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Phytochemicals and plant growth inhibitory activity of *Ageratum conyzoides* L. leaves

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***Abstract**: Ageratum conyzoides leaves have afforded six compounds which were identified as tritriacontane, henitriacontane, 23-pentatetraacontanone, 3,4,-seco-lup- 20 (29)-en-3-OMe, 3°butyl triacosanoate and methyl tetracosanoate. All are being reported first time from this plant. The different fractions viz. hexane, benzene, ethyl acetate and methanol Ageratum conyzoides leaves were tested for their plant growth inhibitory activity against germination of radish seeds. The percent growth inhibition of radish seeds by different fractions are being reported in this paper.*

***Key words**: Leaves, Ageratum conyzoides, Chromatography, IR, [1]HNMR*

I. Introduction

The use of botanicals for the control of insects, pests and weeds has recently advanced to a new stage. The recent frontier has moved from study of synthetic chemicals to study of the thousands of phytochemicals that may have important physiological effects. There is a broad and growing consensus that many phytochemicals found may provide a plant protection umbrella to the crops and versatile protection for human beings . Natural plant products are easily degradable, less persistent in the environment and eco-friendly. The search of plant products is unanimous choice to manage different diseases and to control the development of several herbaceous plants or weeds. *Ageratum conyzoides* L. (Family Asteraceae) - with common name Ajgandha, Ghandhari, Sahadevi - is an annual herb with a long history of traditional medicinal used in many countries in the world, especially in the tropical and subtropical regions **[I].** The plant has been used as purgative, febrifuge, treatment of ulcers and wound dressing **[2]**. In addition to its popular use for skin diseases and wound healing, a decoction of the plant is taken to treat diarrohea and to relieve pain associated with the navel in children **[3]**.

The secondary metabolites of *A. conyzoides* include flavonoids, alkaloids, coumarins, essential oils and tannins. Many of these are biologically active **[4].** These compounds have been shown to affect insect development, as anti juvenile hormones, resulting in sterile adults **[5].** The research was carried out on *A. conyzoides* leaves in a search for new botanicals possessing good biological activity.

II. Materials And Methods

1. Extraction and Isolation:

Plants were collected from Landscape, CCSHAU, Hisar, in the month of March -April 2008. The plant material was washed with tap water and separated into different parts (roots, leaves and stems). The dried leaves (2.5Kg) were chopped into small pieces and put into a round bottom flask (5 L). The extraction was done by boiling with methanol (3 L) by heating the flask on a water bath. After six hours, methanol was removed and the leaves were again boiled with methanol. The procedure was repeated thrice. The methanolic extract was concentrated using distillation over a water bath under reduced pressure. The crude extract was sun dried and divided into two parts. One part (250g) was mixed with silica gel (60 - 120 mesh), again dried on a water bath and subjected to silica gel (60 - 120 mesh) column chromatography. The other part (100g) was fractionated with different solvents of increasing polarity viz. hexane, benzene and ethyl acetate. Fractions so obtained were concentrated over a water bath under reduced pressure. The obtained viscous mass was dried in sunlight for several hours and the completely dried material was used for determining the plant growth inhibitory activity against germination of radish seeds.

The elution of the column was done with solvents of increasing polarity. The eluotropic series with increasing polarity comprising of petroleum ether, benzene, ethyl acetate, methanol and their mixtures were used to isolate the compounds. The column chromatography afforded six compounds labeled as L1 - L6. The characterization of compounds was done with the help of their melting point, recorded at the Ganson Electrical melting point, ^{1}HNMR spectra, recorded on Bruker AC-300F 300MHz NMR spectrometer in $CDCl_3$ and DSMO-d6 using TMS as internal standard, IR spectra recorded on Hitachi 570 infrared spectrophotometer using KBr, and Mass spectra, recorded on VG-70S 11-250J GC-MS-DS mass spectrometer.

