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Orignal Article

PHYTOCHEMICAL ANALYSIS OF *PTEROCARPUS MARSUPIUM* AND *SWERTIA CHIRAYITA* FOR THEIR SECONDARY METABOLITES HAVING ANTIDIABETIC PROPERTIES BY HPTLC

MANJUNATHA, K. P.,[1]* SATHYANARAYANA, M. L.[2] AND SHESHARAO[3]

[1]Assistant Professor Department of Veterinary Pathology, Veterinary College, Hebbal, KVAFSU, Bangaluru. E.mail: ahpdornalli@kvafsu.edu.in , Cell: 8618111083: [2]Professor and Head Department of Veterinary Pathology, Veterinary College, Hebbal, KVAFSU, Bangalore.: [3]Assistant Professor, BRIC Dornhalli Shahapur Yadgir District. E. mail: drraoiahvb@gmail .com ,

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Abstract: *Pterocarpus marsupium and Swertia chirayita are known for their hypoglycemic and antidiabetic properties in Ayurvedic system of medicine. The effects of these plants may delay the development of diabetic complications and correct the metabolic abnormalities. Secondary metabolites are responsible for medicinal activity of these plants. Qualitative phytochemical analysis of these plants confirms the presence of various phytochemicals mainly Antherecene derivatives, Bitter principles, Flavinoids, Coumarins and Glycosides. The results suggest that the phytochemical property of these plants which having bioactive constituents are responsible for their hypoglycemic and antidiabetic effects.*

Keywords: *Pterocarpus marsupium, Swertia chirayita,* antidiabetic.

INTRODUCTION

Diabetes mellitus is a metabolic disorder associated with high blood glucose levels, either due to less production of insulin by the pancreas or due to inability of body cells to respond to the insulin produced. Based on this, there are two types of diabetes: Type I and Type II. Type I is also called as insulin-dependent diabetes mellitus (IDDM) which is mainly due to less production of insulin and type II as non-insulin-dependent diabetes mellitus (NIDDM) which is mainly due to inability of body cells to respond to the insulin produced.

Dr. Manjunatha KP is working as Assistant Professor, (OPG) at Veterinary college Hebbal Bangaluru, KVAFSU Nandi nagar Bidar. He has published 07 Articles in different journals and presented his work in several conferences

Oral hypoglycemic agents such as biguanides, sulphonylureas and thiozolidinediones or insulin therapy are the mainstay of treatment of diabetes and are effectively used in controlling hyperglycemia, but they fail to significantly alter the course of complications and side effects caused by them [1]. Recently, the search for appropriate hypoglycemic agents has been focused on plants used in traditional medicine. Moreover, during the past few years some of the new bioactive drugs isolated from hypoglycemic plants showed antidiabetic activity with more efficacy than oral hypoglycemic agents [2].

Dedication: This work is dedicated to Dr. P.D. Gupta on his 85th birthday.

MATERIALS AND METHODS

Phytochemical analysis of the alcoholic extracts of *Pterocarpus marsupium* and *Swertia chirayita* was carried out using HPTLC technique [3].

Procedure of TLC: Pre-coated silica gel 60F 254 TLC aluminium 10x10 cm (Merck, India) type of plates were used for HPTLC. From each alcoholic extract, 5 µl samples were spotted on a TLC silica gel plate (CAMAG Linomat 5, Germany). Chromatography was performed using solvent systems. This procedure was followed for the analysis of Alkaloids, Anthracene derivatives, Bitter principles, Flavonoids, Coumarins, Saponins and Glycosides.

Alkaloids: The finely ground alcoholic extracts of *Pterocarpus marsupium* and *Swertia chirayita* (0.5 g each) were weighed and mixed with 10 ml of 0.5 N HCl, contents were vortexed and pellets were discarded. To the supernatant, 30 % Na_2CO_3 (pH 10) was added and centrifuged at 2000 rpm for 5 min and supernatant was discarded. The precipitate was washed with chloroform and chloroform extract was collected. Once again the residue was washed with methanol and methanol extract was collected. The chloroform and methanol extract were concentrated to one ml and used for chromatography.

Chromatography solvent: Toluene: Ethyl acetate:

Diethylamine:: 70:20:10.Ethyl acetate: methanol: water:: 100:13.5:10.

Detection: Without chemical treatment: TLC plates were observed under UV-254 nm and UV-366 nm.

Dragendorff reagent: The ready to use reagent (Sd fine-chem Limited, Mumbai) was used. One ml of Dragendorff reagent was diluted with four ml of acetic acid and 20 ml of water. The plate was immersed in the reagent for one second. The plate was examined under white light.

