carum carvi L.)* species by means of supercritical fluid extraction. *PLANT SOIL ENVIRON., 49*, **2003** (6): 277–282.
6. Tetsuko matsumura, Toru ishikawa, and Junichi kitajima. Water-Soluble Constituents of Caraway: Carvone Derivatives and Their Glucosides. *Chem. Pharm. Bull*, **2002,** 50(1) 66-72.
7. Diego S. Pisoni, Douglas Gamba, *et al.* InCl3/NaClO: A Reagent for Allylic Chlorination of Terminal Olefins. *J. Braz. Chem. Soc.*, **2006**, Vol. 17, No. 2, 321-327.
8. Jiaojiao Chen, Min Lu, Yongkui Jing, and Jinhua Dong. The synthesis of L-carvone and limonene derivatives with increased antiproliferative effect and activation of ERK pathway in prostate cancer cells. *Bioorganic & Medicinal Chemistry*, **2006**, 14, 6539–6547
9. Javeed A. W., Rajeshwara N. *et al.* Phytochemical Screening and Aphrodisiac Activity of Asparagus racemosus. *International Journal of Pharmaceutical Sciences and* Drug *Research.* **2011**; 3(2): 112-115
10. http://intranet.wellingtoncollege.org.uk/resource.aspx?id=167862.
11. http://kriemhild.uft.uni-bremen.de/NOP/en/instruction/pdf/2006.
12. A Obadiah, R. Kannan, P. Ramesh. Isolation of carvone and phellandrene from *Murraya koenigii* and study of their antioxidant activity**.** *Chemistry of Natural Compounds*, **2012**, Vol. 48, No. 1.
13. Anand, P., Kunnumakkara, A.B., Sundaram C, Harikumar KB, Tharakan S.T., Lai O.S., Sung, B., Aggarwal, B.B. "Cancer is a preventable disease that requires major lifestyle changes". *Pharm. Res.* 2008, **25** (9) 2097–2116.
14. "Heredity and Cancer". American Cancer Society. Retrieved July 22, 2013.
15. Papazisis, K.T. et al. Optimization of the sulphorhodamine B calorimetric assay. *J.Immun. Methods* **1997**, 208,151-158.
16. Wieland Voigt. Sulphorhodamine B assay and chemosensitivity. *Methods in molecular medicine chemosensitivity*: vol 1: in-vitro assays, vol 110: 39-48.
17. Shankar K Mitra, Neswi S Prakash, Ramachandran Sundaram. Shatavarins (containing Shatavarin IV) with anticancer activity from the roots of*Asparagus racemosus Indian journal of pharmacology*. **2012,** vol 44, 6, 732-736
18. J. Sedlalova, B. Kocourkova, L. Lojkova. The essential oil content in caraway species**.** *7HORT. SCI. (PRAGUE), 30*, **2003**, 73–79
19. Tijare R D, Beknal A B, Mahurkar N, Chandy V. Pharmacognostical and Phytochemical Studies of Root Tubers of *Asparagus gonoclados* Baker. *International Journal of Pharmacognosy and Phytochemical Research*, **2012**; 4(3); 142-145.
20. Kinzler, Kenneth W.; Vogelstein, Bert (2002). "Introduction". *The genetic basis of human cancer* (2nd, illustrated, revised ed.). New York: McGraw-Hill, Medical Pub. Division. p.
21. Peter Houghton et al. The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. *Methods* **2007**, 42, 377-387.

Source of support: Nil, Conflict of interest: None Declared