2. Herbicidal Test

The radish seeds (*Raphanus sativus* L.), procured from Kishan Sewa Kendra, CCSHAU Hisar, were surface sterilized in 95% ethanol for 15 s. Ten seeds were sown in Petri plates of 90 mm diameter containing two layers of ordinary filter papers. Seven ml of test solutions of different fractions of varying concentrations (500, 1000, 2000 ppm) was poured in each Petri plate. The stock test solution was prepared by dissolving the desired amount of dried fraction in 1 ml of ethanol and then mixing with 30 ml of distilled water. The different concentrations of fraction were prepared from stock solution. A mixture of distilled water: ethanol (30:1, 7 ml) was taken as control. Three replicates of each concentration were taken. The radish seeds were allowed to germinate at the 25 ^{o}C in the incubator with 12 h of photoperiod. After 120 h, the number of seeds germinated in each Petri plate was counted and percent seed germination inhibition values were calculated **[6]**.

III. Experimental Section

Compound L1 (Tritriacontane, 1)

The compound was obtained on elution with pure hexane, 25 mg, m.p. 71 °C (literature m.p. 71.8 ^{o}C) **[7]**. IR (KBr) ν_{max} (cm^{-1}):722, 802, 866, 1023, 1097, 1261, 1378, 1466, 2848, 2917; **^{1}H-NMR (δ, CDCl$_3$):** 1.12-1.40 (62H, br, 31x -CH$_2$-), 0.82 (6H, t, *J* 7.5 Hz, 2x -CH$_3$); **MS (m/z, relative intensity):** 464 ((M$^+$, 4.79), 436 (11.98), 414 (11.98), 412 (34.73) 408 (23.95), 380 (82.03), 352 (100), 330 (53.29), 202 (38.32), 174 (17.36).

Compound L2 (Henitriacontane, 2)

It was obtained on elution with pure hexane as white waxy solid, 20 mg, m.p. 70 ^{o}C, (literature m.p. 68 ^{o}C) **[7]**. It was crystallized out from benzene. IR (KBr) ν_{max} (cm^{-1}): 669, 760, 1021, 1096, 1215, 1260, 1377, 1464, 2849, 2917; **^{1}H-NMR (δ, CDCl$_3$)**:1.22 -1.44 (58H, m, 29x -CH$_2$-), 0.84 (6H, t, *J* 7.5 Hz, 2x -CH$_3$); **MS (m/z, relative intensity);** 436 ((M$^+$, 3.38), 419 (12.57), 414 (19.16), 412 (59.88) 408 (31.14), 380 (73.65), 352 (96.41), 330 (100), 310 (7.78), 202 (15.57), 174 (20.36).

Compound L3 (23-Pentatetraacontanone, 3)

The elution by benzene: hexane (1:9) afforded this compound as a white powder, 20 mg, m.p., 80 ^{o}C . IR (KBr) ν_{max} (cm^{-1}): 700, 799, 865, 1021, 1260, 1410, 1715, 2847, 2915, 2962; **^{1}H-NMR (δ, CDCl$_3$):** 2.35 (4H, t, *J* 7.0 Hz, 2x -CH$_2$-CHO-), 1.54 (4H, m, 2x -**CH$_2$**-CH$_2$-CHO-), 1.20 (76H, br, 38x -CH$_2$-), 0.78 (6H, t, *J* 7.0 Hz, 2x -CH$_3$); **MS (m/z, relative intensity):**646 (M$^+$), 618, 582, 558, 542, 510, 482, 458 (6.59), 457 (17.96), 436 (8.98), 412 (18.56), 408 (31.14), 380 (84.43), 352 (100), 330 (39.52), 202 (23.95), 174 (26.95).

Compound L4 (3,4, -Seco-lup- 20 (29)-en-3-OMe, 4)

The compound was obtained on elution with benzene: hexane (1:3), crystallized out from ethyl acetate, 25mg, m.p. 274 ^{o}C, (literature m. p. 275 ^{o}C) **[8]**. The Liebermann-Burchard reaction for this compound hinted the presence of terpenoid. IR (KBr) ν_{max} (cm^{-1}): 800, 1020, 1094, 1261, 1378, 1462, 1734, 2848, 2917, 2960; **^{1}H-NMR (δ, CDCl$_3$):** 4.60 (2H, m, H-20), 3.70 (3H, s,-O-CH$_3$), 2.40 (2H, t, *J* 7.0 Hz, -CH$_2$-CO-), 1.25-1.59 (27H, m, 1x -CH$_3$, 9x -CH$_2$- , 6x >CH-, 0.78-1.02 (18H, m, 6x -CH$_3$); **MS (m/z, relative intensity):**456 (M$^+$, 5.39), 419 (17.36), 414 (25.15), 412 (77.24), 380 (71.86), 352 (100), 330 (44.91), 296 (13.77), 202 (19.16).

