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Herbicidal potential of methanolic extracts of *Pinus merkusii* Jungh. et de Vriese, *Acacia mangium* Willd., *Jatropha curcas* L., *Tectona grandis* L.f. and *Terminalia catappa* L. on *Amaranthus spinosus* L.

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ABSTRACT

We studied the effects of methanolic extracts (10 %, 20 % and 30 %) of Merkus pine (*Pinus merkusii* Jungh. et de Vriese), black wattle (*Acacia mangium* Willd.), Jatropha (*Jatropha curcas* L.), teak (*Tectona grandis* L.f.) and ketapang (*Terminalia catappa* L.) on the seedlings growth of weed spiny amaranth (*Amaranthus spinosus* L.). The herbicide, 2,4-dichlorophenoxy-acetic acid at 0.686 kg a.i.ha^{-1} was used as positive control and distilled water as negative control. The results showed that application of allelopathic extracts of 5-perennial plants at 10 %, 20 % and 30 % concentrations significantly reduced the plant height, number of leaves, stems diameter, weed control, leaf area, root length and shoot and root dry weight of *A. spinosus* weed. At 21 days after application, the methanolic extracts at 30 % concentration of donor plants: *P. merkusii, T. cattapa, T. grandis, J. curcas* and *A. mangium* caused 64.2 %, 51.1 % 50.2 %, 48.2. %, 38.6 % inhibition, respectively, in seedlings growth of *A. spinosus,* while the herbicide 2,4-D at 0.686 kg a.i. ha^{-1} caused 100 % control of test weed *A. spinosus*. The phytochemical test showed that *A. mangium* contained alkaloids and terpenoids, *P. merkusii* and *J. curcas* contained terpenoids and steroids, *T. grandis* contained terpenoids and flavonoids and *T. catappa* contained steroids. Phenolics were present in all five plants extracts. GC-MS analysis showed that leaf extracts of donor plants contained 3 major compounds each as under: *A. mangium* : [isopropyl palmitate (20.51 %), octadecanoic acid (12.63 %), lupeol (11.15 %)], *P. merkusii* [isopropyl palmitate (33.45 %), isopropyl linoleate (11.89 %), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (7.32 %)], *T. grandis* [d:b-friedo-b':a'-neogammacer-5-en-3-ol,(3.beta.)- (13.04 %), stigmasterol, 22,23-dihydro- (13.02 %), n-hexadecanoic acid (8.32 %)], *T. catappa* [lupeol (25.84 %), stigmasterol,22,23-dihydro-(15.43 %), alpha-amyrin (9.81 %)], *J. curcas* [stigmasterol,22,23-dihydro-(24.10 %), cholest-5-en-3-ol,24-propylidene-,(3.beta.)-(15.70 %) and n-hexadecanoic acid (11.74 %)]. The *P. merkusii* provided the greatest control of weed *A. spinosus* (64.2%) due to presence of isopropyl palmitate (33.45 %), isopropyl linoleate (11.89 %) and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (7.32 %).

Keywords: *Acacia mangium, Amaranthus spinosus*, bioherbicide, black wattle, herbicidal potential of methanol extracts, *Jatropha curcas.*, ketapang, marcuspine, phenolic, *Pinus merkusii*, steroid, terpenoid, teak, *Tectona grandis, Terminalia catappa*.

INTRODUCTION

Weeds are serious problem in crop production, they reduce both the yield and quality of crops (57). Weeds (a) competes with crops for growth resources (nutrients, water, light and space), (b) secretes chemical compounds in the environment that adversely affects the growth of other plants and (c) can be alternate hosts for pests and disease (21). Presently weeds are controlled by herbicides; however, their continuous use have caused harmful

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effects viz., (i). development of herbicide-resistant weeds, (ii). adverse effects on human health, (iii). water and soil contamination and (iv). destruction of decomposer organisms) (3,19). Hence, there is need to develop alternate environment friendly methods for weed control. Some efforts have been made to explore the herbicidal potential of plants derived compounds (allelochemicals) (5,6,32,37). Allelopathy is biological process in which plants produce and release one or more allelochemical compounds, which affects the growth or survival of other plants. Allelochemicals are present in all plant tissues (leaves, stems, roots, flowers and fruit). These are byproducts of primary metabolism and called secondary metabolites [Fatty acids, quinone, terpenoids, flavonoids, tannins, cinnamic acid and its derivatives, coumarin, phenols and phenolic acids, non-protein amino acids, sulfides and nucleosides (41,46)]. These allelochemicals are released from plants through root exudates, leaching, volatilization and biomass decomposition (13,46). The allelochemicals can be either advantageous (stimulating) or harmful to target organisms (43,52).

