Evaluation of the Phytotoxicity of Premna Schimperi to the Invasive Weed Parthenium Hysterophorus

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Abstract
Parthenium hysterophorus (*Kinche arem* inAmharic) is one of the worst invasive weeds in Ethiopia. It is known for its toxic effects on plant germination and growth. It is estimated that this weed reduces crop yields by more than 25% in developing countries.
Previous attempts to curb the spread of weeds using synthetic chemicals have not been very successful. On the other hand, allelopathic plants are known to produce natural chemicals that inhibit weed growth. Many biological activities of *Premna schimperi* are reported, except for its herbicidal properties. In this project, we tried to screen the phytotoxic properties of*Premna schimperi* extracts and purified compounds against parthenium weed. Compounds with moderate phytotoxic activity have been isolated from leaf ethanol extracts of *P. schimperi*. Kaempferide (**2**) is an isolated compound with 87% growth inhibition. This is the first report of this kind.
Key words: *P. schimperi*, kaempferide. Labdane diterpene, phytotoxicity,*P. hysterophorus*

- **Introduction**

The agricultural sector is a cornerstone of the economic and social life of the Ethiopian people. Crops such as maize are the country's main production to feed the rapidly growing population (Negasa et al., 2021). The Yield Loss Assessment study shows that weeds, diseases and insect pests are the top challenges affecting crop production.Among these biotic factors, weeds cause about 25% yield loss in least developed countries, including Ethiopia (Adkins & Shabbir, 2014; Belachew & Tessema, 2015).
Parthenium hysterophorus L., also known as Peterson's weed or Santamaria feverfew (Kinche Arem in Amharic), is one of the most devastating and aggressive annual weeds. Due to its reproductive and dispersal potential, it becomes dominant and is considered the worst and most common weed species in areas of eastern, central and northern Ethiopia(Belachew & Tessema, 2015; Talemos et al., 2013).

Parthenium is known for its allelopathic, competitive, and toxic effects on plantsand animals(Adkins & Shabbir, 2014; Talemos et al., 2013; Tamado et al., 2002; Tamado & Milberg, 2000; Tanveer et al., 2015). It also has negative effects on human and animal health and is known to cause asthma, bronchitis, dermatitis and hay fever in humans(M. Kaur et al., 2014; Patel, 2011). As has been claimed in previous research, the above health problems and allelopathic effects of the plant are due to the presence of harmful sesquiterpene lactones and various phenolic acids(Kakad et al., 2020; A. Kaur et al., 2021; Panwar et al., 2015; Wang et al., 2020).

Research into the control mechanism of this weed should be of great importance for the food security of the Ethiopian people. Attempts to curb the spread of weeds using synthetic chemicals have so far been unsuccessful due to their overuse and misuse has caused various disadvantages and problems such as environmental pollution, harmful effects on non-target crops, microorganisms and health concerns.
This underscores the need for affordable alternatives that are convenient to use and environmentally friendly. Plant extracts contain allelopathic secondary metabolites with great potential for use in natural herbicides such as cinmethyline, 1,4-cineole and 1,8-cineole. The use of botanicals for weed control is preferred as they are safe and non-toxic to humans(Bhadoria, 2010; Macias et al., 2003; Masum et al., 2013).
Premna schimperi, which belongs to the family Lamiaceae, includes about more than 200 known species, mostly native to tropical and subtropical Asia, Africa, Australia and the Pacific Islands(Dianita & Jantan, 2017). The plant, called "checho" in Amharic, is a small tree commonly used to treat inflammation, superficial wounds and eye infections in cattle(S. Habtemariam et al., 1990).
A phytochemical review of the literature indicates that the genus Premna is a rich source of iridoid glycosides, diterpenoids and flavonoids(Dianita & Jantan, 2017). In addition, other classes of secondary metabolites such as sesquiterpenoids, triterpenoids, isoflavones, lignans and xanthones are known to be isolated from different species of the genus Premna.