Compound L5 (3° Butyl triacosanoate, 5)

It was obtained on elution with benzene-hexane (1:3) and crystallized out in acetone, 24 mg, m.p. 80 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 702, 800, 864, 1020, 1092, 1260, 1413, 1462, 1734, 2847, 2915: **^{1}H-NMR (δ, CDCl$_3$):** 0.80 - 2.00 (51H, m, 21x -CH2-, 3x -CH3), 0.88 (3H, t, *J* 7.0 Hz, 1x -CH3); **MS (m/z, relative intensity):** 412 (M$^+$, 4.78), 408 (80.24), 380 (81.44), 352 (100), 330 (56.89), 300 (10.18), 202 (22.15), 174 (20.96).

Compound L6 (Methyltetracosanoate, 6)

The compound was obtained on elution with pure benzene and crystallized out in methanol, 24 mg, m.p. 57 ^{o}C, (literature m.p. 59.5 ^{o}C) **[7]**. IR (KBr) ν_{max} (cm^{-1}): 722, 802, 1063, 1261, 1466, 1733, 2848, 2918: **^{1}H-NMR (δ, CDCl$_3$):** 3.50 (3H, s, -COOCH$_3$), 2.20 (2H, br, -CH$_2$-CH$_2$COOMe), 1.24-2.00 (40H, m, 20x -CH$_2$-), 0.86 (3H, t, *J* 7.5 Hz, 1x -CH$_3$); **MS (m/z, relative intensity):** 382 (M$^+$), 174 (50.90), 202 (20.36), 214 (9.58), 295 (8.89), 353 (50.90), 381 (41.32).

IV. Result And Discussion

Compound L1 (Tritriacontane, 1)

The compound was afforded on elution with pure hexane as waxy solid, 20 mg, m.p. 71 ^{o}C (literature m.p. 71.8 ^{o}C) **[7]**. The IR of the compound indicated the absence of functional groups like CO and OH. The MS of the compound showed its molecular weight to be 464 and the molecular formula to be $C_{33}H_{68}$. The ^{1}H NMR

of the compound in $CDCl_3$ showed a triplet (*J* 7.5 Hz) at δ 0.82, integrating to six protons, which could be due to two terminal methyls. A multiple was observed in the range δ 1.12-1.40, for sixty two protons, which represented thirty one methylenes. This data hinted the compound to be tritriacontane. There is an identity between observed data and literature data **[7]** for tritriacontane. The compound L1 was therefore confirmed to be tritriacontane (1). This is the first report of characterization and isolation of tritriacontane from *A. conyzoides* leaves.

$$CH_3\text{-}(CH_2)_{31}\text{-}CH_3$$

1.

Compound L2 (Henitriacontane, 2)

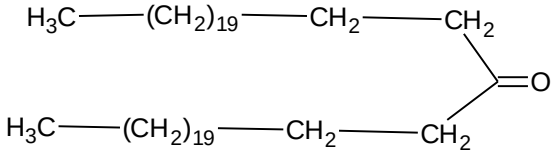
The compound was afforded on elution with pure hexane as waxy solid, 20 mg, m.p. 70 ^oC (literature m.p. 68 ^oC) **[7]**. The IR of the compound indicated the absence of functional groups like CO and OH. The MS of the compound showed its molecular weight to be 436 and the molecular formula to be $C_{31}H_{64}$. The ^1H NMR of the compound in $CDCl_3$ showed a triplet (*J* 7.5 Hz) at δ 0.84, integrating to six protons, which could be due to two terminal methyls. A multiple was observed in the range δ 1.22-1.44, for fifty eight protons, which represented twenty nine methylenes. This data hinted the compound to be henitriacontane. There is an identity between observed data and literature data for henitriacontane The compound L2 was therefore confirmed to be henitriacontane (2). This is the first report of characterization and isolation of henitriacontane from *A. conyzoides* leaves.

$$CH_3\text{-}(CH_2)_{29}\text{-}CH_3$$

2.

