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European Journal of Experimental Biology, 2012, 2 (3):513-519

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Insecticidal Properties of Certain Flora based on Ethnobotanical Records Against Teak defoliator, *H. puera Cramer* (*Lepidoptera: Hybaeidae*)

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ABSTRACT

Plants and their mobile/immobile chemical constituents play an important role in the development of biopesticides. The present study envisaged to assess the effect of plant extracts against the teak larvae, Hyblaea puera (Lepidoptera:Hyblaeidae) which is considered as major pest that strongly influences the development of teak tree. Eight plant species have been selected based on ethnobotanical records for the study. Different organic solvents such as acetone, methanol and ethyl acetate were used for extraction purposes. Higher extract yield and total phenolic content were obtained using organic solvent methanol as compared to other organic solvents. Individual phenolic profiles were estimated from all extracts in which most of the compounds were found responsible for biopesticidal efficacy. The extractive efficiency of individual phenolic compounds were higher in ethyl acetate and methanol extract when compared with acetone extract. Among the eight plant species employed for bioassay study Melia dubia, Briedelia scandens, Adhatoda vasica, Vitex negundo, Strychnos nuxvomica exhibit 100 percent mortality and other plants showed 80 percent mortality at 1000 ppm concentration. The highest insecticidal activity influenced by the presence of phenolic compounds in plant extracts is also discussed in this article.

Key words: Ethnobotanical records, *Hyblaea puera*, plant extracts, phenolics.

INTRODUCTION

In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show the immense potential of plants used in traditional systems [1, 2]. Plants have an almost limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12000 have been isolated, a number estimated to be less than 10 percent of the total. Insecticides of plant origin have been exploited from time immemorial for the management of insect pests of crop plants [3, 4]. Synthetic insecticides have been used excessively with negative consequences such as toxicity towards farmers, consumers, and wild animals, interruption of natural control and pollination, water pollution, and the evolution of resistance pests have acquired to these products [5]. Botanical insecticides have been used in agriculture for at least two thousand years in Asia and the Middle East [6]. With the introduction of integrated pest management concept, botanicals again acquired importance [7]. Bioactive secondary compounds from plants show insecticidal, antifeedant, defence barriers, growth regulating and development modifying properties [8]. Biopesticides produced from plants have been recently attracting the attention of natural product researchers to find the alternate of synthetic compounds and interested in their chemical constituents and biological properties [9].

Therefore researchers all over the world are engaged in a mission to hunt for novel phytochemicals that could potentially be used in the management of insect pests. The present study deals with the bioefficacy of crude extracts against important forest insect pest of Teak defoliator, *Hyblaea puera* larvae mortality.

MATERIALS AND METHODS

Collection of Plant materials

Eight plant species viz., *Adhatoda vasica Nees*, *Aristolochia bracteata* Retz, *Briedelia scandens* Roxb, *Murraya koenigii* L, *Melia dubia* Cav, *Pongamia pinnata* (L) pierre, *Strychnos nuxvomica* L, *Vitex negundo* L were collected based on ethnobotanical survey. The above said species were collected from Coimbatore district of Tamilnadu by interaction with tribal groups of Mudugas, Kurumbas, Irulas, Kotas, Paniyas, Kattunayaks. The collected plant materials were authenticated by a taxonomist at IFGTB, Coimbatore.

Processing of plant materials and preparation of extracts

Fresh leaves of plant samples were air dried and ground into uniform powder. Dry powder of each plant sample was extracted with the organic solvents viz., acetone, ethylacetate and methanol using soxhlet apparatus for 6 hours. Fresh extract was prepared as and when required for further study.

Bioassay Study

Hybleae puera larvae were cultured at entomology laboratory in KFRI sub center, Nilambur. 6 cm diameter of *Tectona grandis* leaf discs were treated with different concentration of extracts ranging from 250 ppm to 1000 ppm. These leaf discs were kept individually in plastic containers after air drying. Pre - starved third instar larvae were released per disc. Observations were made for every 24 hours upto 10 days and results were recorded.

