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Chemical investigation in *Parthenium hysterophorus* – An allelopathic plant+

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ABSTRACT

Chemical investigation on the aqueous and the chloroform extracts of *Parthenium hysterophorus* has resulted in the characterisation of several phenolic acids and pseudoguaianolides. Caffeic acid, *p*-coumaric acid and parthenin were the major constituents. Two parthenin derivatives, 8ß-hydroxyparthenin and anhydroparthenin isolated from the chloroform extract, have not previously been reported in nature. The soil allelochemics extracted from *Parthenium* free soil were composed of the same phenolic acids and pseudoguaianolides as were identified from the aqueous extract of the plant.

Key words : Allelochemics, *Parthenium hysterophorus*, phenolic acids

INTRODUCTION

Parthenium hysterophorus L. (Compositae), endamic to the Amercias, has now been identified as a troublesome weed as it is responsible for allergic contact dermatitis in humans and acute illness in cattle and is a menace to agricultural productivity (14). The weed suppresses crops and plants around it by virtue of its rapid growth and allelopathic effects (3, 6, 7, 14). As a part of our research on the characterisation of bioactive compounds from natural source (1, 2), we have investigated the allelochemicals in *P. hysterophorus*. The soil chemics extracted from *Parthenium* free soil were also identified.

MATERIALS AND METHODS

Air-dried mature whole plants of *Parthenium* (1 kg) were crushed, powdered and extracted with 5.0 l sterilized (autoclaved) water at room temperature (28°C) in the dark for five days. The aqueous extract was freeze-dried and re-extracted with ethyl acetate (3 x 500 ml). The ethyl acetate extract was concentrated to a thick brownish residue (62 ml). The acidic fraction was separated by extracting with 0.1 N sodium bicarbonate solution (3 x 200 ml) and subsequently acidifying the extract with hydrochloric acid to pH 2.5. Finally the acidic compounds were extracted with ethyl acetate (3 x 200 ml). The extract was concentrated, analysed with TLC and the

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components were separated by reverse-phase HPLC using ODS column and methanol-acetonitrile-water (2 : 1 : 3) as the running solvent. The compounds were identified by spectroscopic methods (IR, ^{1}H-NMR and MS) and co-TLC and superimposable IR spectra with the authentic samples.

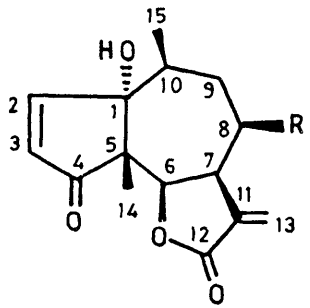
The non-acidic fraction was purified by silica gel column chromatography and then by reverse-phase KPLC. The separated constituents were characterised by detailed spectroscopic analysis (IR, ^{1}H-NMR and MS).

In another experiment, the powdered whole plants of *Parthenium* (1 kg) were extracted with chloroform (3 x 1 l) at room temperature. Each extraction was continued for seven days. The total chloroform extract was concentrated and the components were separated by column chromatography over silica gel followed by reverse-phase HPLC.

The soil chemics were extracted from *Parthenium* free soil (30 cm x 30 cm x 10 cm). The collected soil was shade-dried and ground. The *Parthenium* plant fractions were at first separated from the soil carefully with hands. The material was sieved through 2 mm sieve. The soil produced a colloid suspension when shaken with 5.0 l water. The remaining plant materials present in the soil floated on the suspension and these were separated by filtration through cotton bed. The cotton bed was subsequently washed with water. 4.0 l methanol was added to the combined suspension and the mixture was churned for 24 h. The extract was decanted and the residue was thoroughly washed with methanol (3 x 50 ml) which was also added to the total extract. Methanol was then removed from the extract under reduced pressure. The remaining extract was freeze-dried and re-extracted with ethyl acetate (3 x 200 ml). The acidic and non-acidic fractions were separated from this extract by the method discussed earlier. The components were identified by co-TLC with authentic samples and by analytical HPLC. The co–TLC experiment was done by applying a mixed TLC spot of the components and authentic samples on a TLC plate (precoated with silica gel) where the spots of the components and each authentic sample were also applied separately one after another at the same height in a line by the side of the first spot. Several TLC plates were prepared by applying the similar spots and then run separately in different solvent systems. The components were identified by matching their Rf values with those of authentic samples. Further confirmation of identification of the components was made from an examination of their retention times by analytical HPLC. However, the separation and quantitative estimation of the compounds were not possible due to paucity of the materials.

RESULTS AND DISCUSSION

The aqueous extract of the whole plants of *Parthenium* yielded phenolic acids and pseudoguaianolides (Table 1 and Fig. 1). Six phenolic acids – caffeic acid, *p*-coumaric acid, anisic acid, ferulic acid, *p*-hydroxybenzoic acid and vanillic acid have been earlier isolated (14). Caffeic acid and *p*-coumaric acid were the major phenolic acids in the extract. The pseudoguaianolides were of the sesquiterpene lactone type. Parthenin **(I)** (4, 10), coronopilin **(II)** (9) and damsin **(III)** (13) have been characterised. Parthenin was the major pseudoguaianolide. Previously it was also found as a major sesquiterpene constituent in different populations of *Parthenium* (14).