Anthracene derivatives: The finely ground alcoholic extracts of *Pterocarpus marsupium* and *Swertia chirayita* (0.5 g each) were weighed and extracted by warming for five min on the water bath with five ml of methanol. The clear filtrates were used directly for HPTLC.

Chromatography solvent: A mixture of ethyl acetate: methanol: water:: 100:17:13.

Detection: without chemical treatment: TLC plates were observed under UV-254 nm and UV-366 nm.

Natural products-polyethylene glycol: The plate was heated to 100 °C for three min, then dipped in solution A (1 g diphenylboronic acid aminoether ester dissolved in 200 ml ethyl acetate) and dried, then dipped in solution B (10 g polyethylene glycol 400 dissolved in 200 ml dichloromethane). The plate was observed in white light and UV-366 nm.

Potassium hydroxide: Five per cent ethanolic KOH was prepared and the plate was immersed in the reagent for one second. The plate was observed in white light and UV-366 nm.

Bitter principles: The finely ground alcoholic extracts of *Pterocarpus marsupium* and *Swertia chirayita* (1 g) were extracted separately for 10 min with 10 ml methanol at 60 °C on the water bath. The mixtures were filtered and the filtrates were evaporated to a volume of about two ml.

Chromatography solvent: Ethyl acetate: methanol: water:: 77:15:08.

Detection: Without chemical treatment: TLC plates were observed under UV-254 nm and UV-366 nm.

Vanillin-sulphuric acid: The reagents consisted of 5 % ethanolic sulphuric acid (Solution I) and 1 % ethanolic vanillin (Solution II). The TLC plate was heated at 100 °C for five to10 min and then sprayed vigorously with 10 ml of Solution I**,** followed immediately by five to 10 ml of Solution II. The plate was examined under white light and UV 366 nm.

Flavonoids: The finely ground alcoholic extracts of *Pterocarpus marsupium* and *Swertia chirayita* (1 g) were extracted separately with 10 ml methanol for five min on a water bath at about 60 °C. The clear filtrates were used for chromatography.

Chromatography solvent: Ethyl acetate: formic acid: glacial acetic acid: water:: 100:11:11:27.

Detection: Without chemical treatment: TLC fig. were observed under UV-254 nm and UV-366 nm.

Natural products-polyethylene glycol: The plate was heated to 100 °C for three min, then dipped in solution A (1 g diphenylboronic acid aminoether ester dissolved in 200 ml ethyl acetate), dried in a stream of cold air, then dipped in solution B (10 g polyethylene glycol 400, dissolved in 200 ml dichloromethane). The plate was examined under white light and UV-366 nm.

Fast blue salt B: The spray reagent was prepared by dissolving 0.5 g fast blue salt in 100 ml water. The plate was sprayed and dried. The plate was examined under white light and UV-366 nm.

Coumarins: The finely ground alcoholic extracts of *Pterocarpus marsupium* and *Swertia chirayita* (1 g) were extracted separately by shaking with 10 ml methanol for 30 min on the water bath. The clear filtrates were evaporated to about one ml and 20 µl was applied to TLC.

Chromatography solvent: Toluene: ether (1:1, saturated with 10 % acetic acid).

Detection (without chemical treatment): TLC plates were observed under UV-254 nm and UV-366 nm.

Potassium hydroxide: Ethanolic KOH (5 %) was used as spray reagent. The plate was immersed in the reagent for one second and was observed at UV-366 nm.

Saponins: The finely ground alcoholic extracts of *Pterocarpus marsupium* and *Swertia chirayita* (2 g) were extracted separately by heating for 10 min under reflux with 10 ml of 70 % ethanol, the clear filtrates were evaporated to about five ml for chromatography.

Chromatography solvent: Chloroform: methanol: water:: 64:50:10

Detection (without chemical treatment): With the exception of glycyrrhetic acid, no saponins are detectable by exposure to UV-254 nm or UV-366 nm.

Blood reagent: Ten ml of 3.6 % sodium citrate was added to 90 ml of fresh bovine blood. 0.2 ml of this mixture was mixed with 30 ml phosphate buffer pH 7.4. The plate was sprayed in horizontal position and the plate was observed in visible light.

Vanillin-sulphuric acid: The reagents consisted of 5 % ethanolic sulphuric acid (Solution I) and 1 % ethanolic vanillin (Solution II). The TLC plate was heated at 100 °C for five to10 min and then sprayed vigorously with 10 ml of Solution I**,** followed immediately by five to 10 ml of Solution II. The plate was examined under white light and UV 366 nm.

