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Allelopathic potential of *Jasonia montana* (wild plant) to control weeds of family Convolvulaceae

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ABSTRACT

We studied the allelopathic activity of *Jasonia montana* (Asteraceae) aerial parts against 4 weeds spp: *Convolvulus arvensis*, *Calystegia inflata*, *Portulaca oleracea* and *Arabidopsis thaliana*. Both ethyl acetate and butanol extracts had LD_{50} value 800 µg ml^{-1} and 810 µg ml^{-1}, respectively for *C. arvensis.* These extracts had LD_{50} values of 1000 µg ml^{-1} and 1180 µg ml^{-1} when tested on *C. inflata,* respectively. The active principle was purified by HPLC and the molecules structure was determined by ^{1}HNMR, ^{13}C NMR, MS and UV spectroscopy. Bioassay guided isolation showed that 8 phytotoxic compounds including six flavonoids caused the reductions in *C. arvensis* total biomass fresh weight by: (6,3'-dihydroxy-3, 5,7,4`-tetramethoxyflavone (86.48%), dihydro-quercetin (89.77%), 3, 6, 7, 3`,4`-pentamethoxyflavone (89.95%), 6,4`-dihydroxy-3,7-dimethoxyflavone (91.85%), 6, 4-dihydroxy-3, 5, 7-trimethoxyflavone (90%) and quercetagetin 3, 5, 6, 7, 3`, 4`-hexamethyl ether (83.02%),when compared to its control. Also, one sesquiterpene (Eudesm-4(15), 11(13)-diene-12,5β-oIide) and one polyphenols (3, 5-dicaffeoyl quinic acid) decreased *C. arvensis* total biomass fresh weight by 85.27% and 88.39% over the untreated control. The diverse pre-emergence and post emergence herbicidal activity of *J. montana* indicate that natural herbicides may be used to manage *C. arvensis* weeds.

Keywords: *Arabidopsis thaliana, Calystegia inflata*, *Convolvulus arvensis*, flavonoids, *Jasonia montana*, pre-emergence and post emergence phytotoxicity, *Portulaca oleracea*, sesquiterpene.

INTRODUCTION

Convolvulus species have become an increasing threat to agriculture in Egypt; where it is difficult to control these species without herbicides. Field bindweed (*Convolvulus arvensis)* produces a long taproot that generates many secondary roots. These secondary roots produce buds that can develop into new plants. Bindweed plants also produce many seeds that, with their tough seed coats, can remain viable in the soil for years (25). Hedge bindweed *(Calystegia inflata*) is a robust sprawling plant with large trumpet-shaped pale-lilac to white flowers that are approximately 5cm across. This species may be distinguished from field bindweed by its two leafy, nonlinear bracts that enclose and conceal the sepals (10). The chemical weed control in field crops requires constant innovation. Field bindweed becomes a problem when competes strongly with many crops such as wheat, corn and causes their yield decrease (26). Now we need a better tool,

possessing broader weed control spectra and proper environmental behavior (13). Weed control could be achieved through natural bioactive substances and does not leave any harmful residue to the environment (19). *Jasonia montana* produces a wide variety of bioactive flavonoid compounds (1,2,3,5) and terpenes (7,8,14). Anti bacterial and fungal was recorded (17,22,). The biological importance of this genus prompted us to investigate phytotoxicity of the aerial parts of *J. montana* against different plant species. Now many compounds in plants have proved phytotoxic and are potentially useful in agriculture as natural herbicides. This study aimed to investigate the allelopathic activity of *J. montana* aqueous extracts and organic extracts against convolvulaceae and other weeds. The active components were purified, identified and characterized by spectroscopic methods.

MATERIALS AND METHODS

Plant material

Field bindweed (*Convolvulus arvensis*) seeds and hedge bindweed (*Calystegia inflata* strobl.) were collected from north Sinai and Wadi El-Natroun, respectively, Egypt. *Jasonia montana* (Vahl) Botsch.(Asteraceae) aerial parts were collected in July 2007 at post flowering stage from South Sinai region. Plant specimen was identified by plant taxonomist at Desert Research center according to Tâckholm, (24) and deposited with my collection at plant protection, Desert Research Center, Cairo, Egypt. Seeds of (*Portulaca oleracea*) collected from maize farm in new cultivated land at Wadi El-Natroun, Egypt. Otherwise, wheat seeds (Giza168) were obtained from Agriculture Research Center, Cairo Egypt. *Arabidopsis thaliana* seeds (wild-type *A. thaliana* ecotype Columbia (Col-O)) were obtained from Lehle Seeds (Round Rock, TX), USA.

Biological activity of aqueous extracts concentration

Ten grams of grounded tissue from *J. montana* (aerial parts) were extracted with 100 ml distilled water on a rotary shaker for 4 hours at room temperature. The filtrate was considered as a 100 g dry wt. /liter solution, and diluted to different concentration 0, 1.25, 2.5, 5,10 (g dry wt.100 ml^{-1}) distilled water. Seeds were surface sterilized using sodium hypochlorite (0.3% v/v) for 10-12 min and washed four times in sterile double-distilled water, then ten seeds were placed on two filter papers Whatman # 1 in a sterilized 9-cm petri-dish. Aliquot (10 ml) from each concentration was used for treatments. Sterile water was used as negative controls. *C. arvensis* seeds were exposed to scarification before use to overcome its dormancy. Petri-dishes were sealed with parafilm and incubated in the dark at 25°C. After growth plants were frozen at -10 ^{0}C for 24 h to avoid subsequent growth during the measurement process (19). Germination percentage (G %) was recorded after 7 days of incubation, the experiment was terminated then stem and root lengths of weed seedlings were measured according to Chung and Miller (11).

