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Phytochemical Investigations of *Taverniera Lappacea* Forssk. And its Activity as Herbicides.

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Abstracts: Preliminary phytochemical screening of *Taverniera lappacea* revealed that it contained steroids, terpenoids, saponins, coumarins, flavonoids and phenolics and glycosides and/or carbohydrates. Aqueous extracts of *T. lappacea* vegetative parts added at 8 and 10 g (dry wt) 100 ml^{-1} concentration completely inhibited germination and seedling growth of *Convolvulus arvensis, Abutilon thiophrastes* and *Echinochloa crus-galli*. However, *T. lappacea* complete inhibited *Portulaca oleracea* germination and seedling developing at 10 (g dry wt) 100 ml^{-1}. The most phytotoxic extracts are Ethyl acetate; methanol and methanol 70% completely inhibited all seedling development and germination of the tested weeds at 200 µg ml^{-1} as compared with the control, while acetone extracts inhibited *C. arvensis* germination by (4.54%), shoot length by 53.9%) and root length by 35.29% respectively, as compared with the control treatment, mean while the lowest effect achieved from petroleum ether and diethyl ether in the target weeds germination and seedling development. On the other hand methanolic crude extracts of *T. lappacea* had an LD_{50} calculated from total biomass fresh weight after two weeks for Zea *mays* by (1450 ug ml^{-1}), *E. cure-galli* by (700 µg ml^{-1}) and *A. thiophrastes* by (450 µg ml^{-1}). Investigation of flavonoids, phenolic acids and coumarins revealed the presence of ten compounds of the flavonoids, two compounds of phenolic acids and one compound of coumarin in the plant, where the most active compounds recorded was kaempferol -7- rhamnoside, daidzin and kaempferol -3-O-glucoside with reduction percentage in total biomass fresh weight as fellow: (*A. thiophrastes)* by 56.70%, 70.97% and 54.96%, respectively and (*E. crus-galli)* by 55. 49%, 58.52% and 56.98%, respectively, as compared with the control on the others hands, the second active category appear in quercetin-3, 7- diglucoside, naringenin and caffeic acid depending on the activity against *A. thiophrastes* total biomass fresh weight, whereas, quercetin and scopoletin achieved the second reduction level in *E. crus-galli* as compared with the control.

Key word: *Taverniera lappacea*, flavonoids, phenolic acids, coumarins, Pre-emergance phytotoxicity and weed seeds of Maize (*Zea mays).*

INTRODUCTION

Due to increased awareness about the risks involved in use of synthetic herbicides, much attention is being focused on the alternative methods of weed control. Allelochemicals are plant secondary metabolites, which usually inhibit germination and reduce the growth of the other species. The chemistry of these compounds may be used as newer herbicides with novel molecular sites of action. It is well known that weeds interfere with crop plants causing serious impacts either in the competition for light, water, nutrients, space or allelopathy. Maize (*Zea mays*), is an economic cereal crop as a major source for carbohydrates in Egypt. Competition between maize and weeds can cause total crop loss depending on several weed factors. To achieve high and good quality yields from maize in the future, we hope to introduce natural herbicides from *Taverniera lappacea* and tested to inhibit growing maize weeds. *Taverniera lappacea* (Forssk.) Syn. *Hedsarum lappaceum* (Forssk.) belong to family *Leguminosae*. The genus of *Taverniera* in Egypt includes two species (*Taverniera lappacea* and *Taverniera aegyptiaca*)[1]. *Taverniera lappacea* is a perennial shrub with short branches, altogether densely pubescent. Calyx densely thick-publescent, Corolla yellow. Vexillum 5-6 mm long or oven less. Fruit 1-2-seeded lomentum, l seeded part 6-7 mm wide, densely echinulate, fruit appendages profusely hairy[2].

By tracing the UV triplet of heteroannular dicnes, three novel saikosaponins were isolated from the root bark of *Taverniera aegyptiaca.* They were identified as

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22 β-hydroxyolean-l l, I 3 (18)-dien-3 β- yl- β –D-glucopyranoside. 1β, 22 β -dihydroxyolean- l 1, I3 (I8)-dien-3β- yl- β -D-glucopyranoside and 1β,22β-dihydroxyolcan-l 1,I3(18)-dien-3β-yl-D-xylopyranosyl(1→ 2) β- D-glucopyranoside on the basis of chemical and spectral evidences[3]. A room temperature aqueous extract of the roots of *Taverniera abyssinica* antagonized the contractile responses of the guinea-pig ileum to acetylcholine and histamine. It also relaxed the smooth muscle of the rabbit duodenum, abolished the pendular contractions and antagonized the effects of acetylcholine and histamine on this tissue. The results confirmed that there may be a sound pharmacological basis for the ethnomedical use of the roots of this plant to treat stomach ache[4]. Chemoprofile of *Taverniera cuneifolia* (Roth) Arn. a wild relative of commercial licorice (*Glycyrrhiza glabra* L.) is presented. Both *T. cuneifolia* and *G. glabra* were found to have similar active compounds. At least eighteen chromatophores were found similar in both the plants including the sweetening principle, glycyrrhizin. The extracts of *T. cuneifolia* root, exhibited promising anti-inflammatory, anti-tumor, anti germination tube formation (in *Candida albicans*), protection from mutagen toxicity and cytotoxic activities comparable to that of *G. glabra*. In general, the results suggest that *T. cuneifolia* could be used as substitute of *G. glabra* [5]. From the saponin fraction of the total methanolic extract of the dried root and stem barks of *Taverniera aegyptiaca* Boiss, six new triterpenoidal saponins of oleanane type were isolated and identified as 28-methyl serratagenate-3-β-O- β -xylopyranosyl (1!2)- β -glucopyranoside (2), 28-methyl serratagenate 3- β -O-α-rhamnopyranosyl (1!2)-b-glucopyranoside (3), 3 β -O- α -rhamnopyranosyl (1!2) β -glucopyranosyl-olean-11,13(18)-dien-1 β, 3 β, 22 β -triol (4), 3 β -O- β -glucopyranosyl (1!2)- β -glucuronopyranosylolean-11,13 (18) -dien-1 β,3 β,22 β -triol(5),3 β -O- β -xylopyranosyl(1!2)- β -glucuronopyranosylolean-11,13(18)-dien-1 β,3 β,22 β triol (6), 3 β -O- α -rhamnopyranosyl (1!2)- β -glucuronopyranosylolean -11,13(18)-dien-1 β, 3 β, 22 β -triol (7) together with the known oleanolic acid 3-β -O- β -glucoside (1). The identification of the isolated compounds was done on the basis of chemical and spectral evidences [6].

