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An Isoflavone Glycoside from the Stem of *Euphorbia Hirta* Linn as Antimalarial Compound

M.C. KOLI, RASHMI CHOUDHARY, SOURABH KUMAR†, SUMAN THAKUR, DEEPTI SHUKLA and R.C. SAXENA*

Ayurvedic Drug Research Laboratory, Department of Zoology, S.S.L. Jain P.G. College Vidisha-464 001, India

Euphorbia hirta Linn. of family Euphorbiaceae is found abundant in the plane areas, near rivers and on marshy lands. The whole herbs were collected from a village of Tehsil Multai district Betul a tribal area of Madhya Pradesh region during winter months. The powdered material was extracted in soxhlet uprights which gave maximum 7.66% yield in water and 3.21% in 90% alcohol. The water extract was further purified using column chromatography and TLC. An isoflavone glycoside isolated from the plant showed antimalarial activities at different concentrations.

Key Words: Anti-malarial, Isoflavone, *Euphorbia hirta*, Parasite.

INTRODUCTION

Malaria is caused by the protozoan parasite of the plasmodium family which exists in 4 forms, *viz.*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*; *Plasmodium falciparum* is the most widespread and, if untreated, it can lead to fatal cerebral malaria. Malaria is spread by female anopheles mosquitoes.

The practices of traditional medicine are based on hundreds of years of belief and observations which predate the development and spread of modern medicine. In some countries, traditional medicine remains an integral part of the formal health system and exists on an equal footing with modern medicine[1,2]. The methods of practice of traditional medicine may appear to be numerous and dissimilar but they all represent variations of the basic activities, faith healing, hygienic and drug therapy[3]. Traditional medicine plays an important role in health care in India, also even in the modern societies.

A survey of certain pharmacologically active phytoconstituents in wild plants which are commonly used in folk medicine have proved quite important during the past few decades. The results of these studies have proved to be much significant for their commercial exploitation. Many reports are available now on the phytochemical screening of plants of a particular geographical region[4–7]. How-

†Natural Product Chemistry Lab., Sagar University, Sagar (M.P.), India.

ever, only a few are concerned with such plants used by rural folk and tribal people in malarial fever; hence, the present work was undertaken to isolate the active compound from medicinal plants of tribal areas of Madhya Pradesh (India).

EXPERIMENTAL

Collection and extraction of plant material

Euphorbia hirta of family Euphorbiaceae was collected from near the railway line at tribal areas of Betul of Madhya Pradesh (India). The collection was made in the winter season. The plant parts were dried in shed for 30 days at Pest Control Research Laboratory, S.S.L. Jain P.G. College, Vidisha. A voucher specimen was procured in the laboratory for our herbarium record at S.No. 15.

The known amount of powdered material (200 g) of *Euphorbia hirta* was extracted successively with 90% alcohol and other four solvents in an order of increasing polarity for 60–70 h in a soxhlet apparatus. The crude extracts thus obtained were filtered using Whatman filter paper no. 1 and the solvents were evaporated to dryness under reduced pressure in a vacuum evaporator at 40°C. The percentage yield of the plant extracts in each solvent was calculated (Table-1) and to prevent fungal growth a trace of toluene was added in the crude extracts and were kept in refrigerator till use.

Purification and Isolation Methodologies

The crude extracts obtained from the plant were subjected to purification process by different chromatographic techniques followed by spectroscopic methods.

Column Chromatography: The biologically active compounds were separated from the extracts by column chromatography[8]. A glass column of small size was thoroughly washed with detergent and water to make it grease free and kept for drying in glass drying machine. The packing material silica gel E. Merck (120 mesh) was slurried with ethyl acetate (about one-third full of ethyl acetate). A homogeneous packing of the silica gel was done by maintaining gentle agitation while there was a solvent flow through the column. The column was not allowed to run dry during packing or during separation. For large scale separation of the crude, large sized glass column with sintered disc was used in the present study.

Thin Layer Chromatography: The purified fractions of column chromatography were further assessed on TLC plates for their purity. For this purpose, the glass plates (20 × 5 cms) were cleaned thoroughly before use and the R_f value was determined by the method of Brimley and Barrett[9].

Chromatographic separation of crude 90% methanolic extract

The 90% alcoholic extract (500 mg) was slurried with a small quantity of 90% alcohol (to make a concentrated solution) and allowed to observe on silica gel column (11.85 g) packed with ethyl acetate.