Compound L3 (23-Pentatetraacontanone, 3)

The compound was eluted with ethyl acetate : benzene (1:19) as white powder 20 mg, m.p. 80 ^oC. The IR spectrum of the compound showed the presence of a carbonyl group (1715 cm^{-1}). The M^+ (646) of the compound was observed that is the molecular mass of the compound. The ^1H-NMR of the compound in $CDCl_3$, showed no signal in the aromatic region indicating the alphabetic nature of the compound. A triplet (*J* 7.0 Hz) at δ 2..35, for four protons could be due to methylenes α to keto group. A multiplet at δ 1.54, indicating four protons, was attributable to methylenes β to keto group. A broad signal at δ 1.20, representing seventy-six protons, was assigned to thirty-eight methylenes. A triplet (*J* 7.0 Hz) centered at δ 0.78, integrating to six protons, was assigned to an aliphatic terminal methyl group. Thus the compound could be characterized as 23-Pentatetraacontanone (3). The MS fragmentation pattern lends further support to the proposed structure. A comparison of data on this compound, P with the literature data for 23-Pentatetraacontanone **[9]**, confirmed that identity of the compound as 23-Pentatetraacontanone (3). The compound is first reported from this plant.

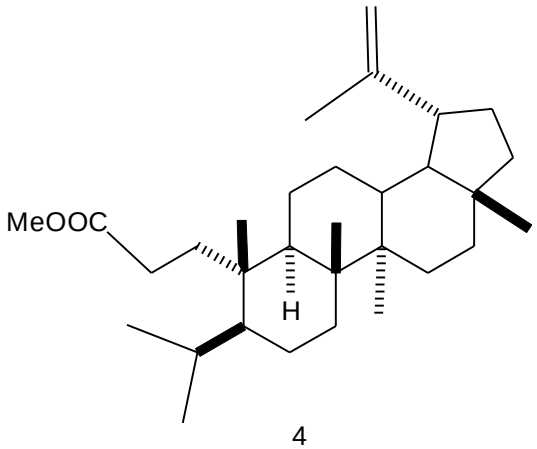


3

Compound L4 (3,4, -Seco-lup- 20 (29)-en-3-OMe, 4)

The compound was obtained on elution with benzene: hexane (1:3), crystallized out from ethyl acetate, 25 mg, m.p. 274 ^oC, (literature m. p. 274 ^oC) **[8]**. The Librman-Burchard reaction for this compound gives brown colour, hinted the presence of terpenoid. The IR of the compound indicated the presence of -CO- group (1734 cm^{-1}). The GC-MS of the compound suggested its molecular mass to be (456). The ^1H-NMR of compound in $CDCl_3$ showed a multiplet for two olefinic protons at δ 4.60. A singlet for three protons was observed at δ 3.70 which could be due to $-OCH_3$ group. There was a triplet for two protons at δ 2.40 which could be due to a methylene α to -CO- group. There was a multiplet in the range δ 1.25- δ 1.59 for twenty seven protons could be due to one methyl, nine methylenes and six methaine. A multiplet in the range δ0.78-1.02 for18 protons represented 6 methyls. This data suggested the compound L4 to be (3,4, -Seco-lup- 20 (29)-en-3-OMe) (4).

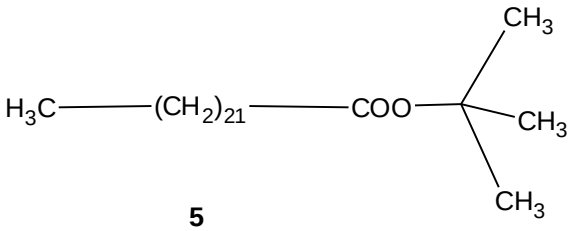
There was a complete consistency in the observed data for compound L4 with literature data for Compound (3,4, -Seco-lup- 20 (29) -en-3-OMe) confirming the identities of the former to be the later.