Several perennial plants have allelopathic influences on the germination and growth of nearby plants. The leaf, seed and root extracts of Jatropha (*Jatropha curcas* L.) inhibited the germination and root growth of corn (*Zea mays* L.*)*, tomato (*Solanum lycopersicum* L.) and upland rice (*Oryza sativa* L.) (42). Aqueous extracts of its leaves and roots at 8 % and 10 % concentrations inhibited the germination, radicle and plumule length of *Phaseolus vulgaris* L., *Z. mays* L., tomato (*Lycopersicon lycopersicum* L.), okra (*Hibiscus esculentus* L.) (1) and tobacco (*Nicotiana tabacum* L.) (29). The extracts of dried jatropha leaves promoted the growth of wheat (*Triticum aestivum* L.) (44). The allelopathic potential of leaves of teak (*Tectona grandis* L.f.) have been reported. Its leaf extracts inhibited the germination of rice (10 %-30 %) and corn [40 %-50 % (28)]. Its 5 % leaves extracts were stimulatory, but higher concentrations (10 %, 25 %, 50 %, 75 %, 100 %) were inhibitory to germination of black gram (*Vigna mungo* L.) and green gram (*Vigna radiate* L.) (33). Pine (*Pinus merkusii* Jungh. et de Vriese) leaf extract at 1000 ppm concentration inhibited the germination of amaranth (*Amaranthus viridis*) and jungle rice (*Echinochloa colonum*) (50). While its 2000 ppm concentration, reduced the plant height (48 %), leaf area (49 %) and dry weight (38 %) of *P. oleracea* (5). The ketapang fruit (*Terminalia catappa* L.) fractions dichloromethane and acetate ethyl at 336 and 383 ppm concentrations, respectively, were most inhibitory to radicle growth of lettuce (*Lactuca sativa* L.) (4). Its leaf extracts at 50 % concentration inhibited the germination of *Cyperus rotundus* L. and *Mimosa pudica* L. (47). The leaf extract of *Acacia mangium* Willd. inhibited the germination and growth of rice (20).

The essential aspect of bioassay test is to detect the allelopathic action of compounds on target species (9,55). In this study spiny amaranth (*Amaranthus spinosus* L.) was selected as the target plant, due to its fast uniform germination and sensitivity and its active competitor with crops (2,38). This study aimed to determine the allelopathic effects of the 5-donor plants and to identify their most potent compounds for developing as bioherbicide to control the *A. spinosus* growth.

MATERIALS AND METHODS

The study was conducted from May to October 2015 in Laboratory of Biology (Herbarium) and Laboratory of Chemistry, Faculty of Mathematics and Natural Sciences-Syiah Kuala University, Integrated Laboratory and Technology Innovation Center, Lampung (*Unila*). The screen house studies were done in the Experimental Farm, Faculty

of Agriculture, Syiah Kuala University (Unsyiah) (95°22ˈ34, 49°T longitude, 5°34ˈ3,44°U latitudes), altitude : 3 m above sea level, Annual rainfall was 1383.6 mm with a maximum temperature : 33.25 °C and minimum temperature : 23.75 °C.

The experimental treatments consisted of two factors, (i). Donor plant spp. : 5 (*A. mangium, P. merkusii, T. grandis, T. catappa* and *J. curcas*.) and (ii). Methanolic extract concentrations (%) : 3 [(10, 20, 30 %) and two controls (negative control (distilled water), positive control (herbicide 2,4-D at 0.686 kg a.i. ha^{-1})]. The treatments were replicated thrice in a completely randomized design.

Preparation of plant extracts

The Leaf samples of the donor plants were collected from different locations (Table 1). We collected the samples of *A. spinosus* samples from Meunasah Gle, Sigli, Pidie and these were identified by Botanist in Herbarium, Unsyiah. The leaf samples of the donor plants were dried for 2-weeks at room temperature, grinded but not sieved. The methanol extract was prepared by soaking the required quantities of dry leaf material of each plant spp. in respective volume of methanol (Table 2) in a glass container for 24 h. The filtrate was then evaporated by a rotary evaporator (R-215, Buchi Corp.) at 40 °C to obtain concentrated extract.

Table 1. Donor plants leaf samples collection details done in May 2015 (Vegetative phase)

S. No.	Donor plant	Age (years)	Location	Altitude (m)
1	*A. mangium*	5	Pocut Meurah Intan Forest Park, Saree, Aceh Besar	700
2	*P. merkusii*	10	Pocut Meurah Intan Forest Park, Saree, Aceh Besar	700
3	*T. catappa*	10	Garden, Faculty of Agriculture, Unsyiah, Banda Aceh	3.0
4	*T. grandis*	10	Garden, Faculty of Agriculture, Unsyiah, Banda Aceh	3.0
5	*J. curcas*	4	Garden, Faculty of Agriculture, Unsyiah, Banda Aceh	3.0

Table 2. Quantity of leaf dry material and methanol used to prepare methanol extracts

	Plant spp.	Leaf Dry material used (kg)	Methanol used (L)
I	*A. mangium*	1.9	9.0
Ii	*P. merkusii*	1.8	8.5
iii	*T. grandis*	1.2	6.0
Iv	*T. catappa*	2.0	10
V	*J. curcas*	2.0	11

Pot culture: We collected soil upto 20 cm depth from Lampakuk Village, Aceh Besar. The soil was dried for seven days, sieved to remove the plant remains and placed into plastic pots (16 cm dia, 13 cm depth) 1.0 kg soil capacity. *A. spinosus* un-sterilized seeds were soaked in water for 2 h and 5-seeds were sown per pot at 2 cm depth on September 9, 2015. Seven days after sowing, thinning was done to keep one healthy plant per pot. After 21 days of sowing (30 of September 2015), the plants were foliar sprayed once with water or plant extracts (10 %, 20 % and 30 %) as per treatment, at 15 ml per pot. The pots were irrigated twice daily with 200 mL tap water.

At 21-days after foliar spray, we recorded *A. spinosus* weed growth parameters [plant height, leaf number, stem diameter, weed control (%), leaf area, shoot weight, root weight and root length)]. Plant height was measured from stem base to top of the plant. For number of leaves, all leaves were counted. Stem diameter (cm) was measured with vernier caliper at 3 cm above the stem base. The weed control (%) of *A. spinosus* was assessed using 0-100 rating system to determine the level of inhibition (Table 3). The leaf area was measured using Leaf Area Meter (Green Leaf Area Meter model GA-5; Tokyo Photo Electric CO, LTD). The Root length was measured (after washing with tap water). Dry weights of shoots and roots were recorded after oven drying at 60 °C for 48 h.