Determination of Total phenolic content

Total phenolic content in the extracts was determined with Folin – Ciocalteu's Reagent (FCR) [10]. 0.5 ml of extract was mixed with 2.5 ml FCR (diluted 1:10 v/v) followed by 2 ml of Na_2Co_3 (7.5% v/v) solution. The tubes were vortexed and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against blank at 650 nm using spectrophotometer (HITACHI U 2000). A calibration curve was constructed using gallic acid as standard and total phenolic content of the extract was expressed in terms of micrograms of gallic acid (µg GAE) per gram of dry weight.

High Performance Liquid Chromatographic (HPLC) analysis

Identification of individual phenolic compounds of the plant extracts were performed by HPLC Hitachi instrument with L-4000 UV detector, L- 6200 intelligent pump and RP- C18 column (150 x0.46 m). A constant flow rate of 1ml/min at a wavelength of 260 nm. The mobile phase containing 32 percent acetonitrile, 0.1M KCl, 0.05M HCl. Phenolic compounds of each sample were identified by comparing their relative retention time (min) with those of standards. The concentration of an individual phenolic compound was calculated on the basis of peak area measurements into mg/100g.

RESULTS AND DISCUSSION

Extraction

Plant matrices contain various solute molecules with more than one functional group. Therefore, it is difficult to predict the solubility of solutes in a particular solvent. An alternative way of considering solubility is to use the concept of polarity. Fig. 1 indicates the percentage yield of extract for plant materials using different polar organic solvents. High yield of extract was found in the order of methanol> acetone> ethyl acetate. In the present study, high amount of extract obtained from *B.scandens* by employing organic solvent methanol which is followed by *M.koenigii, V.negundo, M.dubia, A.bracteata, P.pinnata, S.nuxvomica, A.vasica,*.

Our findings are similar with earlier observation made in a study on high yield of extract obtained by using methanol and ethanol as solvents for extraction [11] . Methanol and ethanol have similar solubility properties because they contain hydroxyl group only. Similarly, previous study [12] had suggested the yield of methanol extract was higher followed by aqueous extract of dry plant material of *Hieracium pilosella* L. An earlier study [13] had stated that methanol and acetone are the suitable solvent for phenols extraction. Extraction of tannins and other phenolics was better in aqueous acetone than in aqueous methanol [14, 15]. The difference in the extract yields from the tested plant materials in the present analysis might be due to the different availability of extractable components, resulting from the varied chemical composition of plants.