MATERIALS AND METHODS

Plant Material: *Taverniera lappacea* vegetative parts were collected from Ras Mohamed protected area, South Sinai, Egypt. Samples were identified [(2)] and deposited with the plant protection collection at the Desert Research Center in Cairo, Egypt. Seeds of weeds and maize were obtained from Desert Research Center, Cairo Egypt.

Preliminary Phytochemical Screening: This includes testing for volatile oil by steam distillation method [7], testing for steroids [8], terpenoids [8], saponins [9], coumarins [10]Also, testing of flavonoid and phenolic compounds were done according to Edeoga *et al*., [8], glycosides and/or carbohydrates [11], and for alkaloids [12] using Dragend-orff's, Mayer's and Wagner's reagents [7].

Aqueous Extraction: Ten grams of air-dried ground of *Taverniera lappacea* were extracted with 100 ml distilled water on a rotary shaker for 4 hours at 25ºC. The mixture was filtered through two layers of cheese cloth to remove debris, and this filtrate was refiltrated through a Whatman No. 4 filter paper and reserved for bioassays. The aqueous filtrate (10g DW L^{-1}) was diluted to the following concentrations: 1%, 2%, 4% 5%, and 10% (v/v). Seeds of Maize (Zea mays*),* Purslane *(Portulaca oleracea*), bindweed (*Convolvulus arvensis)* Barnyard grass (*Echinochloa crus-galli)* and Veltvetleaf (*Abutilon thiophrastes)* were surface sterilized using sodium hypochloride (0.3% v/v) for 10-12 min and washed four times in sterile double-distilled water. Ten seeds from weed species was placed on a filter paper in each of four Petri-dishes (11 cm in diameter), and each treatment consisted of 10 ml of each dilution or undiluted extract added per dish. Sterile water was used as a negative control. Petri-dishes were incubated in the dark at 25°C. Germination was recorded after 7 days of incubation, and the stem and root lengths of weed seedlings were determined at the termination of each experiment.

Pre-emergence Phytotoxicity Bioassay: Powdered air-dried of *Taverniera lappacea* was subjected to extraction with successive selective organic solvents using soxhlet apparatus, in order of increasing polarity including hexane, petroleum ether (b.p. 40-60 °C), diethyl ether, chloroform, acetone, ethyl acetate, methanol and methanol 70%. The obtained residue from each solvent was tested on weed seeds of Maize (Zea mays*), Purslane (P. oleracea*), Barnyard grass (*Echinochloa crus-galli)* and Veltvetleaf (*Abutilon thiophrastes)*. The tested weed seeds were surface sterilized using sodium hypochloride (0.3% v/v) for 10-12 min and washed four times in sterile double-distilled water. The tested weed seeds were sown in 9 cm diameter Petri dishes moistened with 5 ml of the previously isolated extracts of *Taverniera lappacea* with 200 µg ml^{-1}, while the tested weed seeds control were treated with distilled water in equal of the tested extracts. Petri dishes were placed in dark at 20 ± 1 _C for 10 days. Each treatment was replicated three times. Germination and growth length were recorded at the tested end.

Dose –Response Relationship: Seeds of Maize (Zea mays*)*, Barnyard grass (*Echinochloa crus-galli)* and Veltvetleaf (*Abutilon thiophrastes)* were surface sterilized using sodium hypochlorite (0.3% v/v) for 10-12 min and washed four times in sterile double-distilled water. Seeds were placed on static Murashige and Skoog (MS) basal media and allowed to germinate for seven days until roots and shoots emerged. Seven-day-old seedlings were transferred to 24-well plates containing 2 mL of liquid MS media where the roots were submerged. Seedlings were treated with the concentrations with methanolic extracts (100, 200, 400, 800, 1600 µg ml^{-1}). Plant cultures were maintained on growth chamber with a photoperiod of 16h light and 8h dark at 25°C. Total biomass of each seedling was recorded fifteen days after treatment.

Purification and Identification of Active Compounds. Investigation of Flavonoids, Phenolic Acids and Coumarins: Aqueous ethyl alcohol 70 % of *Taverniera lappacea* were evaporated under reduced pressure. The residue was tested on two dimentional paper chromatography performed on Whatman No.1 paper chromatography using the solvent system n-butanol: acetic acid: water (4:1:5, v/v/v) = S1 for the first direction and the solvent system acetic acid: water (15:85) = S2 for the second direction. The developed chromatograms were air dried, examined under (UV) light, then exposed of ammonia and re-examined under UV light, then the residue was applied on the top of a polyamide column. Elution was started with methanol followed by gradually increasing of water as a mixture of methanol / water till finally pure water. The received fractions were evaporated and similar fractions were collected together. The collected fractions were preparative on the precoated silica gel plates using solvent system ethyl acetate: methanol: water (30: 5: 4), where the separated flavonoid and phenolic acid compounds were purified on a Sephadex LH-20 column using 80% methanol system. On the other hand, the separated coumarin compounds were purified on the precoated silica gel plates using solvent system chloroform: methanol (9.5:0.5, v/v) = S3. Each compound was tested against the seeds of Barnyard grass (*Echinochloa crus-galli)* and Veltvetleaf (*Abutilon thiophrastes).*

Physical Analysis:

A. Ultraviolet Spectrophotometric Analysis: Chromatographically pure materials were dissolved in pure methanol and subjected to ultraviolet spectrophotometric investigation using Shimadzu UV visible recording spectrophotometer UV-240.

B. ^{1}H- Nuclear Magnetic Resonance Analysis (^{1}H-NMR): The NMR measurements were carried out on A JEOL EX-270 NMR spectrometer apparatus (270MHz for ^{1}H-NMR and 67.5 MHz for ^{13}C-NMR) as described by Mabry *et al*. [13].

C. Mass Spectrometric Analysis (MS): The mass spectrum was conducted using finnigan SSQ 7000 and MM 7070 E according to the method of Mabry *et al*. [13].

Chemical Reactions:

A. Controlled (Mild) Acid Hydrolysis: The pure material was hydrolyzed using 0.1N HCl under reflux for 1 h. The resultants were traced chromatographically every 5 min. using comparative paper chromatography as stated by Harborne *et al*. [14].

