

Chemistry and bio-efficacy of teak leaf for weed control in wheat

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ABSTRACT

Teak (*Tectona grandis*) leaf formulations (EC, SL and Powder) were prepared as botanical herbicides and evaluated in wheat field in comparison to Pendimethalin during December, 2012 to March, 2013. The highest reduction in weed population (56%) was obtained by methanol extract formulation (SL 20% @ 5 kg/ha, T₈) followed by pendimethalin @ 1.0 and 2.0 kg a.i./ha, T₁₅ and T₁₆ (46-51%) 14 days after treatment application. Significant reduction in weed population was recorded (45%) 21 days after treatment application by leaf powder formulation, T₁₄ while it was 38-60% for Pendimethalin. Defatted methanol extract @ 5 kg/ha (T₆) and powder (refluxed with methanol) @ 50 kg/ha (T₁₄) were the best treatments. Leaf powder without extraction was less efficient than extracted or reflux treatments with methanol. Among seven chromatographic fractions of methanol extract, F₄ exhibited the highest inhibition of weed (*Echinochloa colona* and *Echinochloa crus-galli*) seeds germination (GI = 87-89%) and detected 5-phenolic acids (Gallic acid, Tannic acid, Caffeic acid, Vanillic acid, Benzoic acid and Salicylic acid) by HPLC and confirmed by LC-MS-MS. The results indicated the combination of phenolic acids was responsible for activity.

Key Words: Allelopathy, botanical herbicide, formulation, HPLC, LC-MS/MS, phenolic acids, *Tectona grandis* leaf

INTRODUCTION

While pesticides help in effectively controlling the crop pests (including weeds), their collateral effects are often harmful to human health (1,27) and more generally to biodiversity (29) and/or on non-target organisms (2). Herbicides account for about 40% of the pesticide volume used worldwide. But, over 95% of herbicides reach a destination other than their target species, including non-target species, air, water, bottom sediments and food (22). Besides the health effects caused by herbicides, commercial herbicide mixtures containing other formulation ingredients may have negative impacts on human health (5). Although herbicides will continue to dominate weed-control practices, owing to easy weed control, the sustainability of herbicide use depends on their diversification and integration with non-chemical weed-control strategies (25). Successful breeding for disease and insect resistant cultivars in combination with development of integrated pest

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management systems have led to a reduction in the demand for fungicides and insecticides, while herbicide use is still increasing worldwide (6).

The impact of natural products on the development of new pesticides (including herbicides) may have future prospects for natural products-based pest management (9). Putnam and Duke (28) first explored the possibility of using the allelopathic crops to inhibit the weeds growth in agriculture. The use of allelopathic cover crops, allelopathic intercrops, the inclusion of allelopathic crops in rotation and the use of allelopathic plant residues as mulches are important ways for environment friendly weed management in agricultural systems (13). The phenolic acids are the major allelochemicals (4), which inhibits the germination of other plants (7,18,33). The most significant phytotoxic compounds isolated from the aqueous extracts of *Accacia confusa* were identified as phenolic acids (8). The weed *Parthenium hysterophorus* has toxic and phytotoxic constituents comprising of phenolics and terpenoids- two major chemical classes implicated in toxic and allelopathic interactions of the specie. Among the phenolic acids investigated for inhibitory activity of *Parthenium hysterophorus* on water hyacinth, *p*-hydroxybenzoic acid appeared to be of potential herbicidal activity at 100 ppm, a lethal level for the aquatic weed. Major sesquiterpene lactone parthenin is another allelochemical, which at 100 ppm killed water hyacinth the weed irrecoverably (26). Nine phenolic acids (caffeic acid, gentisic acid, catechol, gallic acid, syringic acid, ellagic acid, resorcinol, *p*-coumaric acid, and *p*-hydroxy benzoic acid) identified by HPLC analysis were mainly responsible for the allelopathic behavior of *C. procera* (12).