Figure 1- Percentage yield of extract for plant species

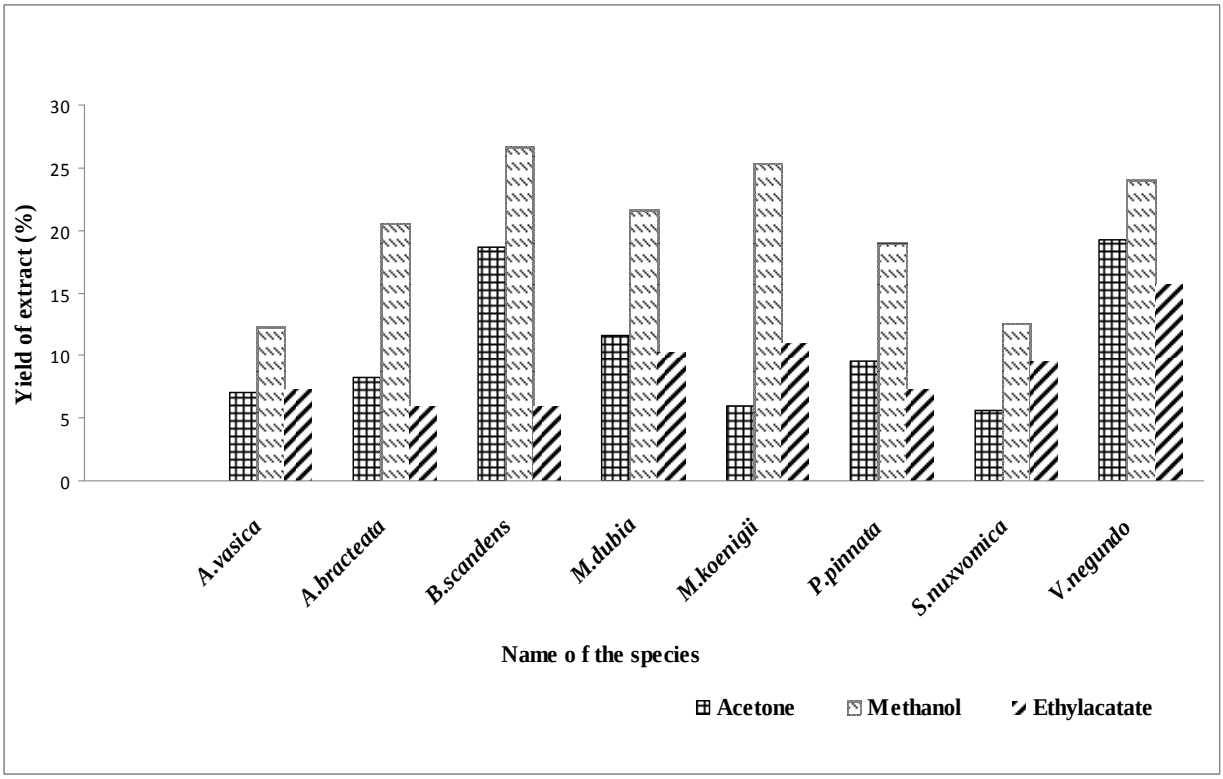

Table 1a: Percent larval mortality of *H.puera* for Acetone extract

Species	Mortality %			
	250ppm	500ppm	750ppm	1000ppm
A.vasica	26.33±6.51^{b}	60.33±2.52^{d}	79.67±1.53^{d}	85.33±4.73^{c}
A.bracteata	25.67±6.03^{b}	44.67±4.51^{c}	46.00±6.00^{c}	65.67±4.93^{b}
B.scandens	25.33±4.62^{b}	47.33±8.08^{c}	65.33±6.81^{c}	85.67±4.93^{c}
M.dubia	26.67±9.87^{b}	65.33±6.11^{d}	68.67±9.02^{cd}	88.67±9.02^{c}
M.koenigii	27.33±7.51^{b}	64.00±4.58^{d}	66.67±7.64^{c}	87.00±6.24^{c}
P.pinnata	21.67±1.53^{b}	41.67±1.53^{c}	62.67±2.31^{c}	82.67±3.06^{c}
S.nuxvomica	40.67±1.15^{c}	62.33±2.52^{d}	79.67±2.52^{d}	86.67±5.86^{c}
V.negundo	22.67±2.31^{b}	27.33±8.08^{b}	31.00±12.77^{b}	81.33±1.15^{c}
P-Control	5.33±1.53^{a}	6.33±2.31^{a}	8.67±2.08^{a}	10.33±2.52^{a}
N-Control	4.00±2.42^{a}	4.00±2.42^{a}	4.00±2.42^{a}	4.00±2.42^{a}

P-Control- Positive control N-Control- Negative control

All values are mean ±SD of five replicates with 20 insects in each replicate (total 100 insects) , values followed by the same alphabets are not significantly different at P<0.05 (DMRT).