B. Complete (Normal) Acid Hydrolysis: The pure material was hydrolyzed using 2N HCl under reflux for 1 h. The released aglycone and sugar were subjected to comparative paper chromatography using authentic samples [14].

C. Enzymatic Hydrolysis: The flavonoid glycoside was enzymatically hydrolyzed through an enzyme corresponds to the type of the sugar in the compound in 0.05 acetate buffer (pH=5.1). The mixture was left at 37-40°C for 24 hours. The hydrolysate was examined through comparative paper chromatography against authentic reference markers as described by Harborne *et al.* [14].

Phytotoxicity Assays for pure compounds: Seeds of Barnyard grass (*Echinochloa crus-galli)* and Veltvetleaf (*Abutilon thiophrastes)* were surface sterilized using sodium hypochlorite (0.3% v/v) for 10-12 min and washed four times in sterile double-distilled water. Seeds were placed on static Murashige and Skoog (MS) basal media and allowed to germinate for seven days until roots and shoots emerged. Seven-day-old seedlings were transferred to tissue culture tube containing1 mL of liquid MS media where the roots were submerged. Seedlings were treated with the concentrations 50 ug ml^{-1} from pure fractions Plant cultures were maintained on growth chamber with a photoperiod of 16h light and 8h dark at 25°C. Total biomass of each seedling was recorded fifteen days after treatment.

All experiments were designed with a randomized complete block design with four replicates. Data were statistically analyzed by ANOVA, according to Snedecor and Cochran[15] and treatment means were compared by LSD test at 5% level of probability.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening of *Taverniera Lappacea:* It is obvious from the obtained results declared that, the plant contained steroids, terpenoids, saponins, coumarins, flavonoids and phenolics and glycosides and/or carbohydrates. Neither volatile oil nor alkaloids were detected.

Bioactivity of Allelochemicals from Aqueous Leachate: Aqueous extracts of *Taverniera lappacea* were bioassayed for their effect on germination and seedling development in Maize (Zea mays*),* Purslane *(Portulaca oleracea*), Bindweed (*Convolvulus arvensis)* Barnyard grass (*Echinochloa crus-galli)* and Velvetleaf (*Abutilon thiophrastes)* species. Filtrates were tested at 0, 1%, 2%, 4% 5%, and 10% (v/v). *Taverniera lappacea* aqueous extracts affected germination and seedling growth parameters of maize (Table-1). At the highest concentration, aqueous extracts of *Taverniera lappacea* decreased *Z. mays* germination by 96.43% and root and shoot length by 98.67% and 94.68% respectively when compared to untreated controls. the extracts at 1%,2%,4,6,8% DW 100 ml^{-1} concentration, of *Taverniera lappacea* decreased *Z. mays* germination by 17.86,32.14,32.14,50.0 and 64.29% and root by 36.17,60.99,68.97,81.38,87.23%and shoot length by 2.5,13.92,30.89,41.77% and 60.76% respectively when compared to untreated controls. In C. *arvensis* aqueous extracts added at 8 %and 10% DW 100 ml^{-1} concentration completely inhibited germination and seedling growth. While 4% and 6% DW 100 ml^{-1} decreased germination by 68.18%and 81.82% relative to controls. Seedling root length was decreased by 26.33% and shoot length by 3% and 23.31%, respectively than untreated control. As for *A. thiophrastes* seeds, *T. lappacea* extracts (8% and 10% DW 100 ml^{-1}) completely inhibited germination and seedling growth. Also aqueous extracts at 4 % and 6% DW 100 ml^{-} reduced *A. thiophrastes* germination by 60% and 75% and root by 31.58% and 93.68%and shoot length by 45.65% and 93.91% respectively when compared to untreated controls. In *E. crus-galli* a complete reduction in germination and seedling mortality was achieved at 8% and 10% DW 100 ml^{-1} of *T. lappacea* aqueous extracts. *T. lappacea* aqueous extracts at 4 % and 6% DW 100 ml^{-1} decreased *Echinochloa crus-galli* germination by 61.54, 80.77%and 64.29% and root by 14.77, 90.91%and shoot length by 7.37 and 17.16% respectively when compared to untreated controls. Finally, *T. lappacea* complete inhibited *P. oleracea* in germination and seedling developing at 10 % DW 100 ml^{-1}. Aqueous extracts of *T. lappacea* were phytotoxic to seedlings of *P. oleracea* at both the 4% and 8% concentrations reduced germination by 63.33 and 86.67%and root length by 41.18,77.65 and 89.41% and shoot length by 31.67 and 70.0%, respectively, than untreated control.

Pre-emergence Phytotoxicity Bioassay: *Tavernaria lappacea* extracted successively with eight solvent, extracts were evaporated and dissolved in methanol for bioassay with two concentrations (0, 200 µg ml^{-1}) the biological a activity of each extracts were tested against target weeds; Barnyard grass (*E. crus-galli)*, Purslane *(P. oleracea*), Bindweed (*C. arvensis)* and Velvetleaf (*A. thiophrastes)* germination and seedling development. The most phytotoxic extracts are Ethyl acetate; methanol and methanol 70% completely inhibited all seedling development and germination of the tested weeds at 200 µg ml^{-1} as compared with the control. Followed with acetone extract at 200 µg ml^{-1} inhibited *E.crus galli* germination (by 74.07%) and shoot length (by 45.88%) but root length was inhibited by roughly (74.28%). Respectively than untreated control, Acetone extracts at 200 µg ml^{-1} reduced *P. oleracea* germination by (44.82%), shoot length by (81.96%) and root length by (88.571%). In *A. thiophrastes*, extracts at 200 µg ml^{-1} decreased *A. thiophrasts* germination by (61.53), shoot length by (50.00%) and root length by 82.97%), respectively than the control. Finally acetone extracts inhibited *C. arvensis* germination by (4.54%), shoot length by 53.9%) and root length by 35.29% respectively, as compared with the control treatment. The lowest effect achieved from petroleum ether and diethyl ether in the target weeds germination and seedling development.

