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Chemical Constitutents from Ajwain Seeds (*Trachyspermum ammi*) and Inhibitory Activity of Thymol, Lupeol and Fatty Acids on Barnyardgrass and Radish Seeds

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The volatile oil of ajwain seeds (*Trachyspermum ammi*) was examined by gas chromatography and the major component thymol (**1**, 53.8%) was identified by spectroscopic studies and separated from volatile oil. After hydro-distillation of the volatile oil of seeds have yielded several compounds lupeol (**2**), linoleic acid (**3**)**,** stearic acid (**4**), eicosanoic acid (**5**), β-sitosterol-3-O-β-D-glucoside (**6**) from hexane : ethyl acetate : methanol extract. The structures of these compounds were elucidated with 500 MHz nuclear magnetic resonance, using 1D and 2D spectral methods, aided by electron ionization mass spectrometry (EI-MS), fast atom bombardment mass spectrometry (FAB-MS), infrared and compared with reported values and authentic samples. Regarding isolation of compounds from ajwain seeds, this is the first chemical investigation report. To the best of our knowledge, lupeol, linoleic acid, stearic acid, eicosanoic acid and β-sitosterol-3-O-β-D-glucoside were identified for the first time in ajwain seeds. In biological activity tests using these identified compounds thymol showed potent inhibitory activity against barnyardgrass (*Echinochloa crus-galli*) and radish seeds (*Raphanus sativus*) and was completely suppressed the germination and growth of shoot and root length. Lupeol exhibited also showed slightly inhibitory effect about 15 to 33% at concentration of 500 ppm to both barnyardgrass and radish seeds. The linoleic acid, stearic acid and eicosanoic acid did not show any strong inhibition effects against barnyardgrass.

Key Words: *Trachyspermum ammi* L, Apiaceae, Seeds composition, Known compounds, Thymol, Lupeol, Linoleic acid, Stearic acid, Eicosanoic acid, β-Sitosterol-3-O-β-D-glucoside, Inhibitory activity, Barnyardgrass, Radish seeds.

INTRODUCTION

The idea of using medicinal plants to treat human being and livestock is not new and in many developing countries their use is still in vogue. Despite the fact that in developed countries modern development in

allopathic medicine is at climax, there is a renewed interest in using medicinal plants to treat humans, pets and livestock. Medicinal plants are being used for therapeutic purposes in several ways. In modern medicine, many drugs are used that are mainly derived from plants *e.g.* digitalis, morphine, atropine, cinchona and vinblastine[1-3]. The interest in plant products has considerably increased all over the world due to the fact that many herbal medicines are free from side effects[2,4,5].

Among the traditional potential herbs, ajwain (*Trachyspermum ammi* L.) belonging to family Apiaceae, is widely used for curing various diseases in both humans and animals. Its other names in literature are, ajwan, ajowan, Bishop's weed, carom, or Ethiopian cumin. A number of medicinal and therapeutic properties have been described to various parts of this plant. For example, its fruit possesses stimulant and carminative properties and is regarded as antispasmodic. It is an important remedial agent for flatulence, atonic dyspepsia and diarrhoea[1]. The seeds of ajwain is bitter, pungent and it acts as anthelmintic, carminative, laxative and stomachic. It also cures abdominal tumours, abdominal pains and piles[2,3]. Ajwain seeds contain an essential oil containing about 50-55% thymol which is a strong germicide, anti-spasmodic, strong anti-bacterial and fungicide. Thymol is also used in antiseptic effect in toothpaste, flavour in cough drops, mouth washes, gargles and perfumery[2,3,6].

Although ajwain, is cultivated in many countries yields an essential oil which is major souce of thymol. The plant is a native of Mediterranean region and is cultivated and found growing wild in south west Asia. In India it is grown in north-western states of Gujrat, Maharashtra, Rajasthan, Madhya Pradesh, Uttar Pradesh and Bihar[7,8].

