*Asian Journal of Chemistry* Vol. 20, No. 2 (2008), 987-991

# Phytochemical Studies on *Parthenium hysterophorus* Linn.

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Various fractions obtained from *Parthenium hysterophorus* contains antiinflammatory activities . The fraction obtained from diethyl ether was found to be a flavonol galangin. Aglycone and glucose fractions were also obtained during fractionation. All the fractions were characterized by UV-spectral analysis. The ethyl acetate fraction obtained was analyzed for its microbial activity, which showed a dose-dependent activity against heamolysis.

**Key Words: Phytochemical, *Parthenium hysterophorus* Linn.**

## INTRODUCTION

*Parthenium hysterophorus* Linn. is a shrub found as weeds throughout Tamilnadu. It belongs to Asteraceae a sub order of compositae, contains 400 species[1]. Crushed leaves of *P. hysterophorus* is used for snake bite and spider bite. The plant is also used for cholagogue[2]. In present studies, the flowers of *P. hysterophorus* have been examined for their polyphenolic constituents and the results are presented.

## EXPERIMENTAL

About 1 kg of fresh flowers of *P. hysterophorus* collected in and around A.V.C. College campus at Mannampandal during October were extracted with 95 % ethanol under reflux. Above 2 L of AnalaR grade ethanol is used for extraction. The alcoholic extract was concentrated *in vacuo* and the aqueous concentrate was fractionated with above 750 mL of benzene, 1 L of peroxide-free diethyl ether and 2 L of ethyl acetate. No crystalline solid could be recovered from benzene fraction.

The residue from diethyl ether fraction was taken up in ice-chest for a few days, when yellow needles were separated. It was identified as a flavonol-galangin. It had $\lambda_{max}^{MeOH}$ nm 267, 305 sh, 359; + NaOMe 280, 327 sh, 412; + $AlCl_3$ 249, 273, 300 sh, 334, 412; + ($AlCl_3$ + HCl) 249, 274, 302 sh, 334, 412; NaOAc 275, 301 sh, 328 sh, 388, + (NaOAc + $H_3BO_3$) 267, 300 sh, 361.

It is soluble in organic solvents and sparingly soluble in hot water. It gave a golden yellow colour with NaOH, orange red colour with Mg-HCl

and violet colour with $FeCl_3$. It responded Wilson's boric acid[3], Horhammer-Hansel[4] and Gibb's[5] tests but did not respond to Molisch's test. $R_f$ values as given in Table-1, which was identified as galangin. This was further confirmed by mixed paper chromatography and m.m.p. with an authentic sample of galangin, isolated from *Alpinia officinarum*.

TABLE-1
$R_f$ (X100) VALUES OF THE CONSTITUENTS OF THE FLOWERS OF *Parthenium hysterphorus*
(Whatmann filter paper No. 1, Ascending 30 ± 2 ºC)

| Compound | Developing solvents* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H |
| Glycoside obtained from ethyl acetate fraction | 18 | 26 | 47 | 69 | 82 | 79 | 60 | 85 |
| Aglycone from the diethyl ether fraction | 8 | - | 9 | 21 | 56 | 96 | 92 | 71 |
| Galangin (authentic) | 8 | - | 10 | 22 | 56 | 95 | 94 | 72 |

*Solvent key; A= $H_2O$; B = 5 % Acetic acid; C =15 % Acetic acid; D = 30 % Acetic acid; E = 60 % Acetic acid; F = *n*-BuOH:AcOH:$H_2O$ = 4:1:5 (upper phase) (BAW); G = Water saturated phenol; H = AcOH:conc. HCl:$H_2O$ = 30:3:10 (Forestal).

The fraction obtained from ethyl acetate was concertrated *in vacuo* and left in an ice for a week. A yellow solid separated was filtered and studied. It was recystallized from methanol to obtain yellow crystals (Yield 0.005 %). It was identified as flavonol glucoside-galangin-3-O-glucoside. It is soluable in ethyl acetate, methanol and sparingly in water. It gave olive green with alcoholic $Fe^{3+}$, deep pink colour with Mg-HCl, yellow colour with NaOH and appeared deep purple under UV as well as on exposure to $NH_3$. It did not respond Horhammer Hansel test but responded to the Wilson's boric acid Gibb's and Molisch's test. It had $R_f$ as indicated in Table-1 and had $\lambda_{max}^{MeOH}$ nm 250, 310sh, 340; + NaoMe 265, 327sh, 356; $AlCl_3$ 270, 320, 385, + $AlCl_3$ + HCl 270, 317, 385; NaOAc 270, 330 and NaOAc/$H_3BO_3$ 251, 315sh, 337.

**Hydrolysis of the glycoside:** The glycoside (0.05 g. 0.02 m mol) was dissolved in hot aqueous methanol (2 mL, 50 %) and an equal volume of $H_2SO_4$ (7 %) was added to it. The reaction mixture was refluxed at 100 ºC for 2 h. The excess of alcohol was distilled off from the hydrolyzate and the resulting aqueous solution was diluted with more water and left under chilled conditions for 2 h. A yellow solid separated was filtered washed and dried. The aqueous filterate and the washings were extracted with diethyl ether. The dry yellow residue on the filter paper was combined with the residue from the dried diethyl ether and studied for the aglycone.