Organic extracts and their biological activity

The dried aerial plant parts samples (500 g) were grounded and soaked for 24 h in 2000 ml of methanol/ water (1:1) at room temperature. The filtrate was heated to 40 ^{0}C to evaporate methanol solvent and the water solution extracted successively with diethyl ether, chloroform, ethyl acetate and n-butanol with partitioning three times with equal

volume (16).The extract was then evaporated to dryness and subjected to bioassays. After dryness 50 mg of ethyl acetate and *n*-BuOH crude extracts dissolved in 10 ml of ethanol and diluted with water to a concentration series of 250, 500, 1000, 1500 and 2000 µg ml^{-1}. All seeds were surface sterilized using sodium hypochlorite (0.3% v/v) for 10-12 min and then washed four times in sterile double-distilled water before the use. Control treatment was treated with ethanol without extracts. Seeds were placed on static Murashige and Skoog (MS) [basal media] and allowed to germinate for seven days in petri dish until roots and shoots emerged. Seven-day-old seedlings were transferred to 24-well plates containing 2 mL of liquid MS media with one seedling in each wall. Seedling was treated with the concentration series of the ethanolic extracts (10, 20, 40, 60 and 80 µl/2 ml^{-1} media (corresponding to the stock solution (5000 ug10 ml^{-1}) in three replicates. Plant cultures were maintained on an orbital platform shaker set at 90 rpm (Lab-Line Instruments, Inc., Melrose Park, IL) with a photoperiod of 16 h and 8 h dark at 25±2 °C. The light intensity in the growth chamber was 24 mol m-2 s-1.Total seedling fresh biomass were recorded 10 days after treatment.

Purification of bioactive phytochemicals

Bioactive extract was subjected to Combi $^{\text{FlashTM RETRIEVE}}$ System (ISCO, Lincoln, NE, USA) using different sizes of normal phase flash columns (120gRed-iSep TM, ISCO).The extracts were added to the top of the column and eluted in successive system (6) with increasing polarity by adding 100 ml each of {hexane, hexane: chloroform (1:1), chloroform, chloroform: EtOAc (1:1), ethyl acetate and methanol} to collect 6 fractions for each solvent system. After evaporation to dryness, 50 µg of each eluted fraction was added to 2 ml-of liquid media petri dish and bioassayed. The active fractions were then subjected to sub-column (40g Red iSep TM, ISCO) and the resulting fractions were purified by HPLC/UV (Dionex, USA) (20) and subject to bioassay against *Convolvulus arvensis* seeding, total biomass fresh weight was recorded after 10 days. Molecular weight was determined by HPLC coupled to a Thermo Finnigan Surveyor MSQ mass spectrometer detector (Dionex, USA). Mass data were collected over the range of the gradient program at a rate of one scan per two seconds. The UV spectrum was determined by HPLC Diode array (Scheme 1).

Supported material for allelopathic compounds identification

Compound 1 (F_{17}) : It was appeared on HPLC profile at retention time (RT): 35.59 min and have where UV spectral data with λmax 334.3 nm with methanol indicated that it is a flavone with 2-OH substitution; the remaining UV spectral data were found to be similar to that of quercetin type compound. The mass spectrum show characteristic fragments at *m/z* (M+1): 375.02 (l00), 373 (69), 359 (24), 181 (4) 151 (16). ^{1}H-NMR (500MHZ, CD_3OD): spectrum of compound (17) gave the following signals: δ (ppm). 7.56 (IH, *d,* J=Hz, H-2'), 7.53 (lH, *dd, J=S, 2H2,* H-6'), 7.08 (lH, *d, J=S* Hz, H-5'),7.05 (lH, s, H-S), 3.75, 3.76, 3.86, 3.92 (3H each, s, OMe), ^{13} C-NMR:153.3(C-2),139.8(C-3),172.1(C-4),152.3(C-5),137.1(C-6),143.8(C-7),95.3(C-8),149(C-9),112.0(C-10), 122.8 (C-1'), 114.8(C-2'), 146.2(C-3'), 149.5(C-4'), 117.7(C-5'), 119.7(C-6'), 61.1(C3-OMe), 59.1(C5-OMe), 56.2(C7-OMe) 55.5 (C4-OMe).it could conclusively identify as 6, 3'-Dihydroxy-3, *5, 7, 4'*-tetramethoxyflavone which has mol. wt. calculated: 374.02 for ($C_{19}H_{18}o_8$).