Several bio-active properties of teak plant (*Tectona grandis* L.) have been reported. Lapachol and 5- hydroxylapachol isolated from the root heart wood of *Tectona grandis* exhibited the cytotoxic properties (15). Methanol extracts of teak bark effectively controls the antibiotic-resistant pathogens, due to the presence of 5-hydroxy-1,4-naphthalenedione, Juglone (24). The antifungal activity of methanolic crude extract of *Tectona grandis*, *Valeriana wallachi* was investigated against *Alternaria cajani*, *Curvularia lunata*, *Fusarium sp.*, *Bipolaris sp.* and *Helminthosporium sp.* at 1000, 2000, 3000, 4000 and 5000 µg/ml concentrations. HPLC analysis of the crude extract of plants showed four phenolic acids (Tannic acid, gallic acid, ferulic acid and caffeic acid). The increase in the production of phenolics in the extract was correlated with the induction of resistance in treated plants against phytopathogenic fungi (30). Some phenolic acids in teak are inhibitory or stimulatory bioactive allelochemicals (16,17,20,23,31). The dry weight of *Vigna mungo* and *Vigna radiata* was increased by 5% aqueous extract of *T. grandis* dry leaves which gradually decreased at higher concentrations (10, 25, 50 and 75%) and the seedlings dry weight was very low at 100% concentration than control (21).

This study aimed to evaluate the herbicidal bio-efficacy of the formulations of teak leaf and its extracts in wheat field; identification and quantification of phenolic acids in teak leaf extract using HPLC and LC-MS/MS and to establish the role of phenolic acids as potential herbicidal chemicals.

MATERIALS AND METHODS

Plant materials

Deciduous leaves of teak (*Tectona grandis*) were collected from the C- Block (Incheck) our University Farm. The leaves were air dried and powdered using a grinder. Seeds of rice (*Oryza sativa*, cv. Satabdi) and greengram (*Vigna radiata*, cv. B-9) and weed species of *Echinochloa* viz. *E. colona* and *E. crus-galli* common in rice fields were collected from our Research Farm. All seeds were sundried and stored in desiccator having CaCl_2 as hygroscopic agent.

Extraction of teak leaves

The powdered teak leaves (2.0 kg) were extracted in Soxhlet apparatus (2 L) in three batches using methanol for 6 h. The solvent extract was evaporated to dryness at 45°C using Rotavapor (R3, Buchi). The residual MeOH extract (200 g) was used for bio-efficacy trial and also for purification through chromatographic separation and for the estimation of phenolic acids using HPLC and LC-MS-MS.

Formulation and Bio-efficacy of teak leaf and its extract

Various formulations of teak leaf and its extract were prepared to understand the pre-emergent weed control efficiency in wheat field. Emulsifiable Concentrate (EC) of leaf extracts in methanol (T_3 - T_4) and in defatted methanol (extraction in hexane followed by methanol, T_5 - T_6) were prepared using surfactants - (a) Na-Alkaline Sulfonate and (b) K-Alkaline Sulfonate in ratio of 3:2 followed by stirring in a homogenizer (IKA, Japan) at 20000 rpm. Soluble Liquid (SL) formulation of leaf extracts in methanol (T_7 - T_8) and in defatted methanol (extraction in hexane followed by methanol, T_9 - T_{10}) were prepared using methanol. The Powder formulation (P) of teak leaf without solvent extraction (T_{11} - T_{12}) and teak leaf after reflux with methanol (T_{13} - T_{14}) was prepared using rice straw dust as the filler.

The bio-efficacy study of the herbicidal treatments (T_3 - T_{14}) was conducted in field study on Wheat (cv. HD 2733) crop in the District Seed Farm, BCKV, Kalyani, West Bengal during December, 2012 to March, 2013 taking Pendimethalin (Stomp 35 EC) as the standard commercial herbicide (@ 1.0 kg a.i./ha (T_{15}) and 2.0 kg a.i./ha (T_{16}) for comparison. Wheat was sown in rows 20 cm apart @ 100 kg seed/ha. Hand weeding (T_1) and untreated control (T_2) plots were also maintained with three replicated plots ($3.6\text{ m} \times 2\text{ m} = 7.2\text{ m}^2$) for each treatment following Randomized Block Design. The treatment details are given in Table 1.

