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Isolation and Characterization Of Some Compound From Ginger

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ABSTRACT

The present research work is mainly concerned with the natural compounds that are obtained from traditional medicinal plants. There are number of natural compounds have been available in the nature. Some natural compounds are very useful to human being and have life potential to save human from many uncurable disease. Keeping view in the mind the research is focused to extract and isolate antioxidant, anti-inflammatory, anticancer, antihyperlipidemic, antidiabetic, antiulcer activities containing compounds are studied. The major natural compounds is ginger, The ginger is obtained from rhizomes of zingiber officinale, and useful anti rhuematic and pungent and spicy contains gingerol and shogaol as active principles. All components are isolated from the concerned extracts. The extraction of all plants are based on successive solvent extraction method for all drugs.The constituents are confirmed by structure elucidation. The structure of each compounds are intrepreted by different spectral techniques like Infrared spectrum, nuclear magnetic spectrum (hydrogen anc carbon thirteen spectra) and mass spectroscopy for molecular formula and molecular weight of the unknown compounds. The biological evaluation of each compound is performed separately. For antioxidant activity DPPH method is used. & the immunomodulatory activity

Keywords: Ginger, isolation ,Antioxidant

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INTRODUCTION

Natural compounds

Natural compounds are obtained in the nature from different medicinal and herbal plants. These are generally secondary metabolites of the various plant parts or waste compound of plant. These are obtained various parts of the herbs, shrubs or plants like stem, root, leaves, fruits, seeds, barks, rhizomes, flowers and entire plants. The particular part of the plant is collected, extracted and isolated for the collection of various types of natural compounds. We include alkaloids, glycosides, terpenoids, resins, tannins, carbohydrates, gums and exudates, liganans. These obtained drugs have various types of pharmacological and therapeutic application.

Introduction:

Ginger (*Zingiber officinale*) is cultivated in some temperate zone or tropical zone countries, such as China & India . It is one of the most widely used spices in the world. Pungency is an important sensory character of ginger & is attributed to the presence of gingerol compounds & their decomposition compound, zingerone, & the dehydration compounds, shogaols. It can also be used in the pharmaceutical industry because they contain active compounds that have effects on certain physiological process The components in ginger include such as extractable oleoresins, many fats, carbohydrates, vitamins, minerals & a potent proteolytic enzyme called gingiberene Oleoresins contributes to sensory perception of ginger There are 5-8% of oleoresins in crude ginger, which consist of two distinct groups of chemicals: volatile oils & non-volatile pungent compounds More than 66 compounds have been identified in volatile oil. They include gingeberene, curcumene & farnesene, β-sesquiphelldren, bisabolene, 1, 8- cineole, linalool, borneol, nerol, geraniol, camphen, limonen, myrcen, β-phelldren, α-pinene, citronellol, geranial, & others Many of these constituents contribute to the distinct aroma & taste of ginger .Non-volatile pungent compounds of ginger consist mainly of gingerols, shogaols, paradols & gingerone These pungent principles produce a “hot” sensation in the mouth.

Many bioactive compounds have been found in ginger, such as gingerols, shogaols & paradols. Gingerols, especially 6-gingerol have been of intense interest because of their biological properties. It has been shown that 6-gingerol has antioxidant activity & anti-inflammation activity, 6-gingerol was also found to have anti-tumor activities which are related to cytotoxic & antiproliferative effects & inhibit angiogenesis in vitro & in vivo

MATERIALS AND METHOD

(a) Procured Material:

Ginger extract was provided by Sabinsa Corp

Solvents, reagents & chromatographic supplies, cell cultures :

Solvents including ethanol, methanol, acetone, ethyl acetate, hexane, *n*-butanol, acetonitrile were purchased from Fisher Scientific & were of HPLC grade. DPPH & HO purchased from Aldrich Chemical Co. Silica gel (60 Å, 32-63 μm) for normal phase chromatography was purchased from Sorbent Technology Inc. Thin Layer chromatography (TLC) plates were purchased from Fisher Scientific. C18 silica gel (60 Å), Sephadex LH-20 & Diaion HP-20 for column chromatography were purchased from Sigma. Lipopolysaccharide (LPS) (*Escherichia coli* 0127: E8), sufanilamide, naphthylethylenediamine dihydrochloride, & dithiothreitol (DTT) were purchased from Sigma Chemical Co. Acrylamide was purchased from E. Merck Co