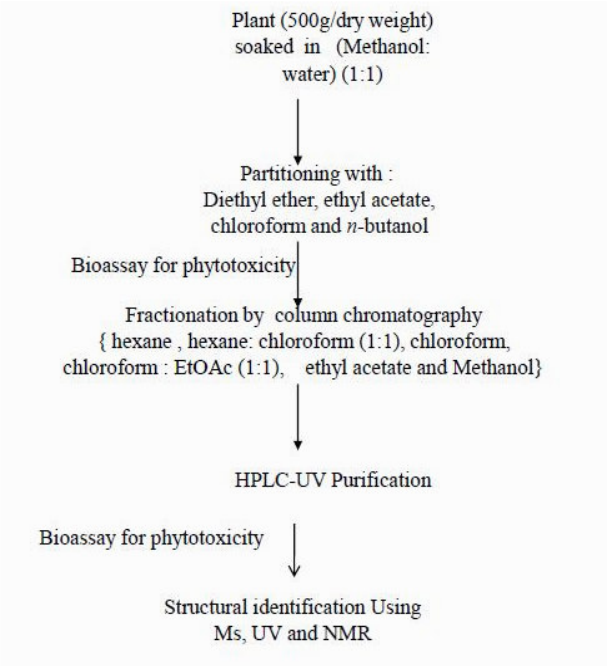


Figure 1. Diagram show isolation and purification of phytotoxin substances from *Jasmonia montana* aerial parts.

Compound 2 (F_{19}) : It appeared on HPLC profile at RT: 36.11 min, with UV absorbance (λmax) reached 275.9and 380 nm in MeOH, where it has mol. wt. (304). The mass spectrum show characteristic fragments at *m/z* (M+1): 305, 289 (100), 233, 205. ^{1}H H^{1}NMR (500MHZ, CD_3OD): 7.66 (1H, *s*, OH on C-5), 7.58 (1H, *s*, OH on C-7), 7.56 (1H, *s*, OH on C-4′), 7.39 (2H, *s*, H-2′ and H-6′), 7.37 (1H, *s*, H-3), 6.66 (1H, *d*, *J*=1.6 Hz, H-8), 6.31 (1H, *d*, *J*=1.7 Hz, H-6), 3.98 (6H, *s*) , 2.42 (3′H, s,OH-8), 2.29 (4′H, s, OH-30),d 2.16 (3H, s). ^{13}C NMR (500MHZ, CD_3OD): 172.17 (C-4), 163.9 (C-2), 163.6 (C-7), 161.4 (C-5), 161.0 (C-9), 157.3 (2C, C-3′ and C-5′), 142.1 (C-4′), 123.5 (C-1′), 104.6 (2C, C-2″ and C-6′), 103.3 (C-3), 98.7 (C-6), according to UV,Ms and NMR compound 2 was identified as dihydro-quercetin.

Compound 3 (F_{21}): It appeared on HPLC profile at RT: 38.34 min, it has a UV absorbance λmax 335.3 nm in MeOH and a molecular mass of 388.2. The mass spectrum show characteristic fragments at *m/z* (M+1): 389.2, 373 (100), 345 (9), 195 (11), 151(19). H^{1}NMR (500MHZ, CD_3OD): δ = 7.74 (dd, *J* =8.5, 2.1 Hz, 1H, 2^{-}-H), 7.69 (d, *J* = 2.1 Hz, 1H, 6-$^{-}$H), 6.99 (d, *J* = 8.5 Hz, 1H,3-$^{-}$H), 6.51 (s, 1H, 8-H), 3.97 (s, 3H, OC*H*3), 3.95 (s, 3H, OC*H*3), 3.93 (s,3H, OC*H*3), 3.87 (s, 3H, OC*H*3), 3.86 (s, 3H, OC*H*3). ^{13}C NMR (500 MHz,CDOD): δ = 55.9 (q, O*C*H3), 56.0 (q, O*C*H3), 56.3(q, O*C*H3), 60.1 (q, O*C*H3), 60.8 (q, O*C*H3), 90.3(d, C-8), 106.5 (s, C-10), 110.8 (d, C-3^{-}), 111.1 (d,C-2^{-}), 122.1 (s, C-1^{-}),

122.8 (d, C-6-), 132.2 (s,C-6), 138.8 (s, C-3), 148.7 (s, C-5-), 151.3 (s, C-4-),152.3 (s, C-9), 152.7 (s, C-5), 55.8 (s, C-2), 158.7(s, C-7), 178.8 (s, C-4).Regardless of the spectroscopic data and review analysis, compound 3 might be 3, 6, 7, 3`, 4`-pentamethoxy quercetin (artemitin*).*

Compound 4 (F_{22}) : It had a UV absorbance ʎ max 335.3 nm in MeOH and a molecular mass of 328.02. The mass spectrum show characteristic fragments at *m/z* (M+1): 329.02 (29), 181 (9) 121 (31). 'H[1]NMR (500MHZ, CD_3OD): 10.13 (IH, s, OH), 9.OO(IH, s, OH), 7.94(2H,d, *J=S* Hz,H-2',6'), 7.07 (lH, s, H-8), 6.93 (2H, *d, J=8* Hz., H-3',5'), 3.92, 3.75, 3.77, (3H each, s, OMe). [13]C NMR (500 MHz,CD_3OD) 152.27(C-2),139.4 (C-3), 61.1(C-3-oMe),178.1(C-4),152.5(C-5), 137.8(C-6), 143.8 (C-7)56.2(C-7-oMe), 95.5(C-8), 149.8(C-9), 112.0(C-10), 120.0(C-1-),129.6(C-2-), 115.4(C-3-), 130.1(C-6-), it could conclusively identify as 6, 4'-Dihydroxy-3, 7-dimethoxyflavone (6-hydroxykaempferol 3, 7-dimethyl ether) reported from *J. montana* .