No information is available on the inhibitory activity of compounds thymol, lupeol and fatty acids. There are no reports in the literature about the chemical constitutents of ajwain seeds except volatile constituents by GC and GC-MS [9], identification of bioactive constitutents with growth or germinating properties is required. Thus the objectives of our research were (1) to isolate and identify bioactive constituents from ajwain seeds and (2) to examine the bioactivity of these compounds. The isolation of compounds from ajwain seeds and their inhibitory activity on barnyardgrass and radish seeds are discussed in this paper. All the isolated compounds including thymol, lupeol and fatty acids are very important compounds in the field of biological activities.

The volatile oil of ajwain seeds was examined by gas chromatography and the main component of volatile oil is thymol (**1**, 53.8%) identified[10], after the separation of oil from seeds, the same extracted with hexane:ethyl acetate:methanol (1:1:1) and isolated six compounds one pentacyclic triperpene alcohol, fatty acids and sterol glycoside *viz.* lupeol (**2**), linoleic

acid (**3**), stearic acid (**4**), eicosanoic acid (**5**), β-sitosterol-3-O-β-D-glucoside (**6**). The chemical investigation of ajwain seeds regarding the isolation of compounds, this is the first report and isolated known compounds and identified by using 500 MHz NMR spectral methods *viz.* ^{1}H and ^{13}C NMR aided by EIMS, FABMS and IR. Earlier these compounds lupeol, lupeol derivatives[11,12] and fatty acids were reported from[13-15]. The possible spectral data of all the isolated compounds (**1-6**) from ajwain seeds are reported in this paper and which should provide valuable information for investigators working in the area of natural products chemistry.

In continuation of our studies on the medicinal plants[10] and ajwain[16], several synthetic and natural derivatives of thymol already known and spectral data are reported in the literature. Although monterpenes inhibit germination of seeds and growth of seedlings[17].

Novel formulations[18] comprising an effective amount of thymol obtained from the plant *Trachyspermum ammi* (Ajwain), appropriate mint oil combination obtained from *Mentha spicata* and *Mentha arvensis* and conventional activities. The invention also provides methods for the preparation of the novel formulation useful in the treatment of drug resistant bacterial infections, the invention provides an anti-bacterial agents comprising an effective amount of thymol, useful in controlling drug resistant bacteria.

Thymol is a monoterpene alcohol and many monoterpenes and other volatile terpenes have a number of widespread medicinal uses. Compounds such as thymol, camphor and menthol are used as counter irritants or cooling, analgesic and antiitching agents and as components of liniments. Many monopenenes have been used anthelmintics, antispasmodic, analgesic mitigative and astringent drugs[17].

Salt stress effects on growth, ion accumulation and seed oil concentration in an arid zone traditional medicinal plant ajwain (*Trachyspermum ammi* [L.] Sprague)[19]. An ethereal extract of omum (*Trachyspermum ammi*; Hindustani: ajwan), a frequently consumed spice and was found to inhibit platelet aggregation induced by arachidonic acid (AA), epinephrine and collagen; in this respect it was most effective against arachidonic acid-induced aggregation[20].

Although triterpnes have been considered to be innocuous plant constituents, several have been established recently to have pronounced physiological activity. The lupeol is a triterpene alcohol also reported as a number of active principles such as antiangiogenic activity, nephrotoxicity, several anticancer against leukemia cells, inhibit skin cancer, anti-inflammatory activity, anti-spasmodic and other activity[17]. This paper deals with isolation and structure elucidation of isolated compounds (**1-6**) and inhibitory activity of (**1-5**) on barnyardgrass and radish seeds.