Dos Response Relationship Between Methanolic Extracts and Plant Seedling Growth Parameters: The methanolic extracts of *T. lappacea* bioassay against maize (*Z.* mays*),* Barnyard grass (*E. crus-galli)* and Velvetleaf (*A. thiophrastes)* after germination in sold media and transfer the seedling (7days age) in liquid media with methanolic crude extracts to calculate dose response relationship. *T. lappacea* had an LD_{50} of *Z. mays* by 1450 ug ml^{-1} in total fresh weight, whereas the highest reduction achieved at 1600 ug ml^{-1} in maize total biomass recoded by 60.31%as compared with the control treatment. As for *E. crus-galli,* while *T. lappacea* extracts at 1600 ug ml^{-} reduced total biomass by 71.91%, than the control. *T. lappacea* had an LD_{50} calculated from total biomass fresh weight after two weeks by 700 µg ml^{-1}; finally methanolic crude extracts of *T. lappacea* had an LD_{50} by 450 µg ml^{-1} In *A. thiophrastes* total fresh weight, whereas, the highest concentration decreased total biomass by 75.41% with the comparison with treated control.

Purification and Identification of Active Compounds. Investigation of Flavonoids, Phenolic Acids and Coumarins: The developed chromatograms of the flavonoids, phenolic acids and coumarins of *Taverniera lappacea* plant **(**after drying and exposing to ammonia then subjecting to ultraviolet light**)** revealed the presence of a complex pattern of flavonoid, phenolic acids and coumarin constituents. To isolate the flavonoid, phenolic acids and coumarin components using preparative TLC (thin layer chromatography) revealed the presence of ten main bands of the flavonoids, two bands of phenolics acids and one main band of coumarin in the plant. Each band was subjected to two dimensional paper chromatography using the solvent systems S1 for the first direction and S2 for the second direction and purificated on a Sephadex LH-20 column using methanol: H_2O (1:1) [16].

Identification of Flavonoids

Identification of Compound (F_1): Compound (F_1) appeared on paper chromatography as deep purple spot, which was changed to yellow on exposure to ammonia vapor and re-examined under UV light with R_f-values 0.18 and 0.62 in systems; S1 and S2 respectively. Complete acid hydrolysis gave an aglycone quercetin and glucose as sugar residue, which was detected by comparing its comparative R_f-values with authentic pure sugars. UV spectral data of compound (F_1) (Table 4) with methanol gave two bands at 356 and 260 nm, band I and band II, respectively indicating that (F_1) was a flavonol with 3-OH substitution, while on addition of NaOAc no shift in band II was observed, thus suggestion the occupation of 7-position. The remaining UV spectral data were found to be similar to that of quercetin type compound.

^{1}H-NMR spectrum of compound (1) gave the following signals: δ (ppm) 7.7(2H, d, J = 8.5 Hz, H2' , H6'), 6.8 (2H, d, J= 8.5, 2.5 Hz, H3', H5'), 6.8 (1H, d, J= 2.5 Hz, H8), 6.4 (1H, d, J= 2.5 Hz, H6), 5.6 (1H, d, J= 2.5 Hz, H1" glucose), 5.3 (1H, d, J= 2.5 Hz, H1'" glucose), 3.4 (m, remaining sugar protons)[13]. From the previous data compound (F_1) was identified as quercetin-3, 7- diglucoside.

Identification of Compound (F_2): Compound (F_2) appeared on paper chromatography as yellow spot, which was unchanged on exposure to ammonia vapor and re-examined under UV light with R_f-values 0.36 and 0.32 in systems; S1 and S2, respectively. Complete acid hydrolysis gave the aglycone kaempferol and rhamnose as sugar residue, which was detected by comparing its comparative R_f-values with authentic pure sugars. UV spectral data of compound (F_2) (Table 4), with methanol gave two bands at 385 and 261 nm, band I and band II, respectively indicating that (F_2) was a flavonol, while on addition of NaOAc no shift was detected in band II, thus suggestion the occupation of 7-position. The remaining UV spectral data were found to be similar to that of kaempferol type compound.

^{1}H-NMR spectrum in DMSO showed signals at: δ 7.8 (2H, d, J= 8.5Hz, H2', H6'), 6.8 (2H, d, J= 8.5Hz, H3', H5'), 6.4 (1H, d, J= 2.5 Hz, H8), 6.1 (1H, d, J= 2.5 Hz, H6), 5.3 (1H, d, J= 8 Hz, H1" rhamnose), 1.2 (3H, d, J= 6 Hz, CH3 rhamnose). From the previous data compound (F_2) was identified as kaempferol -7-rhamnoside.

Identification of Compound (F_3): Compound (F_3) appeared on paper chromatography as purple spot, which was changed to yellow on exposure to ammonia vapor and re-examined under UV light with R_f-values 0.48 and 0.56 in systems; S1 and S2, respectively. Complete acid hydrolysis gave the aglycone quercetin and two sugar residues, which was identified as glucose and rhamnose and detected by comparing its comparative R_f-values with authentic pure sugars. Enzymatic hydrolysis with β-glycosidase gave negative results (no-intermediates), which confirmed that the glucose moiety is directly linked to the flavonoid nucleus and rhamnose is terminally located in compound (F_3). UV spectral data of compound (F_3) (Table 4) with methanol gave two bands at 362 and 257nm, band I and band II, respectively indicating that (F_3) was a flavonol with 3-OH substitution. The remaining UV spectral data were found to be similar to that of quercetin type compound.

^{1}H-NMR spectrum of compound (3) gave the following signals: δ (ppm) 7.6 (1H, d, J = 2.5 Hz, H2), 7.5 (1H, dd, J= 8.5, 2.5 Hz, H6'), 6.8 (1H, d, J= 8Hz, H5'), 6.4 (1H, d, J= 1.5 Hz, H8), 6.2 (1H, d, J= 1.5 Hz, H6), 5.3 (1H, d, J= 8 Hz, H1" glucose), 4.5 (1H, d, J= 2.5 Hz, H1'" rhamnose), 3.4 (m, remaining sugar protons) and 0.8 (3H, d, J= 6 Hz, CH_3 rhamnose) [13]. Mass spectrum showed 610 (M^+, glucose, 60%), 464 (M^+, rhamnose, 191.1%), 302 (quercetin, 100%). From the previous data compound (F_3) was identified as quercetin-3-O-α L-rhamnoside (1-6) β D-glucoside (Rutin).