The following fractions were collected by using different solvent systems; fraction-A [MeOH-H_2O (13 : 7)]; fraction-B [H_2O-n BuOH (1 : 1)]; fraction-C [EtOAc-acetone (4 : 1)]; fraction-D [hexane-MeOH (4 : 1)] and fraction-E [n-

BuOH-H_2O (1 : 1)] (Table-1). The volume of total fractions obtained was 810 mL. All fractions were monitored by TLC until a single spot was obained. In case more than one spot was shown by the fractions, the same was again rechromatographed over silica gel column until a single spot was obtained. These fractions were collected in glass vials and the solvent was allowed to dry and weighed and finally stored in refrigerator for further analysis and bioassay experiments, and named as E_1, E_2, E_3 E_4 and E_5 respectively.

TABLE-1
PERCENTAGE YIELD OF *EUPHORBIA HIRTA* BY SOXHLET APPARATUS IN DIFFERENT SOLVENTS AT 40°C

S.No.	Solvent used	Wt. of the powder (g)	Wt. of the extract (g)	Yield (%)
1.	*n*-hexane (Non-polar)	200	5.620	2.620
2.	Chloroform (Non-polar)	200	6.420	3.210
3.	Ethyl acetate (Non-polar)	200	2.850	1.425
4.	90% Methanol (Polar)	200	5.300	2.650
5.	Water (Polar)	200	15.230	7.660

Thin Layer Chromatography of fractions E_1, E_3 and E_5

Using silica gel 'G' for thin layer chromatography with different solvent system for the different fractions have been mentioned (Table-2). In fraction E_3, spot-1 shows the R_f value as 0.65 which is characterized as saponin extract.

TABLE-2
TLC FRACTION OF *EUPHORBIA HIRTA*

S.No.	Solvent sys.	Fraction code	No. of spot	Behaviour		R_f value of each spot from bottom
				Visible light	U.V. light	
1.	Chloroform	E_1	Spot 1	Invisible	Invisible	0.05
2.	Methanol	*E_3	Spot 2	–	–	0.40
3.	*n*-butane	E_5	Spot 3	Yellow	Florescent	0.50

*This spot was further purified as it has shown 100% insecticidal activities earlier.

Chemical examination and structural elucidation of saponin in plant extract

For chemical examination and structural elucidation, following methods were used:

Isolation and Structural Elucidation of Compound

Spectral studies: Air dried and powdered stems were extracted with 90% MeOH. The methanolic extract was partitioned successively and subjected to silica gel 'G' on GC using $CHCl_3$-MeOH in different proportions. The fraction collected from $CHCl_3$-MeOH (10 : 8) gave 5,4′-dimethoxy-3′-prenyl, 5′-trimethoxy, 7-hydroxy isoflavone 250–254°C (from MeOH) [M+] 545 m/z; UV λ_{max} (MeOH) 260, 309 (84), (+NaOMe) 266,312, (+$AlCl_3$) 268, 320 nm: IR bands (KBr) 1605, 1630 cm^{-1} ^{1}H-NMR of acetate derivative (II) (300 MHz, DMSO-d_6): δ 7.8 (^{1}H, S, H-2); 6.92 (^{1}H, S, H-8), 6.12 (1-H, S, H-6), 7.07 (2-H, S, H-2′ and H-6′), 4.08 (3-H, S, O, Me), 3.89 (3-H, S, O, Me), 3.19 (2-H, d, J 7 hz, H-1″); 5.02 (1-H, t, J 7 hz, H-2″); 1.42 (3-H, S, Me-5″); 1.49 (3-H, S, Me-4″); 2.06 (1.2-H, S, 4 × Ac); 2.38 (3-H, S, Ac); 5.03 (H, d, 7.5 Hz; H-1‴); ^{13}C-NMR ($CDCl_3$); 155.6 (C-2); 164.6 (C-7); 93.6 (C-8); 157.9 (C-9); 104.8 (C-10); 109.2 (C-1′); 154–6 (C-2′); 115.2 (C-3′); 156.5 (C-4′); 146.9 (C-5′); 129.1 (C-6′); 21.8 (C-1″); 122.4 (C-2″); 128.9 (C-3″); 25.8 (C-4″); 19.8 (C-5″); 104.4 (C-1‴); 70.8 (C-2‴); 73.3 (C-3‴); 69.8 (C-4‴); 76.1 (C-5‴); 59.8 (C-6‴).