EXPERIMENTAL

Melting points of isolated compounds were determined on electrochemical engineering apparatus. Thin-layer chromatography (TLC) was carried out on precoated silica gel plates (Merck) with a layer thickness of 0.5 mm, unless otherwise indicated. Spots were detected under UV light (254 and 366 nm) before and after the plates were dipped in a chamber containing 1% vanillin-sulfuric acid (ethanol solution). Column chromatography was carried out on silica gel (70-230 mesh; Merck) and optical rotations were measured on an AA-10 model polarimeter. Both ^{1}H NMR (nuclear magnetic resonance) (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured with a Bruker Avance (DRX-500) spectrometer using deuterated chloroform ($CDCl_3$), methanol (CD_3OD), and pyridine (C_5D_5N) as solvents. Electron ionization mass spectrometry (EI-MS) spectra were recorded with a Jeol JMS-SX 102A spectrometer and fast atom bombardment mass spectrometry (FAB-MS) were recorded with a Jeol JMS-AX 505 WA instrument. Infra red (IR) spectra were recorded with a Thermo-Mattson 60-AR spectrophotometer and ultra violet (UV) spectra were measured with a TU-1800_{PC} UV-VIS spectrophotometer.

Plant material: Ajwain (*Trachyspermum ammi*) seeds were purchased from local market, Lucknow, India, October 2002.

Extraction and isolation: Dried seeds of ajwain (2.0 kg) were hydrodistlled and yielded 56 mL essential oil in the yield of 2.8%. To crystallize thymol, the major component of oil was kept in a freezer and crystals were obtained by passing the crystallized portion through sintered funnel and obtained colourless pure crystals of thymol (**1**, 28 g). After hydro-distillation of essential oil from seeds, the same water in ajwain filtered and seeds after drying and grind in powder form extracted with hexane: ethyl acetate:methanol (1:1:1) to give 19 g extract. The details of hydro-disillation of essential oil and extract preparation are shown in Fig. 1. The extract (19 g) was subjected to normal phase column chromatography over silica gel to yield 34 fractions with the following eluants: fractions 1-4 in hexane, fractions 4-8 in hexane:EtOAc (9:1), fractions 9-12 in hexane:EtOAc (4:1), fractions 13-16 in hexane:EtOAc (7:3), fractions 17-20 in hexane : EtOAc (1:1), fractions 20-24 in EtOAc, fractions 25-28 in EtOAc: MeOH (9.5:0.5), fractions 29-32 in EtOAc:MeOH (9:1) and fractions 33-34 in EtOAc:MeOH (8:2). The initial fractions were either insufficient or complex mixture to purify a compound. while fractions 9-10 after column chromatography with dichloromethane and methanol afforded lupeol (**2**, 1.2 g), white amorphous solid: ^{1}H and ^{13}C NMR data in agreement with those reported in the literature[25,26]. Fractions 12-15 after further separation of yielded linoleic acid (**3**, 110 mg) and stearic acid (**4**, 32 mg). Fractions 16-17 after further separation yielded one compound eicosanoic acid, (**5**, 12 mg) and fractions

23, after CC over silica gel with chloroform and methanol, yielded one pure compound as β-sitosterol-3-O-β-D-glucoside (**6**, 65 mg). All the isolated compounds (Fig.2) were identified with different spectroscopic techniques and with authentic samples of Sigma Aldrich.

