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Preparation and standardization of an antimalarial phytopharmaceutical product from *Swertia purpurescens* (Pan Kha)

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A standardized phytomedicine is locally produced from Pan Kha, *Swertia purpurescens*. Its antimalarial activity and the active principle were approved in 2004. The plant material was extracted with three different concentrations of ethanol; 50%, 70% and 96%. The 96% ethanol extract showed the presence of large amount of flavonoid (xanthone). This herbal drug was physicochemically and phytochemically standardized by using chromatographic and phytochemical methods for its herbal pharmacopoeial quality criteria. This phytomedicine was found to contain 7.82 mg % active principle, bellidifolin by HPLC analysis. The median lethal dose (LD_{50}) was determined as 37 (27.40-49.95) g/kg. Sub-acute toxicity test showed that there was no decrease in body weight of albino rats. No significant changes in weight of the internal organs, the biochemical and haematological profiles of the test groups were observed when compared with the control group. These findings call for a clinical trial of this medicine to be tested on human being.

INTRODUCTION

Myanmar traditional plant, Pan Kha (*Swertia purpurescens*, Gentianaceae) has been used by local people as an antimalarial remedy for many years in Loikaw, Kayah State. Many plants in traditional medicine are usually recognized as major resources of drug development by scientists and researchers. Several disciplinary research works on plants had been done and are still being preceded for their applicability as pharmacological tools. Then, the promising pharmacologically active molecules which can be synthesized in large scale will be used in solving many health problems. An entirely natural pharmaceutical product which contains an active compound together with other supporting compounds (assuming as naturally related compounds for combination therapy), will be very interesting to develop in a finished oral dosage form and to be assessed by clinical trial. The plant, *Swertia purpurescens* had recently focused on *in vitro* and *in vivo* tests for antimalarial activity, acute toxicity test and chemical investigation for identifying an active principle [1]. At this stage, a research to develop a pharmaceutical herbal extract is called for, which can be applied in malarial treatment. A standardized total alkaloid extract namely totaquine produced from cinchona bark has been one of its kind for the treatment of malaria [2]. Development of a standardized xanthone extract from Swertia plant grown in Myanmar will be innovative approach of proving flavonoid as antimalarial remedy apart from an alkaloid or sesquiterpene.

MATERIALS AND METHODS

Swertia purpurescens (Pan Kha) was collected from Loikaw in November. The

whole plant was used for the preparation and standardization of extract. All solvents except locally produced ethanol are of analytical grade reagents from E.Merck, Darmstadt, Germany. Excipients (lactose monohydrate, dicalcium phosphate dihydrate and magnesium stearate) are the pharmaceutical grade products of Germany, China and Thailand.

Preparation of phytopharmaceutical product from plant extract

Before extraction, plant sample was air-dried and cut into small pieces. Exactly 100 g of sample was packed and subjected to solvent extraction by soxhlet assembly. This sample was extracted with three different ratio of ethanol, 50%, 70% and 96% respectively. The extraction time for each extract was six hours. Then the extracts were evaporated to concentrate by means of rotatory evaporator. It was followed by drying in air until solid residues were obtained. These residues were desiccated, weighed and their respective yields of extracts were calculated. The resultant three solid extracts were stored in desiccators. Then these extracts were checked with thin layer chromatography (TLC) for the presence of active principle, flavonoid (xanthone).

The 96% ethanol extract showed the presence of highest amount of flavonoid (xanthone). For large scale production, plant sample was percolated with 96% ethanol for one month. That extract was partitioned with petroleum ether to remove fatty matters and checked with TLC for active ingredient. After removing fatty matters, the extract was dried in air and then it was mixed with pharmaceutical excipients by using a few amount of ethanol and finally dried in air. Trial and error of 12 series of experiments were carried out by mixing varying ratios of three excipients until a thoroughly dried stage of solid powder was obtained. The specific amount of powder was used in capsule filling. Phytopharmaceutical standardization of the prepared powder was also done under controlled condition. In order to have quality assurance of the samples of every batch, TLC scanner (CAMAG TLC scanner III), was used to confirm the presence of active ingredient, bellidifolin.

Standardization of the prepared phytomedicine

The finished product was standardized by general analytical and pharmacopoeial methods (Quality control method for medicinal plants material, W.H.O (1998) [3], Indian Pharmacopoeia (I.P) (2001) [4], and British Pharmacopoeia (B.P) (1996) [5]. The quantity of active ingredient was measured by HPLC.