The liquid formulations (T_3 - T_{10}) and Pendimethalin (T_{15} - T_{16}) were sprayed using 500 L water/ha on 3rd day after sowing i.e. pre-emergence. Therefore, 1.08 L of spray solution containing 5.4 g (single dose) and 10.8 g (double dose) of extract for the liquid (EC and SL) formulations to cover three replicated plots ($7.2\text{ m}^2 \times 3 = 21.6\text{ m}^2$). The powder formulations (100 g, T_{11} - T_{14}) were uniformly applied to cover the plots of 7.2 m^2 each ($3.6\text{ m} \times 2\text{ m}$). Observations on weed population (No./ m^2) were recorded in each

replicated plot on 7, 14, 21 and 30 day after treatment application. Dry matter of weeds (Wt./m²) was recorded on 30 day after treatment application.

Purification of methanol (MeOH) extract

The plant extract was fractionated by Flash Chromatography System (Sepacore, Buchi). The concentrated methanol extract (5 g) dissolved in 20 ml methanol was loaded to the pre-column using syringe (15 ml). The column was then eluted with solvents using hexane, chloroform and methanol at the flow rate of 15 ml/min. All the fractions were concentrated using Rotavapor (R3, Buchi). The concentrated fractions were monitored by Thin Layer Chromatography. Based on TLC behaviour seven fractions (F₁-F₇) were prepared. Solutions of these fractions and the crude methanol extract were subjected to further pre-emergent herbicidal bioassay and to determine the phenolic acid content.

Pre-emergence herbicidal bioassay

Standard solutions (1000 µg/ml) of phenolic acids [viz. Benzoic acid (BA); Salicylic acid (SA); Tannic acid (TA); Caffeic acid (CA); Gallic acid (GA) and Vanillic acid (VA) purchased from ChromaDex, USA] were prepared individually in MeOH and each was diluted into 50 and 100 µg/ml. In addition, a mixture of the phenolic standards was also prepared by taking 1 ml of each phenolic acid standard solution (1000 µg/ml) in a graduated tube and volume was made upto 10 ml, to make 100 µg/ml, which was then further diluted into 10, 25 and 50 µg/ml.

The leaf extract, fractions and the phenolic acids were tested for germination of rice and two weed seeds. Germination test was done in petri plates (10). Two Filter papers (Whatman No.1) were placed into each petriplate (9 cm dia) . Ten seeds of rice and 30 seeds of weed were evenly sown per petriplate. One ml extracts (0.1% w/v) and solution of phenolic acids (50 µg/ml and 100 µg/ml) were added to each petriplate. Control sets received only methanol. The petriplates were incubated in growth chamber [28± 2°C and relative humidity 80% in artificial light]. To keep filter papers moist, 0.5-2.5 ml of distilled water was added after every 24 h, treatment were replicated thrice in Randomized Block Design. Observations on the number of seeds germinated were recorded at 3, 5, 7, 10 and 15 days. The maximum numbers of seeds were germinated 10 days after sowing/application.

The germination data was converted to Germination Inhibition, GI (%) as under (11).

$$\text{Germination Inhibition, GI (\%)} = \frac{X-Y}{X} \times 100$$

Where, X = Maximum number of seeds germinated in control, Y = Maximum number of seed germinated in treated.

Estimation of phenolic acids by HPLC & LC-MS/MS

The analytical method for identification and estimation of the phenolic acids was standardized by HPLC (Shimadzu LC-10AT VP) equipped with Shimadzu SPD-M10A VP Diode Array detector ($\lambda_{\text{max}} = 280 \text{ nm}$). The mobile phase used was acetonitrile:water (1:3) containing 0.1% Orthophosphoric acid with flow rate @ 1 ml/min. Phenolic acids present in each sample were identified by comparing chromatographic peaks with the retention time (R_t) of individual standards. The HPLC chromatogram of a mixed standard solution containing 10 $\mu\text{g/ml}$ of each phenolic acid is presented in **Figure 1** showing the R_t values in the range of 3.6-14.3 minutes. Phenolic acids were also analysed by reversed-phase HPLC with an isocratic elution in less than 25 minutes (32).

