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ISOLATION, CHEMICAL TRANSFORMATION, AND ANTIFUNGAL POTENTIAL OF SESQUITERPENE LACTONES FROM *Inula racemosa*

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The present study reports the isolation of two eudesmanolide type sesquiterpenoid lactones, viz. alantolactone and isoalantolactone, from the roots of Inula racemosa *Hook L. and their chemical transformations using various reagents. All the compounds isolated and synthesized were assessed for their* in vitro *antifungal potential against* Dreschlera oryzae*,* Fusarium moniliforme*, and* Alternaria triticina *using spore germination inhibition method. To understand how the structure of sesquiterpene lactones relates to their antifungal function, structure activity relationships were studied. Isoalantolactone and its derivatives were found to be more toxic as compared to alantolactone and its derivatives. Among the different synthesized compounds, the bromoform derivatives were the most, while the methoxy derivatives were the least effective. All the compounds showed promising results against three test fungi with* ED_{50} *values less than 400 ppm.*

Keywords: *Inula racemosa*, alantolactone, isoalantolactone, antifungal studies, sesquiterpene lactones.

Phytopathogenic fungi are the most damaging pests of cereal crops along with insects and are responsible for decrease in yield as well as quality of harvested crops. *Dreschlera oryzae*, *Fusarium moniliforme*, and *Alternaria triticina* are among the most damaging fungi causing severe damage to rice and wheat crops [1–3]. Despite the availability of synthetic fungicides, there is increased interest in their natural alternatives, which are more environment friendly and cause the least effect on non-target species [4, 5]. In agricultural research, various new compounds have been reported to possess antifungal potential, which are natural products or natural product derived molecules [6].

Sesquiterpene lactones are an expeditiously developing group of natural products with a wide spectrum of biological activities [7]. More than 5000 compounds are isolated from the different members of Asteraceae (Compositae). *Inula racemosa* Hook. f. (*Pushkaramoola* in Hindi), one of the members of the family Asteraceae (Compositae), is a plant of wide medicinal importance and is distributed all over Africa, Europe, and East Asia. The roots of this plant consist mainly of two major sesquterpene lactones, alantolactone and isoalantolactone, which possess numerous biological activities, *viz.* antibacterial, antifungal, nematicidal, plant growth regulating, etc. [8–10].

To the best of our knowledge, although the antifungal activity of alantolactone and isoalantolactone and their derivatives have been reported against many groups of fungi [11], no reports are available against the phytopathogenic fungi under study (*Dreschlera oryzae*, *Fusarium moniliforme*, and *Alternaria triticina*). The present study also describes the structure–activity relationship (SAR) of parent and synthesized compounds based on their antifungal activity against three test fungi.

Alantolactone (**1**) and isoalantolactone (**2**) were isolated from the roots of *I. racemosa* using an earlier described method [11]. Different derivatives (**1a–1f** and **2a–2h**) of isolated compounds (**1** and **2**) were prepared (Scheme 1) and tested for their antifungal potential.

The antifungal activity of isolated as well as synthesized compounds was tested against three phytopathogenic fungi using the spore germination inhibition method, and the ED_{50} was calculated (Table 1). Comparison of the ED_{50} values revealed that isoalantolactone (**2**) possessed more toxic potential in comparison to alantolactone (**1**).

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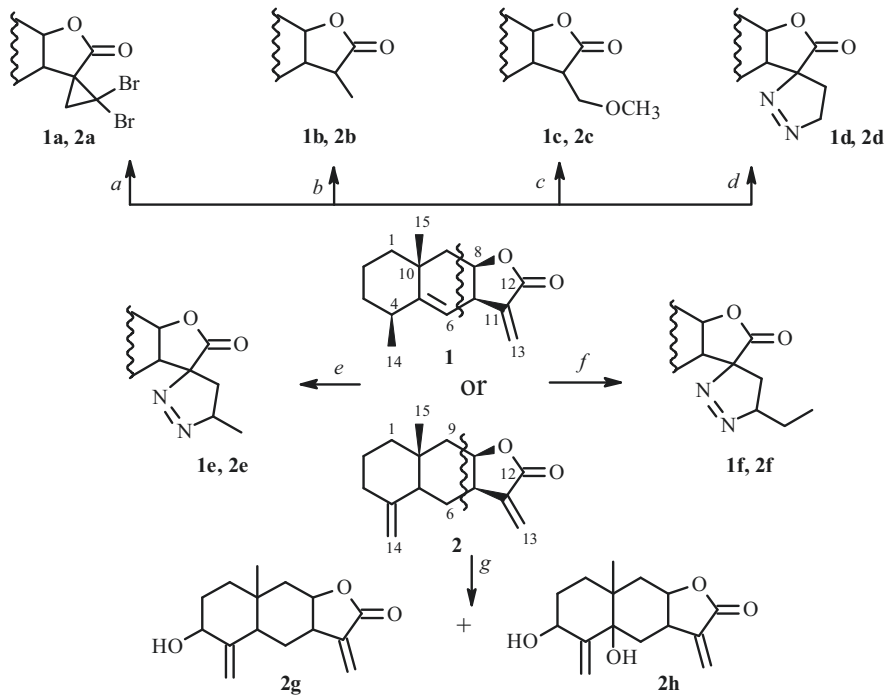
TABLE 1. ED_{50} Values (ppm) of the Tested Compounds against *Alternaria. triticina*, *Dreschlera oryzae*, and *Fusarium moniliforme*