Figure1. *P. schimperi* leaves (Ambo, APPRC garden Ethiopia)

The isolated secondarymetabolites from this genus have been reportedto display interesting biological activitiesincluding antioxidant(Yadav et al., 2013), antibacterial(S. Habtemariam et al., 1990), anti-inflammatory(Al Mahmud et al., 2016), cytotoxic(Soc et al., 1995), antileishmanial (Solomon Habtemariam, 2003) andhepatoprotective(Al Mahmud et al., 2016).To the best of my knowledge, there is no report of the plant's phytotoxic activity. The present study therefore reports the effectiveness of extracts and compounds from *P. schimperi* in controlling of *P. hysterophorus* weed infestation.

- ## Materials and methods

.1. General experimental procedure

The compounds described in this work were isolated by column chromatography on silica gel and alumina (neutral), a medium capable of supporting 80 g of silica gel. The silica gel used for the CC has a particle size of 60-120 mesh. To purify the fractions collected from CC, preparative thin layer chromatography (PTLC) was performed on a 1.0 mm thick pad of silica gel.
TLC was performed on precoated plates (Kieselgel 60 F254, 230-400 mesh, Merck) and alumina plates; Melting points are uncorrected; Detection by UV light at 254 and 366 nm and vanillin-H2SO4 spray reagents;

IR: KBr disk or neat and measured on a Perkin Elmer 1600 and Pye Unicam SP3-300 infrared spectrophotometer. UV spectra were recorded on a Shimadzu UV-VIS recording spectrophotometer, UV-160, Spectronic Genesys spectrophotometer; ^{1}H and ^{13}C NMR were recorded in $CDCl_3$, DMSO, $(CD_3)_2CO$ and CD_3OD using the solvent peak as reference (chloroform: H 7.2 and C 77.2, DMSO, H 2.5 and C 39.5, deuterated acetone: H 2.05 and C 29.5 and). 205.5 methanol: H, 3.3 and C, 49.0). Chemical shift values are reported in (ppm), the solvent signals as internal references; ^{1}H, ^{13}C and 2D NMR spectra were obtained on a Jeol FX 90 spectrophotometer at 90 and 22.5 MHz; Jeol JNM-EX400 instrument at 400MHz and 100MHz; a Bruker UltrashieldTM 400 spectrometer at 400 and 100 MHz with TMS and solvents as internal standard. The values are given in ppm relative to the internal TMS standard.

.2. phytotoxicity screening procedure (*in vitro*)

P. hysterophorus seeds were air dried, hand threshed, and intact and viable seeds were selected. Selected seeds were surface sterilized by shaking in 1% sodium hypochlorite (NaOCl) solution for 5 minutes and washed with distilled water for 3 minutes immediately before use to prevent fungal growth during the screening test. Ten prewashed and disinfected test seeds were sown on each Petri dish connected to filter paper.The plant extract (5 mg) was diluted in 5 ml acetone and then water (95 ml) was added to make a 0.05 mg/ml solution. The final concentration of acetone in water is 5%. The germ-inhibiting effect of the solvent system was examined as a negative control. Each test solution was watered every day until the filter paper was wet for 15 consecutive days. The experiment was performed in triplicate for each extract using a fully randomized design. 5% acetone in water was used as a negative control, while a commercial herbicide (Roundup) was used as a positive control. The seed germination response is the most important deleterious factor for screening plant compounds for their herbicidal activity. After fifteen days, the number of germinated seeds was counted.

Treatment and control tests were performed in triplicate. Petri dish plates were covered and placed in the greenhouse at a temperature of 23-30°C. The inhibition of germination of the extract and the pure compounds is calculated using a standard formula: GI% = [1-(Gt/Gc)] 10% [140], where Gt and Gc are the number of germinated seeds in the treatment and control, respectively.