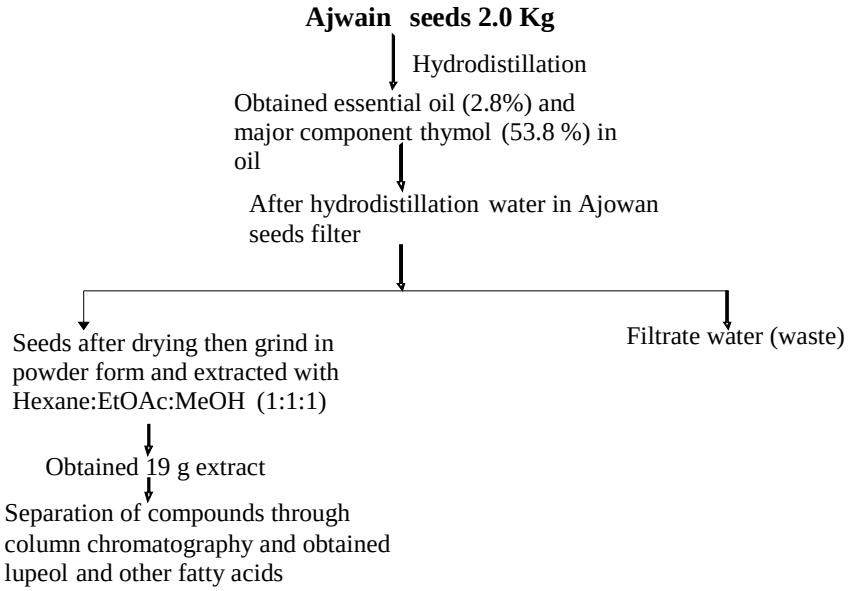
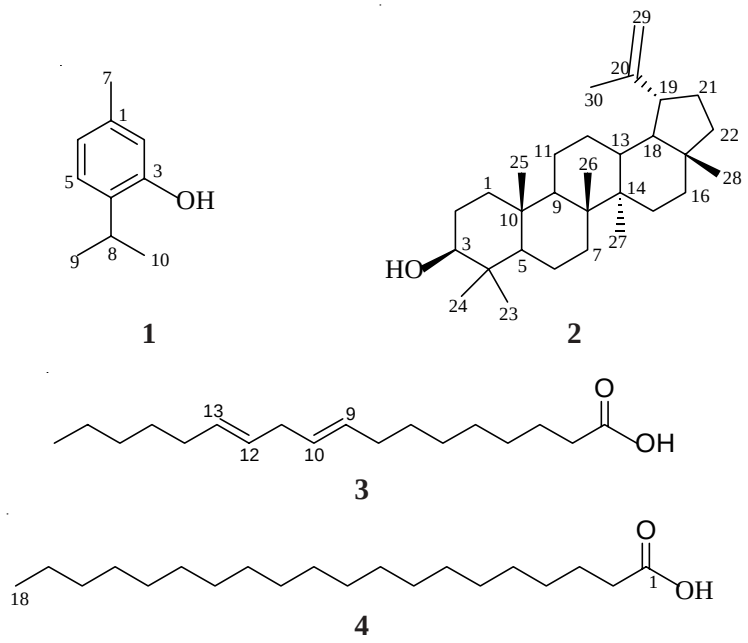


Fig. 1. Flow chart for separation of volatile oil and preparation of extract



1 **2**

3

4

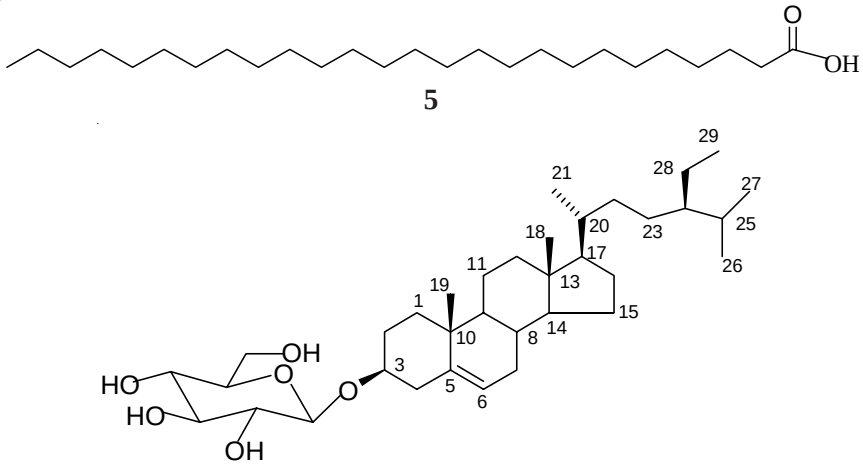


Fig. 2. Structures of thymol (**1**), lupeol (**2**), linoleic acid (**3**), stearic acid (**4**), eicosanoic acid (**5**) and β-sitosterol-3-O-β-D-glucoside (**6**)