Instruments

^{1}H NMR, ^{13}C NMR were recorded on a U-400 instrument. Chemical shifts were expressed in parts per million (δ) using TMS as internal standard. HPLC system was composed of a Hitachi LC 6200A intelligent pump, Waters 490 E programmable multiple wavelength detector,

(b) Method:

Isolation of compounds from Ginger

a) Ginger extract was added to a Sephadex LH-20 column for fractionation. 95% ethanol was used as eluting solvent. The eluate was collected & concentrated to a residue. TLC was used to check the procedure.

b) Residue from step "a" was subjected to Diaion HP-20 column for further isolation.

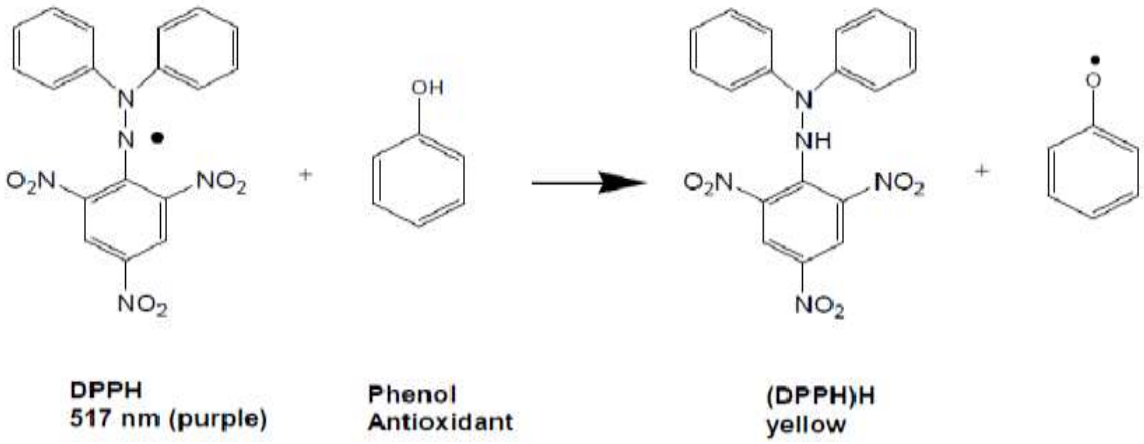
The column was eluted with water first, followed by 50% ethanol,

95% ethanol & the eluents were collected & concentrated. Water fraction, 50% ethanol fraction, 95% fraction were obtained respectively.

c) 95% ethanol fraction from step "b" was subjected to a reverse phase column for further isolation. The column was eluted with methanol/water (3:2). pure compound, were obtained.

Antioxidant assay: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay

The procedure is used to check the antioxidant activity of phenolic compounds or other oxidable products. The diphenyl picryl hydrazyl is blue in color when it is reacted with phenolic compounds this accepts a electron from reducing equivalent hydrogen and become colorless when reaction is complete. The reaction is shown in Figure (Molyneux, 2004; Son *et al*., 2002).



Reaction of DPPH with an antioxidant

The diphenyl picryl hydrazyl DPPH is radical that is purple in colour and maximum absorption indicate at 570nm . When this radical is treated with phenol and phenolic compound it forms a complex whose intentsity is decreased as the colorless. The antioxidant properties of the compound is inversely propotional to the intensity of the colour of the compounds.

The percentage inhibition of DPPH radical by sample was calculated according to formula (Yen & Duh, 1994). % DPPH free radicals scavenge activity = $[(A_0\text{-}At)/A_0] \times 100$

Where Ais the absorbance of the DPPH radical without antioxidant at t=0 min At is the absorbance of the antioxidant at t = 30 minutes.

The method is that 2 mL sample alcohol solution is added to 2 mL DPPH alcohol solution& reacts for 30 min in a dark condition. The DPPH concentration is 100µMIC was obtained by extrapolation from linea rregrestion analysis

Immunomodulatory activity of ginger

The ginger is obtained in India on large scale and this is obtained through the year. The rhizomes of ginger officinale are the biological source of the plant. The rhizomes have pungent taste due to presence of gingerol and anhydrous form of gingerol (shaogol). The color of the ginger is due to carotenoid and this also contain gingiberine that is sesquieterpen. The plant is very important. The rhizomes are used as spicy and gas removal and it is also used in vegetables and tea for making tast. The pungency of the ginger can be destroy with one percent solution of potassium hydroxide.