.3. *In vivo* test procedures

The *in vivo* bioassay tests were performed in the greenhouse according to the standard protocol(R. G. BELZ, 2016).Three types of soil (red soil, sand and compost) were collected, mixed in a 2:1:1 ratio and sterilized. Plastic pots of the same size were prepared and each pot was filled with 3 kg of soil mix. Each pot was watered until wet and placed in a greenhouse at a temperature of 23-30°C. First, 10 viable parthenium seeds were sown in each pot and watered daily for 15 days. After 15 days, the germinated seedlings were thinned to three uniform seedlings for treatment. Test solutions (0.05 mg/mL) of the extract and pure compounds were sprayed using the foliar spray bioassay technique at an average of 5 mL per spray (2 drops per leaf) for 15 days (Javaid et al., 2013). Treatments were ordered using a fully randomized block design with three replicates for each test solution. The solvent (5% acetone in water) and Roundup were used as negative and positive controls, respectively. After two weeks of treatment, the seedlings of the control and treatment groups were gently uprooted and their roots washed with water and allowed to dry separately (Javaid et al., 2013).Seedling dry biomass before and after treatment was recorded and percent growth inhibition was calculated using the standard formula GI % = [1-ADBT/ADBC] x 100%, where ADBT = average treatment dry biomass, ADBC = average dry biomass of control(Inderjit et al., 2008).

.4. Isolation of compounds from *P. schimperi*

The leaves of *P. schimperi* were collected in Ambo, West Shewa Zone, Oromia Region, Ethiopia. The city has a latitude and longitude of8°59′N 37°51′E.It is at an altitude of 2101 meters. The specimen was identified by a plant taxonomist and deposited with the National Herbarium Department of Biology; Addis Ababa University Herbarium with voucher number 97-9G. Based on the preliminary results on the biological activity of *P. schimperi,* three compounds were isolated from the $CHCl_3$ extracts of the aerial part of the plant. *P. schimperi* leaf powder (100 g) was extracted with $CHCl_3$ (500 ml) to give 6 g of black jelly material. It was adsorbed on silica gel and subjected to silica gel (100 g) column chromatography.Elution was performed using hexane:EtOAc solvents of increasing polarity to give 15 fractions. Fraction 5, collected using the hexane:EtOAc (4:1) solvent system as eluent, was allowed to stand overnight and a white powder precipitate formed at the bottom of the vial and was identified as compound 1 (29 mg). The structure of this substance was proposed to be heptacosanol (29 mg) based on various physical and spectroscopic data. Fraction 9 gave a yellow powder in hexane:EtOAc (1:1) solvent system, identified as compound 2 (30 mg). The powder was subjected to physical and spectroscopic examination and identified as kaempferide (30 mg). Fraction 10 (80 mg) was applied to PTLC using hexane:EtOAc (1:1) as eluent and four fractions were collected. Fraction 4 was found to give a clean TLC spot and was labeled as compound 3 (20 mg).

Elucidation and characterization of the compounds**1**, **2**, **3**were carried on the bases of physical and spectroscopic analysis and the data is compared with the literature values. The herbicidal activity of these compounds was studied both *in vitro* and *in vivo* and kaempferide exhibited highest activity with 87% seedling growth inhibition.

• Result and discussion

.1. Bioassay results

The *in vitro* result of $CHCl_3$ extracts of *P. schimperi* showed 78% growth inhibition at 0.05 mg/mL concentration. Following the *in vitro* result, the $CHCl_3$ extract was applied on column chromatography to afford three compounds. These are kaempferide, diterpene, heptacosanol. All the three compounds showed 100% seed germination inhibition *in vitro*at the same concentration.

(Here Table 1) *In vivo* bioassay results displayed that only compound **2** inhibit the growth of the 15-day seedling with 87%. The diterpene compound **1** and compound **3** showed minimum growth inhibition with 29 and 42% respectively. Even though the diterpene compound **1** reported to have strong antibacterial activity, its phytotoxic activity is insignificant.

(Here Table 2)