Table 3. The rating system used to assess weed control (14)

Rating (%)	**Effects**	**Detailed description**
0	No effect	No weedcontrol No crop reduction or injury
10	Slight effects	Very poor weed control Slight crop discoloration or stunting
20		Poor weed control Some crop discoloration, stunting, or stand loss Poor to deficient weed control
30		Crop injury more pronounced, but not lasting
40	Moderate effects	Deficient weed control Moderate injury, crop usually recovers
50		Deficient to moderate weed control Crop injury more lasting, recovery doubtful
60		Moderate weed control Lasting crop injury no recovery
70	Severe effects	Weed control somewhat less than satisfactory Heavy crop injury and stand loss
80		Satisfactory to good weed control Crop nearly destroyed-A few surviving plants
90		Very good to excellent weed control Only occasional live crop plants left
100	Complete effects	Complete weed destruction Complete crop destruction

Phytochemical testing

The phytochemical tests of alkaloids, steroids, terpenoids, saponins and flavonoids were conducted as under:

(i). Alkaloid test: 0.5 g leaves extracted from the donor plant were basified using ammonia, added to 1 mL of chloroform and crushed. The filtrate was added to 10 mL 5 % hydrochloric acid, shaken vigorously and kept until the hydrochloric acid and chloroform separated. The hydrochloric acid layer was taken and divided into three tubes and each was tested for the presence of alkaloids by adding Mayer's, Dragendorff's and Wagner's reagents. The presence of alkaloids was also tested using thin-layer chromatography, in which a small amount of concentrated ammonia was added to 0.5 g of plant extract. This was stirred until mixed, left for 1.0 h and the same amount of dichloromethane was added and left for 30 min until separation occurred. The

dichloromethane fraction was speckled on a chromatography plate and eluted using ethyl acetate and hexane (8:2) and placed into the chamber. After removing it from the chamber, it was placed into an acid cabinet and evenly sprayed with Dragendorff's reagent and then heated on hotplate until the colour changed to brown (17).

(ii). Steroids terpenoids and saponins test: 0.5 g leaves extracted from the source plant were finely crushed and extracted with hot methanol. The obtained filtrate was concentrated to obtain the methanol extract, which was then extracted again with dichloromethane. The dichloromethane phase was tested with the Libermann-Bourchard reagent. A blue or green color indicates the presence of steroids and red indicates terpenoids. The insoluble residue in the dichloromethane was shaken vigorously. The existence of stable foam after 30 min indicated the presence of saponin. This was then hydrolyzed with 4 mL of 2 N hydrochloric acid and filtered and tested with the Libermann-Bourchard reagent. A purple color indicates triterpene saponins, and a green or blue color indicates steroid saponin (17).

(iii). Flavonoid test: 0.5 g samples of leaves, were extracted with methanol and concentrated, which was then extracted again with n-hexane. The residue was extracted with 10 mL of 80 % ethanol and then added to 0.5 mg magnesium metal and 0.5 M HCl. A pink or purple color indicates the presence of a flavonoid (17).

(iv). Phenolics: The presence of phenolic compounds was tested by treatment with FCl_3 solution (17).

GC-MS analysis : GC-MS analysis were performed on Varian CP-3800, manual injection equipped with front injector type 1177. The column specification was VF-5ms 30Mx0,25MM. In this chemical analysis, methanolic extract of donor plants was used. The carrier gas was helium, with a flow rate of 0.9 mL/min. The oven temperature was held at 90℃ for 5 min then programmed at rate of 4℃/min to 250℃ and then held at this temperature for 8 min. Mass spectra were taken at 70 ev. Mass range was from m/z 46-650 amu. Injector port temperature: 280℃, detector: 280℃ and injected volume: 0.1 µL.

Statistical analysis of Data

All Data were analyzed by analysis of variance (F test) and Duncan's new multiple range test at 5 % probability level. The analyses were performed using the SPSS version 16 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

(i). *A. mangium* : The inhibitory and stimulatory influences of extracts on plant height are shown in Figure 1. *A. mangium* 30 % extract was inhibitory (6.0 %) to *A. spinosus* seedlings height. The phytochemical tests showed that *A. mangium* contained the alkaloids, terpenoids and phenolics (Table 4), which are suspected to cause the inhibition in *A. spinosus* weed growth. Further, GC-MS analysis also showed that it also contained the key compound lupeol. The two triterpens (α-amyrin; lupeol) and diterpene (phytol) compounds found in this plant inhibited the germination, shoot length and fresh weight of cowpea (*Vigna unguiculata*) (25). The other compound in *A. mangium* was 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Table 5) which interacts and damages the structure of phospholipid bilayer of cell membrane (7). The 30 % concentration of *A. mangium* extract