Materials:

Animals:

Rat or mice of weight twenty three to twenty seven were taken for the study and these should be six to eight week old. These had been kept under standard environment conditions & had been eaten with standard pellet food purchased by Hind. lever Limited Culcutta, India, & H_2O *ad libitum*.

Medicaments & Chemicals

Ginger extract was provided by Sabinsa Corp A test solution of Ginger Extract had been formulated by dissolution of acacia in H_2O for orally administration to animals. cyclophosphamide in a dosage of twenty five milgram per kilogram per oral had been utilized as a reference standard immune modulator action (Sukla *et. al*, 2009) for comparison. & injecting $0.5x10^9$ S.R.B.C.s is taken from the blood of sheep that is scarified from a local saloughter home. S.R.B.C.s) had been cleaned and washed with 0.9 % NS solution three times without pyrogen. The solution was made to a concentration of 0.5×10^9 cells per ml for immunization& challenge.

Methods:

Delayed Type Hypersenstivity (Puri et.*al.*, 1993):

Each gathering of creature contains six mice or rats. Creatures of is gatherings given as (typical saline of ten milliliter for every kilogram body weight. per oral measurements), mice of second gathering had been administerd with cyclophosphamide (a quarter century for every kilogram body weight. p.o.), mice of third gathering had been administerd with test concentration (one hundred twenty five milligram for every kilogram body weight. p.o.) and mice of fourth gathering had been administerd with test synthetic (two hundred fifty milligram for each kilogram body weight. p.o. p.o.). these all creatures were dealt with from zero to seven dose as given above. vernier caliper was utilized to quantify the thickness of right hand foot cushion. Mice and rats had been then tested by infusion of 0.5×10^9 S.R.B.C.s in ideal in foot cushion. Thickness of foot cushion had been noted for each twenty four hours after the test. The contrast amongst post and pre challenge foot cushion thickness noted in mm had been considered as a measure of (DTH) and The estimation of mean had been gotten to treat bunches and these had been contrasted and that of control gatherings. The ginger Extract had been given per orally on day zero and proceeded till day seven of test the information got had been broke down by statistics..

RESULTS AND DISCUSSION

Isolation of components from ginger & their biological activity study

Following a serious of column chromatography separation of ginger extract

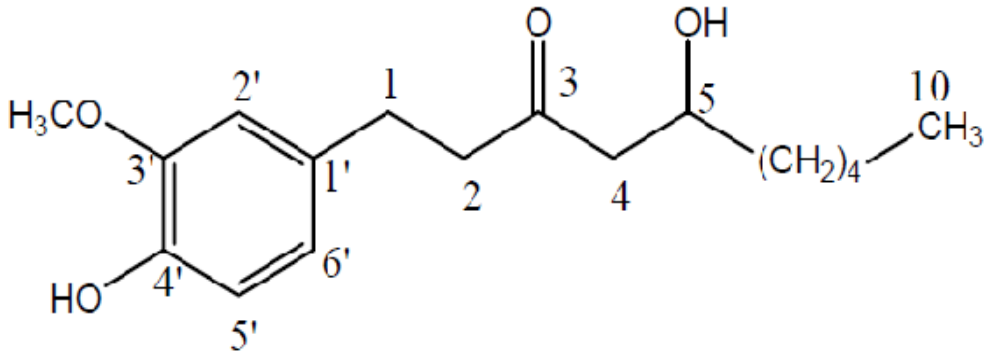
Identification of components isolated from ginger extract.

Identification of 6-gingerol.

Compound 1 from ginger extract is a pale yellow liquid. Its structure was assigned as 6-gingerol on the basis of its ^{1}H NMR &^{13}C NMR (100MHz) data & comparison with the literature data (Agarwal et al., 2001; Shoji et al., 1982).