**Identification of aglycone: (flavonol:galangin):** The yellow coloured aglycone on recrystallization from methanol afforded a yellow crystalline solid, m.p. 278-280 ºC which was identified as galangin by colour reactions, behaviour under UV and $R_f$ (Table-1).

**Identification of sugar: (glucose):** The aqueous hydrolyzate after the removal of the aglycone was neutralized with $BaCO_3$ and filtered. The concentrated filtrate on PC gave $R_f$ values as shown in Table-2 corresponding to those of glucose. The identification of the sugar was further confirmed by direct comparison with an authentic sample of glucose as also by preparation its osazone and recording its m.p. A quantitative hydrolysis of the same by the Folin-Wu's micro method revealed it to be a monoside.

TABLE-2
$R_f$ (X100) VALUES OF THE SUGAR FROM THE GLYCOSIDE OF *Parthenium hysterphorus*
(Whatmann filter paper No. 1, Ascending 30 ± 2 ºC)

| Sugar | Developing solvents* | | | |
|---|---|---|---|---|
| | E | F | G | H |
| Sugar from the hydrolyzate of EtOAc fraction | 77 | 9 | 39 | 90 |
| Glucose (authentic) | 77 | 9 | 39 | 90 |

*Solvent key; E = 60 % Acetic acid; F = *n*-BuOH:AcOH:$H_2O$ = 4:1:5 (upper phase) (BAW); G = Water saturated phenol; H = AcOH:conc. HCl:$H_2O$ = 30:3:10 (Forestal).

**Microbial activity:** Fresh blood was collected from healthy sheep and mixed with equal volume of sterilized solution containing 2 % dextrose, 0.8 % sodium citrate, 0.05 % citric acid and 0.42 % NaCl and used within 5 h. Hyposaline (0.36 % 2 mL), phosphate buffer (0.15 M, pH = 7.4, 1 mL) and SRBC (1 %, 0.5 mL) were taken in a number of tubes. Solutions of different concentrations of the drug were added in all tubes except one tube. The last tube served as control in which instead of the drug, isosaline (0.85 %, 1 mL) was added. The contents were incubated at 37 ºC for 0.5 h and then centrifuged. The intensity of the supernatant was measured at 560 nm using a photoelectric colourimeter. The control tube that contained no drug was taken as representing 100 % SRBC lysis. The percentage of prevention of drug-treated hyposaline-induced SRBC lysis was calculated from the difference in absorbance readings of the control and drug-treated tubes using the following relation:

$$100 - \frac{\text{Reading corresponding to drug treated sample}}{\text{Reading corresponding to control}} \times 100$$

The results are given in Table-3.

TABLE-3
EFFECT OF ETHYL ACETATE ISOLATE AGAINST HYPOTONICITY INDUCED HAEMOLYSIS

| Conc. of the drug (µg) | Prevention (%) | Conc. of the drug (µg) | Prevention (%) |
|---|---|---|---|
| 15 | 82 | 65 | 74 |
| 25 | 85 | 75 | 67 |
| 35 | 85 | 85 | 60 |
| 45 | 82 | 95 | 53 |
| 55 | 79 | 105 | 45 |

## RESULTS AND DISCUSSION

The fresh flowers of *P. hysterophorus* have been found to contain galangin and its 3-O-glucoside.

The UV spectrum of the flavonol aglycone obtained from $Et_2O$ fraction exhibited two major peaks at 359 nm (band I) and 267 nm (band II) which showed flavonol skeleton. A bathochromic shift of 53 nm on addition of NaOMe revealed the absence of a free 4'-OH in the B-ring. A shift of 53 nm on the addition of $AICl_3$/HCl showed the presence of a free 5-OH in the A-ring. The presence of a free OH at C-7 was ascertained by a shift of + 8 nm (band II) on the addition of NaOAc. The $AlCl_3$ spectrum was almost same as that of $AlCl_3$/HCl revealing the absence of a catechol type of aubtitution in B-ring. The $H_3BO_3$ spectrum also confirmed it as there was only + 2 nm shift on the addition of NaOAc/$H_3BO_3$.

The UV absorption of the glycoside is at 340 nm. A comparison of band l absorption of the glycoside and the aglycone reveals that there may be 3-glycosylation in the flavonol. A bathochromic shift of only 25 nm (band I) ascertained the absence of a free OH at C-3. The presence of 5-OH groups was confirmed by the bathochromic shift of + 45 nm on the addition absorption peaks to reveal the absence of an *ortho*-dihydroxy grouping in B-ring. The presence of a free OH at C-7 was evidenced from the + 20 nm the required bathochrmic shift indicating the absence of catechol type of substitution in B-ring. On this basis the identify of the pigments obtained from $Et_2O$ and EtOAC solubles can be confirmed as galagin and its 3-glucoside respectively.

The SRBC membrane stabilization effects of EtOAc fraction can be inferred from the data presented in Table-3 which showed a dose-dependent activity against heamolysis. It has maximal inhibition of hypotonicity (haemolysis) induced SRBC lysis at a concentration of 30 µg.

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(*Received*: 23 December 2006; *Accepted*: 29 September 2007) AJC-5928