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Antimalarial activity and related chemical constituents of Swertia species from Kayah State

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The most promising activity was found in ethanol extracts of *Swertia purpurescens* (whole plant) at the dosage of 2.8 g/kg/day both in suppressive and therapeutic tests when various extracts of these three plant specimens were preliminary screened for antimalarial activity, using in *in vivo* model. The active ethanol extract observed to have significant antimalarial activity in *in vivo* model was subjected to *in vitro p. falciparum* system. It's antimalarial activity was found starting from the dosage of 1000 μg/ml. Chemical separation of the active extracts was serially carried out by means of solvent–solvent partition, column chromatographic and thin layer chromatographic techniques. Out of seven components yielded, only one showed to have suppressive activity against malaria parasite in both experimental models. The active compound was identified as a xanthone molecule by means of UV, FTIR, ^{1}HNMR (600MHz), DQF-COSY, HSQC, HMBC and NOE spectroscopic techniques. *Swertia chirata* and xanthone molecules have known to be antimalarial agents in recent years. Therefore, this study could structurally elucidate an active compound Bellidifolin from *S. purpurescens* and for the first time, the possessing of antimalarial activity in *S. purpurescens* could be reported.

INTRODUCTION

Plant-based Antimalarial drugs such as quinine and artemisinin were discovered in the natural product recourse area. Medicinal plants and the knowledge of traditional medicine are the prime recourses for the development of anti-malarial agents. In Myanmar, it is observed that traditional and herbal medicine are used and heavily relied upon by most people for health purposes. Therefore, a study was conducted on that plant to investigate the chemical constituent and to evaluate its activity for further development if a useful anti-malarial agent derived from Myanmar plant sources.

This paper represents a critical account of crude extracts and the active constituents with the chemical structure possessing antimalarial activity against *P. falciparum* and *P. berghei* experimental models.

MATERIALS AND METHODS

Preparation of plant extracts and phyto-chemical tests

Three species of the same genus commonly known as Pan Kha were collected from Loikaw in November because it was reported by the local people that the plant was most active in the specified season. These species were identified and confirmed by their specific botanical names by a competent taxonomist from the Department of Botany, Mandalay University. The whole plant was used for the chemical extraction.

Each plant species was extracted successively with the solvents such as chloroform, ethanol and water to yield nine extracts. Preliminary phytochemical investigations were carried out for three plants to determine the presence of the organic constituents (Physico-chemical standard of Unani Medicine) [1].

Acute toxicity test

Ten albino mice in each group were fasted overnight before administration of each plant extract. Increasing doses of three species of chloroform extracts, 0.6, 1.2 and 2.4g/kg and alcohol and watery extracts, 0.7, 1.4 and 2.8 g/kg were orally administered. The mice were housed separately in individual cage with free access to food and water and observed clinically for 1 week. LD_{50} of three plants extracts were deter-mined after performing the acute toxicity test [2].

Screening of plant extracts for antimalarial activity in in vivo and in vitro model

Plasmodium berghei and Dutch Denken Yoken (DDY) mice for *in vivo* testing and *Plasmodium falciparum* parasites for *in vitro* testing were used. Nine extracts were initially screened for antimalarial activity by applying *in vivo* model using *P. berghei*. Both suppressive and therapeutic tests were performed [3, 4]. One of the ethanol extract which showed outstanding antimalarial activity in both *in vivo* tests, was subjected to *in vitro* during testing system [5]. To be able to attain homogeneous solution, chloroform extracts of all three plants were dissolved in olive oil. Forty percent alcohol was used to dissolve alcohol extracts in *in vivo* models. Aqueous extracts were easily dissolved in water. For *in vitro* experiments, the ethanol extract which was found to be the most active ingredient was dissolved in RPMI medium. Twenty percent alcohol was used to produce homogeneous solution of ethanol extract in *in vitro* models.