^{1}H NMR (400 MHz, Appendix 1) δ: 0.88 (3H, t, J=6.8 Hz, H-10), 1.23-1.68 (8H,m,H-6~H-9), 2.52 (1H, d, J=8.4 Hz, H-4a), 2.54 (1H, d, J=3.6 Hz, H-4b), 2.73 (2H, brd, J=6.8 Hz, H-2), 2.81 (2H, brd, J=6.8 Hz, H-1), 3.84 (3H, s, OCH), 4.03 (1H, m, H-5), 6.64 (1H, dd, J=8, 2 Hz, H-6'), 6.67 (1H, s, H-2'), 6.81 (1H, d, J=8 Hz, H-5')

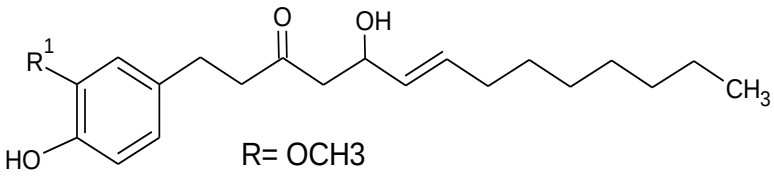
^{13}C NMR (100 MHz, Appendix 2) δ: 13.8 (C-10, q), 22.3 (C-9, t), 24. 9 (C-7, t),29.0(C-8, t), 31.1 (C-1, t),, 36.2 (C-6, t), 45.2 (C-2, t), 49.1 (C-4, t), 55.6 (OCH 3,q)67.5 (C-5, d), 110.9 (C-2', d), 114.3 (C-5',d), 120.4 (C-6', d), 132.4 (C-1', s), 143.7 (C-4',s), 146.3 (C-3', s), 211.3 (C-3, s)



Structure of 6-gingerol

Identification of 6-shogaol

Compound 4 from ginger extract is a pale yellow liquid. It was elucidated as 6-shogaol (Figure 4.7) byH NMR &C NMR, & confirmed by comparing with literature data (Connell & Sutherl&, 1969).



Structure of 6-shogaol

^{1}HNMR (400 MHz, Appendix 7.) δ: 0.88 (3H, t, J=6.8 Hz, H-10), 1.26-1.31 (4H, m, H-8 & H-9), 1.42 (2H, m, H-7), 2.17 (2H, m, H-6), 2.84 (4H, m, H1 & H-2), 3.82 (3H, s, OCH), 6.09 (1H, dt, J=16, 1.6 Hz, H-4), 6.66 (1H, dd, J=8, 2Hz, H-6'), 6.70 3 (1H, d, J= 1.6 Hz, H-2'), 6.81 (1H, d, J= 7.6 Hz, H-5') 6.83 (1H, d, J=16.0 Hz, H-5).

^{13}C NMR (100 MHz, Appendix 8) δ: 13.7 (C-10, q), 22.2 (C-9, t), 27.5 (C-7, t), 29.6(C-8, t), 31.1 (C-1, t), 32.2 (C-6, t), 41.6 (C-2, t), 55.5 (OCH 3,q), 111.0 (C-2', d),114.2 (C-5',d), 120.5 (C-6', d), 130.8 (C-4, d) 132.8 (C-1', s), 143.7 (C-4',s), 146.3 (C-3', s), 147.4 (C-5, d), 199.9 (C-3, s)

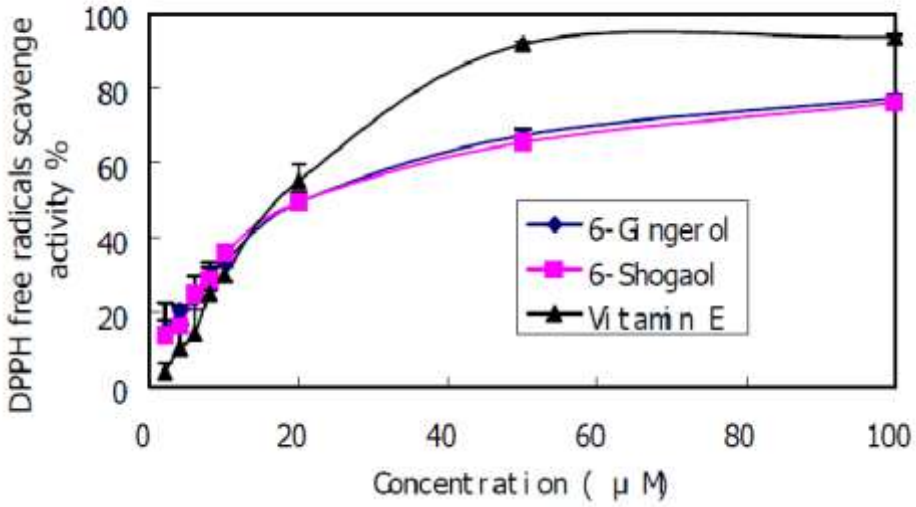
Antioxidant activity of 6-gingerol & 6-shogaol