Glycosides: The finely ground alcoholic extracts of *Pterocarpus marsupium* and *Swertia chirayita* (1 g) were extracted separately by shaking with 10 ml of methanol for 30 min on the water bath. The clear filtrates were evaporated to about one ml and 20 µl was applied to TLC plates.

Chromatography solvent; Toluene: ether (1:1, saturated with 10 % acetic acid).

Detection: Without chemical treatment: TLC plates were observed under UV-254 nm and UV-366 nm.

Aniline-diphenylamine phosphoric acid: The spray reagent was prepared using four gram of diphenylamine and four ml of aniline which were dissolved in 160 ml of acetone. To this 30 ml of O-phosphoric acid was carefully added. The plate was immersed in the reagent for one sec and then heated at 120 °C. The plate was examined under white light.

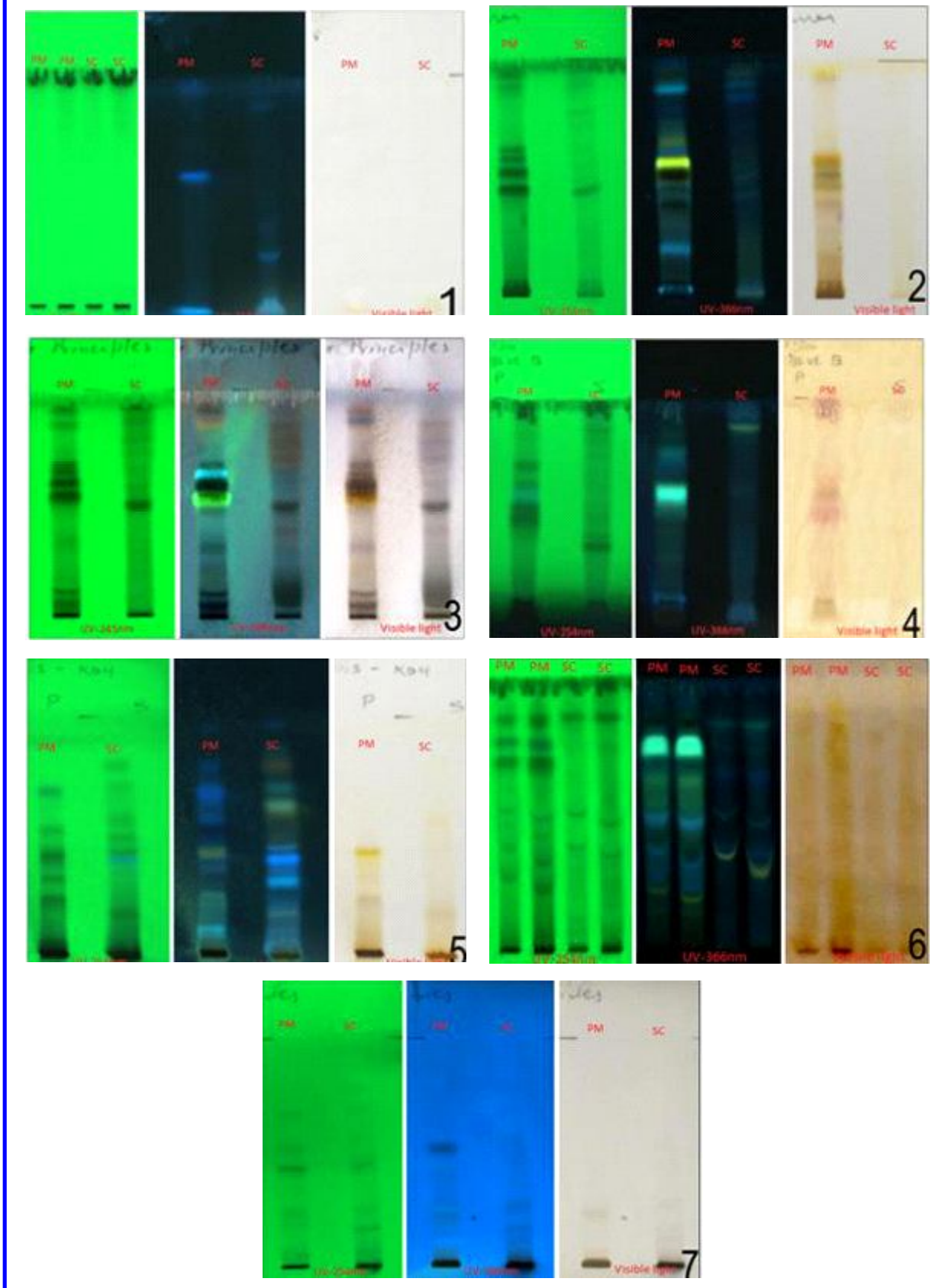
RESULTS

Phytochemical analysis of *Pterocarpus marsupium* and *Swertia chirayita* extracts were carried out in the present investigation by High Performance Thin Layer Chromatography Technique (HPTLC) and the results are presented as here under.