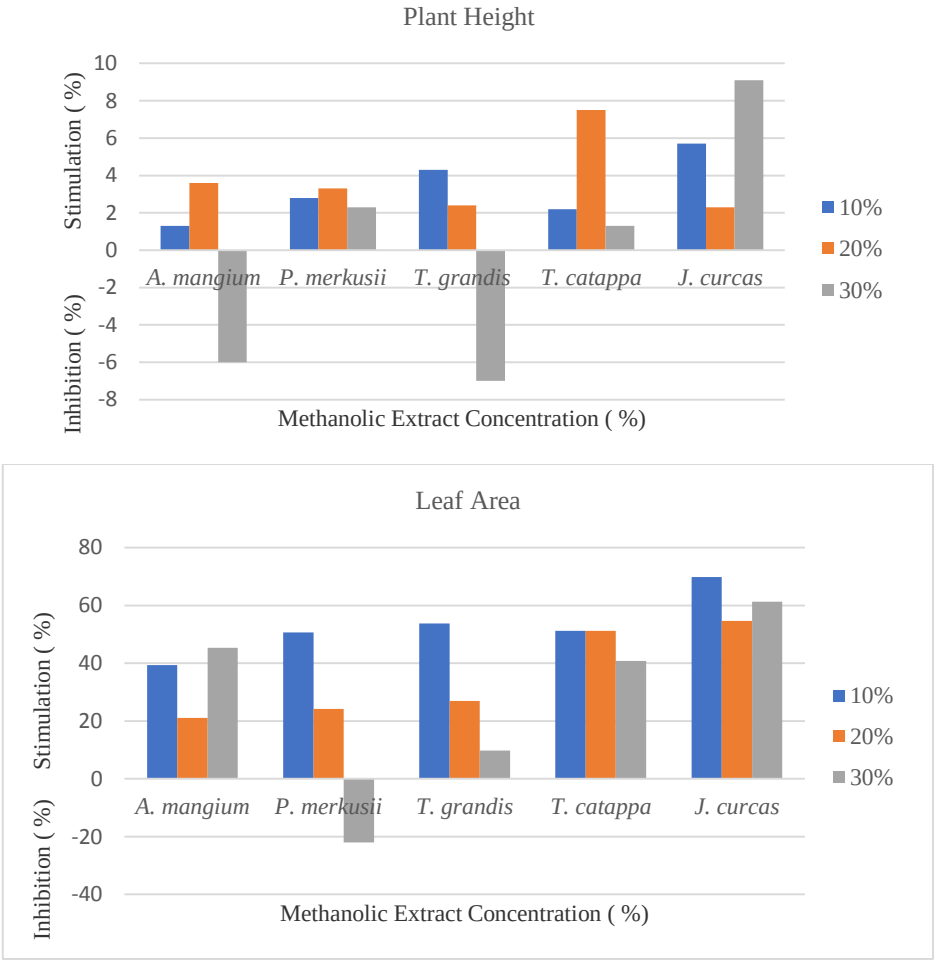

Figure 1. Effects of donor plant methanolic extracts on plant height and leaf area of *A. spinosus* at 21 days after foliar application.

Table 4. Phytochemical analyses of the 5- donor plants extracts

	Phytochemical Content					
Plant Species	**Alkaloid**	**Terpenoid**	**Saponin**	**Steroid**	**Flavonoid**	**Phenolic**
A. mangium	+	+	-	-	-	+
P. merkusii	-	+	-	+	-	+
T. grandis	-	+	-	-	+	+
T. catappa	-	-	-	+	-	+
J. curcas	-	+	-	+	-	+

+/- means the presence or absence of the group of compounds.

caused drastic decrease (58.3 %) in dry weight of *A. spinosus* shoots (Figure 2) and was similar to 30 % extract of *P. merkusii* (53 %). Allelochemical compounds at high concentrations inhibits the formation of nucleic acids, proteins and adenosine triphosphate (ATP) (50). If ATP is reduced, then cell metabolism will be also decreased (51). Neophytadiene, a diterpene terpenoid present in *Nepeta rtanjensis* and *Nepeta cataria*, inhibits the shoot growth of ragweed (*Ambrosia artemisiifolia*) (11).

Table 5. Main compounds identified in the methanol extracts of *A. mangium* leaves by GC-MS.

No.	Retention time (min)	Compound name	Compound content (%)	Similarity quality (%)*
1	19.467	phenol,2,4,6-tris(1-methylethyl)-	3.73	75.45
2	28.633	3,7,11,15-tetramethyl-2-hexadecen-1-ol	3.37	72.68
3	32.061	isopropyl palmitate	20.51 **I**	91.7
4	32.306	2-mercaptobenzothiazole	3.82	98.52
5	36.389	phytol	3.30	87.28
6	38.837	octadecanoic acid	12.63 **II**	92.33
7	51.908	alpha.-Amyrin	3.19	97.19
8	52.76	lupeol	11.15 **III**	95.58
9	56.571	squalene	8.94	86.85
10	72.881	androstan-17-one,3-[tributylsilyl)oxy]-,(3.beta.,5.alpha.)-	4.22	93.25

*: % similarity of the fragmentation MS of identified molecules as compared to the database in MS (mass spectroscopy).

(ii). *P. merkusii* : The 30 % extract of *P. merkusii* at 21 DAA significantly reduced the *A. spinosus* number of leaves (71.5 %), the stem diameter (39.6 %) and shoot dry weight (53.0 %) (Figure 2). The leaves of *P. merkusii* contains secondary metabolites (terpenoids, steroids and phenolic), which may have roles in leaf shedding. *P. merkusii* leaf extract application on *Amaranthus viridis* and *Echinochloa colonum* showed differences in the number of leaves in the seedlings (50). *P. merkusii* has pinene (terpenoid derivatives), it affects the plant's metabolism, which can cause functional disorders in cells. Pinene compounds upon entering the cell are oxidized immediately and affect the cell metabolism (34). Besides the pinene compounds, the other toxic compounds found in *P. merkusii* are tannins, which inhibit the hypocotyl growth and control the respiration in mitochondria. Tannin compounds deactivate the enzymes (amylase, proteinase, lipase and urease) and also inhibit the activity of gibberellin hormone (34). The decrease in leaf area also corresponds to the previous results, where *P. merkusii* leaf extract significantly inhibited the leaf width (49 %) of *Portulaca oleracea* (5); the litter of *P. merkusii* also decreases (69.5 %) the leaf area of turfgrass (37). The *Ageratum conyzoides* extracts reduce the chlorophyll content of mungbean plants by inhibiting the biosynthesis and enhancing its degradation (22). The *Senecio salignus* extract adversely affected the photosynthesis in *Physalis ixocarpa* and *Echinochloa crus-galli* due to 2-sesquiterpenes: β-caryophyllene and caryophyllene oxide (49).