The antioxidant activity of 6-gingrol & 6-shogaol isolated from ginger extract was assayed by DPPH free radicals scavenge. Various concentrations of 6-gingerol, 6-shogaol & Vitamins E were mixed with 100 μM (final) DPPH dissolved in 95 % alcohol. Absorption at 517 was measured after 30 minutes. 6-gingerol & 6-shogaol showed concentration-dependent free radical scavenging ability (shown in Figure). The antioxidant activity of 6-shogaol is comparable to 6-gingerol. At the concentration under 12 μM, they are more potent than Vitamin E. While at concentration higher than 13 μM, their antioxidant activities are not as good as Vitamin E. The 50% DPPH scavenge concentrations are: 6- gingerol: 21 μM, 6-shogaol: 21 μM, Vitamin E: 18 μM

Phenolic substances are the most effective antioxidants from natural sources. The phenolic antioxidants include alkoxyl phenols that containing one free & one alkylated hydroxyl group, usual methoxy, or polyphenols with ortho- or para- dihydroxylic groups, or phenols containing condensed ring The antioxidant activity of phenolic compounds is related to the hydroxyl group & the presence of a second hydroxyl group in the *ortho* or *para* postition Furthermore, the antioxidant efficiency of phenolics is increased substantially by methoxy substitution in position *ortho* to the hydroxyl group because the electron-donating methoxy group allows increased stabilization of the resulting aryloxyl radical through electron delocalization after hydrogen donation by hydroxyl group (Rice-Evans *et al*, 1996). Both of the 6-gingerol & 6-shogaol contain a same aromatic moiety with a free hydroxyl group & a methoxyl group in the position *ortho* to hydroxyl group. Therefore, it is no surprise that they have strong antioxidant activity.

The structure difference between 6-gingerol & 6-shogaol is that 6-gingerol has a hydroxyl group on decane while 6-shogaol is dehydrated form of 6-gingerol, resulting from the elimination of OH group at C-5 with the formation of a double bond between C-4 & C-5. According to Sekiwa, side chain of phenol does not influence the antioxidant activity (Sekiwa *et al.*, 2000). This supports our

results that 6-gingerol &6-shogaol almost have the same antioxidant activities



DPPH free radicals scavenge activity of 6-gingerol, 6-shogaol &Vitamins E. Data represent one of three similar results.