4

Compound L5 (3° Butyl triacosanoate, 5)

It was obtained on elution with benzene : hexane (1:3) as waxy solid, 24 mg. It crystallized out from benzene as a white solid, m.p. 80 ^{o}C. The IR of the compound indicated the presence of CO group (1734 cm^{-1}). The M^{+} (410) of the compound was not observed but M^{+} +2 (412) was observed. The elemental analysis of the compound also suggested its molecular mass (410) and molecular formula $C_{27}H_{54}O_2$. The ^{1}H NMR of the compound in $CDCl_3$ depicted a multiplet at the range δ 0.80-2.00, integrating fifty-one protons, indicating the presence of three methyl groups attached to 3° carbon and twenty-one methylene. A triplet (*J* 7.5 Hz) was observed at δ 0.88, integrating to three protons, hinting a terminal methyl group. This data suggested the compound to be 3° Butyl triacosanoate (5). After a survey of the literature it was reveled that this is a hitherto unreported compound.



5

Compound L6 (Methyltetracosanoate, 6)

This compound was obtained on elution with benzene: hexane (1:1). It crystallized out from methanol as a white solid, m.p. 57 ^{o}C, (literature m.p. 59.5 ^{o}C) **[7]**, 24mg. The IR of the compound indicated the presence CO group (1733 cm^{-1}). The MS of the compound suggested its molecular mass to be 382 and the molecular formula to be C_{25} H_{50} O_2. The ^{1}H NMR of the compound in $CDCl_3$ depicted a singlet at δ 3.50, integrating to three protons, indicating the presence of an -OMe group. There was a broad signal at δ 2.20, integrating to two protons, hinting a methylene group α to a carbonyl group. There was a multiplet in the range of δ 1.24-2.00 for forty two protons, representing twenty one methylenes. A triplet (*J* 7.5 Hz) was observed at δ 0.86, integrating to three protons, hinting a terminal methyl group. This data suggested the compound to be methyl tetracosanoate. A comparison of the data of the compound L6 with the literature data **[7]**, for methyl tetracosanoate, confirmed the identity of the compound as methyl tetracosanoate (6). It is the first report of characterization and isolation methyl tetracosanoate from *A. conyzoides* leaves.

CH_3-$(CH_2)_{21}$-CH_2COOCH_3

6.

PLANT GROWTH INHIBITORY ACTIVITY

The Methanolic extract and four different fractions (hexane, benzene, ethyl acetate and methanol) of the leaves of *A. conyzoides* were tested for their plant growth inhibitory activity against the germination of seeds of radish at 100, 500, 1000, 1500 and 2000 µg / ml concentrations. The data - presented in Table 1 - revealed that maximum inhibition i.e. 60 % was shown by methanolic extract followed by hexane fraction i.e. 50 % at 2000 µg/ml. The benzene, ethyl acetate and methanol fractions showed maximum inhibition of seeds germination i.e.10 %, 23.4 %, and 13.4 % at 2000 µg / ml. The seeds of radish were found sensitive to germinate at 2000 µg / ml concentration of methanolic extract followed by the hexane fraction of the leaves. The seeds were also found sensitive to germinate at 1500 µg / ml concentrations of hexane, ethyl acetate and methanol fractions of leaves.

In all the tested extracts and fractions of roots, leaves and stems of *A. conyzoides*, the methanolic extract of the leaves showed significant inhibition i.e. 60 % at 2000 µg / ml followed by the hexane fraction of the leaves and methanolic extract of roots i.e. 50 %. All other fractions and extracts were found less active against the germination of seeds of radish.

Table 1: Effect of different extract / fractions of *Ageratum conyzoides* leaves on germination of radish seeds, 120 h of sowing

Extract/Fraction	**Concentration (µg/mL)**	**Leaves**	
		Germination (%)	**Inhibition (%)**
Hexane fraction	**100**	100	0
	500	96.6	3.4
	1000	86.6	13.4
	1500	70	30
	2000	50	50
Benzene fraction	**100**	100	0
	500	100	0
	1000	100	0
	1500	93.3	6.7
	2000	90	10
Ethyl acetate fraction	**100**	100	00
	500	96.6	3.4
	1000	93.3	6.7
	1500	86.6	13.4
	2000	76.6	23.4
Methanol fraction	**100**	100	0
	500	100	0
	1000	100	0
	1500	96.6	3.4
	2000	86.6	13.4
Methanol extract	**100**	100	0
	500	83.3	16.7
	1000	73.3	26.7
	1500	73.3	26.7
	2000	40	60
Control		100	00

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