PHYTOCHEMICAL INVESTIGATION AND ANTIPLASMODIAL ACTIVITY OF LEAF EXTRACT OF *Cassia obtusifolia* Linn.

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SUMMARY

The methanol extract of fresh leaves of *Cassia obtusifolia* Linn. (Caesalpiniaceae) was investigated for its antiplasmodial activity against chloroquine-resistant strains of *Plasmodium faliciparum* (Malaria causing protozoan). *In vitro* activity against *P. falciparum* strain K-1 was assessed using the Parasite Lactate Dehydrogenase assay method. The main anti-plasmodial compound 1, 3, 8, trihydroxy-6 methyl-9, 10 anthracenedione has been isolated from *Cassia obtusifolia* Linn. leaves. Concentration of the compound in the leaf was 1.5%. The leaf extract was effective to check the incidence of disease about 80%.

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Cassia obtusifilia Linn. (Caesalpiniaceae) is commonly known as "Chakawar". It is widely distributed along road side and other fallow lands through out the India and the other tropical and temperature region of Asia, Africa and America. It's leaves are diuretic, anthelminitic, hepatoprotective, antiplasmodic, stomachic, useful in half headache, leprosy, snake bite, asthma, proriasis, hepatitis-B, stomach ulcers and in the treatment of malarial fever.

The antiulcer properties of the aqueous and methanol extracts of fresh leaf of *Cassia obtusifilia* Linn. have been reported by (Akah *et al*., 1984 and Nwafor and Okwuasaba, 2001). The present study was undertake to evaluate the antiplasmodial effects of the methanol extracts of fresh leaf of *C. obtusifolia* Linn. against *Plasmodium faliciparum*. This note describes the isolation and activities of 1, 3, 8-trihydroxy-6 methyl-9, 10, anthracenedione the major antimalarial principle of the plant.

MATERIALS AND METHODS

Plant Material:

The mature fresh leaves of *Casia obtusifolia* Linn. was collected from the rural areas of Jaunpur district (U. P.) and identified with the help of flora (Duthie, 1960).

Extraction and Isolation :

The dried powdered leaf of *Cassia obtusifilia* Linn. (100 gm) was exhaustively extracted with 90% methanol using Soxhlet apparatus. The extract was concentrated to a small volume in vacuo and this gave yield of 20-28% w/w. Analytical silica get 150 A (Whatman) 250 mm thick was activated at 80-100°C. The solvent system used was hexane ethylacetate (80-20 V/V). The crude methanol extract (4 ml) was made into a slurry with silica get (20 gm), dried in an oven and fractionated using Accelerated Gradient Chromatography (AGC) for gradient elution as follows : hexane, hexane ethyl acetate, ethyl acetate, ethyl acetate ethanol and methanol to complete elution. A total of 114 fractions were obtained after complete elution. Each fraction was examined using analytical Thin Layer Chromatography (TLC) and those fractions with similar spots were pooled together, resulting in 5 fractions coaded A, B, C, D and E. Compound C_1 recrystallizes out from the hexane : ethyl acetate (85 : 15V/V) portion, giving an orange amorphouspowder (50 mg). The UV, IR, MS, ^{1}H and ^{13}C-NMR Spectra of C_1 (Fig. 1) were in accordance with previously reported data of emodine isolated from some *Cassia* sp. (Lemli and Cuvelle, 1967 and Gritranapan *et al*., 1983).

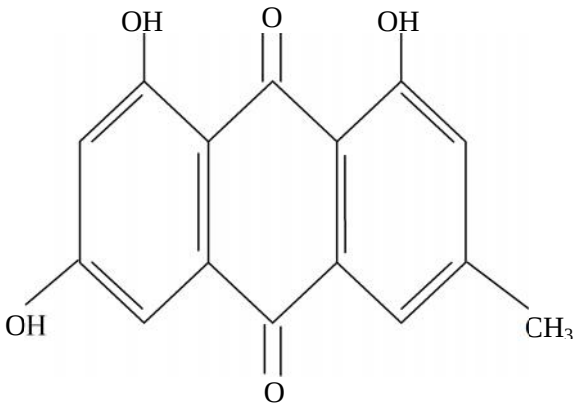

Fig. 1 : Compound C_1 : 1, 3, 8-trihydoxy-6 methyl 9, 10-anthracenedione (Emodin)