Identification of Compound (F_4): Compound (F_4) appeared on paper chromatography as purple spot, which was changed to yellow-green on exposure to ammonia vapor and re-examined under UV light with R_f-values 0.63 and 0.23 in systems; S1 and S2, respectively. Complete acid hydrolysis gave the aglycone apigenin and one sugar residue identified as glucose. UV spectral data of compound (F_4) (Table 4) with methanol gave two bands at 335 and 267nm, band I and band II, respectively indicating that (F_4) was a

flavone, while on addition of NaOAc no shift was detected in band II, thus suggestion the occupation of 7-position. Addition of H_3BO_3 no shift was detected in band I, which was proved the absence of catecholic hydroxyl groups. Addition of $AlCl_3$ caused a bathochromic shift in band I, thus indicating the presence of free OH at C-5 [13].

^{1}H-NMR spectral data: Aglycone moiety: δ (ppm) 7.95. (d, j =7.5 Hz H-2̀ and H-6̀) . 6.95 (d, j= 7.5 Hz H-3̀, H-5̀) 6.8 (d, j =2.5 Hz H-8), 6.75 (S, H-3) and 6.4 (d, j =2.5 Hz, H-6). Sugar moiety: δ (ppm) 5.1 (d, J = 7.5 Hz H" -glucose), 3.2-3.8 (m, glucose protons). From the previous data compound (F_4) was identified as apigenin -7-O-glucoside.

Identification of Compound (F_5): Compound (F_5) appeared on paper chromatography as light blue spot, which was changed to fluorescent light blue on exposure to ammonia vapor and re-examined under UV light with R_f-values 0.63 and 0.69 in systems; S1 and S2 respectively. UV spectral data of compound (F_5) (Table 4) with methanol exhibited a major band at λ_{max} 253 and shoulder at λmax 315 (characteristic range of isoflavanone), while on addition of NaOAc no shift was detected in band II, thus suggestion the occupation of 7-position. Addition of H_3BO_3 no shift was detected in band I, which was proved the absence of catecholic hydroxyl groups. No shift on addition of $AlCl_3$ indicating the abscence of free OH at C-5 [13].

^{1}H-NMR spectral data δ (ppm): 9.56(s,OH), 8.38 (1H,s,H-2), 8.05(1H,d, J=8.78Hz,H-5), 7.42 (2H, d, J= 8.61Hz, H-2', H-6'), 7.25 (1H, d, J= 2.21Hz, H-8), 7.13 (1H, dd, J= 2.3, 8.74 Hz, H-6), 6.81 (2H, d, J= 8.59 Hz, H-3', H-5'), 4.65 (1H, d, J= 5.33 Hz, H-1"). Mass spectrum showed molecular ion peak (M^+) at 416. From the previous data compound (F_5) was identified as daidzein 7-O-glucoside (daidzin).

Identification of Compound (F_6): Compound (F_6) appeared on paper chromatography as brown spot, which was changed to yellow on exposure to ammonia vapor and re-examined under UV light with R_f-values 0.80 and 0.55 in systems; S1 and S2, respectively. Complete acid hydrolysis gave the aglycone kaempferol and one sugar residue, which was identified as glucose. UV spectral data of compound (F_6) (Table 4) data with methanol gave two bands at 350 and 265nm, band I and band II, respectively indicating that (F_6) was a flavonol with 3-OH substitution, while on addition of NaOAc a bathochromic shift in band II which indicated the presence of free OH at 7- position. Addition of H_3BO_3 no shift was detected in band I, which was proved the absence of catecholic hydroxyl groups. Addition of $AlCl_3$ caused a bathochromic shift in band I, thus indicating the presence of free OH at C-5 (13).

^{1}H-NMR spectral data δ (ppm): 7.9 (2H, d, J = 9 Hz, H 2̀ and H 6̀) , δ 6.7 (2H, d, J = 9 Hz, H3 ' and H5'), δ 5.6 (I H , d, J = 1.5 Hz, H8), δ 5.5 (l H , d , J = 1.5 H z , H 6) , δ 5.1 (d , J = 7 H z , H 1"glucose) and δ3.1-3.8 (m, remaining sugar protons). From the previous data compound (F_6) was identified as kaempferol -3-O-glucoside.

Identification of Compound (F_7): Compound (F_7) was obtained as yellow color under UV no changed on exposure to ammonia vapor and re-examined under UV light with R_f - values 0.81 and 0.25 in S1 and S2, respectively, which revealed that compound (F_7) may be flavonoid aglycone. UV spectral analysis of the compound (F_7) in methanol gave two bands at 375 and 253 nm, band I and band II, respectively indicating that (F_7) was a flavonol compound with free 3-OH group, where on addition NaOAc a bathochromic shift in band II which indicated the presence of a free 7-OH group, while addition of NaOAc / H_3BO_3 caused bathochromic shift in band I which indicated the presence of O- dihydroxy group in B-ring. The bathochromic shift of band I in $AlCl_3$ indicates the presence of 3 and 5-OH group, while the hypsochromic shift with $AlCl_3$ / HCl in band I after addition of HCl indicates the presence of O-dihydroxy group in B-ring where these results were in agreement with Mabry *et al*., [13].

^{1}H-NMR spectrum in DMSO showed signals at: δ 7.6 (1H, dd, J= 8.5, 2.3 Hz, H2'), 7.5 (1H, dd, J= 8.5Hz, H6'), 6.89 (1H, d, J= 8.5 Hz, H5'), 6.4 (1H, d, J= 2.5 Hz, H6) and 6.2 (1H, d, J= 2.5 Hz, H8) (13). EI-Mass spectrum of compound (F_7) showed molecular ion peak M^+ at 301.9 m/z. The previous obtained data when compared with published data it could be concluded that compound (F_7) can be identified as quercetin.

Identification of Compound (F_8): Compound (F_8) appeared on paper chromatography as yellow spot, which was changed to florescence yellow on exposure to ammonia vapor and re-examined under UV light with R_f -values 0.84 and 0.15 in systems; S1 and S2, respectively. Complete acid hydrolysis gave an aglycone kaempferol and one sugar residue, which was identified as glucose. UV spectral data of compound (F_8) (Table 4) in methanol gave two bands at 367 and 268 nm, band I and band II, respectively indicating that (F_8) was a flavonol, while on addition of NaOAc a bathochromic shift in band II which indicated the presence of free OH at 7- position. Addition of H_3BO_3 no shift was detected in band I, which was proved the absence of catecholic hydroxyl groups Addition of $AlCl_3$ caused a bathochromic shift in band I, thus indicating the presence of free OH at C-5 [13].

