



International Journal of Emerging Technologies in Computational and Applied Sciences (IJETCAS)

www.iasir.net

Isolation, characterization of sesquiterpene parthenin and its estimation from *Parthenium hysterophorus* pollen

U. G. Basarkar* and A. A. Saoji
P. G. Department of Botany
HPT Arts /RYK Science College
Prin. T. A. Kulkarni Vidyanagari
College Road Nashik – 422 005 (MS) India

Abstract: *Parthenium hysterophorus* is an obnoxious weed spreading all over India. It causes severe allergic diseases like Hay-fever, asthma and dermatitis. The whole plant is allergenic especially pollen grains of this plant are responsible for several allergic diseases. The meager amount of work is available on chemical nature of pollen. Therefore in the present study, isolation, characterization and estimation of sesquiterpene lactone, parthenin from pollen was carried out. From IR, UV, Mass and PMR spectra and Co-TLC, isolated compound was confirmed as parthenin. It is found to be present in considerable amount in pollen grains of *Parthenium*.

I. INTRODUCTION

Parthenium is an obnoxious weed of worldwide distribution and tops the seven most dangerous weeds in the world. In India it is an introduced weed. It has covered about five million hectares of land in different parts of India replacing many dominant plants in wide range of habitats including cultivated fields. The *Parthenium* weed is known to cause tremendous health hazards. A health hazard caused by *Parthenium* is reached to such an extent that about 12 deaths are reported in severely affected patients by recurrent infection (Lonkar et al., 1974). Ranade (1975) reported that pollen of this weed was the main cause for eczematoid dermatitis.

Another important aspect of *Parthenium* toxicity is concerned with human health. The sesquiterpene lactones of plant are secreted in milk. When ingested by the milch animals due to which milk becomes bitter in taste and parthenin exerted in milk if consumed by susceptible human patients produces similar types of allergy. The whole weed is allergenic, especially the pollen grains which float freely in air, polluting it and causing severe allergic diseases like asthma, dermatitis and hay fever. All parts of the plant contain toxic substances such as parthenin, sesquiterpene lactone and ether phenolics. Toxicity of the plant is said to be mainly due to these substances.

From the available literature it was observed that a meager amount of work is carried on chemical nature of pollen. Considering allergenic importance of *Parthenium* pollen, attempts were made to isolate sesquiterpene lactone, parthenin from pollen grains. Though some workers have reported its presence from other parts of the plant, this is the first report of isolation and estimation of parthenin from the pollen grains of *Parthenium hysterophorus*.

II. MATERIAL AND METHODS

100 grams of the pollen grains of *Parthenium* were collected and extracted with the solvents to get ingredients as follows.

The pollen grains were extracted continuously with methanol in a Soxhlet's apparatus for 24 hours. The resulting extract was dark brown. This extract was then evaporated to dryness in a flash evaporator at room temperature and the residue obtained was refluxed with petroleum ether. The refluxed petroleum ether extract was designated as fraction – I. The portion which did not go into the solution with petroleum ether was successively extracted with chloroform and ethanol. The chloroform extract was evaporated to dryness, residue was dissolved in hot ethanol and the volume was made up to 200 ml. After cooling, equal volume of 4% aqueous lead acetate solution was added to this ethanol extract. The mixture was allowed to stand at room temperature for 20 minutes. This gave the precipitate which was removed by filtrations through a pad of diatomaceous earth and the clear filtrate was concentrated to half its volume. This removes most of the ethanol. The aqueous solution thus left was extracted with 50 ml portions of chloroform. All the chloroform extracts were combined and then dehydrated with anhydrous sodium sulphate. Then evaporated to dryness in *vacuo*. This gave dried mass. This dried mass was dissolved in 15 ml of benzene and acetone (2:1) and subjected to silica gel column chromatography. The column (3 x 55 cm) was first eluted with benzene (200 ml) then it was eluted with 200 ml portions of a solvent containing increasing amounts of acetone in benzene (10 – 100%). The fractions (200 ml of each) were evaporated to dryness and analyzed by thin layer chromatography (TLC) on

silica gel G plates using benzene: acetone (4: 1) as a solvent system. The compounds were located on TLC plates by exposing to iodine vapors. This yielded one predominant spot and a few minor spots. The predominant spots having the same R_f value were pooled and subjected to preparatory thin layer chromatography using benzene: acetone (4: 1) as the solvent system. The area corresponding to the predominant spot was scrapped and eluted with chloroform. The chloroform elute was evaporated and the residue was dissolved in minimal amount of ethyl acetate and crystallized. The compound was again twice crystallizes by drop wise addition of cyclohexane. This crystallizes compound was designated as P – 1.