Thymol (1): R_f 0.60 (Hexane : EtOAc; 8:2), m.p. 51-52ºC; IR (KBr, ν_{max}, cm^{-1}): 3464, 3361, 2963, 2872, 2367, 1719, 1582, 1513, 1420, 1299, 1225, 1154, 1099, 1060, 730; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.50 (1H, br, s, H-2), 7.07 (1H, d, J = 7.8, H-5), 6.71 (1H, d, J = 7.8, H-6), 2.30 (3H, s, br, H-7), 3.16 (1H, m, H-8), 1.20, 1.22 (3H, d, each, J = 6.9, H-9, 10), 5.09 (1H, s, aromatic OH). δ ^{13}C NMR (125 MHz, $CDCl_3$): δ 136.6 (C-1), 116.3 (C-2), 152.5 (C-3), 131.7 (C-4), 126.3 (C-5), 121.9 (C-6), 20.8 (C-7), 26.7 (C-8), 22.7 (C-9, 10); EI MS m/z (rel. int.): 150 $[M]^+$ ($C_{10}H_{14}O$); FABMS (positive mode) m/z 151 $[M + H]^+$; negative mode m/z 149 $[M - H]^-$. The spectral data of compound 1 were compared to the published data of thymol derivatives[16,21].

Lupeol (2): White amorphous solid, m.p. 215-216ºC; $[\alpha]_D^{20}$ + 27.80 (*c* 4.7 $CHCl_3$); IR (KBr, ν_{max}, cm^{-1}): 3402, 2980, 2880, 2820, 2380, 1760, 1643, 1470, 1380, 1020. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.12 (1H, m, H-1α), 1.82 (1H, m, H-1β), 1.55 (1H, m, H-2α), 1.92 (1H, m, H-2β), 4.45 (1H, dd, J = 4.0, 11.5, H-3), 1.30 (1H, m*, H-5), 1.48 (2H, m*, H-6), 1.43 (2H, m*, H-7), 1.36 (1H, m*, H-9), 1.26 (1H, m*, H-11α) 1.42 (1H, m*, H-11β), 1.04 (1H, m*, H-12α), 1.70 (1H, m*, H-12β), 1.64 (1H, m*, H-13), 1.30 (1H, m*, H-15α), 1.57 (1H, m*, H-15β), 1.82 (2H, m*, H-16), 1.40 (1H, m*, H-18), 2.50 (1H, td, 11.5, 5.5, H-19), 1.40 (1H, m*, H-21α), 1.98 (1H, m*, H-21β), 1.28 (1H, m*, H-22α), 1.65 (1H, m*, H-22β), 1.13*, 0.98*, 1.01*, 0.89*, 0.98*, 0.76* (all singlets, H-23 - H-28 Me), 4.70 (1H, dd, J = 2.0, 0.7, H-29α), 4.60 (1H, br d (2.5), H-29β), 1.64 (3H, br, s, Me -30), ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.3 (C-1), 23.7 (C-2), 79.0 (C-3), 38.1 (C-4), 55.3 (C-5), 23.2 (C-6), 24.2 (C-7), 45.6 (C-8), 49.8 (C-9), 36.6

(C-10), 20.5 (C-11), 25.1 (C-12), 37.2 (C-13), 44.2 (C-14), 29.9 (C-15), 37.1 (C-16), 42.7 (C-17), 50.4 (C-18), 48.3 (C-19), 150.9 (C-20), 30.0 (C-21), 40.1 (C-22), 25.2 (C-23), 14.5* (C-24), 15.4 (C-25), 16.0 (C-26), 16.1 (C-27), 18.0 (C-28), 109.3 (C-29), 19.4 (C-30); EI MS m/z (rel. int.): 426 $[M]^+$ ($C_{30}H_{50}O$); FAB MS (positive ion mode) m/z 427 $[M + H]^+$; negative mode m/z 425 $[M - H]^-$.

Linoleic acid (3): ^1H and ^{13}C NMR are similar to reported values and Co-TLC comparable with authentic sample of sigma[13].