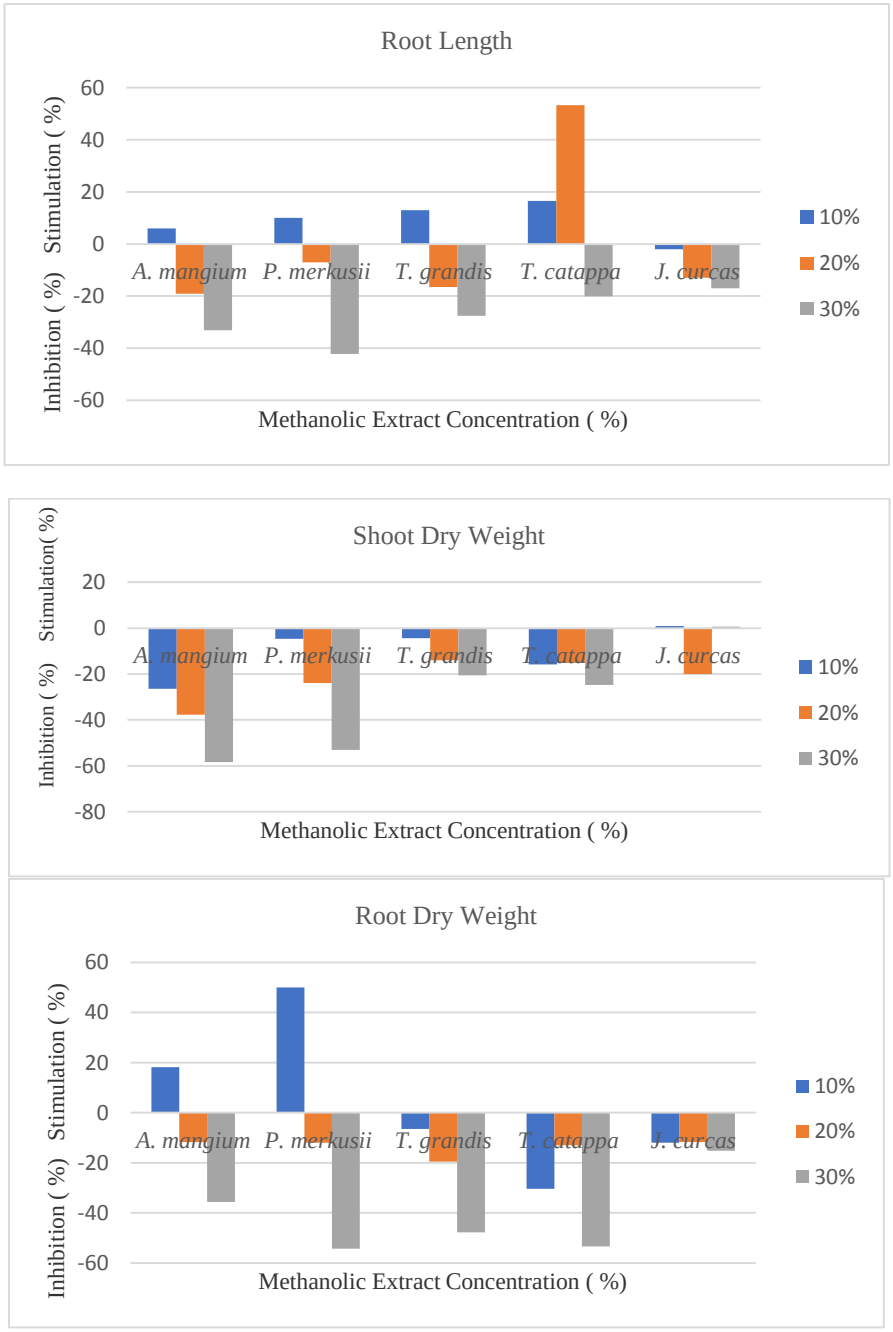

Figure 2. Effects of donor plant methanolic extracts on root length, shoot and root dry weight of *A. spinosus* at 21 days after foliar application.

The 30 % *P. merkusii* extract at 21 DAA provided greater weed control (64.20 %) than other treatments (Figure 3). Based on the field observations, the 30 % *P. merkusii* extract foliar spray after 21-days caused yellowing of all leaves but did not kill the weed. Besides the *A. spinosus* stem shrank in diameter and became pale. This agrees with results of Cahyanti *et al.* (5), in which *P. merkusii* leaf litter was toxic to pigweed (*Portulaca oleraceae*). *P. merkusii* produces secondary metabolites as terpenoids compounds (monoterpenes α-pinene and β-pinene) that are toxic for plants and animals (53).