Compound 5 (F_{26}) : It appeared on HPLC profile at RT: 44.95 min, which subjected to UV and Ms Fragment as well as NMR and showed the following data. The compounds had a UV absorbance λmax 281.2 nm in MeOH and a molecular mass of 232.2. The mass spectrum showed fragments characteristic at *m/z* (M+1): 233.2 [M]' (l00), 217(14), 181 (84) 95 (64); 22.4. H[1]NMR (500MHZ, CD_3OD): 6.48(d, J=1.5 Hz), 5.55(13H,dd,J=1 Hz), 5.21(15H,d,J=2 Hz), 2.91(H-7,d,J=3 Hz), 2.53 (6H,m,J =1.4 Hz), 1.97(8H,m,J=3 Hz),1.65(2H,m,J=1.3 Hz), 1.552.34(3H,d,J=5 Hz), 0.97(H-15,s),[13]CNMR (500MHZ, CD_3OD): (C-1-C-15): 34.5, 29.3, 31.8, 146.7, 87.1, 32.7, 34.6, 22.1, 32.8, 39.1, 138.9, and 165.6, 127.3, 108.1, corresponding to UV,Ms and NMR spectra it could conclusively identify as Eudesm-4(15), 11(13)-diene-12,5β-oIide (7)Eudesm-4(15), 11(13)-diene-12,5β-oIide .

Compound 6 (F_{28}) : It appeared on HPLC profile at RT: 45.83 min and showed the following mass spectrum m/z (M+1): 345.02 [M] + (1oo), 343 (67), 329 (29), 181 (9) 121 (31). UV nm: 485. H.NMR (400MHZ, CD_3OD): 10.13 (IH, s, OH), 9.OO (IH, s, OH), 7.94(2H, d, J=S Hz, H-2', 6'), 7.07 (l H, s, H-8), 6.93 (2H, d, J=8 Hz., H-3',5'), 3.92, 3.75, 3.77, (3H each, s, OMe). [13]CNMR (500MHZ, CD_3OD):153.2(C-2),139.4(C-3), 61.1(C-3-oMe), 172.1(C-4), 152.5(C-5),137.1(C-6), 143.8(C-7) 59.1(C-5-oMe), 95.5(C-8), 149.8(C-9), 112.0(C-10), 120.0(C-1-), 129.6(C-2-), 115.4(C-3-), 129.1(C-6-), the calculated molecular mass by 344.02. Corresponding to the mentioned data and comparing with the published data, compound6 identified as 6, 4-Dihydroxy-3, 5, 7-trimethoxyfavone (6-hydroxykaempferol 3, 5, 7-trimethyl ether).

Compound 6 (F_{29}) : It appeared on HPLC profile at RT: 48.72 min, it could conclusively identify as 3, 5-dicaffeoyl quinic acid, have mol. wt. 515, with λmax 381,404 nm in MeOH. The mass spectrum show characteristic fragments at m/z (M+1):516.02, 441.4(100), 387.2(65), 359.2 (23), 341 (5), 312(61), 261.3(4), 219 (80), 205 (18), 192 (10), 179 (13).

Compound 7 (F_{30}) : It appeared on HPLC profile at RT: 50.30 min, the compounds had a UV absorbance λmax 335.3 nm in MeOH and a molecular mass of 402. The mass spectrum was showing characteristic m/z (M+1): 403 (M)' (75), 387 [M-151]' (l00%), 195[A-15] + (l0%), 165 (B)' (15%). 'H[1]NMR (500MHZ, CD_3OD): 7.7 (2 H. *m,* H-6' and H-2'), 7.0 (I H, *d,* H-5'), *6.8*(1 H, s, H-8). 4.02 (3 H, s, OMe), 3.9 (9 H, s, 3-OMe, OMe and 4'-OMe), 3.92 (3H, s, 7-OMe) and 3.8 (3H, s. 3'-OMe), 4.1 (3H, s. 5-OMe), 3.88 (6H, s, OMe), 3.80 (3H, s, 3-OMe), 3.60 (3H. s, 4'-OMe), 3.42(3H. s, 3'-OMe), and 3.26 (3 H. s. 7-OMe) could conclusively identify as quercetagetin 3, 5, 6, 7, 3', 4'-hexamethyl ether.

Post emergence activity of *J. montana* crude extracts.

Convolvulus arvensis and *Calystegia inflata* seeds were sowed in pots filled with Peat moss soil, after emergence only two seedlings were kept in each pot. Pots had been initially watered two times weekly until 4-6 leaves stage in the greenhouse. The spray treatment was prepared from *J. montana* extracts at 5, 10 and 20 mg ml^{-1}applied for *C. arvensis* and at 10, 20, 30 and 40 mg $ml^{-1}$for *C. inflate.* The spray solution was prepared as follows: appropriate amount of extract initially dissolved in ethanol and then distilled water containing Tween20 at 0.5% (v/v) were added and give the chance for ethanol to evaporate. Sprays were applied with a glass sprayer to provide 5 ml of liquid solution to each pot. Control pots were similarly sprayed with distilled water containing 0.5%Tween 20. The sprayed pots were arranged in a randomized complete block in greenhouse. The data were obtained in four replicates, the survival of seedlings. Total fresh weight and dry weight for aboveground parts were recorded after two weeks of spraying (16).