Stearic acid (4): Colourless solid; R_f 0.41 (Hexane : EtOAc, 7:3), m.p. 69-70ºC; IR (KBr, ν_{max}, cm^{-1}): 3020, 2918, 2890, 1702, 1407, 1295, 1094, 758; ^1H NMR (500 MHz, $CDCl_3$): δ 2.36 (t, J = 5.6 Hz, H-2), 1.65 (m, H-3), 1.38 (br s, H-4 to H-17), 0.88 (t, J = 5.2 Hz, H-18); ^{13}C NMR (125 MHz, $CDCl_3$): δ 179.75 (C-1), 34.28 (C-2), 32.24 (C-3), 30.02 (C-4), 30.00 (C-5), 29.99 (C-6), 29.97 (C-7), 29.96 (C-8), 29.91 (C-9), 29.76 (C10), 29.68 (C-11), 29.56 (C-12), 29.39 (C-13), 25.03 (C-14), 23.02 (C-15), 24.92 (C-16), 22.91 (C-17), 14.31 (C-18); EIMS m/z (rel. int.) 284 $[M]^+$ ($C_{18}H_{36}O_2$)(5.2), 256 (100), 238 (12), 213 (39),185 (22), 129 (48), 57 (76).

Eicosanoic acid (5): Colourless solid; R_f 0.41 (Hexane : EtOAc, 7:3), IR (KBr, ν_{max}, cm^{-1}): 3020, 2918, 2890, 1702, 1407, 1295, 1094, 758; ^1H NMR (500 MHz, $CDCl_3$): δ 2.36 (t, J = 5.6 Hz, H-2), 1.65 (m, H-3), 1.40 (br s, H-4 to H-19), 0.98 (t, J = 5.2 Hz, H-20); ^{13}C NMR (125 MHz, $CDCl_3$): δ 179.75 (C-1), 34.28 (C-2), 32.24 (C-3), 30.02 (C-4), 30.00 (C-5), 29.99 (C-6), 29.97 (C-7), 29.96 (C-8), 29.91 (C-9), 29.76 (C10), 29.68 (C-11), 29.56 (C-12), 29.39 (C-13), 25.03 (C-14), 23.02 (C-15), 24.92 (C-16), 22.91 (C-17), 23.12 (C-18), 24.21 (C-19), 14.31 (C-20); EIMS m/z (rel. int.) 312 $[M]^+$ ($C_{20}H_{40}O_2$).

β-sitosterol-3-O-β-D-glucoside (6): ^1H and ^{13}C NMR are similar to reported values and Co-TLC comparable with authentic sample of sigma[22,23].

Inhibitory activity determination method: Five compounds thymol, lupeol, linoleic acid, stearic acid and eicosanoic acid were used to test their inhibitory effects. Barnyardgrass (*Echinochloa crus-galli*) and radish seeds (*Raphanus sativus*) were selected for bioassay. In this experiment, two compounds (**1-2**) were tested with both barnyardgrass and radish seeds and three compounds (**3-5**) were tested with barnyardgrass. Commercial seeds of radish and seeds of barnyardgrass were collected in field of Konkuk University farm in 2004. Empty and undeveloped seeds were discarded by floating in tap water. The remaining seeds were then air-dried and hermetically stored at -20°C to keep seeds fresh. Seeds of barnyardgrass were treated with solution of H_2SO_4 (90%) for 30 s to loosen to their seed coats, and washed many times with distilled water. In germination test, germination ratio of both seeds were shown to be greater than 80%.

The isolated compounds **1-5**, were dissolved in acetone with 5% and

distilled water 95% to obtain the following concentrations: 50, 100, 200, and 500 ppm. Treatment with distilled water in acetone 5% were used as the control. Twenty seeds of each one were put in Petri dish (6 cm in diameter) line with filter paper (Whatman) and added 5 mL of diluted solvent in each, respectively. After that, Petri dishes were placed in the growth chamber under following condition: 25°C, humidity of 75%, lighted time of 9.00-17.00. After one week, percentage of germination, growth of shoot and root length were measured.