^{1}H-NMR spectral data δ (ppm): 8.0 (2H, d, J= 8 Hz, H2', H6'), 6.9 (2H, d, J= 8 Hz, H3', H5'), 6.4 (1H, d, J= 2.5 Hz, H8), 6.2 (1H, d, J= 2.5 Hz, H6). EI-mass spectrum of compound (F_8) revealed a molecular ion peak (M^+) at m/e 286 and other important ions. m/e 285, 258, 229, 121 and 93. From the previous data compound (F_8) was identified as kaempferol.

Identification of Compound (F_9): Compound (F_9) appeared on paper chromatography as deep purple spot, which was changed to greenish purple on exposure to ammonia vapor and re-examined under UV light with R_f-values 0.86 and 0.34 in systems; S1 and S2, respectively. UV spectral data of compound (F_9) (Table 4) in methanol exhibited a major band at λ max 290 and shoulder at λ max 325 (characteristic range of flavanone), while On addition of NaOMe, it exhibited a bathochromic shift indicating the presence of free OH group at C7 and C4'. On addition of NaOAc/ H_3BO_3 the spectrum was identical to that in methanol. On addition of $AlCl_3$, the spectrum exhibited a bathochromic shift which was not affected by the addition of HCl, indicating the presence of free OH group at C-5 [13]. 1H-NMR spectral data δ (ppm): 7.3 (2H, dd, J= 8.5, 2.3 Hz, H2', H6'), 6.8 (2H, dd, J= 8.5, 2.3 Hz, H3', H5'), 6.0 (1H, d, J= 2.5 Hz, H8), 5.8 (1H, d, J= 2.5 Hz, H6), 5.2 (1H, dd, J= 5, 11 Hz, H2), 2.5 (cis and trans, 2H, 2d, J= 17 Hz, H3) (13). From the previous data compound (F_9) was identified as naringenin.

Identification of Compound (F_{10}): Compound (F_{10}) was obtained as purple color under UV, which was changed to yellow green on exposure to ammonia vapor and re-examined under UV light with R_f 0.86 and 0.11 in S1 and S2, respectively, these results revealed that the compound may be flavonoid aglycone. UV spectral analysis with methanol gave two bands at 337 and 266 nm, band I and band II, respectively, indicating that the compound (F_{10}) was a flavone compound, where on addition of NaOMe caused bathochromic shift, thus indicating that the 4′ position to be free hydroxyl group, while addition of NaOAc exhibited a bathochromic shift so proving that the 7 position to be free hydroxyl. Mean while addition of H_3BO_3 caused no shift so proving the absence of catecholic hydroxyl groups. On other hand addition of $AlCl_3$ caused a bathochromic shift of which was not changed after addition of dilute HCl, so further confirming the absence of catecholic hydroxyl groups which agreed the conclusion of Mabry *et al.,* [13].

1H-NMR spectral data showed signals at: δ (ppm) 7.95(d, J=7.5 Hz for H-2, H-6′), 6.9 (d, J=7.5 Hz for H-3′, H-5′), 6.75 (S, H-3), 6. 5 (d, J=2.5 Hz for H-8and 6.1 (d, J=2.5 Hz for H-6). EI-Mass spectrum of compound (10) showed molecular ion peak M+ at 270 m/z and fragments 242 (19%), 153 (22%), 118 (14%), 121 (6%). The previous obtained data when compared with published data it could be concluded that compound (F_{10}) was identified as apigenin.

Identification of Phenolic Acids

Identification of Compound (P_1): Compound (P_1) appeared on paper chromatography as blue spot, which was changed to bright blue on exposure to ammonia vapor and re-examined under UV light with R_f- values 0.77 and 0.25 in systems; S1 and S2, respectively. UV spectral data of compound (P_1) (Table 4). Mass spectrum showed a molecular ion beak (M^+) at 181. From the previous data compound (P_1) was identified as caffeic acid.

Identification of Compound (P_2): Compound (P_2) appeared on paper chromatography as blue spot, which was changed to Fluorescence blue on exposure to ammonia vapor and re-examined under UV light with R_f- values 0.85 and 0.57 in systems; S1 and S2, respectively. UV spectral data of compound (P_2) (Table 4). Mass spectrum showed a molecular ion beak (M^+) at 194. From the previous data compound (P_2) was identified as ferulic acid.

Identification of Coumarins

Identification of Compound (C_1): Compound (C_1) was detected as blue color under UV, which was changed to fluorescence blue on exposure to ammonia vapor and re-examined under UV light with R_f 0.75 in system S3. Examination of compound (C_1) in MeOH using UV spectrophotometer gave two bands at 225 and 350 nm; a where in addition of NaOAc gave a bathochromic shift, which indicates the presence of free OH at position 7. The obtained UV spectral data was similar to those reported for 7-hydroxy-6-methoxycoumarin compounds [17]. Mean while ^{1}H-NMR spectral data showed signals at: δ 7.6 (1H, d, J=9 Hz, H-4), 6.9 (1H, s, H-5), 6.7 (1H, s, H-8), 6.3 (1H, d, J=9 Hz, H-3) and 3.9 (3H, s, OCH3). The presence of pair of doublets at δ 7.6 and 6.3 ppm indicates that compound (C_1) is unsubstituted coumarin pyrone ring [17]. Mass spectrum showed fragments at m/z 193 (M^+, 100%), 192 (M-1) 178 ([M-CH3]$^+$, 7%), 163 ([M-OCH3]$^+$, 9%), 82([M-4CO]$^+$, 8%), 69 ([M-4CO-CH3]$^+$, 7%). The previous obtained data, when compared with published data [17] concluded that compound (C_1) could be identified as 7- hydroxyl -6- methoxycoumarin (scopoletin).