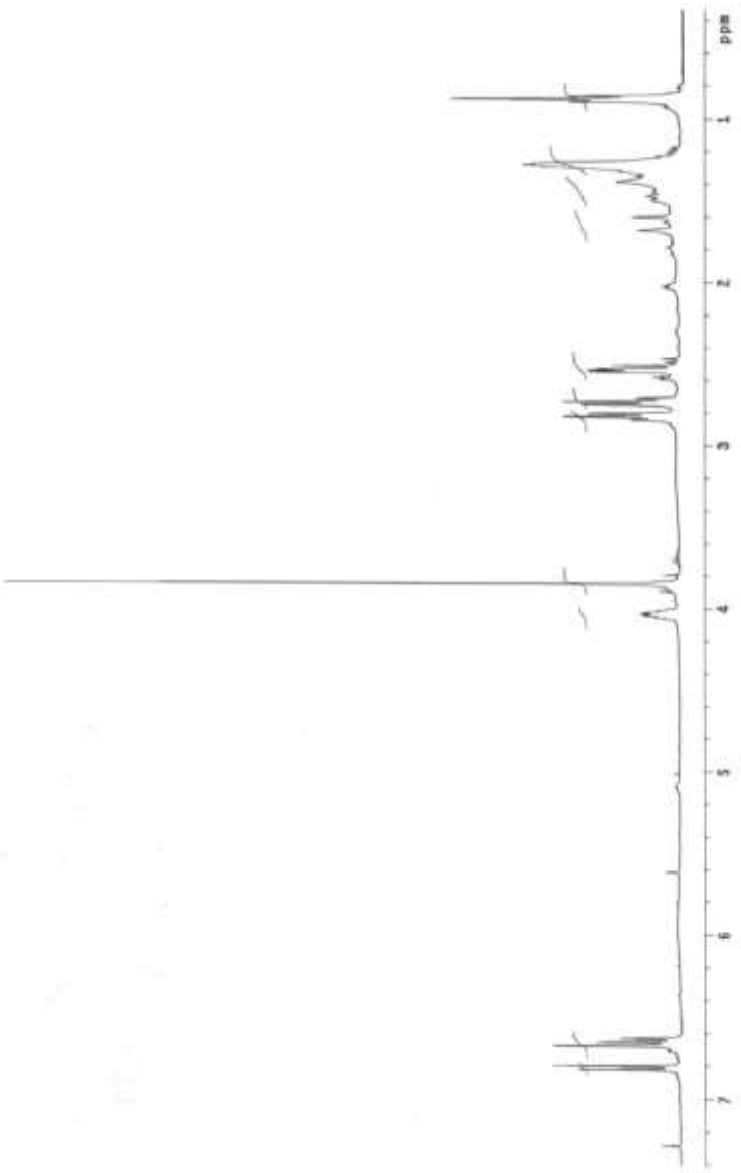
Delayed Type Hypersensitivity Test

Effect of ginger Ext on cell mediated immune response by DTH induced footpad oedema is shown in Table 1 Apart from humoral response there was significant increase in DTH response or cell mediated immunity indicated of increase mean paw edema value. Ginger Ext showed increase in DTH in all treated group when compared with control group.

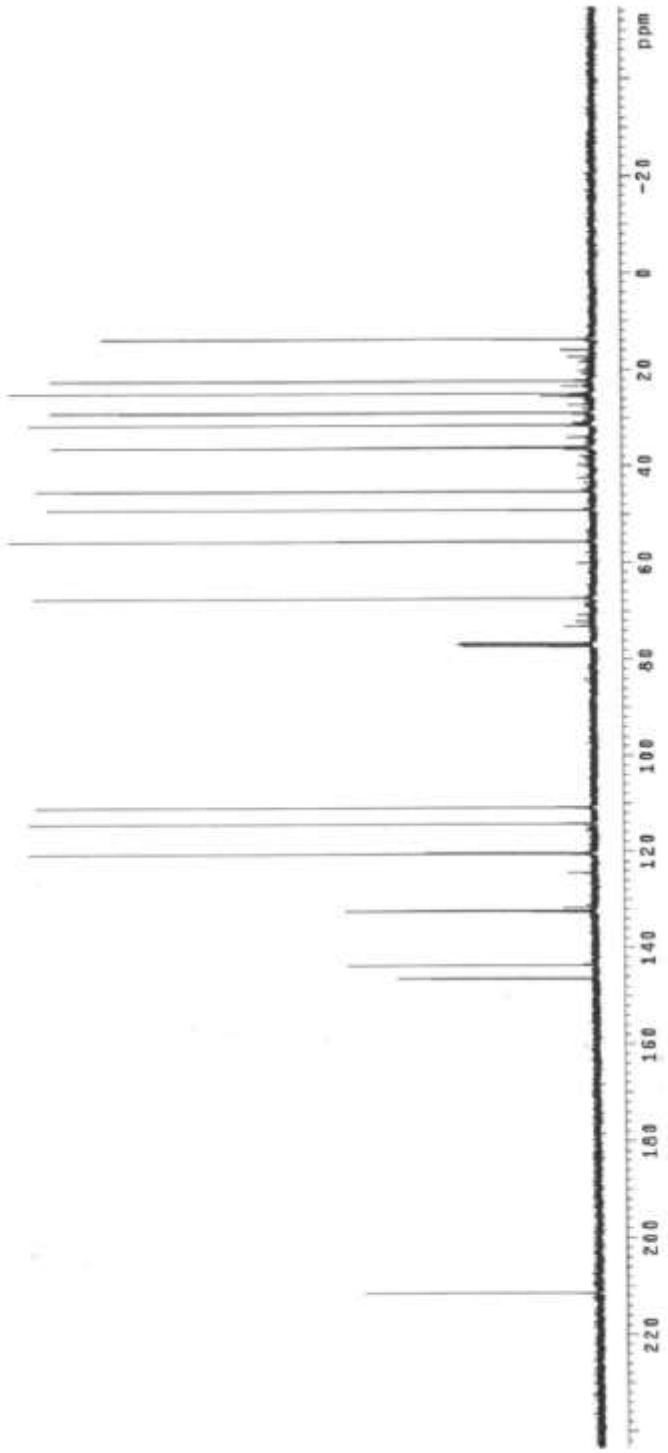
Table 1: Effect of Ginger Ext treatment in Delayed type hypersensitivity test by induced Foot pad oedema