(iii). *T. grandis*: The *T. grandis* 30 % extracts at 21 DAA significantly controlled 50.23 % *A. spinosus* weed (Figure 3). The 10 % and 20 % leaves extract of *T. grandis* were stimulatory to plant height (4.3 % and 2.4 %) (Figure 1) and 10 % leaves extract was stimulatory to root length (13 %) of *A. spinosus* weed (Figure 2), while the higher concentration of extracts were inhibited the growth of *A. spinosus*. Extracts at all concentration (10 %, 20 % and 30 %) reduced the number of leaves, stem diameter and seedling dry weight (Figure 2), but increased the leaf area of *A. spinosus* (Figure 1). Its leaf extract at 40 % - 50 % concentration suppressed the stems and roots growth in corn seedlings (28). Shrinking of stem diameter might be due to loss of water in *A. spinosus* weed stem. It is implied that allelopathic compounds in the extracts of *T. grandis* might have inhibited the absorption of water by the roots leading to decreased turgor pressure in *A. spinosus* stem. Based on the previous research, *T. grandis* allelopathic effects were due to the presence of phenolic and terpenoid compounds, which inhibits the photosynthesis and cell division in plants (30). GC-MS analysis detected two key compounds lupeol and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol in *T. grandis*, these could adversely affect the plant growth.

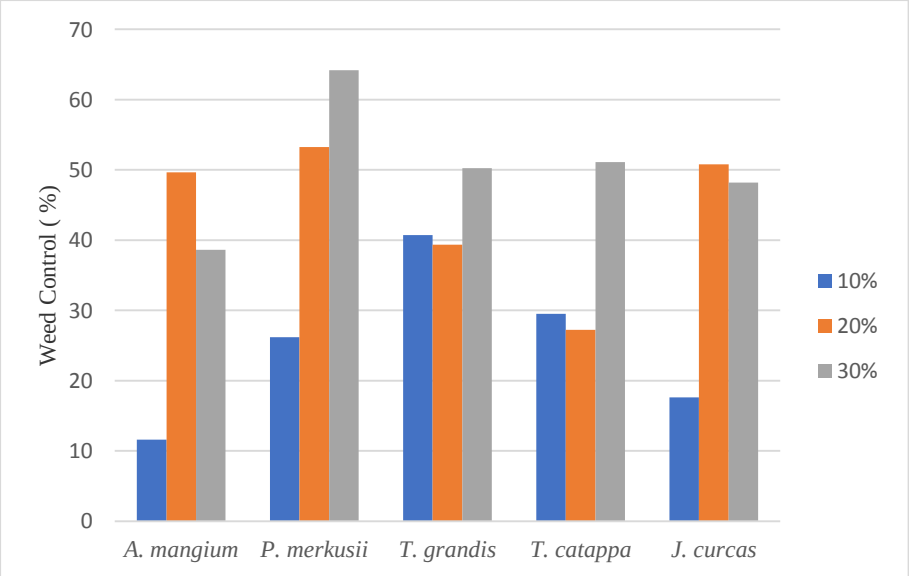

Figure 3. Effects of donor plant methanolic extracts on *A. spinosus* weed control at 21 days after foliar application.

(**iv). *T. catappa*:** The 30 % extract of *T. catappa* provided 51.13 % control of *A. spinosus* weed at 21 DAA (Figure 3). The application of *T. catappa* extract curled the *A. spinosus* leaves at the edges and eventually dried. The *T. catappa* leaf extract at 50 % concentration killed the nut grass by 72.50 % (50). *T. catappa* contained secondary metabolites, Steroid and phenolics (Table 5). Besides, its leaf extracts also have secondary metabolites, (alkaloids, flavonoids, tannins, terpenoids, resins and steroids) which causes the abnormal leaf colour, leaf baldness and death of nut grass (47). The GC-MS analysis (Table 8) showed that *T. catappa* also contained stigmasterol and lupeol compounds. The major compounds (squalene, stigmasterol, linoleic acid, palmitic acid, stearic acid, lupeol and phytol) in *Excoecaria agallocha* leaf leachates are inhibitory to seeds germination and seedlings growth of *Eleusine coracana* (finger millet) (10).

(v). *J. curcas*: Its extract (30 %) increased the plant height (9.1 %) at 21 DAA, but was not significant than negative control (distilled water) (Figure 1). It seems there are regulating substances in *J. curcas*, which increased the test weed height. Allelochemicals can act simultaneously on several processes, producing varied responses for each one, depending on the concentration of compound (45,46). However, this increase was not always directly proportional to the other parameters of growth.

The influence of the 5-donor plant species leaves extracts were variable, depending on how their substances influences the weed growth. The leaf extracts of *J. curcas* also increased the shoot growth of cauliflower (*Brassica oleracea* var. botrytis), depending on the crude concentration (36). The leaf extracts of the 5- donor plants decreased the growth but did not kill the *A. spinosus* weed. It is assumed that the concentration of extracts of these donor plants was not high enough to completely kill the weed, although their extracts inhibited the growth (metabolism, photosynthesis, respiration, etc.) of *A. spinosus*. The contents of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol compound in the 5 donor plant were: *A. mangium* (3.37 %), *P. merkusii* (7.32 %), *T. grandis* (3.80 %), *T. catappa* (3.26 %) and *J. curcas* (3.26 %) (Table 5-9). The other common compound was lupeol and its contents were : *A. mangium* (11.15 %), *P. merkusii* (4,19 %), *T. grandis* (4,96 %), *T. catappa* (25.84 %) and *J. curcas* (0.75 %). The contents of stigmasterol were *A. mangium* (0.40 %), *P. merkusii* (0.45 %), *T. grandis* (13.02 %), *T. catappa* (15.43 %) and *J. curcas* (24.10 %). These compounds could possibly interfere the growth of *A. spinosus* weed.