1, 3, 8-Trihydrxy-6 methyl-19, 10-anthracenedione (Emodine) :

Compound C_1 C_{15} H_{10} O_5, HRMS M^+ atm/Z 270.05282. An amorphous power with melting point range of 245-252^{0}C has characteristic IR absorption at 3500 cm^{-1} due to OH and 1650 cm^{-1} due to carbonyl. The ^{1}HNMR Spectrum showed absorption at L_F 2.45(CH_3), L_F 7.5(1H), L_F 7.24(1H), L_F 7.12 (1H). ^{13}C NMR repealed the presence of two carbonyl at L_F 192.16 and L_F 182.73. It also the presence of 12 olefinic/aromatic carbons at L_F 109.44, 110.39, 110.89, 115.00, 122.00, 125.03, 134.74, 134.74, 137.10, 150.08, 163.83, 166.85, 167.22 and one aliphatic carbon at L_F 22.64 (CH_3). The Distortion enhancement by polarization (DEPT) and Correlation Spectroscopy (cosy) revealed the presence of 4 methinene (*i.e.* CH) and 1 CH_3. From the coupling experiment, it showed coupling between the CH_3, hydrogen and the proton at L_F 7.5, comparison of these data with that in the library revealed compound C_1 as emodin.

In Vitro Antiplasmodial Activity:

Antiplasmodial activity was determined *in vitro* against a chloroquine resistant strain K1 of *Plasmodium falciparum* and the control drug chlorquine diphosphate using the parasite lactate dehydrogenase assay method. For vitro assays, we used *P. falciparum* parasites (strain K1) that are chloroquine resistant. Malarial parasites were maintained in human A^+ erythrocytes suspended in RPMI 1640 – medium supplemented with A^+ serum and glucose according to previously publication method (Trager and Jensen, 1976 and Fairlamb *et al.*, 1985). Cultures containing predominantly early ring stages were used for testing chloroquine diphosphate was used as a positive control and uninfected and infected erythrocytes without compound extracts were include in each test.

RESULTS AND DISCUSSION

In continuing the search for antimalarial compounds from this medicinal plants, the methanol extract of *Cassia obtusifolia* Linn. was subjected to bioactivity guided fractionation. The methanol extract (Crude), fraction (A – E) and compound C_1 were screened for antiplasmodial activity against chloroquine resistant strain of *P. Falciparum*. The fraction C showed a moderate antiplasmodial activity, the highest activity being observed in the compound C_1. Compound C_1 showed a higher antiplasmodial activity than the methanol extract from which it was obtained. Using spectroscopic studies (MS, ^{1}H NMR and ^{13}C MR supported by ^{1}H ^{1}H Cosy and $_1$H ^{13}C Cosy expriments), the structure of 1, 3, 8, trilydroxy-6 methyl-9, 10 anthracenedione was elucidated.

In vitro activities of the crude extract, fractions, compound C_1 and chloroquine diphosphate against *Plasmodium falciparum* (Strain K1). $^aIC_{50}$, Concentration that inhibits 50% growth of parasite.

The HRMS of C_1 showed a M^+ peak at m/z 270.05282 corresponding to a molecular formula of C_{15} H_{10} O_5 (Fig. 1). However, the above data were found to correspond to that of emodin, which had previously been isolated from *Cassia obtusifolia* Linn. other *Cassia* sp. (Lemli and Cuvelle, 1967 and Gigranapan *et al*., 1983). However, this is the first time that a particular compound has been isolated in *Cassia obtusifolia* Linn. and shown to possess antiplasmoclial activity. It is used in the treatment of malaria fever (very commonly accurs in M.P., U.P., Bihar, Delhi and other densely populated cities of India).

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