Alkaloids:

a) Without chemical treatment: There was a pronoun-ced quenching of fluorescence on TLC plates at UV-254 nm, intense blue fluorescence at UV-366 nm for Pterocarpus marsupium and Swertia chirayita extracts on TLC plates at UV-254 nm and UV-366 nm (Fig. 1).

b) Dragendorff reagent: There were blue or brown bands on TLC plates at UV-254 nm and on TLC plates at UV-366 nm for Pterocarpus marsupium extract (Plate 5). Intence blue bands appeared on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TLC plates for Swertia chirayita extract at any UV wave lengths (Fig. 1).

Inference: The alcoholic Pterocarpus marsupium seed extract and Swertia chirayita leaves extracts are negative for the presence of alkaloids.

Anthracene derivatives

a) Without chemical treatment:There was pronounced quenching on TLC plates at UV-254 nm and development of blue bands on at UV-366 nm with brown and light yellow bands at visible light for alcoholic Pterocarpus marsupium seed extract and alcoholic Swertia chirayita leaves extracts (Fig. 2).

b) Natural products: polyethylene glycol Bluish brown bands appeared for alcoholic Pterocarpus marsupium seed extract and alcoholic Swertia chirayita leaves extract on TLC plates in visible light and UV - 366 nm. (Fig. 2).

Inference: The alcoholic Pterocarpus marsupium seed extract and alcoholic Swertia chirayita leaves extract were positive for the presence of anthracene derivatives.

Bitter principles:

a) Without chemical treatment :There was pronounced quenching of fluorescence for alcoholic Pterocarpus marsupium seed extract but there was no pronounced quenching for alcoholic Swertia chirayita leaves extract on TLC plates at UV-254 nm and UV- 366 nm (Fig. 3).

b) Vanillin-sulphuric acid: Greenish fluorescence appeared for alcoholic Pterocarpus marsupium seed extract but there was no fluorescence appeared for alcoholic Swertia chirayita leaves extract on TLC plates at UV-366 nm (Fig. 3). Inference: The alcoholic Pterocarpus marsupium seed extract consists of Bitter principles, whereas alcoholic Swertia chirayita leaves extract is positive for the presence of Bitter principles.

Table 1: Showing analysis of bioactive compounds from Pterocarpus marsupium and Swertia chirayita by HPTLC

Sl No.	Bioactive compounds	*Pterocarpus marsupium*	*Swertia chirayia*
01	Alkaloids	Absence	Absence
02	Antherecence derivatives	Presence	Presence
03	Bitter principles	Presence	Presence
04	Coumarins	Presence	Presence
05	Flavinoids	Presence	Presence
06	Glycosides	Presence	Presence
07	Saponin	Absence	Absence

Flavonoids (Without chemical treatment): Fluorescence quenching for alcoholic Pterocarpus marsupium seed extract and alcoholic Swertia chirayita leaves extract on TLC plates at UV-254 nm was observed and light blue fluorescence appeared on TLC plates at UV-366 nm. (Fig. 5).

b**) Natural products** – polyethylene glycol intense blue fluorescence appeared for alcoholic Pterocarpus marsupium seed extract and alcoholic Swertia chirayita leaves extract on TLC plate at UV-366 nm ((Fig. 3). and brown bands appeared in visible light (Fig. 4).

c) Fast blue salt B Blue fluorescence appeared for alcoholic Pterocarpus marsupium seed extract and alcoholic Swertia chirayita leaves extract on TLC plates at UV-366 nm.

Explanation of figures:

Fig. 1: Phytochemical analysis of *Pterocarpus marsupium* and *Swertia chirayita* for alkaloids not showed pronounced quenching of fluorescence on TLC plates at UV-254 nm and intense blue fluorescence at UV-366 nm.

Fig. 2: Phytochemical analysis *Pterocarpus marsupium* and *Swertia chirayita* for anthracene derivatives showing pronounced quenching on TLC plates at UV-254 nm and development of blue bands at UV-366 nm with brown and yellow bands at visible light.