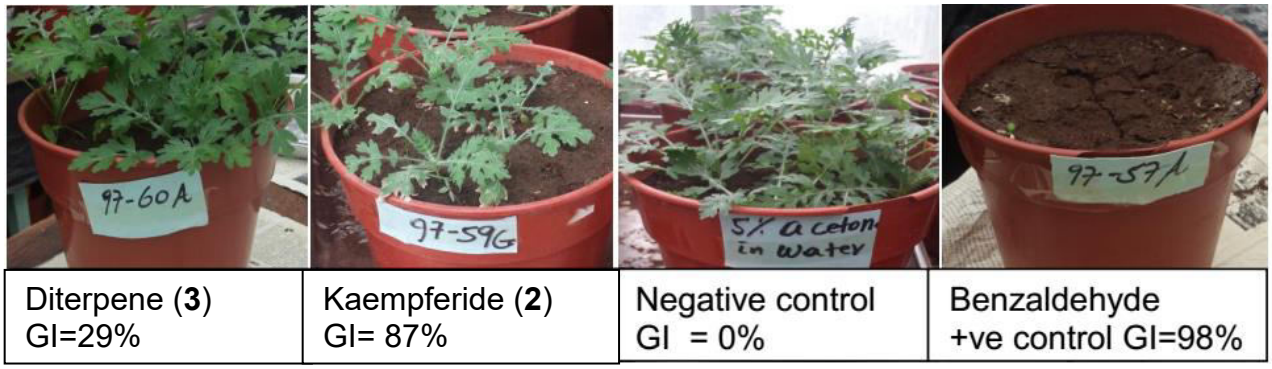
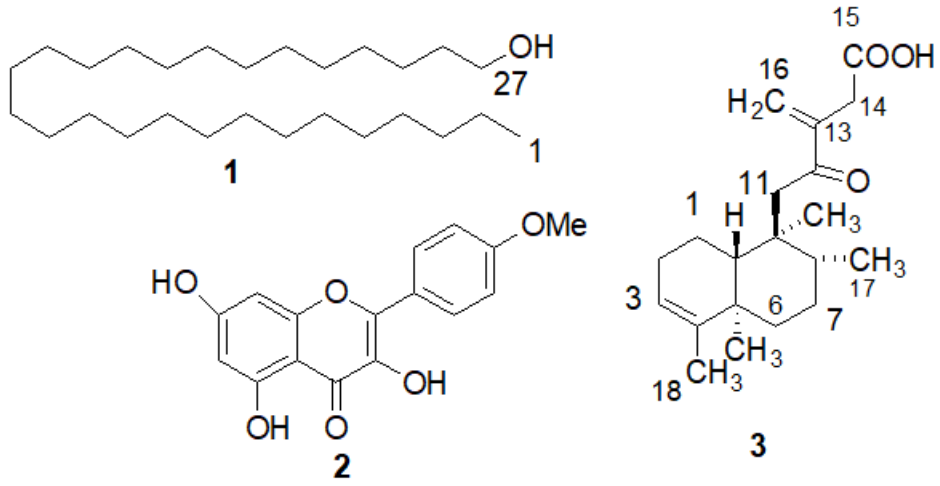


Figure 2.*In vivo* results of compound 3 and 2 in comparison with the standard

.2. Characterization of compounds isolated from *P. schimperi*



Compound 3

Compound **3** was isolated from fraction 10. Fraction 10 (80 mg) was applied on PTLC and compound **3** (20 mg) was obtained as a jelly pure material. The TLC profile of this compound developed using hexane: EtOAc (3:2) solvent system and vanillin in sulfuric acid as a spraying agent showed a single pink spot (Rf 0.54). The UV-Vis spectrum of compound **3**showed no absorption bands in the UV-Visible region. It is, therefore, suggestive to disclose the absence of any conjugated chromophore in the compound.

The IR spectrum of compound **3**showed a broadband at 3429 cm^{-1}indicatives for the presence of O-H stretching. The methyl C-H stretching is displayed at 2924 cm^{-1}. The sharp peak displayed at 1632 cm^{-1} is evident for the presence of carbonyl functional group in the molecule. IR band at 1384 cm^{-1} is directive of the presence of geminal dimethyl stretching.

In the ^{1}H-NMR spectrum of compound **3**, the presence of exocyclic protons is confirmed by the downfield signals appeared at δ 6.19 (1H, *s*) and δ 5.92 (1H, *s*). The HH-COSY experiment showed the correlation of these exocyclic protons with isolated methylene protons appeared at δ 3.30 (2H, *s,* H-14). The other downfield signal observed at δ 5.15 (1H, *m*) is evident for the presence of an olefinic methine proton which is correlated with protons appeared at δ 1.57 (3H, *s*, H-18) and 1.96 (2H, *m*, H-2). Two doublets of doublet signals showed at δ 2.65 (1H, *d*, *J* = 15.6) and δ 2.85 (1H, *d*, *J* = 15.2) are assigned from two diastereotopic protons (H-11). The ^{1}H-NMR experiment of compound **3**disclosed the presence of four terminal methyl protons each appeared at δ 1.57 (3H, *s*), δ 0.99 (3H, *s*), δ 0.86 (3H, *d*, *J* = 6.8 Hz) and δ 0.82 (3H, *s*).