Table1b: Percent larval mortality of *H.puera* for methanol extract

Species	Mortality %			
	250ppm	500ppm	750ppm	1000ppm
A.vasica	27.33±11.02^{b}	65.00±6.24^{e}	69.67±10.60^{d}	84.67±5.03^{c}
A.bracteata	27.33±11.85^{b}	31.67±15.31^{bc}	44.67±6.43^{b}	65.33±4.62^{b}
B.scandens	24.33±5.86^{b}	29.00±9.54^{b}	42.33±2.52^{b}	84.33±3.79^{c}
M.dubia	23.00±4.36^{b}	32.33±17.21^{bc}	50.67±15.95^{bc}	99.00±1.73^{d}
M.koenigii	43.67±6.35^{c}	48.67±11.72^{cde}	66.00±8.72^{d}	87.00±7.00^{c}
P.pinnata	41.33±1.15^{c}	46.67±7.02^{bcd}	64.00±3.46^{cd}	83.33±3.06^{c}
S.nuxvomica	43.33±3.06^{c}	63.00±4.36^{de}	67.00±6.56^{d}	86.00±5.29^{c}
V.negundo	24.00±3.61^{b}	42.00±1.73^{bc}	64.00±3.61^{cd}	69.33±9.50^{b}
P-Control	4.67±0.58^{a}	6.33±1.15^{a}	8.67±1.53^{a}	10.33±1.15^{a}
N-Control	4.00±2.42^{a}	4.00±2.42^{a}	4.00±2.42^{a}	4.00±2.42^{a}

P-Control- Positive control N-Control- Negative control

All values are mean ±SD of five replicates with 20 insects in each replicate (total 100 insects) values followed by the same alphabets are not significantly different at P<0.05 (DMRT).

Table1c: Percent larval mortality of *H.puera* for ethyl acetate extract

Species	Mortality %			
	250ppm	500ppm	750ppm	1000ppm
A.vasica	27.33±11.02^{b}	65.00±6.24^{e}	69.67±10.60^{d}	84.67±5.03^{c}
A.bracteata	27.33±11.85^{b}	31.67±15.31^{bc}	44.67±6.43^{b}	65.33±4.62^{b}
B.scandens	24.33±5.86^{b}	29.00±9.54^{b}	42.33±2.52^{b}	84.33±3.79^{c}
M.dubia	23.00±4.36^{b}	32.33±17.21^{bc}	50.67±15.95^{bc}	99.00±1.73^{d}
M.koenigii	44.67±6.35^{c}	48.67±11.72^{cde}	66.00±8.72^{d}	87.00±7.00^{c}
P.pinnata	41.33±1.15^{c}	46.67±7.02^{bcd}	64.00±3.46^{cd}	83.33±3.06^{c}
S.nuxvomica	43.33±3.06^{c}	63.00±4.36^{de}	67.00±6.56^{d}	86.00±5.29^{c}
V.negundo	24.00±3.61^{b}	42.00±1.73^{bc}	64.00±3.61^{cd}	69.22±9.50^{b}
P-Control	4.67±0.58^{a}	6.33±1.15^{a}	8.67±1.53^{a}	10.33±1.15^{a}
N-Control	4.00±2.42^{a}	4.00±2.42^{a}	4.00±2.42^{a}	4.00±2.42^{a}

P-Control- Positive control N-Control- Negative control

All values are mean ±SD of five replicates with 20 insects in each replicate (total 100 insects) values followed by the same alphabets are not significantly different at P<0.05 (DMRT).

Bioassay study

Teak (*Tectona grandis*) is one of the most important tropical hardwood forest species in the international market because of its high quality timber [16]. The most serious pest is teak defoliator, *H.puera*. The data summarized on table 1a, 1b and 1c represent the mortality of *H.puera* larvae varied from 60 to 100 percent at different concentrations of extracts. Methanol extracts were found to show high mortality, ranging from 80 to 100 percent, followed by ethyl acetate with 60 to 100 percent and acetone with 60 to 80 percent at 1000 ppm concentration.