Group	Dose	DTH response mm Mean±SD	
		4 hr	24hr
I-Control	10 ml/kg normal saline	0.339±0.031	0.17±0.015
II-St&ard	cyclophosphamide(25mg/kg)	0.833±0.047	0.81±0.032
III- Dose I	125 mg	0.592±0.043	0.36±0.049
IV-Dose II	250mg	0.75±0.052	0.58±0.038

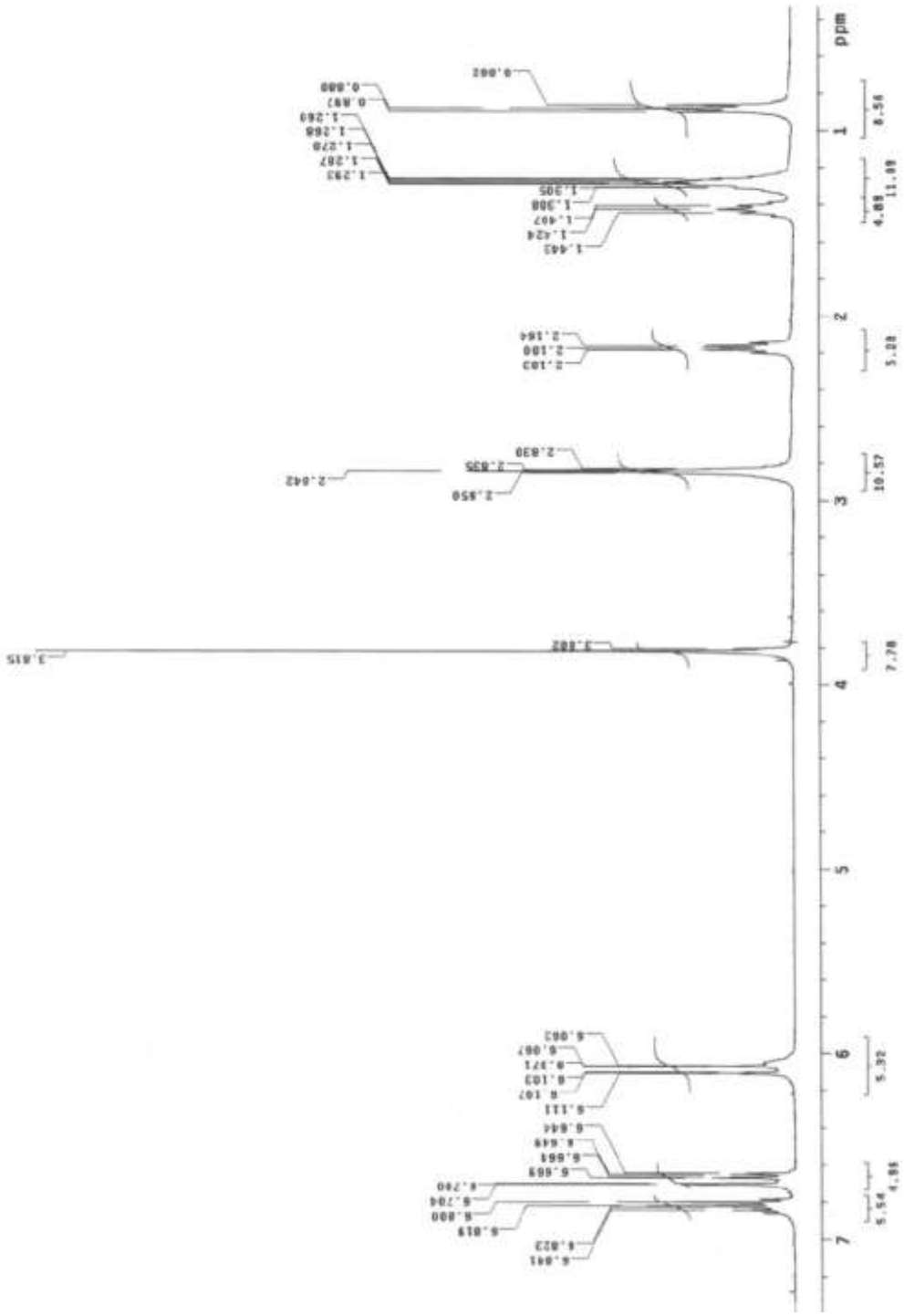