Data were statistically analyzed by ANOVA, according to Snedecor and Cochran (23) and treatment means were compared by LSD test at 5% level of probability.

^{1}H- and ^{13}C-Nuclear Magnetic Resonance Analysis (NMR).

The NMR measurement was carried out on JEOL EX-500 NMR spectrometer apparatus (500 MHz for ^{1}H- NMR and 500 MHz for ^{13}C-NMR) as possible. Solvent: Deuterium methanol (CD_3OD) as described by Mabry (18).

RESULTS AND DISCUSSION

Aqueous leachate as a primary test guided for *J. montana* herbicidal activity

Germination and seedling length of the tested species inhibited under different concentration of *J. montana* (aerial parts) aqueous extracts (Figure 2). Percentage germination and seedling length inhibition increased as the concentration of water extract increased. The highest applied concentration of 10 g DW 100 ml^{-1} completely inhibited germination of *C. arvensis* and *C. inflate*. The LD_{50} values of aqueous extracts were 3.75 g DW 100 ml^{-1} for shoot length, 2.8 g DW 100ml^{-1} for root length, and 3.0 g DW 100ml^{-1} for germination in *C. arvensis* (Figure 2). In *C. inflate* its LD_{50} values were 2.5 g DW 100ml^{-1} for shoot length, 2.15 g DW 100ml^{-1} for root length, and 2.7 g DW 100ml^{-1} for germination (Figure1). Finally, at the highest concentration of 10 g DW 100ml^{-1}, aqueous extracts of *J. montana* decreased *T. aestivum* germination by 65.52% and root length by 85.16% and shoot length by 64.59% when compared to the untreated controls. Dose

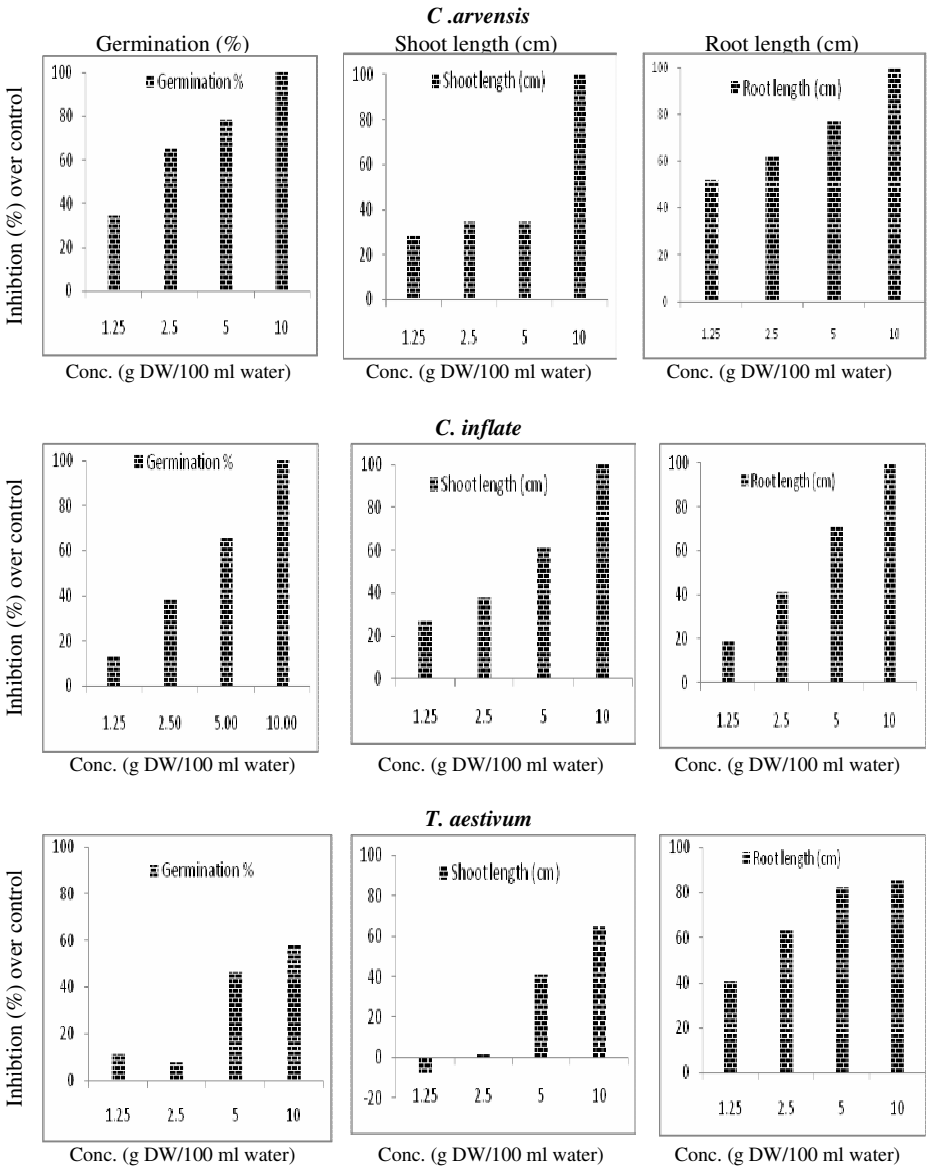


Figure 2. Allelopathic potential of *Jasonia montana* aerial parts aqueous extracts on germination and seedling length of the study species.

response curve of *J. montana* aqueous extracts showed that LD_{50} values in *T. aestivum* were 5.25, 3.22, and 6.5 g DW 100 $ml^{-1}$for shoot length, root length, and germination, respectively (Figure 2).