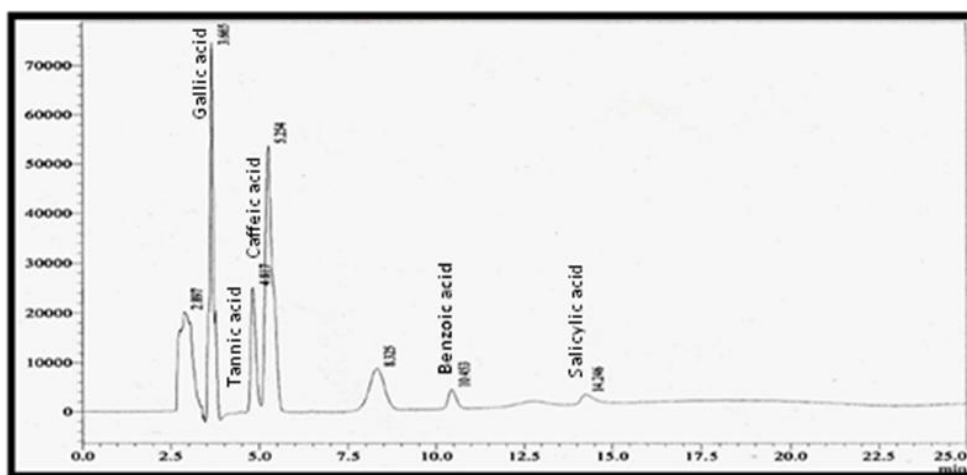


Figure 1. HPLC Chromatogram of mixture of standard phenolic acids

The concentration of the identified phenolic acids was quantified by comparing the area of respective HPLC peaks of the samples and confirmed by LC-MS (Waters 2695) equipped with Xterra MS C18 column (3.5 μ , 2.1 \times 50 mm), ESI interface and Micromass Quattro MicroTM detector. The mobile phase used was methanol:water (9:1) adding 0.1% acetic acid and 5 mM ammonium acetate with flow rate @ 0.3 ml/min. Vanillic acid could not be analysed in HPLC due to poor resolution with caffeic acid. However, the presence of Vanillic acid was confirmed by LC-MS/MS according to their mass, but tannic acid was not detected. Barros *et al.* (3) also analyzed phenolic compounds by high performance liquid chromatography coupled to photodiode array detector and mass spectrometer (HPLC-DAD-ESI/MS).

Statistical Analysis

The laboratory data were subjected to Multiple Regression Analysis followed by Principal Component Analysis to establish the relationship of phenolic acid content in teak leaf extract and their inhibitory effect on seed germination.

RESULTS AND DISCUSSION

Field study

The bio-efficacy of teak leaf formulations as pre-emergent herbicide was evaluated in terms of reduction in weed population and dry matter content (Table 1). The dominant weed species in the experimental fields were *Cynodon dactylon*, *Echinochloa colona*, *Cyperus rotundus*, *Cyperus difformis* (monocots) and *Amaranthus viridis*, *Chenopodium album*, *Melilotus alba* (dicots). However, there was no adverse impact on wheat establishment and growth in the herbicide (chemical and botanical) treated plots.

Table 1. Effects of teak leaf extracts and herbicides on weed population and weed biomass in wheat field