Compound	*A. triticina*	*D. oryzae*	*F. moniliforme*	Compound	*A. triticina*	*D. oryzae*	*F. moniliforme*
1	270	250	280	**2b**	184	160	205
1a	160	120	180	**2c**	298	270	314
1b	220	190	235	**2d**	278	260	287
1c	385	360	395	**2e**	206	172	218
1d	370	355	380	**2f**	215	185	235
1e	267	258	284	**2g**	257	230	287
1f	180	165	196	**2h**	270	234	300
2	220	200	235	Carbendazim	08	18	15
2a	155	140	168	Propiconazole	12	15	8



a. $CHBr_3$, KOH; *b*. $NaBH_4$ or $Li(LiAl[OC(CH_3)_3]_3$; *c*. Mg/CH_3OH; *d*. CH_2N_2, $(CH_3)_3N$; *e*. CH_3CHN_2, $(CH_3)_3N$; *f*. $CH_3CH_2CHN_2$, $(CH_3)_3N$; *g*. SeO_2/TBHP

Scheme 1. Reaction scheme of alantolactone (**1**) and isoalantolactone (**2**) with different reagents and their products (**1a**–**1f** and **2a**–**2h**)

A similar trend was observed in their derivatives. Among the various synthesized derivatives, dibromocyclopropane derivatives (**1a** and **2a**) were the most toxic, whereas 13-methoxy-11,13-dihydro derivatives (**1c** and **2c**) were the least toxic. Comparison of different pyrazoline derivatives (**1d–1f** and **2d–2f**) showed varying toxic effects, and, among these, diazopropane derivatives (**1f** and **2f**) were found to be most effective against all the fungi under study in comparison with diazomethane (**1d** and **2d**) and diazoethane derivatives (**1e** and **2e**). The hydroxyl derivatives of isoalantolactone (**2g** and **2h**) showed less toxic effect in comparison with parent compound (**2**) against all test fungi. On comparison of the three fungi, the compounds showed the most toxic effect against *D. oryzae* and the least effect against *F. moniliforme*. All the compounds showed less toxic effect as compared to commercial standard fungicides, carbendazim (**3**) and propiconazole (**4**). However, both the isolated compounds (**1** and **2**) as well as their derivatives (**1a–1f** and **2a–2h**) showed ED_{50} values less than 400 ppm against all the three fungi (Table 1).

Structure–activity relationship (SAR) may be defined as the correlation between the structure of the compound and its biological activity. SAR relates to the particular functional groups responsible for evoking a response in the test organism or the site in the test organism. It is well known fact that the change in structure of the compound results in either an increase

or a decrease in its biological activity. Alantolactone and isoalantolactone, the two isomeric sesquiterpene lactones isolated from the roots of *I. racemosa*, were tested for their antifungal activity against three phytopathogenic fungi. Isoalantolactone was found to be more toxic as compared to alantolactone against all the tested fungi.