Fraction – I and successively extracted ethanol were evaporated. They gave meager quantity of residue which could not be crystallized. So they were discarded. Isolated compound P – 1 was identified and confirmed by melting point, IR, UV, Mass, NMR and Co – TLC.

Co – TLC and estimation of parthenin from pollen of *Parthenium*:

Thin layer chromatography (Egon Stahl, 1969) involves preparation of plates, activation and storage of plates, spotting of test substance, developing and visualization of spots. The plates of thin layer were prepared on 20 x 20 cm sq. glass plates as follows.

30 grams of silica gel G with calcium sulphate (13%) as binder were weighed and then taken in a beaker. 60 ml of distilled water added to it and shaken continuously for 45 seconds to obtain slurry of silica gel. This slurry was poured into applicator adjusted to a thickness of 0.25 mm. the slurry was spread over plates gently and uniformly. After spreading, the plates were allowed to dry in situ at room temperature for 30 minutes. The plates after air drying were placed in aluminum racks. The racks were then transferred to hot air oven. The plates were activated in an oven at 110 °c for one hour. The plates were removed and stored in desiccators 2 – 5 mm margin from thin layer of all sides was stripped off before use.

Spot of 0.01 ml of authentic parthenin and P – 1 compound were applied at a distance of 20 mm from the edge of the plate and 2 cm apart from adjacent spot. The plate was then kept in a chamber containing benzene : acetone (4:1) as solvent system for ascending chromatography. The chamber was saturated with benzene acetone (4:1) with the help of filter paper linings on either side. It was ensured that only 10 mm of the plate dipped in solvent. The plate was developed for about 30 minutes and solvent was allowed to travel 12 cm from starting point which was duly marked. The plate was then taken out from developing chamber. The developing solvent on the plate was allowed to evaporate at room temperature.

The plate was then exposed to detecting reagent, iodine vapors and sprayed evenly with sulphuric acid: nitric acid (1:1). The plates were then activated at 110 o c for 10 minutes in hot air oven.

The color of the spots applied on plate was appeared after activation. The R_f value was calculated as follows: -

$$R_f = \frac{\text{Distance of spot centre from starting point}}{\text{Distance of solvent front from starting point}}$$

Estimation of parthenin was carried out by using standard curve (Fig. –1) prepared by measuring optical densities of different concentrations of standard (authentic) Parthenin at 340 nm on UV spectrophotometer.

III. RESULTS

Fractionization of methanolic extract of air dried pollen powder of *Parthenium hysterophorus* resulted in isolation of solid crystallizes substance named as P – 1. Co – TLC of the P – 1 compound showed single yellow colored spot having R_f value 0.50 (chromatogram – I) in benzene: acetone (4:1) solvent system. The melting point of the compound was found to be 162 – 164 o c. the concentration of parthenin was found to be 0.038% in pollen.

UV (Me OH) 215, 340: (Fig. – 2)

UV band at 340 nm indicating the presence of lactone ring while peak at 215 nm assigned for hydroxyl group.

IR Spectrum KBr cm⁻¹: (Fig. 3)

IR spectrum showed following bands 3391-2967, 2937, 1737-1714, 1650, 1480-1450, 1405-1321, and 1165-1025----- cm⁻¹. In the IR spectrum, band at 3391-2967 cm⁻¹ accounted for the presence of –OH group. Band in the region of 2937 cm⁻¹ is assigned to C – H stretching of alkane and the band at 1737-1714 cm⁻¹ was due to conjugated C = O stretching of five membered lactone ring. Band at 1650 cm⁻¹ was due to C = C of five membered ring while band at 1480-1450 cm⁻¹ was assigned to C – H bending of alkane. Band at 1405-1321 cm⁻¹ were due to O – H bending vibration. Band at 1156-1025 cm⁻¹ were C – O of lactone ring.

Mass spectrum: (Fig. – 4)

Molecular ion peak was observed at M⁺ at 264. Other fragments were observed at M/e 249, 234, 216, 161, 123, 105 -----, Molecular ion peak at M⁺ 264 indicated molecular weight of the compound. Peak at M/ e 249 was due to loss of methyl (-CH₃) group. Another peak at M/e 216 was due to loss of water molecule (-H₂O). Peak at M/e 161 and M/e 161 and M/e 123 were due to loss of carboxyl (C=O) group.