The ^{13}C-NMR experiment with the aid of Dept-135 revealed the presence of six quaternary, three methine, seven methylene and four methyl carbons. The most downfield signal observed at δ 201.2 is assigned for α, β-unsaturated ketone carbon. The ^{13}C-NMR spectrum with the help of HMBC experiment confirmed presence of α, β-unsaturated acid functional group appeared at 176.1. The HMBC experiment showed correlation with an isolated methylene proton appeared at 3.3. The ^{13}C spectrum of compound **3** exhibited four olefinic carbons appeared at δ 143.8, δ 143.3, δ 126.8 and δ 120.5. Carbon signals at δ143.8 and δ 126.8 are due to exocyclic double bond in consistence with proton spectrum. Compound **3** is compared with literature values of the clerodane diterpene and all the NMR data of this compound is found in a close agreement with it (S.

Habtemariam et al., 1990). This compound was previously isolated from *P. schimperi* and *P. oligotricha*(Solomon Habtemariam, 2003; Soc et al., 1995).and reported to exhibit strong antibacterial and antileishmanial activities(Solomon Habtemariam, 2003).
(here Table 3)

Compound **3** is compared with literature values of the clerodane diterpene previously isolated from *P. schimperi* and *P. oligotricha* andfoundin a close agreement(Solomon Habtemariam, 2003; Soc et al., 1995).This compound was reported to exhibit strong antibacterial and antileishmanial activities(Solomon Habtemariam, 2003).

Compound 2

Compound **2** (30 mg) was obtained as a yellowish crystal from fraction nine and ten. The TLC profile of compound **2** showed a yellow spot in hexane:EtOAc (3:2) solvent system with an Rf value of 0.51 when it sprayed with vanillin in sulfuric (1%).This compound melts at 219-220°C (lit. 227-23°C) [132]. The UV-Vis spectral analysis indicated a characteristic of flavonoid chromophore with λmax at 268 and 364 nm. The IR spectrum of compound 100 showed three bands at 3301, 3455 and 3515 cm^{-1} each assigned for O-H stretching of a hydroxyl group of a flavonoid found in the different environment. The bands appeared at 1667, 1512 and 1162 $cm^{-1}$are interpreted for the presence of carbonyl group, aromatic group C=C stretching and C-O stretching respectively.

The ^{1}H-NMR spectrum of compound **2** (Appendix 18) displayed clearly well-resolved signals in the aromatic region. The most downfield signal appeared at δ 12.44 (1H, *s*) is a result of strong hydrogen bonding between hydroxyl group at C-5 and a carbonyl group at C-4. The other downfield signals at δ 10.83 (1H *br s*) and δ 9.47 (1H, *br s*) are indicative for the presence of two more hydroxyl groups in the compound. The doublet signals appeared at δ 8.12 (2H, *d*, *J*= 8.8 Hz), δ 7.09 (2H, *d*, *J* = 8.8 Hz) are suggestive for the presence of symmetrical aromatic *ortho* coupled protons (H-2'/6' and 3'/5'). These doublet signals are also suggestive the fourth position is substituted by a certain group; most probably the methoxy group appeared at δ 3.83 (3H, *s*). The ^{1}H NMR spectrum of compound **2** also showed the presence two additional aromatic protons, *meta*-coupled doublet at 6.45 (1H, *d*, *J* = 2 Hz, H-8) and δ 6.19 (1H, *d*, *J* = 2 Hz, H-6). The presence of all these aromatic protons (two *ortho* coupled and two *meta* coupled) are suggestive the presence of a *tetra*-substituted and a 1,4-di-substituted phenyl rings. The later ring was further confirmed to be *p*-hydroxyphenyl system from the ^{13}C-chemical shift of the carbon signals at δ 129.7 (C-2', 6') and δ 114.4 (C-3', 5').