Kataria and Chahal [11] also reported the higher fungitoxic potential of isoalantolactone than alantolactone against three phytopathogenic fungi, *Alternaria brassicae*, *Penicillium italicum,* and *Rhizoctonia solani.* Earlier, the biological activities of these sesquiterpene lactones were purely attributed to the presence of the α-methylene-γ-lactone moiety [12]. But the difference in the activity revealed that the other structural features were also important. A comparative analysis of the structure of these compounds showed that these compounds differ only in the position of the additional double bond. This information implies that the exocyclic double bond may be responsible for the higher toxicity of the isoalantolactone. These findings prompted us to explore the role of the conjugated double bond in determining any biological property. Therefore, we attempt to modify the conjugated double bond in both the sesequiterpene lactones (**1** and **2**) using different reagents. The reduced derivatives (**1b** and **2b**) showed only a small increase in toxicity. The addition of the methoxy group and pyrazolines decreases the activity of the synthesized derivatives (**1c–1f** and **2c–2f**) as compared to the parent compounds (**1** and **2**), which revealed that the conjugated double bond plays an important role in the inhibition of spore germination in the test fungi. Further increase in antifungal activity of pyrazoline derivatives was also supported by previous study [11] which showed that extension of the carbon chain in the lactone ring increased the inhibition potential of the compound. The increase in activity due to cycloaddition of bromine (**1a** and **2a**) might be due to the inhibitory effect of halogens. On comparing alantolactone (**1**) and isoalantolactone (**2**) and their derivatives, (**1a–1f** and **2a–2f**), the latter were found to be more toxic as compared to their alantolactone analogues, which confirmed the importance of exocyclic double bond for the activity.

In general, polar groups like the hydroxyl group (OH) are responsible for the increased activity of the molecules. Therefore, hydroxyl derivatives of isoalantolactone (**2g** and **2h**) were also prepared. A comparison of ED_{50} values showed that hydroxyl derivatives were less active against fungus as compared to parent compound **2**, which revealed that addition of the HO group decreased the antifungal potential instead of increasing it. Nidiry et al. [13] also reported that the presence of an additional hydroxyl group in alcohol has an adverse effect on the antifungal activity of parent compounds.

EXPERIMENTAL

General. Melting points were determined in open capillaries on a Buchi B-545 melting point apparatus and are uncorrected. FT-IR spectra were measured on a Perkin Elmer, Model RX-1 FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC (400 MHz) spectrometer or mentioned otherwise as solutions (in $CDCl_3$) using TMS as an internal reference.

Extraction and Isolation of Pure Compounds from Roots of *I. racemosa*. The dried and powdered roots (250 g) of *Inula racemosa* were subjected to Soxhlet extraction using chloroform (1.0 L) as the solvent. The chloroform extract was distilled to yield a yellow semisolid crude extract (5.0 g). The concentrated extract was subjected to column chromatography using silver nitrate impregnated silica gel. Alantolactone (**1**, mp 78°C) and isoalantolactone (**2**, mp 108°C) were isolated from hexane (100%) and hexane–dichloromethane (5%) fractions, respectively.

Compound 1. IR (KBr, ν, cm^{-1}): 1745, 1657, 1457, 1370, 1261, 890, 811. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.09 (3H, d, J = 7.6, H-14), 1.19 (3H, s, H-15), 3.57–3.61 (1H, m, H-7), 4.81–4.84 (1H, m, H-8), 5.16 (1H, d, J = 4.1, H-6), 6.19 (1H, d, J = 1.9, H-13), 5.63 (1H, d, J = 1.7, H-13). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 39.46 (C-1), 22.56 (C-2), 37.56 (C-3), 32.86 (C-4), 148.94 (C-5), 118.83 (C-6), 41.70 (C-7), 76.45 (C-8), 42.63 (C-9), 32.69 (C-10), 139.84 (C-11), 170.45 (C-12), 121.63 (C-13), 16.74 (C-14), 8.58 (C-15).

Compound 2. IR (KBr, ν, cm^{-1}): 1755, 1641, 1447, 1262, 887, 818. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.82 (3H, s, H-15), 2.95–3.01 (1H, m, H-7), 4.44 (1H, d, J = 1.4, H-14), 4.77 (1H, d, J = 1.52, H-14), 4.48–4.51 (1H, m, H-8), 5.59, 6.13 (each 1H, d, J = 1.52, H-13). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 36.81 (C-1), 17.68 (C-2), 40.52 (C-3), 148.98 (C-4), 46.15 (C-5), 22.69 (C-6), 41.35 (C-7), 76.85 (C-8), 42.18 (C-9), 32.28 (C-10), 142.22 (C-11), 170.68 (C-12), 120.08 (C-13), 106.41 (C-14), 27.48 (C-15).