Among the eight plant species evaluated for bioassay study, *M.dubia* was found to be more effective with LC_{50} value 120.465 ppm which is followed by *M.koenigii* (135.482 ppm), *A.vasica* (188.413 ppm), *B.scandens* (189.413 ppm), *A.bracteata* (244.397ppm), *S. nuxvomica* (252.181 ppm), *v.negundo* (278.810 ppm), *P.pinnata* (316.922 ppm) were represented in table 2. The larval mortality is due to the presence of phenolic compounds identified in plant extracts. Catechol is a phenolic compound which is present in all of the plants employed in this study.

Table 2: Probit analysis to test the efficacy of plant extracts against larvae of *H.puera*

Species	Extract	Heterogeneity (χ2)	Regression Equation	LC_{50} ppm
A. vasica	Acetone	1.086	Y = -0.919 + 0.002 x	428.266
	Methanol	3.817	Y= -0.203 + 0.002 x	188.172
	Ethyl acetate	3.710	Y= -0.637 + 0.002 x	313.788
A.bracteata	Acetone	1.605	Y = -0.710 + 0.001 x	619.310
	Methanol	0.851	Y= -0.372 + 0.002 x	244.397
	Ethyl acetate	1.408	Y= -0.472 + 0.001x	549.465
B. scandens	Acetone	0.761	Y = -1.118 + 0.002 x	474.027
	Methanol	3.960	Y= -0.821 + 0.003 x	189.413
	Ethyl acetate	17.961	Y= -1.158 + 0.002 x	613.611
M. dubia	Acetone	5.580	Y = -0.632 + 0.002 x	330.134
	Methanol	4.734	Y= -0.785 + 0.003 x	120.465
	Ethyl acetate	13.393	Y= -1.457 + 0.003 x	480.305
M. koenigii	Acetone	4.809	Y = -0.836 + 0.002 x	377.376
	Methanol	6.287	Y= -0.311 + 0.002 x	135.482
	Ethyl acetate	3.592	Y= -0.531 + 0.002 x	287.708
P. pinnata	Acetone	0.008	Y = -1.298 + 0.002 x	587.563
	Methanol	1.040	Y= -0.507 + 0.002 x	316.922
	Ethyl acetate	1.250	Y= -0.635 + 0.002 x	415.339
S.nuxvomica	Acetone	0.173	Y = -0.717 + 0.002 x	366.339
	Methanol	3.738	Y= -0.692 + 0.003 x	252.181
	Ethylacetate	1.126	Y= -0.629 + 0.002 x	348.848
V. negundo	Acetone	9.176	Y = -1.339 + 0.002 x	669.499
	Methanol	2.094	Y= -0.105 + 0.002 x	278.810
	Ethyl acetate	0.216	Y= -1.108 + 0.002 x	572.824

*Y=Probit kill; LC_{50}= Concentration to give 50 percent mortality, *All data were found to be significantly heterogeneous at 5% level.*

Catechol acts as precursor for the production of pesticides [17]. Ferulic acid, vanillin, gallic acid, syringaldehyde, vanillic acid were the phenolic compounds identified in this study. The results of present study compared with investigation of [18] carried out bioassay study to assess the effect on the behaviour and survival of beetles. They observed caffeic and Ferulic acids, vanillin and luteolin-7- o glucoside, gallic acid, Quercetin, naringin, syringaldehyde, vanillic acid induced knock down effect. Seed extract of *Azadrachita indica* followed by leaf extract of *Cassia sianea*, *Strychnos nuxvomica* and tuber extract of *Amarphophallus componata* were found to be most effective against third instar larvae and eggs of teak defoliator *H.puera* [19]. The mode of action of azadirachtin

lies in effects on deterrent and other chemoreceptors resulting in antifeedancy and direct effects on most other tissues studied resulting in an overall loss of fitness of the insect [20,21]. Betulinic acid shows an effective insect growth regulating activity and exhibits great promise in suppressing the population of pest, Tribolium confusum [22].The effect of different phenolic compounds of plant extracts on larval mortality of *H.puera*, the present investigation provides a new avenue to develop eco-friendly pesticides for the management of insect pests of teak.