Chemical separation of active extract and confirmatory test for antimalarial activity

The active extract which appeared in both *in vivo* and *in vitro* experiments was further analysed to isolate the individual chemical constituents. Preliminary investigation was carried out by thin layer chromatography (TLC) [6] with the solvent system of hexane: ethyl acetate (7:3). On the basis of TLC findings, column chromatographic separation was further carried out by using hexane and ethyl acetate (9:1, 7:1, 6:1, 4:1, 7:3, 3:2, 1:1) as eluent. Thus a sufficient amount of the major constituent form, this active extract was obtained for further chemical analysis.

Chemical structure elucidation

Literature background [7, 8] was initially reviewed to find out the main class of chemical compounds present in the plant of the genus *Swertia*. It is found to be xanthone as the main compound by means of FTIR, ^{1}HNMR (600 MHz) and NOE spectronic techniques.

RESULTS

Botanical investigation revealed, three species namely – *Swertia affinis*, *Swertia angustifolia* and *Swertia purpurescens.* The yield percentage of chloroform were 3.85 %, 6.42 %, 5.14%, 95% ethanol extracts were 15.25 %, 18.98 %, 16.67 % and water extracts were 5.49 %, 6.45 %, 11.28 % from *Swertia affinis, Swertia angustifolia* and *Swertia purpurescens*, respectively.

According to acute toxicity test conducted on experimental mice, none of the nine extracts produced toxicity if given in suppressive as well as therapeutic dose. LD_{50} values of chloroform extracts of *Swertia purpurescens, Swertia angustifolia* and *Swertia affinis* were the same ie, 2.4g/ kg/ day. The other two, ethyl alcohol and aqueous extracts of these three species were the same ie. 2.8g/kg/day.

Phytochemical test showed glycoside, carbohydrate, flavonoid, polyphenol, steroid, terpene, saponin, lipophilic group and volatile oil were highly positive but alkaloid, protein and amino acid were not present in three species.

In vivo suppressive test, therapeutic test and in vitro test

Preliminary screening of different extracts of all medicinal plants were done by performing *in vivo* suppressive test (Table1).

Table 1. *In vivo* parasite suppression of different extracts of three Swertia species on day 4 observed in suppressive test

(gm/ kg/ day)	Percent parasite suppression on day 4		
	S.purpurescens	*S.angustifolia*	*S.affinis*
Chloroform extract			
2.4	47.19	33.69	36.05
1.2	38.30	15.74	34.19
0.6	25.16	5.96	32.61
Ethanol extract			
2.8	65.2	55.3	34.7
1.4	51.48	41.65	33.62
0.7	44.74	41.65	Nil
Aqueous extract			
2.8	15.28	5.5	13.99
1.4	14.52	Nil	Nil
0.7	8.56	Nil	Nil

The experimental results were expressed in terms of percent parasite suppression. Percent parasite suppression means percentage of parasitaemia reduced in treated group of mice due to tested drug compared to that of untreated control of mice. Percent parasite suppression was determined on Day 4. Ethanol extract in varying doses namely, 0.7g/ kg/day, 1.4g/ kg/ day and 2.8g/ kg/ day showed suppressive effect in all three Swertia species, being highest suppression (44.74% to 65.20%) in *Swertia purpurescens.* Therapeutic effect of ethanol extract of three swertia species on *Plasmodium berghei* were investigated (Fig.1) and *Swertia purpurescens* extracts gave 44% suppression and *Swertia affinis* gave 31.38% suppression. Therapeutic activities of 95% ethanol extract of *Swertia purpurescens* was significantly more than those of two *Swertia* species ($p<0.5$).

Therefore, the authors chose 95% ethanol extracts of *Swertia purpurescens* for structure elucidation whereas watery and chloroform extract showed no suppressive activity in *in vivo* models.