General Procedure for Reaction of Alantolactone or Isoalantolactone with $CHBr_3$. To $CHBr_3$ (15 mL), aq. KOH (10 mL, 50%) was added in the presence of phase transfer catalyst (TEBAC, 100 mg), and the mixture was homogenized for 2 min. Alantolactone or isoalantolactone (**1** or **2**, 1.0 g) was added to the same reaction mixture and stirred for 5 h. The completion of reaction was monitored by TLC. The reaction mixture was diluted with water and extracted with ethyl acetate

(3 × 50 mL) and dried (Na_2SO_4). Excess solvent was removed under vacuum. The solid pure compounds **1a** (yield 0.91 g, mp 58°C) or **2a** (yield 0.92 g, mp 149°C) were obtained separately in each reaction.

Compound 1a. IR (KBr, ν, cm^{-1}): 1767, 1458, 1356, 894, 688. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.05 (3H, d, J = 7.6, H-14), 1.19 (3H, s, H-15), 1.92 and 2.18 (each 1H, d, J = 8, H-13), 3.16–3.18 (1H, m, H-7), 4.95–4.98 (1H, m, H-8), 4.91 (1H, d, J = 3.5, H-6). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 40.64 (C-1), 22.83 (C-2), 38.10 (C-3), 32.95 (C-4), 151.38 (C-5), 115.29 (C-6), 41.26 (C-7), 76.79 (C-8), 42.26 (C-9), 32.83 (C-10), 42.65 (C-11), 171.84 (C-12), 27.27 (C-13), 16.80 (C-14), 28.54 (C-15), 29.66 (C-16).

Compound 2a. IR (KBr, ν, cm^{-1}): 1762, 1643, 890, 650. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.86 (3H, s, H-15), 1.92–2.29 (each 1H, d, J = 7.9, H-13), 2.59–2.65 (1H, m, H-7), 4.80–4.82 (1H, m, H-8), 4.79 (1H, d, J = 1.28, H-14), 4.45 (1H, d, J = 1.32, H-14). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 41.23 (C-1), 22.64 (C-2), 36.70 (C-3), 148.85 (C-4), 46.43 (C-5), 23.74 (C-6), 42.77 (C-7), 76.73 (C-8), 42.09 (C-9), 25.67 (C-10), 42.68 (C-11), 172.11 (C-12), 34.64 (C-13), 17.85 (C-14), 106.69 (C-15), 28.97 (C-16).

General Procedure for Reaction of Alantolactone or Isoalantolactone with $NaBH_4$. Alantolactone or isoalantolactone (**1** or **2**, 1.0 g) was taken in round bottomed flask and dissolved in methanol (20.0 mL). To this, sodium borohydride (2.0 g) was added in batches. The reaction mixture was stirred for 25 min. The extent of completion of the reaction was monitored by TLC. For workup, a few drops of acetic acid was added, and the reaction mixture was extracted with water and then with dichloromethane (3 × 50 mL). The organic layer was dried (Na_2SO_4), and excess of solvent was distilled off to get pure crystals of compound **1b** (yield 0.92 g, mp 172°C) or **2b** (yield 0.93 g, mp 288°C) separately in each reaction.

Compound 1b. IR (KBr, ν, cm^{-1}): 1728, 1599, 1456, 112, 889. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.24 (3H, d, J = 7.1, H-13), 1.26 (3H, s, H-15), 1.14 (3H, d, J = 7.6, H-14), 3.02–3.07 (1H, m, H-7), 4.74–4.77 (1H, m, H-8), 5.18 (1H, d, J = 3.2, H-6). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 38.76 (C-1), 23.07 (C-2), 38.52 (C-3), 33.05 (C-4), 150.61 (C-5), 115.56 (C-6), 42.92 (C-7), 76.98 (C-8), 40.38 (C-9), 32.91 (C-10), 42.25 (C-11), 179.33 (C-12), 10.73 (C-13), 16.88 (C-14), 28.75 (C-15).

Compound 2b. IR (KBr, ν, cm^{-1}): 2934, 1756, 1640, 1168, 884. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.83 (3H, s, H-15), 1.22 (3H, d, J = 7.16, H-13), 2.79–2.82 (1H, m, H-11), 2.95–3.01 (1H, m, H-7), 4.44–4.51 (1H, m, H-8), 4.77 (2H, br.s, H-14). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 40.50 (C-1), 22.69 (C-2), 36.80 (C-3), 148.96 (C-4), 46.50 (C-5), 34.82 (C-6), 42.24 (C-7), 76.84 (C-8), 41.75 (C-9), 27.47 (C-10), 41.57 (C-11), 179.46 (C-12), 9.29 (C-13), 106.59 (C-14), 17.79 (C-15).