All compounds extracts were evaporated, then the residues were dissolved in 350 ul methanol for testing with 40 ug ml^{-1} media on Barnyard grass (*E. crus-galli)* and Velvetleaf (*A. thiophrastes)* seedling. Total

biomass data was recorded after 15 days. The most active fractions recorded from kaempferol -7-rhamnoside (F_2), daidzin (F_5) and kaempferol -3-O-glucoside (F_6) with reduction percentage in total biomass fresh weight as fellow: (*A. thiophrastes)* by 56.70%, 70.97% and 54.96%, respectively and (E. *crus-galli)* by 55. 49%, 58.52% and 56.98%, respectively, as compared with the control on the others hands, the second category appear in quercetin-3, 7- diglucoside (F_1), naringenin (F_9) and caffeic acid (P_1) depending on the activity against *A. thiophrastes* total biomass fresh weight, whereas, quercetin (F_7) and scopoletin (C_1) achieved the second reduction level in *E. crus-galli* as compared with the control (Table 5 and Fig 1).

Phytotoxic activity from *T. lappacea* methanolic extracts and purified compounds against *A. thiophrastes* weeds. Values are presented as mean for total biomass fresh weight as compared to the control. Errors bars are the S.D of the mean. N=3, values are significantly different from those of respective control at the levels of *P < 0.05 according to LSD test.

Summarizing *T. lappacea* herbicidal activity by both aqueous and organic extracts in inhibiting the growth of most tested weeds, aqueous extracts and three extracts starting from ethyl acetate, methanol and methanol 70% extracts significantly inhibited most of tested weed emergence and decreased germination and seedling growth depending on the concentration. In the other issues the most active and pure compound

Table 1: Effect of aqueous leachate of *T. lappacea* on germination and seedling development of some weeds

		Test plants				
Conc.		Z. maize	*C. arvensis*	*A. theophrastes*	*E. crus-galli*	*P. oleracea*
Control	S.L (cm)	4.39	7.00	7.67	3.17	2.00
	RL(cm)	12.53	7.17	3.17	2.93	2.83
	Germ	9.33	7.33	6.67	8.67	10.00
1%	S.L (cm)	4.28	10.33	8.00	3.17	2.83
	RL(cm)	8.00	9.10	3.01	2.37	2.00
	Germ	7.67	6.00	5.33	8.33	10.00
2%	S.L (cm)	3.78	10.17	6.87	3.60	2.77
	RL(cm)	4.89	7.17	3.83	2.67	2.63
	Germ	6.33	4.33	4.33	6.33	7.67
4%	S.L (cm)	3.03	6.79	4.17	2.93	2.80
	RL(cm)	3.89	5.28	2.17	2.50	1.67
	Germ	6.33	2.33	2.67	3.33	7.00
6%	S.L (cm)	2.56	5.33	0.47	2.62	1.37
	RL(cm)	2.33	2.93	0.20	0.27	0.63
	Germ	4.67	1.33	1.67	1.67	3.67
8%	S.L (cm)	1.72	0.00	0.00	0.00	0.60
	RL(cm)	1.60	0.00	0.00	0.00	0.30
	Germ	3.33	0.00	0.00	0.00	1.33
10%	S.L (cm)	0.23	0.00	0.00	0.00	0.00
	RL(cm)	0.17	0.00	0.00	0.00	0.00
	Germ	0.33	0.00	0.00	0.00	0.00
LSD (0.05)	S.L (cm)	1.260	3.659	3.359	1.179	0.961
	RL(cm)	5.342	2.819	1.537	1.379	1.017
	Germ	3.544	3.988	3.470	4.534	4.546

Table 2: Pre-emergence Bioassay of *T. lappacea* successive extracts on germination and seedling development of some weeds.

		Test plants			
Conc.		*C. arvensis*	*A. theophrastes*	*E. crus-galli*	*P. oleracea*
Control	S.L (cm)	4.267	2.800	2.833	8.500
	RL(cm)	1.700	1.567	2.333	2.333
	Germ	7.333	8.667	9.000	9.667
Hexane	S.L (cm)	3.633	1.267	2.567	2.267
	RL(cm)	2.133	0.633	1.667	0.500
	Germ	7.333	4.000	6.333	9.667
Petroleum ether	S.L (cm)	2.267	1.367	2.200	2.100
	RL(cm)	0.867	0.533	1.400	0.267
	Germ	6.000	4.667	6.000	6.333
Diethyl ether	S.L (cm)	2.267	1.833	2.500	1.833
	RL(cm)	0.433	0.933	0.533	1.267
	Germ	6.667	6.667	6.000	6.667
Chloroform	S.L (cm)	1.933	1.667	2.500	1.767
	RL(cm)	0.633	0.633	1.433	0.333
	Germ	6.667	6.333	6.667	8.333
Acetone	S.L (cm)	1.967	1.400	1.533	1.533
	RL(cm)	1.100	0.267	0.600	0.267
	Germ	7.000	3.333	2.333	5.333
Ethyl acetate	S.L (cm)	0.000	0.000	0.000	0.000
	RL(cm)	0.000	0.000	0.000	0.000
	Germ	0.000	0.000	0.000	0.000
Methanol	S.L (cm)	0.000	0.000	0.000	0.000
	RL(cm)	0.000	0.000	0.000	0.000
	Germ	0.000	0.000	0.000	0.000
Methanol 70%	S.L (cm)	0.000	0.000	0.000	0.000
	RL(cm)	0.000	0.000	0.000	0.000
	Germ	0.000	0.000	0.000	0.000
LSD (0.05)	S.L (cm)	0.780	0.499	0.940	0.533
	RL(cm)	0.450	0.436	0.211	0.332
	Germ	3.012	2.116	2.498	3.109

Table 3: Dos response relationship between *T. lappacea* methanolic extracts and plant total biomass fresh weight gm).

Tested plant	Conc.	Control	100	200	400	800	1600	LSD0.05
Zee maize		0.925	0.816	0.618	0.604	0.529	0.367	0.68
Abutilon theophrastes		0.084	0.057	0.054	0.037	0.032	0.021	0.056
Echinochloa crus-galli		0.148	0.11	0.098	0.087	0.068	0.042	0.1087

Table 4: UV-spectral data (λ_{max}, nm) of the isolated flavonoid and coumarin compounds