Validation of high-performance thin-layer chromatography method for determination of bellidifolin in herbal extract and pharmaceutical dosage form

A new, simple, sensitive, precise and robust high performance thin-layer chromatographic (HPTLC) method for analysis of bellidifolin was developed and validated for the determination of bellidifolin in herbal extracts and in pharmaceutical dosage form. Analysis of bellidifolin was performed on TLC aluminum plates pre-coated with silica gel 60F-254 as the stationary phase. Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase consisting of n-hexane-ethyl-acetate (3:2v/v) at room temperature (25 ± 2Ċ). CAMAG TLC scanner III was used for spectrodensitometric scanning and analysis in absorbance mode at 237nm.

Acute and sub-acute toxicity tests

Acute toxicity test

Eight groups of 10 adult mice of both sexes weighing 25 ± 5 g were kept in cages of five each. The mice were treated with seven different doses i.e. 4, 8, 16, 20, 24, 28 and 32 g/kg of prepared antimalarial phytopharmaceutical product dissolved in water by using feeding nozzle. They were observed daily for one week. The lethality (LD_{50}) was calculated using the method of Lichfield & Wilcoxon [6].

Sub-acute toxicity test

For sub-acute toxicity test, four groups of six albino rats (Wistar strain), each weighing 140 ± 10 gm of both sexes were kept in separate cages. The rats were treated with 1 g/kg, 2 g/kg and 4 g/kg antimalarial phytopharmaceutical product using feeding nozzle for two weeks [7, 8, 9]. They were closely observed for two weeks. After that they were held another 24 hours before sacrificed. Then, the animals were sacrificed; the blood and the internal organs were collected.

Laboratory investigations

After two weeks of drug administration, biochemical tests, liver function tests and haematological tests (complete picture) were done and histopathological examination were made on heart, liver, lung, kidney, spleen, stomach and small intestine by a competent pathologist.

Statistical analysis

Comparative data of control and experimental animal test groups investigated in sub-acute toxicity test was analyzed by using Student's t test.

RESULTS

Yield percentage (w/w) of alcoholic plant extracts and corresponding defatted sample

The yield percentages of 96%, 70% and 50% alcoholic plant extracts were 18.1%, 19.1% and 11.2% respectively. Among them, 96% ethanol extract showed large amount of active principle, bellidifolin (Fig.1). After partition of the 96% alcohol extract of the plant with petroleum ether in order to remove fatty matter and to obtain naturally dry extract powder, the extract of defatted sample was 11.0% and the fat content was 7.1 %. Then, it is required to add or mix with an appropriate pharmaceutical excipient. Various kinds of pharmaceutical excipient which are available were tested for the chemical properties to attain dry extract powder. The ratio of extract and excipients are 1:1.

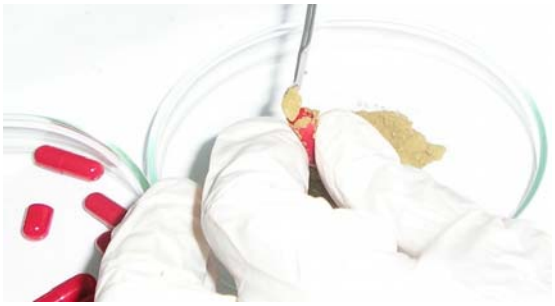

Fig. 1. The prepared capsule

Determination of active compound from prepared phytomedicine

Active ingredient (AI) bellidifolin from the prepared phytomedicine was determined to be 7.82 % by HPLC analysis. A capsule contained 400 mg of phytopharmaceutical powder in which 200 mg was active plant extract. 31.28 mg of the flavonoid, bellidifolin was found to contain in a capsule.

Table 1. Pharmacopoeial specifications and quality criteria of capsule

Test parameter	Results
Appearance	Dark brown powder in red hard gelatin capsule
Average weight	400 mg/ cap
Uniformity of weight of capsule	Comply with Indian and British Pharmacopoeias
Disintegration time (limit not more than15 mins)	(6-9) mins

R_f value of bellidifolin and analyzed spots of upper and lower layers of Swertia purpurescens extracts

The same Rf values of defatted (2) and active ingredient, AI, 0.6. Upper layer of fat sample 1 was found to be lack of bellidifolin while lower layer 2 showed the presence of bellidifolin (Fig. 2). The HPTLC analysis was found to give compact spots for bellidifolin in pharmaceutical form batch 1 to 5, R_f value of 0.6 ± 0.01 (Fig. 3).