Total phenolic content

Total phenolic content of different plant materials were presented in table 3.The total phenolics content of extracts were determined from regression equation of calibration curve (Y=0.005x+0.19, R^2=0.992) and expressed in Gallic acid equivalents (GAE). Table 3 represent the total phenolic content of *B.scandens* methanol extract was found to be highest with the concentration of 197.53 ± 5.84 µg/g, which is followed by *P. pinnata* (193.93±8.72 µg/g), *M.koenigii* (174.66±9.53 µg/g), *M.dubia* (170.06±15.71 µg/g), *A.vasica* (167.13±4.69 µg/g), *A.bracteata* (140.16±8.41µg/g), *V.negundo* (137.46±13.5 µg/g), *S.nuxvomica* (125.26±9.65 µg/g). The determined amounts of total phenolics from *M.koenigii* acetone extract (174.66±9.53 µg/g) in the present study were higher than that reported for *M.koenigii alcohol:water extract* (168µg/g)[23].

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Table 3. Total phenolic contents of plant materials (µg/g)

Species	Acetone extract	Methanol extract	Ethylacetate extract
A. vasica	133.86±8.50	145.2±22.19	167.13±4.69
A. bracteata	140.16±8.41	151.73±8.42	120.06±7.58
B. scandens	179.33±13.11	197.53±5.84	171.33 ±20.67
M. dubia	170.06±15.71	149.13±9.53	132.6±28.25
M. koenigii	174.66±9.53	150.33±7.40	163.13±7.43
P. pinnata	193.93±8.72	144.06±10.62	130.0±9.61
S. nux vomica	125.26±9.65	149.66±9.44	107.4±5.07
V.negundo	137.46±13.5	128.06±20.49	92.73±7.90

Total phenolic content of *V.negundo* ethanolic extract was found to be high 249.96± 8.34 GAE/g dry weight of extract [24] when compared with present investigation. Aqueous extract, hydro alcohol and petroleum ether extract of *A.vasica* was found to be 92.4±0.14 µg/g, 81.51±2.7 µg/g, 63.95±2.1 µg/g respectively [25] which was lower than the acetone, methanol and ethylacetate extracts of present study. An earlier study by Gupta *et al*., 2011[26] reported that *P.pinnata* had a total phenolic content of 8.64 mg/g. With reference to the above reports results of the present study strongly suggests that phenolics are important components of the plants and among three solvent extracts analysed methanol extract had the highest total phenolic content. This may be due to the fact that phenolics are often extracted in higher amounts in more polar solvents [27, 28].

Table 4: Concentration of phenolic compounds present in acetone extract (mg/100g)

Name of the compound	*A.vasica*	*A.bracteata*	*B.scandens*	*M.dubia*	*M.koenigii*	*P.pinnata*	*S.nuxvomica*	*V.negundo*
Phloroglucinol	0.01	0.1	-	0.03	0.06	0.03	-	0.06
Catechol	0.4	0.16	0.9	0.3	0.1	0.03	0.1	0.2
Ferulic acid	0.3	-	1.8	-	0.02	0.02	-	-
Syringic acid	-	0.2	0.37	0.8	0.2	0.2	0.2	1.6
Morpholin	0.1	0.6	-	0.6	0.5	-	0.4	0.4
Vanillin	0.03	-	-	0.03	-	-	-	-
Resorcinol	0.03	-	-	-	-	0.2	-	-
Syringaldehyde	-	-	0.8	-	-	-	-	-

Table 5: Concentration of phenolic compounds present in methanol extract (mg/100g)