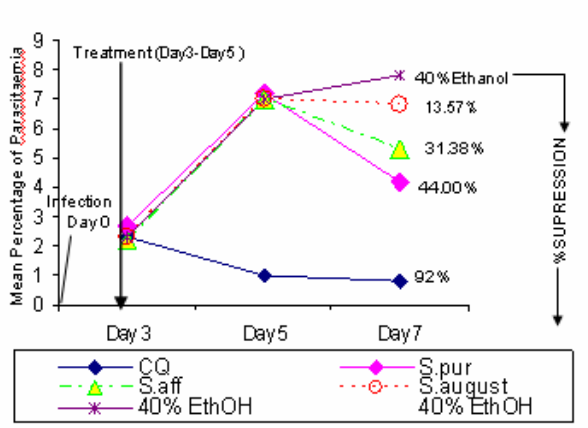
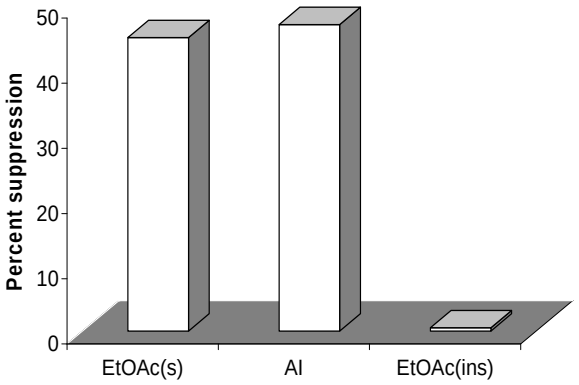


Fig. 1. Therapeutic effect of ethanol extracts of 3 species of Swertia plant on *P. berghei* infection in mice

For the chemical separation, the active ethanol extract of *Swertia purpurescens* was dissolved in ethyl acetate solution to provide non polar and polar fractions. These two fractions were again tested in *in vivo* model and it was found that only ethyl acetate soluble fraction was active as shown in Fig. 2.



EtOAc(s) = Ethyl acetate soluble fraction
AI = Active ingredient
EtOAc(ins) = Ethyl acetate insoluble fraction

Fig. 2. Parasite Suppression (%) of different fractions of *S. purpurencens* tested in mice

In the *in vivo* experiment, the activities of both ethanol extract and ethyl acetate fraction were found to be active with the dosage of 2.8 g/kg/day and 0.7784 g/kg/day

respectively. Out of seven fractions tested, the *in vitro* results showed that fraction 3 possessed highest activity with ED_{50} of 175 µg/ml. The TLC study of the most active ethyl acetate soluble fraction of ethanol extract indicated two major spots together with several minor spots under UV light.

Structure elucidation of compound

Among the six possible xanthone structures (Fig. 3) which had been already reported to present in the genus *Swertia* in the literature [9], the structure of the active crystal from *Swertia purpurescens* was elucidated as follow. According to ^{1}H-NMR spectrometer (600 MHz), only one aromatic methoxy proton δ = 3.9079 ppm was detected in the active crystal of *Swertia purpurescens* so that structure I and II were possible structures. Among the two possible structures I and II, I was confirmed by Nuclear Over hauser Effect (NOE). Structure II was not revealed by NOE due to lack of one proton adjacent to methoxy groups (Fig. 3).