General Procedure for Reaction of Alantolactone or Isoalantolactone with $LiAl[OC(CH_3)_3]_3$. Alantolactone or isoalantolactone (**1** or **2**, 2.0 g) was dissolved in dry THF (20.0 mL) and to this was added lithium tri-tert-butoxyaluminohydride (1.0 g). The reaction mixture was stirred for 15 min and after completion of reaction, THF was removed under vacuum. For workup, a few drops of glacial acetic acid was added, and the reaction mixture was extracted with water and then with diethyl ether (3 × 50 mL). Evaporation of solvent afforded a pure compound, **1b** (yield 1.91 g, mp 172°C) or **2b** (yield 1.93 g, mp 288°C) for each reaction.

General Procedure for Reaction of Alantolactone or Isoalantolactone with Mg/Methanol. To dry methanol (50.0 mL), alantolactone or isoalantolactone (**1** or **2**, 0.5 g) and active Mg turnings (0.5 g) were added with constant stirring under dry conditions. A mild exothermic reaction was observed after 3 h with the evolution of H_2 gas. The progress of the reaction was monitored by TLC. Unreacted metal was then separated, and the mixture was added to dilute HCl (150 mL) to get a clear solution; pH was adjusted to around 8.0–9.0 by adding ammonia solution. The reaction mixture was then concentrated, diluted with water, and extracted with dichloromethane (4 × 20 mL). The organic layer was dried (Na_2SO_4) to afford a mixture. In the case of alantolactone, the mixture (0.441 g) was chromatographed over silica gel to get pure compound **1c** (yield 0.32 g, mp 86°C), whereas in the case of isoalantolactone the mixture (0.436 g) obtained on chromatographic isolation yielded the compound **2c** (yield 0.34 g, mp 91°C).

Compound 1c. IR (KBr, ν, cm^{-1}): 1750, 1660, 1450, 1370, 1262, 1138, 890, 810. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.04 (3H, d, J = 3.8, H-14), 1.15 (3H, s, H-15), 2.91–2.95 (1H, m, H-7), 3.29 (3H, s, OCH_3), 4.79–4.82 (1H, m, H-8), 5.10 (1H, d, J = 3.6, H-6). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 38.75 (C-1), 22.72 (C-2), 37.89 (C-3), 32.90 (C-4), 148.90 (C-5), 119.96 (C-6), 42.85 (C-7), 76.74 (C-8), 42.00 (C-9), 32.72 (C-10), 50.43 (C-11), 177.94 (C-12), 72.43 (C-13), 16.84 (C-14), 28.86 (C-15), 59.21 (C-16).

Compound 2c. IR (KBr, ν, cm^{-1}): 1770, 1660, 1470, 1380, 1140, 1110, 895, 730. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.80 (3H, s, H-15), 2.93–2.95 (1H, m, H-7), 4.42 (1H, d, J = 1.4, H-14), 4.73 (1H, d, J = 1.52, H-14), 4.43–4.45

(1H, m, H-8), 3.27 (3H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 37.21 (C-1), 19.65 (C-2), 40.52 (C-3), 149.24 (C-4), 47.45 (C-5), 23.62 (C-6), 40.25 (C-7), 76.25 (C-8), 41.26 (C-9), 33.75 (C-10), 44.78 (C-11), 170.59 (C-12), 75.30 (C-13), 106.41 (C-14), 27.48 (C-15), 59.30 (C-16).

General Procedure for Reaction of Alantolactone or Isoalantolactone with Diazomethane, Diazoethane, or Diazopropane. To a solution of lactone (**1** or **2**, 2.0 g each) in ether containing 2–3 drops of triethylamine was added an excess ethereal solution of diazomethane, diazoethane, or diazopropane separately. It was kept overnight. After completion of reaction, the solvent was evaporated to afford crystalline compounds in each case, which were identified as pyrazoline **1d** (yield 1.81 g, mp 98°C), **1e** (yield 1.82 g, mp 70°C), or **1f** (yield 1.88 g, mp 55°C), or **2d** (yield 1.65 g, mp 118°C), **2e** (yield 1.72 g, mp 104°C), or **2f** (yield 1.80 g, mp 73°C), respectively, for each reaction.