Name of the compound	*A.vasica*	*A.bracteata*	*B.scandens*	*M.dubia*	*M.koenigii*	*P.pinnata*	*S.nuxvomica*	*V.negundo*
Pyrogallol	0.5	-	0.27	-	0.7	-	1.1	-
Benzoic acid	0.4	-	0.19	0.6	-	-	-	-
Catechol	-	0.06	-	-	0.4	-	0.2	-
Ferulic acid	-	-	1.82	0.1	-	0.3	0.1	1.5
Syringic acid	0.01	0.2	-	-	0.2	-	0.03	0.03
Morpholin	-	0.02	-	0.2	-	-	-	0.1
Vanillin	-	-	-	-	-	0.1	-	-
Syringaldehyde	0.1	-	-	0.7	-	-	-	-
Gallic acid	-	-	-	-	-	0.3	-	-
Cinnamic acid	--	-	-	-	0.1	-	-	-
Chlorogenic acid	-	-	-	-	-	-	-	0.5

Phenolic compounds

Fifteen phenolic compounds were identified from plant extracts presented in table 4, 5 and 6.These constituents were Pyrogallol, Benzoic acid, Catechol, Ferulic acid, Syringic acid, Phloroglucinol, Morpholin, Vanillin, Resorcinol, syringaldehyde, Ellagic acid, Gallic acid, Vanillic acid, Cinnamic acid, Chlorogenic acid. Eight plant species taken for this study show great variations in their concentration of different phenolic compounds.

Table 6: Concentration of phenolic compounds present in ethyl acetate extract (mg/100g)

Name of the compound	*A.vasica*	*A.bracteata*	*B.scandens*	*M.dubia*	*M.koenigii*	*P.pinnata*	*S.nuxvomica*	*V.negundo*
Phloroglucinol	0.02	-	-	-	-	0.02	0.1	-
Pyrogallol	-	1.06	1.8	-	-	--	-	-
Benzoic acid	-	0.7	2.3	-	-	-	0.1	-
Catechol	1.0	1.1	1.04	0.6	0.3	1.1	0.1	0.16
Ferulic acid	0.3	-	0.3	0.08	0.03	0.3	0.2	0.2
Syringic acid	0.1	0.2	0.78	0.09	0.7	0.2	0.6	1.4
Morpholin	0.4	0.8	0.5	0.9	2.6	0.6	0.1	0.1
Vanillin	0.02	0.08	0.1	0.04	-	0.03	-	-
Resorcinol	-	-	0.3	-	-	-	-	-
Cinnamic acid	-	-	-	-	-	-	0.04	-

Catechol and Ferullic acid was the major phenolic compounds identified which is followed by Syringic acid, Morpholin, Vanillin, Phloroglucinol, Pyrogallol and Benzoic acid. The least concentration of phenolic compounds identified were Resorcinol, Syringaldehyde, Gallic acid, Chlorogenic acid, Vanillic acid and Cinnamic acid. Difference in phenolic compounds depends upon their genus, species, varieties and cultivars [29].

CONCLUSION

Based on the results of present study we conclude that organic solvent methanol is found to yield higher amount of extract. Estimation of phenolic content in different extracts of eight plant samples showed methanol extracts has maximum phenolic content followed by acetone. The phenolic compounds such as Ferulic acid was found to be more in *B.scandens* and *V.negundo* followed by Catechol in *P. pinnata*, *A. bracteata* ; Pyrogallol in *B. scandens* and Syringic acid in *V. negundo*. Bioassay studies on *H.puera* using the extracts at different concentrations viz., 250, 500, 750 and 1000 ppm showed promising results. A range between 60 and 100 percent mortality was observed. Methanol extracts were found to show good mortality ranges between 80 and 100 percent followed by ethyl acetate with 60 and 100 percent and acetone with 60 and 80 percent. Of the eight plant species used *M. dubia* was found to be more effective followed by *M. koenigii*, *A.vasica*, *B .scandens*, *V. negundo*, *S. nuxvomica*. Based on the study it is revealed that plant extracts may be considered as potential biopesticides against early developmental stages of *H.puera*.

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