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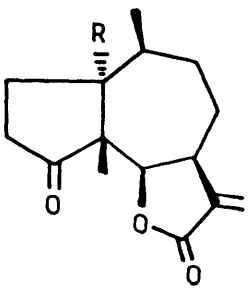
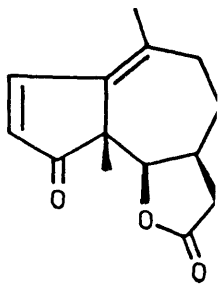
IV R = OH



II R = OH
III R = H



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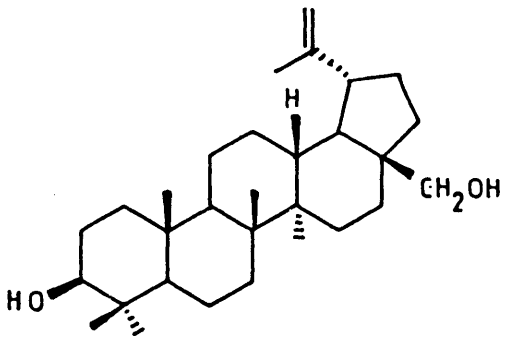

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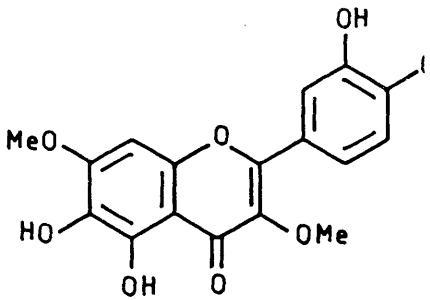

VII

Fig. 1. Structures of complex organic compounds isolated from *P. hysterophorus* - **I** : Parthenin; **II** : Coronopilin; **III** : Damsin; **IV** : 8 β-Hydroxyparthenin; **V** : Anhydroparthenin; **VI** : Betulin and **VII** : Quercetagetin-3, 7-dimethylether.

TABLE 1. Compounds isolated from the whole plants of *Parthenium*

Compound	Yield (mg/kg of the plant)
A. Aqueous extract	
(a) Phenolic acid	
(i) Caffeic acid	102
(ii) *p*-coumaric acid	56
(iii) Anisic acid	41
(iv) Aerulic acid	38
(v) *p*-hydroxybenzoic acid	25
(vi) Vanillic acid	17
(b) Pseudoguaianolide	
(i) Parthenin	78
(ii) Coronopilin	14
(iii) Damsin	3
B. Chloroform extract	
(a) Pseudoguaianolide	
(i) Parthenin	876
(ii) Coronopilin	132
(iii) Damsin	19
(iv) 8 β-hydroxyparthenin	7
(v) Anhydroparthenin	16
(b) Sterol	
β-sitosterol	287
(c) Triterpenoid	
Betulin	12
(d) Flavonoid	
Quercetagetin-3, 7-dimethylether	14
C. Compounds in *Parthenium* soil	
Same as shown in A	Quantitative estimation of the compounds was not possible due to paucity of the material.

From the chloroform extract, five pseudoguaianolides – parthenin **(I)** (4), coronopilin **(II)** (9), damsin **(III)** (13), 8 β-hydroxyparthenin **(IV)** and anhydroparthenin **(V)** have been isolated alongwith β-sitosterol, a triterpenoid, betulin **(VI)** (8) and a flavonoid, quercetagetin-3, 7-dimethylether **(VII)** (12). The last two pseudoguaianolides, 8ß-hydroxyparthenin **(IV)**, $C_{15}H_{18}O_5$, m.p. 155-156°C (MeOH) and anhydroparthenin **(V)**, $C_{15}H_{16}O_3$, m.p. 127-128°C (C_6H_6) were not previously reported. The structure of these two compounds was established from detailed NMR spectral studies (Table 2) and by comparison of their spectral data with those of parthenin (4) and 8 β-hydroxycoronopilin **(II)**. The acid catalysed dehydration of parthenin **(I)** to anhydroparthenin **(V)** could be effected (4). As *Parthenium* contains different acids, the possibility of **(V)** as an aertefact during extraction cannot be ruled out.

TABLE 2. NMR spectral data of 8 β-hydroxyparthenin (IV) and anhydroparthenin (V) [in ppm]

Position	^{1}H-NMR (400 MHz, d_4-MeOH)		^{13}C-NMR (100 MHz, d_4-MeOH)	
	Compound (IV)	Compound (V)	Compound (IV)	Compound (V)
1			84.6	133.4
2	7.54 (d, J=6.0 Hz)	7.87 (d, J=6.0 Hz)	161.7	158.2
3	6.15 (d, J=6.0 Hz)	6.11 (d, J=6.0 Hz)	129.6	128.7
4			208.3	210.1
5			58.6	60.4
6	4.83 (d, J=7.5 Hz)	4.45 (d, J=7.2 Hz)	81.2	80.7
7	3.35 (m)	3.32 (m)	45.8	44.8
8	3.97 (brs)	2.62 - 1.78 (m)	80.7	27.4
9	2.82 - 2.02 (m)		29.4	28.6
10			42.5	145.4
11			142.6	141.7
12			171.9	171.8
13	6.18 (d, J=3.0 Hz) & 5.62 (d, J=3.0 Hz)	6.27 (d, J=3.0 Hz) & 5.58 (d, J=3.0 Hz)	123.0	123.4
14	1.28 (s)	1.34 (s)	15.6	16.5
15	1.16 (d, J=8.0 Hz)	2.02 (s)	17.2	20.8

The soil allelochemics extracted from *Parthenium* freed soil were the same six phenolic acids and three pseudoguaianolides which were characterised from the aqueous extract of the whole plant. However, the quantitative determination of the compounds was not possible as the amount of the total material was very low. This observation indicated that the compounds from *Parthenium,* which were extractable with water, were absorbed on the soil.

The sesquiterpenes, parthenin **(I)**, coronopilin **(II)** and damsin **(III)** and the phenolic acids isolated from *P. hysterophorus* are well known as allelopathic agents (3, 5, 7, 14). Thus, the allelopathic activity of the weed is due to the presence of these chemicals in the weed and in the soil close to the weed.

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