Protone magnetic resonance (PMR): (Fig. – 5)

PMR of P – 1 compound showed signals at delta (CDCl_3) 7.64 (d, H_2), 6.1 (t, H_3), 5.65 (d, H-13 b), 4.8 (s, H-7), 4.95 (d, H – 5), 1.65 (g, C-Me). In PMR spectrum of compound a broad doublet at delta 7.64 was assigned for three methyl protons. Another doublet at delta 5.65 indicating seven protons of six membered rings while quadrate at delta 1.65 indicating total protons of methyl group.

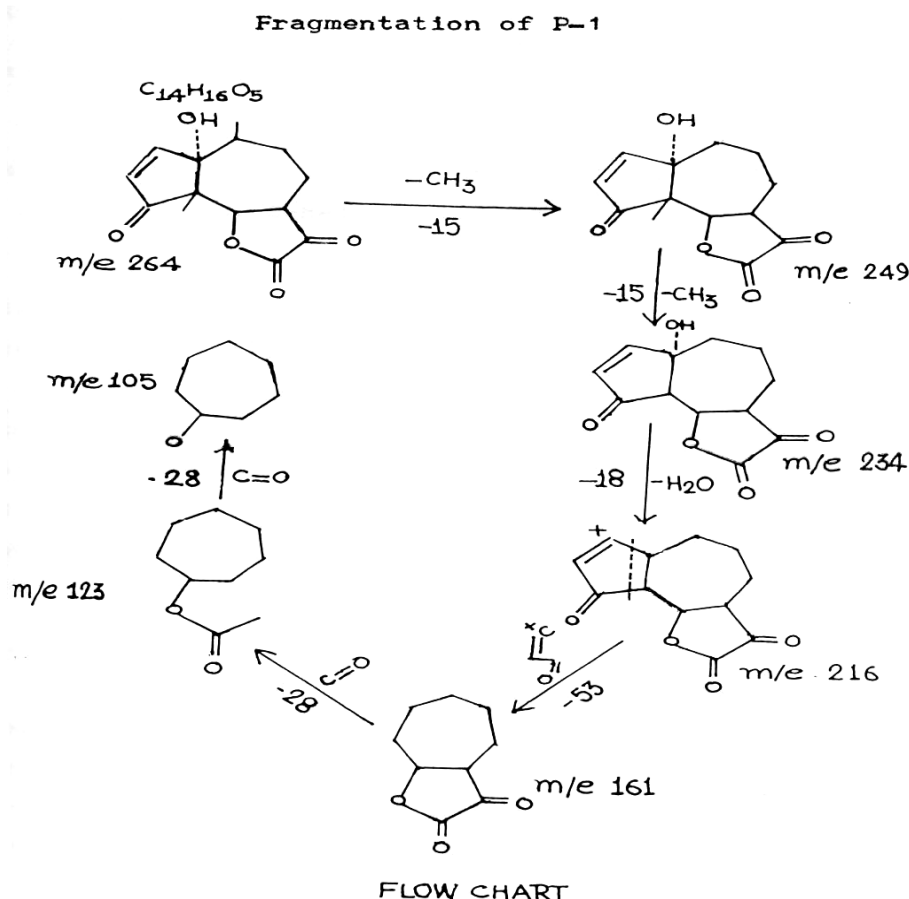
Thus from the above data of melting point, TLC and spectrum analysis such as IR, UV, Mass and PMR of P – 1 compound, were found to be identical with the authentic parthenin. Therefore P – 1 compound was identified and confirmed as parthenin.

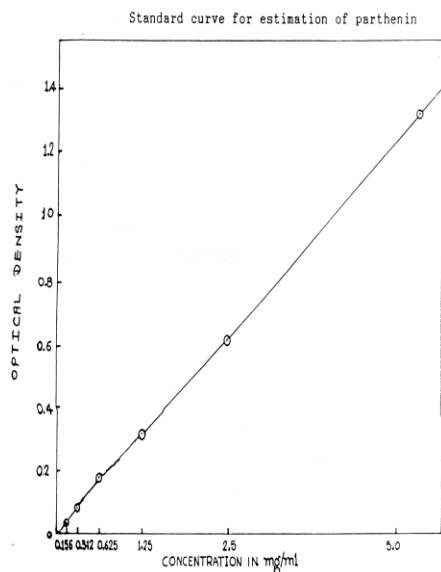
IV. DISCUSSION

When revealed through literature, it was observed that data is not available on quantitative estimation of sesquiterpene lactone, parthenin from the pollen grains of *Parthenium*.