Treatments	Weeds population (N) and Reduction % (R) over untreated control (T ₂)								Weeds biomass (DW) and Reduction (%)	
	7 DAA		14 DAA		21 DAA		30 DAA		30 DAA	
	N	R	N	R	N	R	N	R	DW	R
T ₁ Hand Weeding	5.0	16.7	8.6	36.8	13.0	16.7	5.3	65.4	0.3	75
T ₂ Untreated Control	6.0	-	13.6	-	15.6	-	15.3	-	1.2	-
T ₃ Methanol Extract EC (20% a.i) 2.5*	4.3	28.3	8.6	36.8	13.3	14.7	13.6	11.1	1.0	16.7
T ₄ Methanol Extract EC (20% a.i) 5.0*	7.0	-17	9.6	29.4	13.0	16.7	14.3	6.5	1.1	8.3
T ₅ Defatted methanol extract EC (20%) 2.5*	4.6	23.3	9.3	31.7	13.6	12.8	13.6	11.1	1.0	16.7
T ₆ Defatted methanol extract EC (20%) 5.0*	4.3	28.3	8.3	39	11.6	25.6	12.6	17.6	0.9	25
T ₇ Methanol Extract SL (20%) 2.5*	6.6	-11	8.6	36.8	12.3	21.2	13.0	15.0	1.0	16.7
T ₈ Methanol Extract SL (20%) 5.0*	3.0	50	6.0	55.9	11.6	25.6	13.6	11.1	1.0	16.7
T ₉ Defatted methanol extract SL (20%) 2.5*	5.0	16.7	9.6	29.4	11.0	29.5	12.6	17.6	0.9	25
T ₁₀ Defatted methanol extract SL (20%) 5.0*	5.3	11.7	8.0	41.2	12.6	19.2	15.0	2.0	1.1	8.3
T ₁₁ Leaf (without extraction) P (36%) 25*	8.0	-33	12.3	9.6	13.3	14.7	15.6	-0.02	1.2	0
T ₁₂ Leaf (without extraction) P (36%) 50*	6.6	-11	8.6	36.8	12.3	21.2	15.3	0	1.2	0
T ₁₃ Leaf (after reflux with MeOH) P (36%) 25*	5.3	11.7	11.3	16.9	12.6	19.2	15.0	2.0	1.1	8.3
T ₁₄ Leaf (after reflux with MeOH) P (36%) 50*	5.0	16.7	12.6	7.35	8.6	44.9	12.0	21.6	0.9	25
T ₁₅ Pendimethalin (Stomp) EC (35%) 1.0*	5.3	11.7	7.3	46.3	9.6	38.5	11.3	26.1	0.8	33.3
T ₁₆ Pendimethalin (Stomp) EC (35%) 2.0*	4.0	33.3	6.6	51.5	6.3	59.6	7.6	50	0.5	58.3
LSD (5%)	0.54	-	0.70	-	0.69	-	0.73	-	0.28	-

*Dose : kg a.i./ha (a.i. = leaf extract/powder/pendimethalin); EC : Emulsifiable Concentrate; SL : Soluble Liquid; P : Powder; DAA: Day after treatments application, N : Number of weeds/m²; R : Reduction (%) of weeds population/Dry matter; – (Minus) values indicate increase in population/dry matter

The Pendimethalin and other treatments reduced the weed population (2-60%) during 30 days of observation. The maximum reduction was observed between 14-21 days. The teak leaf formulations, T₈ (SL 20% of Methanol Extract) gave highest reduction in weed population (56%) which was higher than pendimethalin, T₁₅, T₁₆ (46-51%) at 14 days after treatment application. After 21 days, Pendimethalin exhibited 38-60% weeds reduction, whereas T₁₄ caused 45% reduction followed by T₆, T₈ and T₉ (26-30% reduction).

At 30 days after application, pendimethalin (T₁₅ and T₁₆) significantly reduced (35-58%) the weed biomass (dry matter), while it was 25% in T₆, T₉ and T₁₄ (Table 1). For better visual comparison, the inhibitory effects of teak leaf extracts and herbicides on weeds population and weeds biomass at 30 days after application are presented in Figure 2. The leaf powder without MeOH extraction (T₁₁ & T₁₂) did not suppress the weeds population and weeds biomass. Among the extracted treatments (T₃-T₁₀) and the reflux treatments (T₁₃ & T₁₄) with methanol, T₁₄ exhibited comparable activity with the recommended dose of pendimethalin (T₁₅) followed by T₆ and T₉. Considering the sustained weed inhibitory activity (Table 1 and Figure 2). It may be inferred that the double doses of defatted methanol extract (T₆ @ 5 kg/ha) and leaf powder after reflux with methanol (T₁₄ @ 50 kg/ha) are the best treatments. The teak leaf methanol extract also controlled the weeds in transplanted rice field with standing water (14), which was comparable with the standard herbicide Pretilachlor.