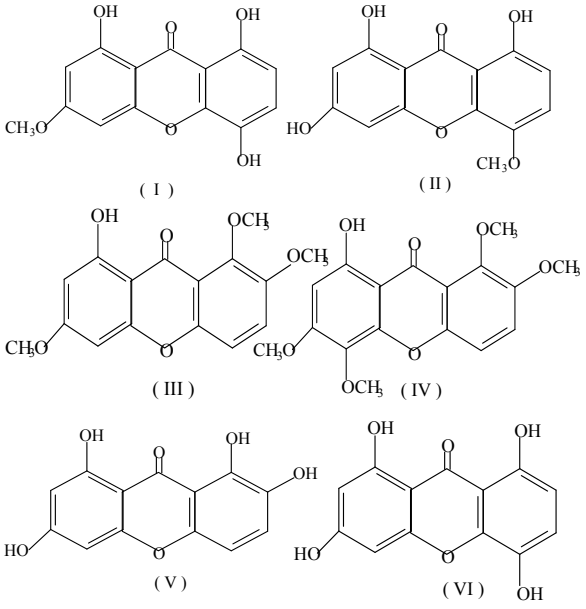


Fig. 3. Six possible xanthone structures of *Swertia purpurescens*

In this experiment, irradiation of methoxy methyl protons (δ = 3.9079 ppm) with low radio frequency responses the two aromatic protons (δ = 6.663 ppm) and (δ = 6.42 ppm) respectively, and the coupling constant of both of these aromatic protons (J= 1.8 Hz) as meta to each other (Fig. 4).

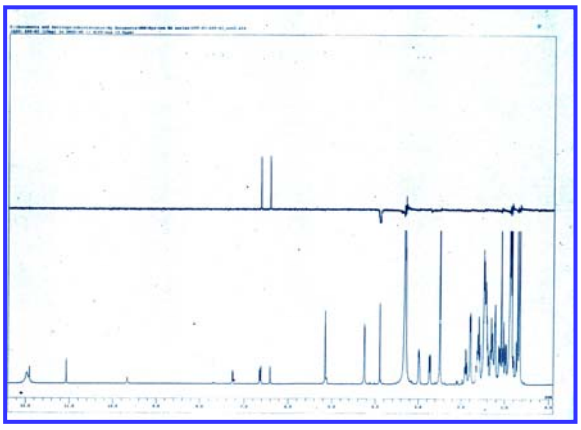


Fig. 4. Nuclear over hauser effect structure

It gave rise to the evidence that only structure I, Bellidifolin (1, 5, 8-trihydroxy-3-methoxy xanthone), was determined for the chemical configuration of active crystal of *Swertia purpurescens* in this study. NOE spectrum is shown in Fig. 4.

DISCUSSION

Although a single local name, Pan Kha was known by the local people of Kayah State, this study could reveal three species of genus *Swertia* as *S. affinis, S. angustifolia* and *S. purpurescens*. Since there is no report on Myanmar resource of Swertia species for the botanical identity before this study, the clarification of botanical species of Pan Kha is the first report that *Swertia* as *S. affinis, S. angustifolia* and *S. purpures-cens* are available in the Kayah State of Myanmar. On the use of these plants by local people for health purpose, it could be reported that these plants were not acute toxic at the dose of 2.8g/kg/day (ethanol extract and H_2O) and 2-4 g/kg/day ($CHCl_3$) in this study. Regarding the antimalarial activity, some extracts of each plant were observed to be active at certain extent, of which ethanol extract of *S. purpurescens* was reported to be the most active as the

findings in *in vivo* experiment model. Serial chemical separation of ethanol extracts of *S. purpurescens* by TLC and column chromatographic techniques provided a significant amount of a crystalline substance together with minor constituents. The promising activity of this crystalline substance was interestingly found when it was retested in both of *in vivo* and *in vitro* models. Therefore, this study could have revealed the antimalarial active principle of *S. purpurescens*. Finally, the antimalarial active crystalline compound could have been identified as Bellidifolin by means of advanced analytical instruments such as FTIR, ^{1}HNMR and NOE spectronic techniques. According to the literature available which had reviewed the xanthone and antimalarial activity [7, 10], synthetic xanthone compounds and some natural xanthones from *Swertia* species (not from *S. purpurescens*) were reported to possess antimalarial activity. However, *S. purpurescens* and its chemical compound, Bellidifolin, were not seen for their antimalarial activity in the available previous reports. Therefore, this study could structurally elucidate an active compound, Bellidifolin, from *S. purpurescens* and for the first time, the possessing of antimalarial activity in *S. purpurescens* could be reported.

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