Statistical analysis: Data were analyzed using SAS version 6.12[24]. All of the treatments were replicated thrice with completely randomized design. The pooled mean values were separated based on the least significant difference (LSD) at the 0.05 probability level.

RESULTS AND DISCUSSION

Due to medicinal importance of ajwain (*T. ammi*) and because there are no reports in literature of the chemical constituents of seeds. After hydrodistillation of volatile oil from seeds and the same extracted with solvents. The chemical investigation of ajwain seeds after separation of volatile oil from seeds and isolated six compounds including one pentacyclic triperpene alcohol as lupeol (**2**) and three fatty acids *viz.* linoleic acid (**3**), stearic acid (**4**), eicosanoic acid (**5**), sterol glucoside as β-sitosterol-3-O-β-D-glucoside (**6**), and identified by using 500 MHz NMR spectral methods *viz.*: ^{1}H, ^{13}C, ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR aided by EIMS, FABMS and IR spectroscopy. Due to known compounds there is no need to describe all compounds and all possible spectroscopic data are given in the experimental section and compared Co-TLC with authentic standard of Sigma, ^{1}H and ^{13}C-NMR of data thymol, lupeol and fatty acids in agreement with those reported in the literature[14,16,25,26]. All the isolated compounds including thymol, lupeol and fatty acids are very important in the field of biological activities as above discussed. Regarding ajwain seeds constituents, this is the first chemical investigation.

Inhibitory effects of compounds on barnyardgrass and radish seeds: The inhibitory activity of these compounds against barnyardgrass and radish seeds and the results of the germination assays are shown in Tables 1 and 2. Thymol (**1**) showed completely suppressed the germination and growth of shoot, root length of barnyardgrass and radish (100% inhibition), respectively at the concentration of 500 ppm. Furthermore, decreasing the concentration of this compound showed the inhibitory effects 84.1% and 95% of shoot and root of barnyardgrass and complete inhibition to radish at 200 ppm. At the concentration of 50 ppm, thymol was also significantly exhibited inhibition to root length of barnyardgrass 53.1% and 62% to shoot length of radish, respectively.

Lupeol (**2**) exhibited a slightly inhibitory effect about 15 to 33% at 500 ppm to both growth of barnyardgrass and radish (Table-1). Linoleic acid, stearic acid and eicosanoic acid did not show any strong inhibition. In contrast, they were slightly stimulated barnyardgrass growth (Table-2), eicosanoic acid which was promoting 6.7 and 45% of barnyardgrass growth at 500 ppm. Stearic acid showed negligible suppression to shoot length of barnyardgrass and promoting root elongation. Especially, at 200 ppm, linoleic acid showed dramatically suppression at 39.2 and 95.5% to root and shoot length of barnyardgrass (Table-2).