Dose response curve of ethyl acetate and n-butanol extracts against weed seedlings

Growth inhibitory test of ethyl acetate and n-butanol after partition with *J. montana* methanol: water aerial parts extract on seedling development of convolvulaceae weeds and other weeds, revealed that the both extracts remarkably inhibited weeds total biomass as compared with controls. The LD_{50} values of ethyl acetate and n-butanol extracts were 800 µg $ml^{-1}$and 810 µg $ml^{-1,}$ for *C. arvensis,* 1000 µg ml^{-1} and 1180 µg ml^{-1} for *C. inflate ,* 680 µg $ml^{-1}$and 750 µg ml^{-1} for *A. thaliana* and 500 µg $ml^{-1}$and 600 µg ml^{-1} for *P. olreacea,* respectively (Figure 3). There a difference was found between the effect of ethyl acetate extracts in depressing weed total biomass and n-butanol extracts, however the inhibitory effect of ethyl acetate was clearly pronounced than n- butanol with weeds which was entirely affected.

Post emergence activity of *J. montana* crude extracts against family Convolvulaceae weeds

Post emergence activity of ethyl acetate and n-butanol extracts was striking against convolvulaceae seedling (5-6 leaves stage) on greenhouse trails. The symptoms of injury were very similar on both *C. arvensis* and *C. inflate* seedlings. Both the ethyl acetate and the n-butanol extracts showed potent activity against *C. arvensis* seedling by decreasing the plant dry weight by 16.88, 55.64% and 75.88%, and 45.73% and 67.24%, respectively at 5, 10 and 15 mg ml^{-1} compared to control. These extracts at 10, 20, 30 and 40 mg ml^{-1} showed a significant level of herbicidial activity on C. *inflate* seedling by decreasing the plant dry weight as follows: 6.75%, 52.36%, 71.62% (ethyl acetate) and 75.67% and 16.37%, 34.38%, 61.50% and 69.95% (n-butanol) respectively compared to control (Figure 4).

Fractionation of ethyl acetate and n-butanol and bioassay on *C. arvensis* seedlings

Two active fractions (hexane: chloroform and chloroform) were obtained from ethyl acetate extracts. The bioassay of these fractions at 40 µg ml^{-1} significantly reduced *C. arvensis* total biomass fresh weight by 85.94% and 86.23% respectively, than the controls. Meanwhile, n-butanol extracts showing two active fractions (chloroform and chloroform: ethyl acetate) were detected from bioassay guided fractionation. The bioassay of these fractions reduced C. *arvensis* total biomass fresh weight by 83.01% and 78.62%, respectively at 40 µg ml^{-1}, than the controls (Table 1).

Purification and characterization of active compounds.

Developing the active fraction (chloroform fractions in both ethyl acetate and n-butanol extracts) for further purification on HPLC/UV machine showed that 32 compounds were found in its profile. The active compounds had a greater inhibitory effect on total biomass of *C. arvensis* seedlings (Table 2). The structures of the active compounds were elucidated by comprehensive spectroscopic analysis and also by directed comparison with the respective published data (Figure 5). These phytotoxic compounds