The ^{13}C-NMR data together with Dept-135 displayed the presence of nine quaternary, four methine and one methyl carbons. The most downfield (at δ 176.4) and the most upfield (at δ 55.8) signals are assigned for α, β-unsaturated carbonyl carbon the flavonol and methoxy carbon of compound 100 respectively. The methine carbons at δ 129.7 (C-2', C-6') and δ 114.5 (C-3', C-5') were due to symmetrically placed aromatic carbons on asymmetrically *para* substituted B-ring of the flavonoid. The compound exhibited oxygenated aromatic quaternary carbons at δ 146.4 (C-2), δ136.5 (C-3), δ 161.1 (C-5), δ 164.4 (C-7), δ 159.6 (C-9) and δ 160.9 (C-4'). The additional quaternary carbons observed were at 123.7 and 103.5 due to C-1'and C-10 respectively. All physical and spectroscopic data were compared with literature reports of kaempferide isolated from the same plant and was found in a good agreement (Shehu et al., 2016).

Table 4 (here)

Based on all physical and spectroscopic data, compound **2**was proposed to be kaempferide.

Compound 1

In the investigation of secondary metabolites from *P. schimperi* and other biologically active plants, compound **1** was isolated from fraction 5 as a white powder (29 mg) melting at 75-76ºC. The TLC developed using mobile phase hexane: EtOAc (4:1) was visualized as a pink spot (Rf 0.5) after spraying with vanillin in sulfuric acid. The UV-Vis spectrum (in $CHCl_3$) showed absorption neither in the UV nor in the visible region. The IR spectrum displayed signals at 3395 cm^{-1} (for O-H stretching), 2924 and 1470 cm^{-1} (for C-H stretching and bending) and 708 for C-C stretching. The 1H-NMR spectrum (in CDCl3, Appendix 10) demonstrated a triplet signal at δ3.66 (J=6.4 Hz) assigned to methylene protons on oxygenated carbon. The quintet signal at δ 1.58 is due to methylene protons on carbon flanked between two methylene groups. A broad signal at δ1.27 (48H) is a characteristic signal for several overlapping methylene protons. The presence of terminal methyl group in the compound is confirmed with an upfield triplet signal appeared at δ 0.90 (3H, J=6.7 Hz). The ^{13}C-NMR spectrum showed a downfield signal at δ63.1 corresponds to an oxygenated methylene carbon. Furthermore, the carbon signal observed at δ14.1 is a characteristic signal for terminal methyl groups. The ^{13}C-NMR data with the help of DEPT-135 indicative the compound is a straight chain alcohol. The number of methylene groups may be revealed by generation of MS data.

Compound 1(29 mg): oil like jelly material; soluble in $CHCl_3$; Rf 0.68 (mobile phasehexane: EtOAc, (4:1); UV (EtOH) λmax: noabsorption; Mp: 75-76ºC; IR υ_{cm-1}: 3395 cm^{-1} (O-H), 2924 and 1470 for C-H stretching and bending and 708 for C-C stretching; ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift δin ppm, coupling constant J in Hz: δ_H 3.66 (2H,*t, J*=6.6 Hz, H-1), 1.59 (2H, *m*, H-2), 1.27 (48H, *br s,*H-3 to H-26), 0.90 (3H, *t*, *J*=6.7 Hz, H-27); ^{13}C NMR (100 MHz, CDC_3): δ_C 63.12 (C-1), 32.83 (C-2), 31.94 (C-3), 29.71-29.37 (C4-C24), 25.76 (C-25), 22.70 (C-26) and 14.11 (C-27).

- **Conclusion**

Plant material and extracts have been used to control invasive weeds. The use of plant materials as herbicides is considered to be environmentally safe. Furthermore, plant materials are readily available, renewable and chances ofweeds developing resistance are negligible. Kaempferide is isolated as promising phytotoxic compound for the control of *p. hysterophorus* weed. Future studies aimed at isolating other minor compound and determining synergism and antagonism effects of the bioactive compounds from *P. schimperi* are necessary to determine the combinations that offer the best control against P. hysterophorus weed.