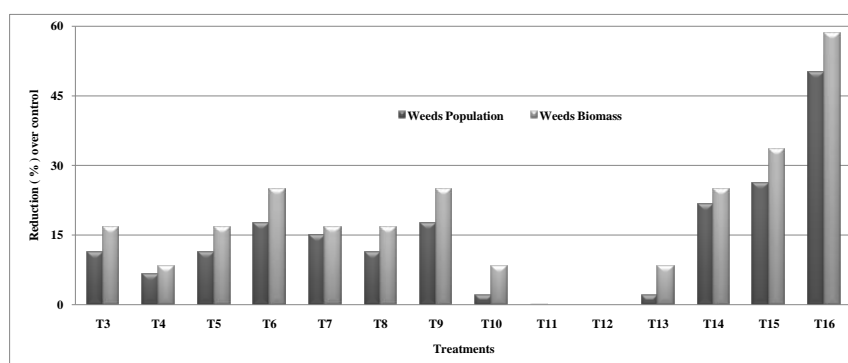


Figure 2. Inhibitory effects of teak leaf extracts and herbicides on weeds population and weeds biomass at 30 days after application

[T₃ : Methanol Extract EC (20% a.i) 2.5*; T₄ : Methanol Extract EC (20% a.i) 5.0*; T₅ : Defatted methanol extract EC (20%) 2.5*; T₆ : Defatted methanol extract EC (20%) 5.0*; T₇ : Methanol Extract SL (20%) 2.5*; T₈ : Methanol Extract SL (20%) 5.0*; T₉ : Defatted methanol extract SL (20%) 2.5*; T₁₀ : Defatted methanol extract SL (20%) 5.0*; T₁₁ : Leaf (without extraction) P (36%) 25*; T₁₂ : Leaf (without extraction) P (36%) 50*; T₁₃ : Leaf (after reflux with MeOH) P (36%) 25*; T₁₄ : Leaf (after reflux with MeOH) P (36%) 50*; T₁₅ : Pendimethalin (Stomp) EC (35%) 1.0*; T₁₆ : Pendimethalin (Stomp) EC (35%) 2.0*; *Dose: kg a.i./ha (a.i.=leaf extract/powder/pendimethalin); EC: Emulsifiable Concentrate; SL: Soluble Liquid; P: Powder]

Laboratory study

The results of pre-emergence herbicidal activity of the selected phenolic acids, combination of phenolic acids and teak leaf methanol (T_{MeOH}) extracts and its fractions on greengram, rice, *E. colona* and *E. crus-galli* are given in Table 2. The mean value of germination inhibition (GI) by six phenolic acids at 50 µg/ml (T₁) was 9.4% on *E. colona* and 6.3% on *E. crus-galli* seeds. At 100 µg/ml (T₂) the mean values of GI increased to 21.8% and 18.7% respectively for *E. colona* and *E. crus-galli*. Among the standard phenolic acids, the maximum inhibitory activity was exhibited by Benzoic acid on *E. colona* (44.8%) and on *E. crus-galli* (34.5%) at 100 µg/ml. The mixture of phenolic acids (@ 25 µg/ml), however exhibited significant effect on weed germination (GI = 60-65%) comparable with the T_{MeOH} extract (GI = 57-62%). The activity was increased with increase in concentration of the phenolic acids mixture. None of the compound or their combinations nor the teak leaf extract exhibited significant effect on green gram and rice germination even at higher concentrations (Table 2).