Phytochemical testing

The phytochemical tests showed that phenolics were present in all plant extracts and alkaloids were only in *A. mangium* (Figure 2). Likewise, Flavonoids were present only in *T. grandis. A. mangium, P.merkusii, T. catappa, and J.curcas* contain terpenoids except *T. catappa*. Steroids were present only in *P. merkusii, T. catappa* and *J. curcas*. *T. grandis* contained terpenoids, flavonoids and phenol. These metabolic compounds often act as allelopathic agents, e.g. phenolics and terpenoids in leaves of *A. mangium* and *T. grandis* (30), terpenoid derivative tannins and steroids in *P. merkusii* (36,37), steroids in *T. catappa* (47) and phenolics in all donor plants (23).

Chemical Analysis by GC-MS

The anlaysis by GC-MS of dry leaf methanol extracts, detected the relatively high concentrations of compounds in extracts of 5-donor plants (Tables 5-9).

Table 6. Main compounds identified in the methanol extracts of *P. merkusii* leaves by GC-MS.

No.	Retention time (min)	Compound name	Compound content (%)	Similarity quality (%)
1	28.647	3,7,11,15-tetramethyl-2-hexadecen-1-ol	7.32 **III**	78.52
2	32.129	isopropyl palmitate	33.45 **I**	92.26
3	37.614	9,12-octadecadienoic acid (Z,Z)-	4.88	71.14
4	37.891	isopropyl linoleate	11.89 **II**	57.18
5	38.817	octadecanoic acid	5.91	70.37
6	53.738	lupeol	4.19	72.17
7	84.414	d-friedoolean-14-en-3-one	3.68	93.1

Table 7. Main compounds identified in the methanol extracts of *T. grandis* leaves by GC-MS.

No.	Retention time (min)	Compound name	Compound content (%)	Similarity quality (%)
1	18.505	2,6-di-tert-butyl-4-(dimethylaminomethyl)phenol	3.22	96.90
2	19.488	2,4,6-Tris(1,1-dimethylethyl)-4-methylcyclohexa-2,5-dien-1-one	3.22	83.64
3	28.646	3,7,11,15-tetramethyl-2-hexadecen-1-ol	3.80	73.66
4	31.978	n-hexadecanoic acid	8.32 **III**	94.90
5	38.754	octadecanoic acid	4.66	92.17
6	43.154	stigmasterol,22,23-dihydro-	13.02 **II**	73.31
7	47.408	cholest-5-en-3-ol.24-propylidene-,(3.beta.)-	9.79	66.09
8	50.657	lup-20(29)-en-3-one	6.49	92.57
9	54.117	lupeol	4.96	82.50
10	54.935	d:b-friedo-b':a'-neogammacer-5-en-3-ol,(3.beta.)-	13.04 **I**	93.35
11	64.466	d:b-friedo-b':a'-neogammacer-5-en-3-ol,(3.beta.)-	4.12	73.75

Table 8. Main compounds identified in the methanol extracts of *T. cattapa* leaves by GC-MS.

No.	Retention time (min)	Compound name	(Compound content (%)	Similarity quality (%)
1	28.645	3,7,11,15-tetramethyl-2-hexadecen-1-ol	3.26	84.94
2	31.958	n-hexadecanoic acid 5	8.20	83.78
3	37.692	9,12,15-octadecatrienoic acid,2,3-dihydroxypropyl ester,(Z,Z,Z)-	3.25	72.35
4	43.022	stigmasterol,22,23-dihydro-	15.43 **II**	97.74
5	47.511	.beta.-amyrin	7.90	93.56
6	48.807	germanicol	5.67	93.17
7	53.175	.alpha.-amyrin	9.81 **III**	92.03
8	54.089	lupeol	25.84 **I**	84.92

(i). *A. mangium* : GC-MS analysis identified 10 key compounds in the methanol extract of *A. mangium* leaves. Primary metabolite compounds were: isopropyl palmitate (fatty acid) (20.51 %), octadecanoic acid (fatty acid) (12.63 %), and secondary metabolites consisted of lupeol (triterpenoid) (11.15 %), squalene (triterpenoid) (8.94 %), androstan-17-one,3-

[(tributylsilyl)oxy]-,(3.beta.,5.alpha.)-(steroid) (4.22 %),2-mercaptobenzothiazole(alkaloid) (3.82 %), phenol,2,4,6-tris (1-methylethyl)-(phenolic) (3.73 %), 3,7,11,15-tetramethyl-2-hexadecen-1-ol, (terpenoid) (3.37 %), phytol (diterpenoid) (3.30 %) and alpha-amyrin (triterpenoid) (3.19 %).

(ii). *P. merkusii* : Seven key compounds were identified in the methanol extracts of *P. merkusii* leaves. Primary metabolite compounds were: isopropyl palmitate (fatty acid) (33.45 %), isopropyl linoleate (fatty acid) (11.89 %), octadecanoic acid (fatty acid) (5.91 %) 9,12-octadecadienoic acid (Z,Z)- (fatty acid) (4.88 %) and the secondary metabolites consisting of 3,7,11,15-tetramethyl-2-hexadecen-1-ol (triterpenoid) (7.32 %), lupeol (triterpenoid) (4.19 %), d-friedoolean-14-en-3-one (triterpenoid) (3.68 %) .