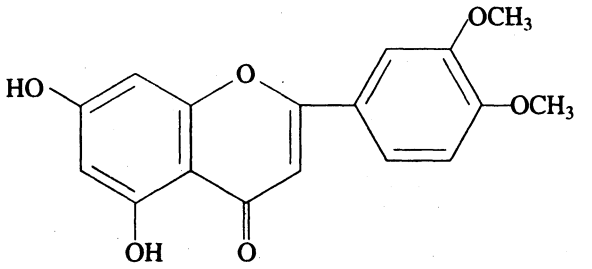
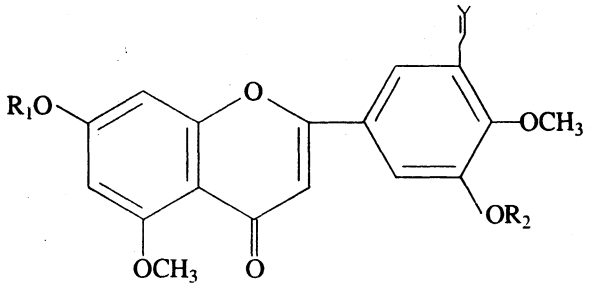


Fig. 1. 3,5,7-Trihydroxy-3′,4′-dimethoxy flavone

Acid hydrolysis of compound: Acid hydrolysis of E_3 gave (Fig. 1) and galactose moeity.

Permethylation followed by acid hydrolysis yielded-5,4′, dimethoxy, 3′ prenyl, 5′,7 hydroxy isoflavone III (Fig. 2) mp 202°C, [M^+] 396. UV max MeOH: 265, 317 (sh); (+NaOMe) 265, 309; (+NaOAC) 268, 349, 359; (+$AlCl_3$) 268, 321 nm; IR bands (KBr) 1.645 cm^{-1}; ^{1}H-NMR (300 MHz$CDCl_3$); δ 8.01 (1H, S, H-2); 6.42 (1H, d, J 2.5 Hz, H-8); 6.24 (1H, d, J 2.5 Hz, H-6); 7.38 (2H, S, H-2′ and H-6′), 3.98 (3H, S, OMe), 3.92 (6H, S, 2 × OMe), 9.08 (2H, S, OH exchangeable with D_2O); 3.59 (2H, d, J 7 Hz, H-1″); 5.42 (1H, 2, J 7.1 Hz, H-2″); 1.59 (3H, S, Me-5″); 1.78 (3H, S, Me-4″); ^{13}C-NMR (DMSO-d_6): 155.4 (C-2); 119.5 (C-3); 182.4 (C-4); 162.9 (C-5); 99.6 (C-6); 163.9 (C-7); 90.1 (C-8); 157.8 (C-9); 102.8 (C-10); 109.8 (C-1′); 154.9 (C-2′); 114.9 (C-3′); 156.4 (C-4′) (C-1′); 154.9 (C-2′); 114.9 (C-3′); 156.4 (C-4′); 148.1 (C-5′); 129.8 (C-6′); 22.8 (C-1″); 122.9 (C-2″); 128.9 (C-3″); 25.5 (C-4″); 18.9 (C-5).

Antimalarial activity: The extracts which were tested for their Schizonticidal activity showed varying results. The parasitaemia on day 4 is shown in Table-3. Even though 100 mg/kg body weight showed negligible effect in all the three groups, 300 and 500 mg/kg body weight showed a reduction of



(I)

Fig. 2. 5,4′-Dimethoxy, 3′-prenyl, 5′-7-hydroxy isoflavone

50–70% parasitaemia. There was not much difference between 500 and 100 mg/kg body weight, except in fraction E_3 which showed better results. E_3 in a dose of 1000 mg/kg body weight showed a reduction of 37.29%. The animals in the control group, which received chloroquine as positive control were found negative.

The results thus showed that fraction E_3 of *Euphorbia hirta* which contains an isoflavone glycoside can be used as antiplasmodial compound.

The neem tree gained a worldwide reputation as an antimalarial plant. Sharma *et al.*[10] also listed several plants showing anti-plasmodial activity from crude extracts to isolated compound. Khalil[11] from Jordan reported the plants used in traditional medicine including malarial fever.

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