SPECTRA



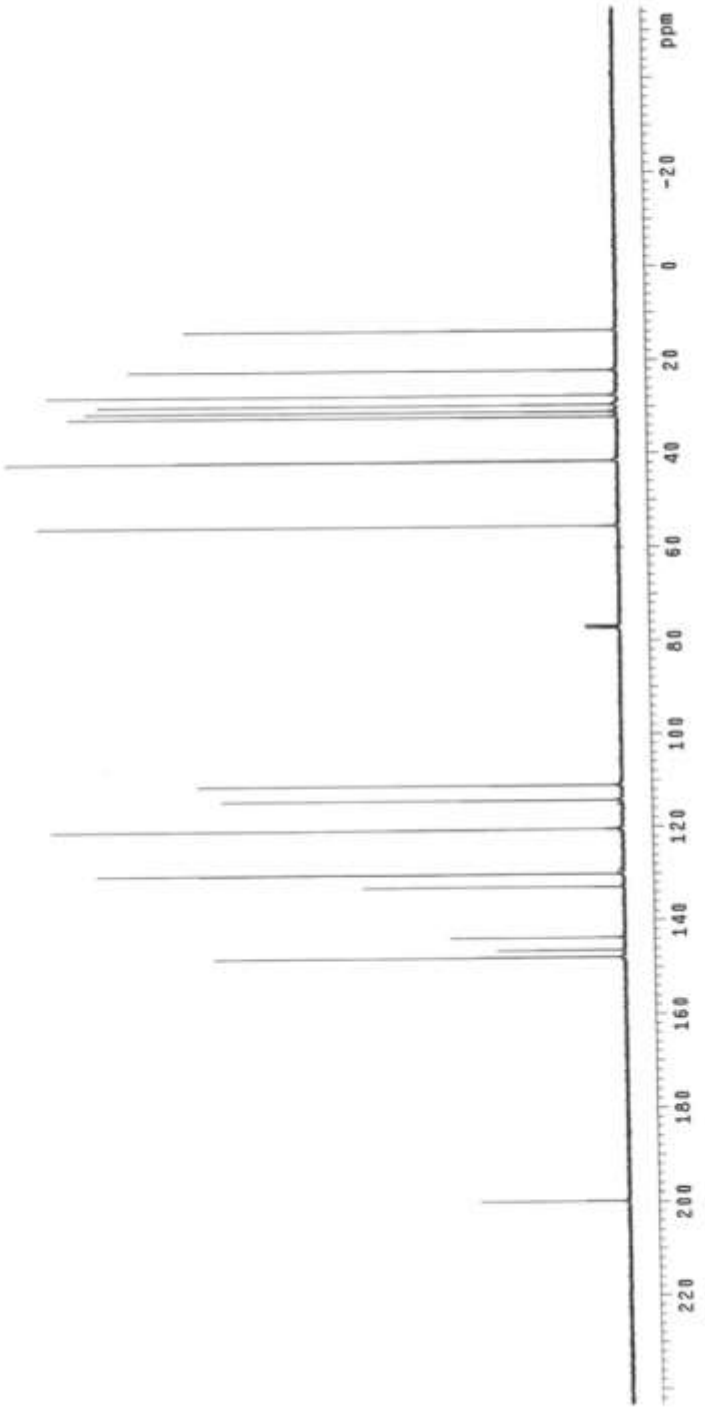
Spectrum 1.7.1: ^{1}H-NMR of 6-gingerol



Spectrum 1.7.2: ^{13}C-NMR of 6-gingerol



Spectrum 1.7.3: ^{1}H-NMR of 6-shogaol



Spectrum 1.7.4:^{13}C-NMR of 6-shogaol

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