Compounds	Reagents					
	MeOH	NaOMe	NaOAc	NaOAc + H_3BO_3	$AlCl_3$	$AlCl_3$ + HCl
Quercetin-3, 7- diglucoside.	260, 270(sh), 356	269, 290(sh), 390	260, 293(sh) 375, 420(sh)	260, 388	269, 295(sh), 333, 430	265, 296(sh), 356(sh), 400
kaempferol 7- rhamnoside	245, 261, 334, 385 240, 314 (sh), 362, 432	240, 266, 335 (sh), 398		262, 320, 388, 402	261, 323, 375	240, 315 (sh), 362, 432
Rutin	257,267 (sh),300 (sh), 362	270, 310 (sh), 410	270, 310 (sh), 385	260, 300 (sh), 385	274, 300(sh), 335(sh), 420	274, 305(sh), 342 (sh), 409
Apigenin-7-glucoside	226,267,335	240,271,304(sh),401	253(sh),264,351,388(sh)	267,339	274,300(sh),344,387	275,292(sh),341,383
Daidzin	253, 315 (sh)	254, 269(sh), 322(sh)	253, 322(sh)	250, 316(sh)	255, 310(sh)	255, 300(sh), 359 (sh)
Kaempferol-3- O glucoside	265, 325,350	275, 325,400	223, 275, 304(sh), 375	224, 274, 377	265, 290, 420	265, 300, 420
Quercetin	253,272 (sh), 300 (sh), 375	266, 330 (sh), 436	258, 332 (sh), 387	263, 305 (sh), 385	230, 320, 445	265, 305 (sh), 358 (sh) , 421
Kaempferol	253 (sh),268, 342 (sh), 367	280,318,420	275, 302(sh),385	267, 296(sh), 320 (sh), 370	266, 305 (sh),350, 422	266, 305 (sh),350 , 422
Naringenin	290, 325(sh)	247, 267(sh), 327	290 (sh), 327	292, 327 (sh),330 (sh)	310, 375	310 , 375
Apigenin	266,298(sh),337	277,325,394	276,301,375	367,301,338	276,301,346,385	276,299,342,384
Caffeic acid.	224, 330	224, 355				
Ferulic acid	285, 312	250(sh), 290, 319				
Scopoletin	225, 350	232, 416				

Table 5: bioassay for the purified compounds on weed total biomass by (gm) fresh weight.

Fractions	Cont.	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	P_1	P_2	C_1	LSD
A. thiophrastes	0.071	0.0414	0.0321	0.0458	0.0509	0.0207	0.0308	0.0388	0.0617	0.0368	0.0541	0.0394	0.0451	0.0595	0.0298
E. crus-galli	0.0078	0.0060	0.0034	0.0057	0.0069	0.0032	0.0035	0.0043	0.0043	0.0051	0.0070	0.0047	0.0050	0.0039	0.00362

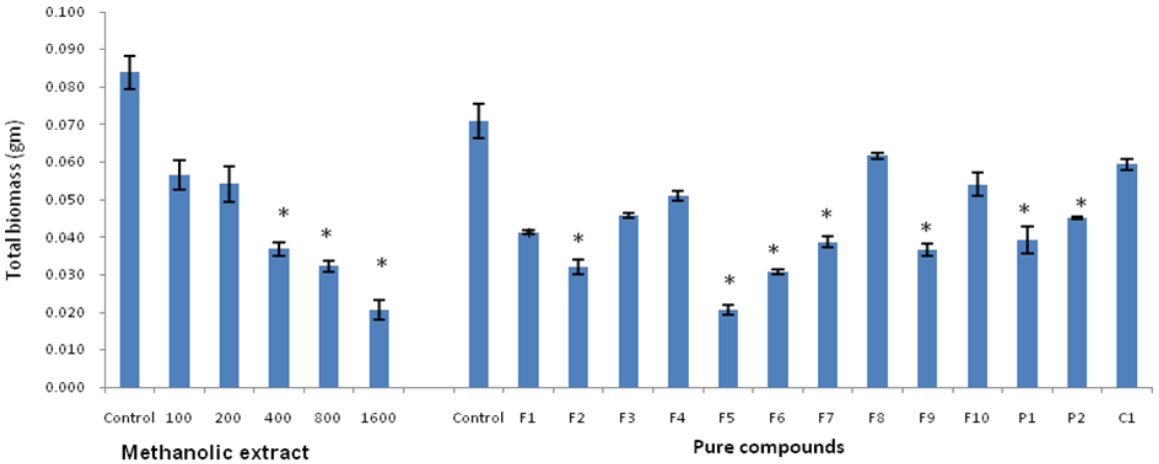

Fig. 1: Phytotoxic activity of of *T.lappacea methanolic* extract and *fractions* on *A. Thiophrasres* total biomass fresh weight (gm)

reduced fresh total biomass ranged from 49 % to 70 % as comparable with its control. The phytotoxicity of *T. lappacea* allelochemicals to the maize crop is not high only appearing in the highest concentration from the total extracts. *T. lappacea* did not show any post emergence activity at 5000, 10000 and 20000 µg ml-1 from methanolic extracts against maize (*Z. mays),* barnyard grass (*E. crus-galli)* and veltvetleaf (*A. thiophrastes)* at (3-4) leaves stage in the greenhouse, after treatment with spray solution, total fresh weight and dry weight for vegetative parts were recorded after two weeks and survival of seedling was determined. Finally we come to conclusions, the toxicity of aqueous and organic extracts increasing with increasing the concentration[18], our results indicated that most allelochemicals able to extracts with water, however the toxicity of the organic extracts more than aqueous extracts toxicity, as well as the activity. From LD_{50} value, A. *thiophrastes* was more sensitive than *E. crus-galli* to the two extracts, as observed results form it's LD_{50}. The fact that flavonoids are a natural product with potent herbicidal activity makes this topic more worthy of future investigation, phytotoxic activity of many flavonoids compounds were reported [19],[10],]21],[22]. We have explored the allelopathic activity of wild herbs as allelopathic plants to purify many

allelochemicals and introduce for use in weed control, which have the potential role of use directly as herbicide substitute or as structural leads for new synthetic herbicide.

Conclusion: The objective of the present work focuses on identifying natural chemicals components from *Taverniera lappacea* that are responsible for phytotoxic effects against Maize (Zea mays *)* weeds such *as: Purslane (P. oleracea*), bindweed (*Convolvulus arvensis)* Barnyard grass (*Echinochloa crus-galli)* and Veltvetleaf (*Abutilon thiophrastes)*. A bioassay guided fractionation was used to identify phytotoxic fractions of crude extracts and dose-response relationships between allelochemical concentration and the weed species growth parameters were evaluated. Purified active components were identified by spectroscopic methods and herbicidal activity was confirmed by its pre emergence activity, which have the potential to be developed as natural herbicides that can be used to effectively control target weeds.

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