Table 2. The activity of the standard phenolic acids and teak leaf extract on test weeds

Treatments	Germination Inhibition, GI (%)*			
	Green Gram	Rice	<i>E. colona</i>	<i>E. crus-galli</i>
Phenolic acids (50 µg/ml, T ₁)				
Gallic acid (GAT ₁)	0	0	0	0
Tannic acid (TAT ₁)	0	0	14.28	6.67
Caffeic acid (CAT ₁)	0	0	7.40	3.48
Vanillic acid (VAT ₁)	0	0	6.90	3.44
Benzoic acid (BAT ₁)	0	0	14.29	13.33
Salicylic acid (SAT ₁)	0	0	13.79	11.11
Mean	0	0	9.44	6.34
Phenolic acids (100 µg/ml, T ₂)				
Gallic acid (GAT ₂)	0	0	10.34	6.89
Tannic acid (TAT ₂)	0	0	17.24	16.67
Caffeic acid (CAT ₂)	0	0	13.79	16.67
Vanillic acid (VAT ₂)	0	0	13.33	14.28
Benzoic acid (BAT ₂)	0	0	44.82	34.48
Salicylic acid (SAT ₂)	0	0	31.03	23.33
Mean	0	0	21.76	18.72
Mixture of phenolic acids				
M ₁ (10 µg/ml)	0	0	59.26	51.85
M ₂ (25 µg/ml)	0	0	65.38	60.74
M ₃ (50 µg/ml)	0	0	78.57	75
M ₄ (100 µg/ml)	0	0	92.86	89.29
T _{MeOH} Extract	0	0	62.07	57.14
Mean	0	0	71.63	66.80

*10 days after application

The germination inhibition (GI) by the flash chromatographic fractions of T_{MeOH} extract was increased from 62.1% to 65.4-88.9% in F₂ - F₅ fractions on *E. colona* seeds and from 57.1% to 63.0-87.5% on *E. crus-galli* seeds. The maximum activity was observed in F₄ (87.5-88.9%) and the increase in activity in different fractions followed (Table 3) the order of F₄> F₅> F₃> F₂> T_{MeOH}. The activity in the polar fractions (using methanol) decreased to 48.1-61.5 % in the in order of F₇< F₆< T_{MeOH}. However, F₄ and F₅ exhibited inhibitory effect on germination of rice by 27.6% and 13.3% respectively (Table 3). There was no inhibitory effect on germination of green gram seeds by teak leaf extract or its fractions.

Table 3. The activity of the fractions of T_{MeOH} extract of teak leaf on test plants

Fractions of T _{MeOH}	Germination Inhibition, GI (%) 10 days after application			
	Green gram	Rice	<i>E. colona</i>	<i>E. crus-galli</i>
F ₂	0	0	65.38	62.96
F ₃	0	0	73.33	75.86
F ₄	0	27.59	88.89	87.5
F ₅	0	13.33	81.48	75
F ₆	0	0	61.53	53.57
F ₇	0	0	53.85	48.15

Phenolic acids

The phenolic acids are the major allelochemicals (4), which inhibits the germination of plants (7). Teak leaf extracts also inhibited the germination and seedling growth of weed species (viz. *Echinochloa colona*, *Echinochloa crus-galli*, soyabean seeds/seedlings, etc.) due to the phenolic acids as inhibitory or stimulatory bioactive allelochemicals (16,20,31). In the present investigation, six phenolic acids were identified in teak methanol extract and its fractions by HPLC (Table 4). Gallic acid, Tannic acid and Caffeic acid were detected in all samples in range of 0.20-10.78; 0.06-6.82 and 0.02-0.76 µg/ml, respectively. The highest concentration of these acids was found in F₆. Salicylic acid was detected in only one fraction F₄ (12.99 µg/ml). Benzoic acid was present in two fractions F₃ (0.31 µg/ml) and F₅ (10.41 µg/ml). The occurrence of salicylic acid and benzoic acid (in combination with other phenolic acids) might be responsible for the increased activity in F₄ and F₅ fractions (Table 3 and 4).