In the acute toxicity test**,** (LD_{50}) was determined as 37 (27.40 - 49.95) g/kg. In sub-acute toxicity test, all tested rats were maintained the normal physical appearance

Table 2. Physico-chemical data of finished drug powder

Test parameter	Results
Organoleptic charactertics	
Appearance	powder
Colour	dark brown
Odour	characteristic
Taste	very bitter
Loss on drying at 105 ¯C	4.0%-4.30%
Ash values	
Total ash (%w/w)	5.30-6.25
Water soluble ash (%w/w)	0.5
Acid insoluble ash (%w/w)	0.35
Extractable matters	
Water soluble matters (hot –extract)	75-80 %
Ethanol (96%) soluble matters (cold maceration)	40-45 %
Bitterness value	20.50 unit/g
Foaming index (height 16 cm, diameter 6 mm)	250-333.3

TLC data of reference bellidifolin and Swertia purpurescens extracts

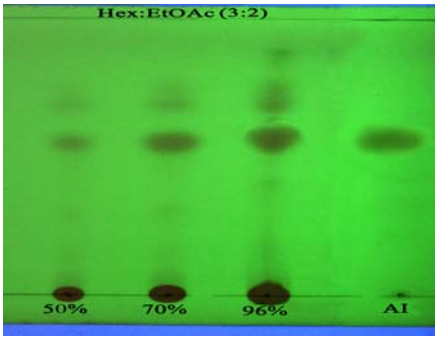

Fig. 2. Thin layer chromatogram of 50%, 70% and 96% *Swertia purpurescens* ethanol extracts and active ingredient (AI)

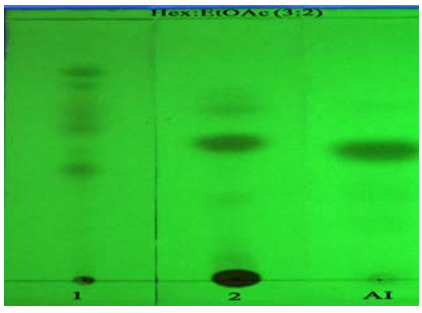

Fig. 3. Thin layer chromatogram of upper (1), lower layer (2) and active ingredient (AI)

and there were no gastrointestinal side-effects during the administration of drug observed. The body weight gains in all the

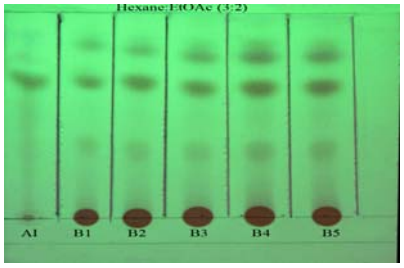

Fig.4. Active ingredient (AI) and batch 1 to 5 of finished drug, solvent system, Hexane: EtOAc (3:2 v/v)

three tested groups were similar to the control group. No significant changes in weight of the internal organs such as heart, liver, lung, kidney, spleen, stomach and intestine were observed.

Laboratory investigations

No significant differences in biochemical, liver function and haematological parameters were observed between test and control groups of laboratory animals. Mild congestions were marked in kidney and lung at the high dose of 4g/kg.

DISCUSSION

The study brings out a promising herbal product, which will hopefully be useful for the treatment of malaria. This phytomedicine has actually been developed basically on Myanmar traditional therapeutic uses and experimentally proven data. In the first phase of the project, *in vitro* as well as *in vivo* antimalarial activity and related active chemical constituent of the *Swertia purpurescens* had been already reported in 2005 [1]. This second phase has dealt with preparation of standardized herbal extract from this plant by applying phytopharmaceutical technology. This phytomedicine also fulfills the needs of scientific approach to traditional medicine by the virtue of its easy manufacturing process, ready to use, economically affordable and therapeutically reliable for treating malaria. It is expected that this phytochemical compound would be applicable as reference substance for further production of this phytomedicine. The results of acute and sub-acute toxicity

studies have also indicated its safety. Now a scientifically proven herbal extract medicine had been already prepared and determined its quality, safety, and experimental effectiveness. Therefore, the authors would like to commence and encourage to do a case-control clinical trial research for the practical applicability of this standardized herbal medicine and that only thing necessary and last phase is to assess its "efficacy on human".

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