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Conflict of interest

There is no known financial and any other conflicts between authors.

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Table 1. ***In vitro*** **phytotoxicity results of compounds isolated from** ***P. schimperi***

compound	number of germinated seeds					
	T1	T2	T3	mean	STDV	%GI
1	0	0	0	0	0	100
2	0	0	0	0	0	100
3	0	0	0	0	0	100
Roundup®	0	0	0	0	0	100
5% acetone	8	7	9	8	1	20%

Table 2. ***In vivo*** **result of pure compounds from** ***P. schimperi***

compounds	ADB of seedlings before treatment (mg)				ADB of seedling after treatment(mg)				Mass difference	
	T1	T2	T3	M1	T1	T2	T3	M2	M2-M1	%GI
1	96	100	104	100	1990	1995	2015	2000	1900	42
2	96	100	104	100	540	532	488	520	420	87
3	96	100	104	100	2455	2420	2475	2450	2350	29
Roundup®	96	100	104	100	140	141	139	140	40	98
5% acetone	96	100	104	100	3400	3444	3436	3426	3326	0

Table 3. NMR data table comparison of compound 3 with known diterpene

Experimental data of compound **3**		Literature data of clerodane diterpene(S. Habtemariam et al., 1990)	
^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
1 19.6		19.7	
2 36.1		36.3	
3 120.5	5.15 (1H, *s*)	120.6	5.1 (1H, *s*)
4 143.3		143.4	
5 38.3		38.5	
6 27.4		27.6	
7 26.5		26.6	
8 37.1	1.96 (1H, *m*)	37.0	1.88 (1H, *m*)
9 42.1		42.3	
10 46.6	1.68 (1H, *dd*)	46.8	1.68 (1H, *dd, J* = 12.1)
11 43.3	2.85, 2.61 (2H, *dd*, *J*=15.2)	43.5	2.88, 2.59 (2H, *dd*, *J*=15.2Hz)
12 201.3		201.2	
13 143.8		143.8	
14 36.1	3.30 (2H, *s*)	36.3	3.27 (2H, *s*)
15 176.1		176.1	
16 126.6	5.92 (1H,*s*), 6.19 (1H,*s*)	126.6	5.87 (1H, *s*), 6.14 (1H, *s*)
17 17.6	0.86 (3H, *d, J=6.8 Hz*)	17.6	0.83 (3H, *d*, *J* = *6.8Hz*)
18 19.9	1.57 (3H, *s*)	20.0	1.53 (3H, *s*)
19 17.9	0.96 (3H, *s*)	18.0	0.96 (3H, *s*)
20 16.6	0.82 (3H, s)	16.6	0.78 (3H, s)

Table 4. ^{1}H and ^{13}C-NMR data of compound 2 and literature report of kaempferide

Experimental data of compound **2**		Literature data of Kaempferide(Shehu et al., 2016)	
^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
2 146.4		146.3	
3 136.5		136.1	
4 176.4		176.1	
5 161.1		160.8	
6 98.7	6.20 (1H, *s*)	98.3	6.18 (1H, *d*)
7 164.4		164.3	
8 93.9	6.45 (1H, *s*)	93.6	6.37 (1H, *d*)
9 156.6		156.3	
10 103.5		103.7	
1' 123.7		123.3	
2' 129.7	8.12 (1H, *d, J=8.8*)	129.4	8.12 (1H, *d*)
3' 114.4	7.09 (1H, *d*)	114.1	7.09 (1H, *d*)
4' 160.9		160.6	
5' 114.4	7.09 (1H, *d*)	114.1	7.09 (1H, *d*)
6' 129.7	8.14 (1H, *d*)	129.4	8.12 (1H, *d*)
O-CH_3 55.8	3.83 (3H, *s*)	55.4	3.83 (3H, *s*)
3-OH	9.47 (1H, *s*)		9.45 (1H, *s*)
5-OH	12.45 (1H, *s*)		12.32 (1H, *s*)
7-OH	10.83 (1H, *s*)		10.57 (1H, *s*)