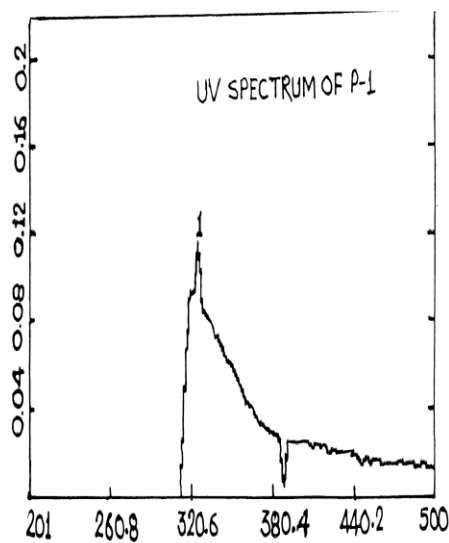
Army (1897) isolated an apparently non alkaloidal, non glycosidal substance, parthenin by extracting the plant with dilute alcohol. This crystalline material concentrated in the leaves and reached at peak in late summer. Trichomes of *Parthenium* contain allergenic sesquiterpene lactone as high as 8 % of the dry weight. Parthenin being the major component of it (Rodriguez *et al.*, 1971). Kanchan (1975) reported that concentration of growth inhibitors namely parthenin was highest in the leaves followed by inflorescence, fruits, and stems. Lonkar *et al.*, (1976) analyzed the fractions containing parthenin and compounds of similar polarity are the active allergens. Lonkar (1976) applied the patch test and found that all patients presenting the typical picture of *Parthenium* dermatitis showed positive results to patch test with flower, leaf and also with parthenin.

Previous reports have implied that sesquiterpene lactones are synthesized in Trichomes (Rodriguez *et al.*, 1975), Wicham *et al.*, (1980) reported that these compounds may be produced elsewhere e. g. chloroplast (Goodwin, 1967) and probably transported to Trichomes. They further reported that parthenin was not detected in roots of mature plant but was present in callus cultures derived from same plant. Our investigation revealed that parthenin is present in *Parthenium* pollen in considerable amount (0.038%), may be the principle cause in precipitating human allergy due to pollen grains.

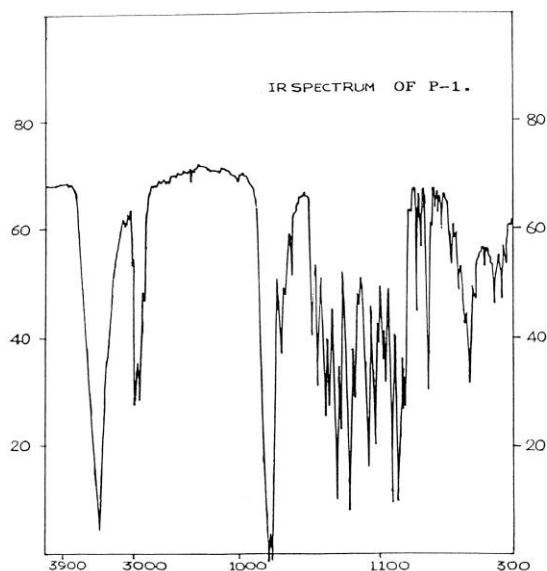




Co - TLC (Fig. -1)

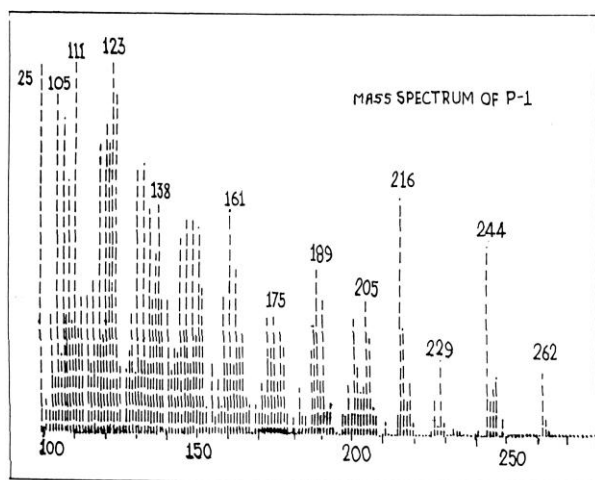


UV (Me OH) 215, 340: (Fig. - 2)