Compound 1d. IR (KBr, ν, cm^{-1}): 2924, 1767, 1646, 1554. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.13 (3H, d, J = 7.6, H-14), 1.28 (3H, s, H-15), 1.54–1.65 (4H, m, H-13, 16), 4.78–4.86 (1H, m, H-8), 5.04 (1H, d, J = 3.4, H-6). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 42.19 (C-1), 21.28 (C-2), 38.58 (C-3), 33.01 (C-4), 152.81 (C-5), 113.39 (C-6), 42.07 (C-7), 101.55 (C-8), 42.66 (C-9), 28.56 (C-10), 78.38 (C-11), 172.94 (C-12), 32.80 (C-13), 16.80 (C-14), 22.82 (C-15), 78.12 (C-16).

Compound 1e. IR (KBr, ν, cm^{-1}): 2929, 1763, 1670, 1554, 1170, 888. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.23 (3H, d, J = 7.8, H-14), 1.26 (3H, s, H-15), 1.57 (3H, d, J = 7.3, H-17), 4.73–4.76 (1H, m, H-8). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 38.53 (C-1), 22.74 (C-2), 37.55 (C-3), 33.04 (C-4), 145.48 (C-5), 118.81 (C-6), 37.87 (C-7), 86.59 (C-8), 40.79 (C-9), 32.76 (C-10), 76.46 (C-11), 172.75 (C-12), 42.02 (C-13), 18.74 (C-14), 24.68 (C-15), 43.12 (C-16), 16.83 (C-17).

Compound 1f. IR (KBr, ν, cm^{-1}): 2965, 1745, 1656, 1555, 1169, 870. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.96 (3H, t, J = 7.2, H-18), 1.22 (3H, d, J = 7.7, H-14), 1.27 (3H, s, H-15), 1.33–1.37 (2H, m, H-17), 1.52–1.84 (2H, m, H-13), 4.74–4.76 (1H, m, H-8), 5.37 (1H, d, J = 3.7, H-6). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 38.5 (C-1), 20.10 (C-2), 36.42 (C-3), 35.70 (C-4), 144.20 (C-5), 122.80 (C-6), 36.97 (C-7), 81.60 (C-8), 41.21 (C-9), 30.6 (C-10), 76.90 (C-11), 172.33 (C-12), 46.00 (C-13), 19.92 (C-14), 25.4 (C-15), 59.77 (C-16) 28.13 (C-17), 9.00 (C-18).

Compound 2d. IR (KBr, ν, cm^{-1}): 3084, 1761, 1643, 1549, 1212, 891. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.86 (3H, s, H-15), 1.23–1.33 (2H, m, H-16), 2.31–2.37 (2H, m, H-13), 4.45 (1H, d, J = 1.20, H-14), 4.79 (1H, d, J = 1.35, H-14), 5.52–5.54 (1H, m, H-8). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 42.02 (C-1), 20.42 (C-2), 41.30 (C-3), 149.02 (C-4), 46.29 (C-5), 21.86 (C-6), 34.58 (C-7), 78.03 (C-8), 43.33 (C-9), 22.64 (C-10), 106.55 (C-11), 173.11 (C-12), 36.72 (C-13), 17.80 (C-14), 103.15 (C-15), 78.75 (C-16).

Compound 2e. IR (KBr, ν, cm^{-1}): 3078, 1763, 1676, 1549, 1216, 891. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.10 (3H, s, H-15), 1.50 (3H, d, J = 7.2, H-17), 3.94–3.96 (1H, m, H-16), 4.50–4.52 (1H, m, H-8), 4.75 (2H, br.s, H-14). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 41.54 (C-1), 24.83 (C-2), 40.51 (C-3), 149.40 (C-4), 46.40 (C-5), 26.22 (C-6), 36.83 (C-7), 76.64 (C-8), 42.01 (C-9), 34.34 (C-10), 106.62 (C-11), 173.46 (C-12), 43.57 (C-13), 120.11 (C-14), 22.69 (C-15), 46.24 (C-16), 19.03 (C-17).