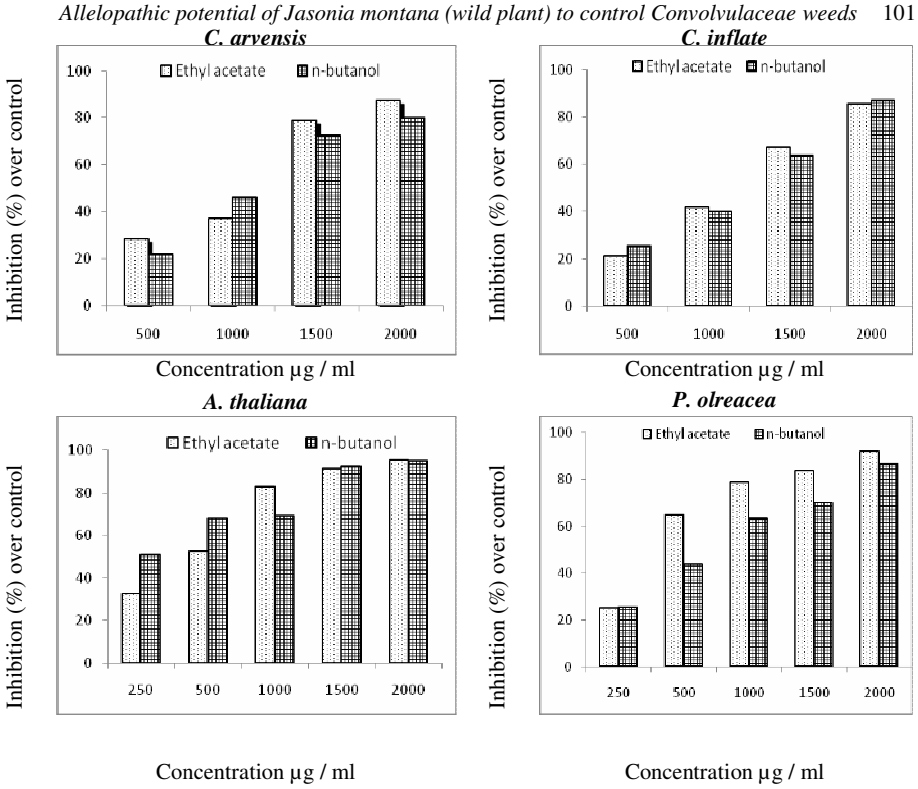


Figure 3. Allelopathic potential of *Jasonia montana* aerial dried parts organic crude extracts on the tested weeds seedling total biomass fresh weight.

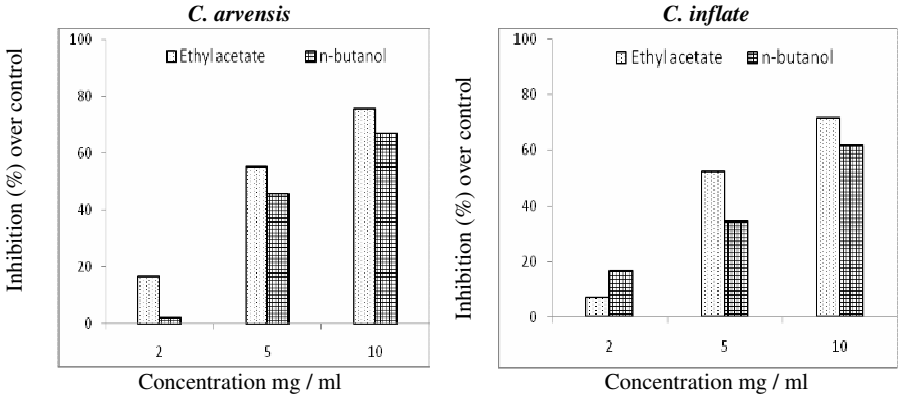


Figure 4. Post emergence activity of *Jasonia montana* aerial parts organic crude extracts on convolvulaceae weeds above ground parts dry weight (gm).

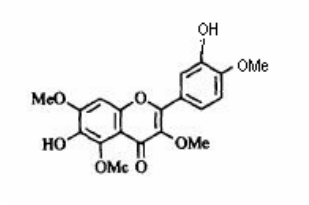
Table 1. The activity of fractions separated by Combi ^{Flash} column chromatography against *C. arvensis* seedling total biomass fresh weight

Fraction	Ethyl acetate	n-Butanol
Control	0.230±0.007	0.175±0.009
Hexane	0.211±0.004	0.173±0.008
Hexane :chloroform	0.032±0.009	0.118±0.005
Chloroform	0.032±0.004	0.030±0.007
Chloroform :ethyl acetate	0.118±0.007	0.037±0.004
Ethyl acetate	0.213±0.005	0.170±0.006
Ethyl acetate: methanol	0.208±0.004	0.189±0.009
Methanol	0.180±0.008	0.131±0.007
LSD(0.05)	0.099	0.0644

Table 2. The activity of *Jasonia montana* purified compounds separated from HPLC profile against *C. arvensis* seedling total biomass fresh weight

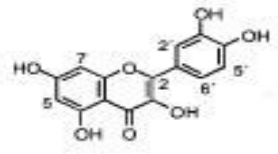
Purified compounds (µg/ml)	Biomass
Control	0.192±0.008
F1	0.182±0.013
F2	0.190±0.023
F3	0.168±0.012
F4	0.166±0.011
F5	0.181±0.010
F6	0.112±0.006
F7	0.166±0.012
F8	0.182±0.009
F9	0.137±0.007
F10	0.185±0.007
F11	0.189±0.009
F12	0.136±0.009
F13	0.116±0.005
F14	0.112±0.011
F15	0.094±0.003
F16	0.162±0.014
F17	0.026±0.014
F18	0.034±0.005
F19	0.020±0.002
F20	0.029±0.005
F21	0.019±0.003
F22	0.016±0.003
F23	0.125±0.009
F24	0.154±0.0012
F25	0.111±0.005
F26	0.028±0.002
F27	0.027±0.005
F28	0.019±0.003
F29	0.022±0.003
F30	0.033±0.003
F31	0.051±0.002
F32	0.093±0.004
LSD (0.05)	0.0763

1



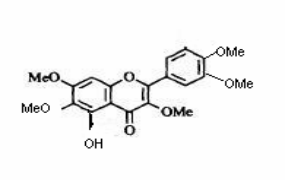
6,3'-dihydroxy-3,5,7,4'-tetramethoxy flavone