TABLE-1
GERMINATION AND GROWTH INHIBITORY EFFECTS OF IDENTIFIED COMPOUNDS (**1**), (**2**) ON BARNYARDGRASS AND RADISH

	Concentration (ppm)	Germination (%)	Shoot length (mm)	Root length (mm)	Germination (%)	Shoot length (mm)	Root length (mm)
			Barnyardgrass			Radish	
Thymol(**1**)	500	0.0d(100.0)	0.0d(100.0)	0.0c(100.0)	0.0d(100.0)	0.0c(100.0)	0.0d(100.0)
	200	80.0c(18.4)	0.54d(84.1)	0.16c(95.0)	0.0d(100.0)	0.0c(100.0)	0.0d(100.0)
	100	88.0cb(10.0)	1.4c(58.8)	0.58c(81.9)	84.0a(16.0)	1.3b(69.0)	1.4c(73.1)
	50	95.0ab(3.1)	2.1b(38.2)	1.5b(53.1)	85.0a(15.0)	1.6b(62.0)	2.8b(46.2)
	Control	98.0a(0.0)	3.4a(0.0)	3.2a(0.0)	100.0b(0.0)	4.2a(0.0)	5.2a(0.0)
	LSD(0.05)	8.8	0.7	0.6	18.6	0.6	0.9
			Barnyardgrass			Radish	
Lupeol (**2**)	500	90.0b(10.0)	3.3b(15.4)	3.2b(20.0)	96.7a(3.3)	2.6a(21.2)	2.8b(33.3)
	200	97.0ab(3.0)	3.5ab(10.3)	3.0b(2.5)	96.7a(3.3)	2.4a(27.2)	3.0b(28.6)
	100	98.3ab(1.7)	3.8ab(2.7)	3.3b(17.5)	100.0a(0.0)	2.9a(12.1)	2.9b(30.9)
	50	100.0a(0.0)	3.6ab(7.7)	3.4ab(15.0)	98.3a(1.7)	3.0a(9.0)	2.8b(33.3)
	Control	100.0a(0.0)	3.9a(0.0)	4.0a(0.0)	100.0a(0.0)	3.3a(0.0)	4.2a(0.0)
	LSD(0.05)	8.81	0.6	0.6	4.1	1.0	1.1

Means with the same letters in a column are not significantly at p=0.05
Values in the parentheses are inhibition percentages over the control
Values in the parentheses with (-) are stimulation percentage over the control

TABLE-2
GERMINATION AND GROWTH INIHIBITORY EFFECTS OF IDENTIFIED COMPOUNDS (**3**), (**4**), (**5**) ON BARNYARDGRASS AND RADISH

Compound	Treatment			
	Concentration (ppm)	Germination (%)	Shoot length (mm)	Root length (mm)
Linoleic acid (**3**)	500	96.7a(-5.5)	2.4ab(14.3)	1.1bc(50.0)
	200	78.3ab(14.6)	1.7b(39.2)	0.1c(95.5)
	100	93.3ab(-1.7)	2.7a(3.6)	2.2ab(0.0)
	50	96.7a(-5.5)	3.0a(-7.1)	2.6a(-18.1)
	Control	91.7ab(0.0)	2.8a(0.0)	2.2ab(0.0)
	LSD(0.05)	18	0.7	1.1
Stearic acid (**4**)	500	90.0a(8.4)	3.0b(19.0)	2.9a(-3.6)
	200	96.7a(1.6)	3.4ab(8.1)	2.8a(0.0)
	100	95.0a(3.4)	3.3ab(10.1)	2.9a(-3.6)
	50	91.7a(6.7)	3.1b(16.2)	2.7a(3.5)
	Control	98.3a(0.0)	3.7a(0.0)	2.8a(0.0)
	LSD(0.05)	9.1	0.6	1.0
Eicosanoic acid (**5**)	500	96.7a(-3.6)	3.2a(-6.7)	2.9a(-45)
	200	95.0a(-18)	3.1a(-3.3)	2.7a(-35)
	100	90.0a(3.5)	3.1a(-3.3)	2.7a(-35)
	50	91.7a(1.7)	3.0a(0.0)	2.8a(-40)
	Control	93.3a(0.0)	3.0a(0.0)	2.0a(0.0)
	LSD(0.05)	9.1	0.9	9.1

Means with the same letters in a column are not significantly at p=0.05
Values in the parentheses are inhibition percentages over the control
Values in the parentheses with (-) are stimulation percentage over the control

Conclusion

There is no available report in the literature for either growth or germination inhibition of the aforementioned compounds. The secondary compounds were known to be exudated from natural plants and inhibited or stimulated nearby plants in ecosystem. To date, many identified allelochemicals such as phenolic acid, terpenic acid, flavonoids, and alkaloids are responsible for allelopathic activity[27,28]. These tested compounds showed various both inhibition and stimulation. Especially, thymol may be utilized for reducing weed interference and diminishing the dependency on synthetic agricultural chemicals, labor cost and environmental pollution concern. However, using this compound in practice, it needs to be further study and examine exactly how much ppm can inhibit weeds in natural condition and whether this concentration may make environmental contamination with its toxicity.

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