(iii). *T. grandis* : Ten key compounds were identified in the methanol extracts of *T. grandis* leaves. Primary metabolite compounds were: n-hexadecanoic acid (fatty acid) (8.32 %), octadecanoic acid (fatty acid) (4.66 %) and secondary metabolites consist of d:b-Friedo-b':a'-neogammacer-5-en-3-ol, (3.beta.)-(triterpenoid) (13.04 %), stigmasterol,22,23-dihydro-(steroid) (13.02 %), cholest-5-en-3-ol,24-propylidene-, (3.beta.)-(steroid) (9.79 %), lup-20(29)-en-3-one (triterpenoid) (6.49 %), lupeol (triterpenoid) (4.96 %), 3,7,11,15-tetramethyl-2-hexadecen-1-ol, n-(triterpenoid) (3.80%),2,6-di-tert-butyl-4-(dimethylamino-methyl) phenol (alkaloid) (3.22 %) and 2,4,6-tris(1,1-dimethylethyl)-4-methyl- cyclohexa-2,5-dien-1-one (3.22 %).

(iv). *T. catappa* : Eight key compounds were identified in the methanol extracts of *T.catappa* leaves . Prymary metabolite compounds were: n- hexadecanoic acid (fatty acid) (8.20 %), 9,12,15-octadecanoicacid acid,2,3-dihydroxypropyl ester,(Z,Z,Z)- (fatty acid) (3.25 %). The secondary metabolites found in the methanol extract of *T. catappa* leaves were lupeol (triterpenoid) (25.84 %), stigmasterol,22,23-dihydro-(steroid) (15.43 %), alpha.-amyrin (triterpenoid) (9,81 %), beta.-amyrin (triterpenoid) (7.90 %), germanicol (triterpenoid) (5.67 %), and 3,7,11,15-tetramethyl-2-hexadecen-1-o(triterpenoid) (3.26 %).

Table 9. Main compounds identified in themethanol extracts of *J. curcas* leaves by GC-MS.

No.	Retention time (min)	Compound name	Compound content (%)	Similarity quality (%)
1	19.474	2,4,6-tris(1,1-dimethylethyl)-4-methylcyclohexa-2,5-dien-1-one	4.47	99.58
2	28.639	3,7,11,15-tetramethyl-2-hexadecen-1-ol	5.29	98.48
3	31.932	n-hexadecanoic acid	11.74 **III**	84.0
4	32.254	2-mercaptobenzothiazole	3.56	90.65
5	38.682	octadecanoic acid	3.49	72.13
6	43.085	stigmasterol,22,23-dihydro-	24.10 **I**	87.97
7	47.113	cholest-5-en-3-ol,24-propylidene-,(3.beta.)-	15.70 **II**	79.31

(v). *J.curcas* : Seven key compounds were identified in the methanol extract of *J.curcas* leaves. Primary metabolite compounds were: n-hexadecanoic acid (fatty acid) (11.74 %), octadecanoic acid (fatty acid) (3.49 %). The secondary metabolites in *J. curcas* leaves consisted of stigmasterol,22,23-dihydro-(steroid) (24.10 %), Cholest-5-en-3-ol,24-propyli - dene-,(3.beta.)-(steroid) (15.70 %), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (triterpenoid)

(5.29 %), 2,4,6-tris(1,1-dimethylethyl)-4-methylcyclohexa-2,5-dien-1-one (4.47 %) and 2-mercaptobenzothiazole (alkaloid) (3.56 %).

CONCLUSIONS

At 21-days after foliar application of extracts, all extracts at 30 % concentration, inhibited plant height, leaf number, stem diameter, leaf area, root length and shoot and root dry weight of *A. spinosus* plants. The Inhibition followed the order: *P. merkusii* (64.20 %) > *T. cattapa* (51.13 %) > *T. grandis (*50.23 %) > *J. curcas (*48.20 %) and *A. mangium* (38.63 %). The herbicide 2,4-D applied at 0.686 kg a.i. ha^{-1} caused 100% inhibition at 21 days after application. The GC-MS chemical analysis identified 7 to 11 secondary metabolites compounds in methanol extracts of each donor plants spp. However, the 3- major compounds in the leaf extracts of *A. mangium*, were: isopropyl palmitate (20.51 %), octadecanoic acid (12.63 %) and lupeol (11.15 %). In *P. merkusii* leaf extracts the compounds present were: isopropyl palmitate (33.45 %), isopropyl linoleate (11.89 %), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (7.32 %). In *T. grandis* leaf extracts the compounds present were: d:b-friedo-b':a'-neogammacer-5-en-3-ol,(3.beta.)-(13.04 %), stigmasterol,22, 23-dihydro-(13.02 %), n-hexadecanoic acid (8.32 %). In *T. catappa* leaf extracts the compounds present were: lupeol (25.84 %) and in *J. curcas* leaf extracts the compounds present were: stigmasterol,22,23-dihydro- (15.43 %), alpha-amyrin (9.81 %). stigmasterol,22,23-dihydro-(24.10 %), cholest-5-en-3-ol,24-propylidene-,(3.beta.)-(15.70 %) and n-hexadecanoic acid (11.74 %). The phytochemical test showed that *A. mangium* contained alkaloids and terpenoids, *P. merkusii* contained terpenoids and steroids, *T. grandis* contained terpenoids and flavonoids, *T. catappa* contained steroids and *J. curcas* contained terpenoids and steroids. Phenolics were present in all 5-plant extracts. The *P. merkusii* provided the greatest control of weed *A. spinosus* (64.2%) due to presence of isopropyl palmitate (33.45 %), isopropyl linoleate (11.89 %), and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (7.32 %).

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