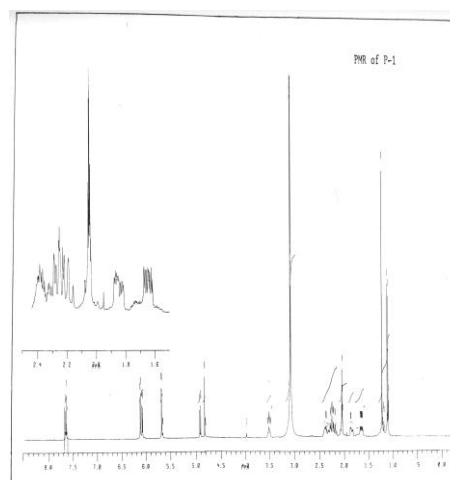


IR Spectrum KBr cm⁻¹: (Fig. 3)

PEAK	THRESHOLD	2% T		
CM-1	% T		CM-1	% T
3391.0	4.12		616.0	42.04
2967.0	27.55		597.0	31.07
2937.0	28.54		557.0	46.51
2331.0	68.41		505.0	52.26
1737.0	- 1.60		450.0	45.94
1714.0	- 1.37		412.0	46.50
1658.0	36.97		389.0	53.17
1593.0	51.14			
1480.0	49.34			
1450.0	30.91			
1405.0	25.05			
1384.0	27.19			
1342.0	9.74			
1321.0	22.44			
1276.0	7.54			
1247.0	28.67			
1221.0	45.69			
1156.0	15.82			
1122.0	19.78			
1097.0	38.92			
1075.0	35.50			
1060.0	31.49			
1023.0	9.00			
989.0	7.21			
962.0	26.37			
952.0	27.06			
880.0	44.67			
856.0	56.15			
825.0	29.77			
816.0	36.77			
781.0	63.18			
763.0	62.28			
742.0	59.13			
687.0	52.86			
649.0	48.22			



Mass spectrum: (Fig. - 4)



Protone magnetic resonance (PMR): (Fig. - 5)

REFERENCES

- [1]. Arny, H. V. *Parthenium hysterophorus*. Am. J. Pharm. 69:168, 1897.
- [2]. Egon Stahl, Thin layer chromatography. A laboratory handbook. Springer – Verlag Berlin Heidelberg, New York, Toppan Co. Ltd., Tokyo, Japan, 135, 1969.
- [3]. Goodwin, T. W., Regulation of terpenoid synthesis in plants IN : T. B. Pridham ed. Terpenoids in plants, Academic Press, New York. 57 : 1967.
- [4]. Kanchan S. D., Growth inhibitors from *Parthenium hysterophorus* L. Curr. Sci. 44:358-359, 1975.
- [5]. Lonkar, A., Mitchell, J. C. and Calman, C. D. Contactdermatitis from *Parthenium hysterophorus* Trans. St. Johns. Hosp. Derma. Soc. (Lond.) 60(1) : 43-53, 1974.
- [6]. Lonkar, A., Nagasampagi, B. A., Narayan, C. R., Landge, A. B. and Salvaikar, D. D. Contact dermatitis. 2:151-153, 1976.
- [7]. Ranade, S. Results of newly synthesizes vaccine in cases of congress eczema in India. Paper read at Aswin Naval Bose Hospital, Bombay, 1975.
- [8]. Rodriguez, E., Yashika, H. and Mabry, J. J. The sesquiterpene lactone chemistry of the genus *Parthenium* (Compositae). Phytochemistry. 10:114, 1971.
- [9]. Rodriguez, E. Chemistry and distribution of sesquiterpene lactone and flavonoides in *Parthenium* (Compositae) systematic and ecological implication. Ph. D. Thesis., Univ. of Texas, Austin, 1975.
- [10]. Wicham, K., Rodriguez, E. and Joseph, A. Comparative phytochemistry of *Parthenium hysterophorus* L. (Compositae). Bot. Gaz. 141 (4): 435-439, 1980.