Table 4. Phenolic acids profile of teak leaf extract

Treatments	Phenolic acid content (µg/ml)					
	Gallic	Tannic	Caffeic	Vanillic	Benzoic	Salicylic
T _{MeOH}	0.31	0.61	0.03	0	0	0
F ₂	0.20	0.13	0.06	0	0	0
F ₃	0.23	0.36	0.17	0	0.31	0
F ₄	0.21	2.51	0.67	4.32	0	12.99
F ₅	2.50	1.73	0.49	0	10.41	0
F ₆	10.78	6.82	0.76	0	0	0
F ₇	2.39	0.06	0.02	0	0	0

The presence of phenolic acids in teak leaf extract was further confirmed by their appearance of molecular ion peaks and their fragment ions produced by Multiple Reaction Monitoring (MRM) using LC-MS/MS. The retention time, molecular ions, and their fragment ions (including quantifier and qualifier ions) are presented in Table 5. The molecular ions at m/z 168.86, 178.87, 166.80, 120.74 and 137.13 were obtained for Gallic acid, Caffeic acid, Vanillic acid, Benzoic acid and Salicylic acid respectively with corresponding quantifier ions at m/z 124.8, 122.8, 123.0, 120.74 and 93.01 and qualifier ions at m/z 78.5, 76.5, 107.9, 91.61 and 64.53. However, Tannic acid could not be analysed under the operating conditions of LC-MS/MS.

Table 5. LC-MS characteristics of the phenolic acids detected in teak leaf extract

Phenolic acid	Retention Time (min)		MS Parameters		
	HPLC	LC-MS	MRM (m/z)	Collision Energy (eV)	Cone Voltage (kV)
Gallic Acid	3.66	0.56	168.86→52.4 168.86→78.5 168.86→124.8	21.0 23.0 15.0	34.0
Tannic Acid	4.82	-	-	-	-
Caffeic Acid	5.24	0.50	178.87→76.5 178.87→122.8 178.87→132.9	33.0 21.0 19.0	38.0
Vanillic Acid	5.58	0.49	166.80→90.8 166.80→107.9 166.80→123.0	19.0 17.0 13.0	30.0
Benzoic Acid	10.45	0.77	120.74→53.2 120.74→76.6 120.74→91.6	17.0 11.0 21.0	28.0
Salicylic Acid	14.25	0.53	137.13→64.5 137.13→75.1 137.13→93.0	40.0 38.0 20.0	27.0

Total phenolic acid and flavonoid content have been reported as the medicinal principle in the leaves of *Tectona grandis* (23), while four phenolic acids (Tannic acid, Gallic acid, Ferulic acid and Caffeic acid) present in the crude extract of teak and some medicinal plants were correlated with plants resistance against phytopathogenic fungi (30).

This study revealed the field bio-efficacy of methanol extract or reflux of teak leaves on weed control in wheat cultivation. Our previous study explored the possibility of using these teak leaf extract in rice field for control of mainly the monocot weed species, viz. *Cynodon*, *Echinochloa*, *Digitaria*, *Eleusine*, *Cyperus*, etc. (14). The laboratory studies confirmed the occurrence of some phenolic acids in the extract (Table 4) which was also reported in previous studies (16). The inhibitory activity of the phenolic acids was also evaluated on germination of weed seeds (Table 2). The purified fraction with maximum activity (F_4) exhibited the highest concentration level of 12.9 $\mu\text{g/ml}$ (Table 3). But, none of the phenolic acids could produce a comparable activity to F_4 even at 100 $\mu\text{g/ml}$ concentration for the individual acid and at 10 $\mu\text{g/ml}$ for 6-phenolic acids mixture (Table 2

and 3). Phenolic acids are responsible for allelopathic activity by involvement in various mechanisms. The allelopathic activity of wheat has been attributed to phenolic acids, hydroxamic acids and the related compounds (18), the salicylic acid (SA) inhibits the seed germination in maize and barley (33). However in most cases, phenolic compounds appear as mixture and not as single substance. Hence, the contribution made to allelopathy by phenolic compounds was probably never due to a single substance (19).

The statistical analysis of the concentration of phenolic acids and their activity under the present investigation also indicated the role of combination of phenolic acids responsible for the activity rather than the individual one. Moreover, involvement of the phenolic acids could not explain the total observed activity of the extract. Therefore, the role of other phenolic compounds or those belonging to other allelochemicals need to be explored for understanding the observed biological activity of the teak leaf extract.

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