Compound 2f. IR (KBr, ν, cm^{-1}): 3082, 2964, 1769, 1707, 1527, 1216, 893. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.65–1.89 (3H, m, H-13, 16), 1.10 (3H, t, J = 5.6, H-18), 1.24 (3H, s, H-15), 4.47, 4.79 (each 1H, br.s, H-14), 4.84–4.88 (1H, m, H-8), 5.16 (2H, d, J = 3.8, H-6), 6.66 (dq, J = 1.96, 7.7, H-17). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 38.58 (C-1), 22.69 (C-2), 37.79 (C-3), 149.07 (C-4), 56.4 (C-5), 24.16 (C-6), 33.76 (C-7), 77.37 (C-8), 42.73 (C-9), 29.69 (C-10), 106.58 (C-11), 171.21 (C-12), 45.69 (C-13), 109.43 (C-14), 21.65 (C-15), 58.95 (C-16), 23.10 (C-17), 10.39 (C-18).

General Procedure for Allylic Oxidation of Isoalantolactone Using SeO_2/TBHP. Selenium dioxide (10.0 mg) was added to *tert*-butyl hydroperoxide (3.0 mL, 70%), and the mixture was stirred for 30 min to which a solution of isoalantolactone (**2**, 2.0 g) in dichloromethane (25.0 mL) was added. The reaction mixture was stirred for 4 h followed by dilution with cold water and extraction with dichloromethane (2 × 25 mL). The combined organic extracts were washed with water and dried (Na_2SO_4). Evaporation of the solvent afforded a mixture (1.9 g) of two compounds, which were chromatographed over silica gel to give pure compounds **2g** (yield 0.6 g, mp 144°C) and **2h** (yield 0.2 g, mp 161°C).

Compound 2g. IR (KBr, ν, cm^{-1}): 3454, 2935, 1747, 1662, 1263, 1162, 1084, 952, 906, 820. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.82 (3H, s, H-15), 2.98–3.04 (1H, m, H-7), 4.58 (1H, t, J = 7.52, H-3), 4.50–4.53 (1H, m, H-8), 5.61 and 6.13 (each 1H, br.s, H-14), 4.33 and 4.99 (each 1H, br.s, H-13). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 37.00 (C-1), 35.68 (C-2), 73.26 (C-3), 150.13 (C-4), 40.98 (C-5), 29.04 (C-6), 40.21 (C-7), 76.91 (C-8), 40.43 (C-9), 27.05 (C-10), 142.09 (C-11), 170.74 (C-12), 120.31 (C-13), 109.83 (C-14), 16.94 (C-15).

Compound 2h. IR (KBr, ν, cm^{-1}): 3436, 2930, 1729, 1659, 1420, 1264, 1162, 822. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.94 (3H, s, H-15), 3.32–3.38 (1H, m, H-7), 4.53–4.58 (1H, m, H-8), 4.43 (1H, br.s, H-3), 4.89 and 5.10 (each 1H, br.s, H-14), 5.65 (1H, d, J = 2.28, H-13), 6.13 (1H, d, J = 2.76, H-13). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 29.06 (C-1),

30.28 (C-2), 74.90 (C-3), 147.90 (C-4), 75.34 (C-5), 32.96 (C-6), 36.86 (C-7), 77.11 (C-8), 36.97 (C-9), 35.45 (C-10), 141.99 (C-11), 171.00 (C-12), 120.64 (C-13), 112.69 (C-14), 21.65 (C-15).

***In vitro* Screening of Compounds for Fungitoxicity**. Antifungal activity was tested by the spore germination inhibition technique. Spore suspension was prepared from a ten-day-old culture of the test fungi (*Alternaria triticina*, *Fusarium moniliforme*, and *Drechslera oryzae*) with sterilized water. The experiment was replicated three times for each concentration as well as control. A negative control containing only water was also maintained. These slides were placed in Petri plates lined with moist filter paper and were incubated at 24 ± 1°C. The number of spores germinated was counted, and the percent spore germination inhibition was calculated by the formula

% Spore germination inhibition = (Spore germination in control – Spore germination in treatment/ Spore germination in control) × 100.

The fungicides carbendazim (Bavistin 50WP) and propiconazole (Tilt 25 EC) were used as standards, and activity was expressed in terms of ED_{50} values.

In general, we can establish that alantolactone and isoalantolactone may act as good starting molecules to prepare natural product based fungicides. Hence it can be concluded that the sesquiterpene lactones present in *I. racemosa* root extract possess significant antifungal activity, and with a small modification in the structures we can enhance the activity of these natural derived biomolecules to get a better natural pesticide molecule.

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