2



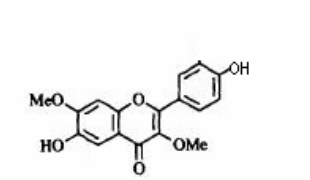
dihydro-quercetin

3



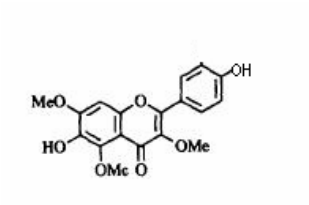
3, 6, 7, 3`,4`-pentamethoxy flavone

4



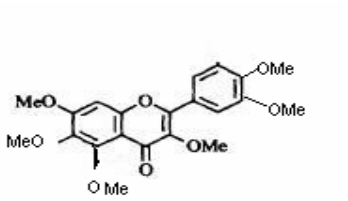
6, 4'-dihydroxy-3, 7-dimethoxy flavone

5



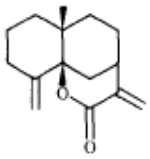
6, 4-dihydroxy-3, 5, 7-trimethoxyflavone

6



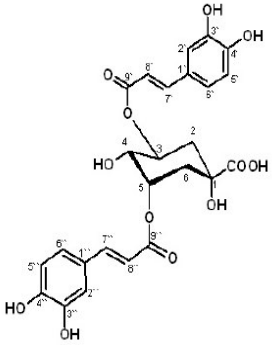
quercetagetin 3, 5, 6, 7, 3', 4'-hexamethyl ether

7



Eudesm-4(15), 11(13)-diene-12,5β-oIide

8



3, 5-dicaffeoyl quinic acid

Figure 5. Structure of phytotoxic compounds from *Jasonia montana* aerial parts

are included six flavonoids, causing the reductions in *C. arvensis* total biomass fresh weight by: (6,3`-dihydroxy-3, 5,7,4`-tetramethoxyflavone(86.48%), dihydro-quercetin (89.77%), 3, 6, 7, 3`,4`-pentamethoxyflavone (89.95%), 6,4'-dihydroxy-3,7-dimethoxy-flavone (91.85%), 6, 4-dihydroxy-3, 5, 7-trimethoxyflavone (90%) and quercetagetin 3, 5, 6, 7, 3`, 4`-hexamethyl ether (83.02%),when compared to its control (Figure 6). Also, one sesquiterpene (Eudesm-4(15), 11(13)-diene-12,5β-oIide) and one polyphenols (3, 5-dicaffeoyl quinic acid) decreased *C. arvensis* total biomass fresh weight by 85.27% and 88.39% than the untreated control (1,4,5,7).

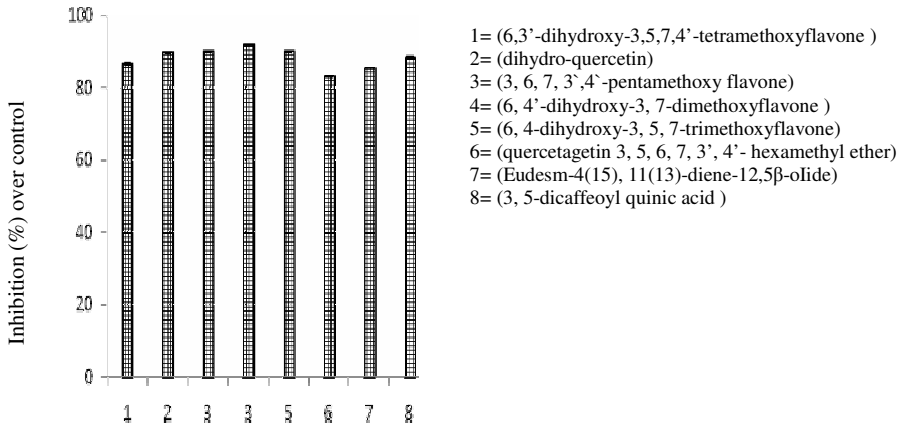


Figure 6. Phytotoxic compounds from *J. montana* aerial parts against *C. arvensis* total biomass.

The phytotoxicity value has been reported that weed roots were more sensitive than shoot may be due to fact that they were in direct contact with the allelochemicals. Various phenolic compounds inhibited root cell division (9).Corresponding to bioassay weed control, *J. montana* aerial parts shown pre and post emergence activity on *C. arvensis* and *C. inflata* seedling growth and germination. *A. thaliana* seedling was more sensitive to *J. montana* allelochemicals than other weeds seedling followed with *C. arvensis* and *P. olreacea,* while *C. inflate* seedling moderately affected by the extracts at the tested concentration. Post emergence activity could be attributed to the presence of flavonoids and terpenes compound and many type of its have a high yield in *J. montana.* The data reported that the most phytotoxic compound identified by spectroscopic analysis was *6*, 4-dihydroxy-3, 5, 7-trimethoxyflavone, however, the presence of dihydro-quercetin recorded the lowest phytotoxic activity on *C. arvensis* seedling as compared with other identified compounds. *J. montana* has anti bacterial and fungal activity as well as medicinal activity (hypoglycemic, antidiabetic, and antimicrobial activities) (22,17). Thus *J. montana* allelochemicals may be potentially used as natural herbicides beside therapeutic agent diseases caused by these microorganisms, the allelopathic plant products are known to offer a vast array of secondary compounds which have the potential role of use directly as herbicide substitute or as structural leads for new synthetic herbicides (12,15).

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