Fig. 3: Phytochemical analysis *Pterocarpus marsupium* and *Swertia chirayita* for bitter principles showing pronounced quenching on TLC plates at UV-254 nm and blueish fluorescence at UV-366 nm with brow bands at visible light.

Fig. 4: Phytochemical analysis *Pterocarpus marsupium* and *Swertia chirayita* for coumarins showing pronounced quenching on TLC plates at UV-254 nm and blue fluorescence at UV-366 nm.

Fig. 5: Phytochemical analysis *Pterocarpus marsupium* and *Swertia chirayita* for flavonoids showing pronounced quenching on TLC plates at UV-254 nm and intense blue fluorescence at UV-366 nm.

Fig. 6: Phytochemical analysis *Pterocarpus marsupium* and *Swertia chirayita* for saponin showing pronounced quenching on TLC plates at UV-254 nm and intense blue fluorescence at UV-366 nm with brownish yellow zones at visible light.

Fig. 7: Phytochemical analysis *Pterocarpus marsupium* and *Swertia chirayita* for glycosides showing pronounced quenching on TLC plates at UV-254 nm and UV-366 nm with dark brown bands.

Inference: The alcoholic Pterocarpus marsupium seed extract and alcoholic Swertia chirayita leaves extract contain flavonoids.

Coumarins: (Without chemical treatment: There was distinct fluorescence quenching for alcoholic Pterocarpus marsupium seed extract and alcoholic Swertia chirayita leaves extract on TLC plate at UV-254 nm and blue fluorescence appeared on TLC plate at UV-366 nm (Fig. 5).

Pterocarpus marsupium seed extract and alcoholic Swertia chirayita leaves extract on TLC plates at UV366 nm (Fig. 5).

Inference: The alcoholic Pterocarpus marsupium seed extract and alcoholic Swertia chirayita leaves extract are positive for the presence of coumarins.

Saponins: With the exception of glycyrrhetic acid, no saponins are detectable by exposure to UV-254 nm or UV-366 nm. Phytochemical analysis for saponins showed no white or blue zones on the visible light (Fig. 6).

I**nference:** The alcoholic Pterocarpus marsupium seed extract and alcoholic Swertia chirayita leaves extract are positive for presence of saponins.

Glycosides: a) Without chemical treatment: There was pronounced fluorescence quenching for alcoholic Pterocarpus marsupium seed extract and alcoholic Swertia chirayita leaves extract on TLC plate at UV-254 nm (Fig. 6).

b) Aniline-diphenylamine phosphoric acid Dark blue bands appeared for alcoholic Pterocarpus marsupium seed extract and alcoholic Swertia chirayita leaves extract on TLC plate at UV-366 nm (Fig. 7).

Inference: The alcoholic Pterocarpus marsupium seed extract and alcoholic Swertia chirayita leaves extract are positive for presence of glycosides.

DISCUSSION

The alcoholic extract *Pterocarpus marsupium* and *Swertia chirayia* were found to be positive for Anthracene derivatives, Bitter principles, Flavonoids, Coumarines and Glycosides and negative for Alkaloids and Saponins.

Pterocarpus marsupium is known to contain marsupin, pterostilbene and epicatechin, which have been shown to possess antidiabetic along with antioxidant activity [4].

Chakravarthy et al. [5] extracted the flavonoid fraction (XE) from the bark of *Pterocarpus marsupium* and demonstrated that drug XE reversed the alloxan induced changes in the blood sugar level and the beta-cell population in the pancreas. They also isolated epicatechin, a pure flavonoid component from the bark of the plant and claimed it to be the antidiabetic principle of the plant and suggested that the compound acts by regeneration of pancreatic beta cells.

Manickam et al. [6] reported that the flavonoids marsupin and pterostilbene present in the aqueous extract of *Pterocarpus marsupium* possessed antihyperglycemic activity in alloxan induced diabetic rats. Anandarajan et al. [7] isolated isoflavone from the methanolic extract from the hardwood of *Pterocarpus marsupium* and reported that this compound increased glucose uptake in skeletal muscle.

The phytochemical investigation of genus *Swertia,* carried out so far has afforded around 200 compounds with varying structures. Among these constituents xanthonoids, terpenoids, flavonoids, irridoid and seco-irridoid glycosides and few alkaloids and miscellaneous compounds form the major classes [8]. In the present study, majority of the classes were detected except the alkaloids and saponins.

Swertia chirayita extracts contained many naturally occurring polyhydroxy xanthones and flavonoids that have been associated with wide range of biological and pharmacological activities including antidiabetic and antioxidant activities [9]. Swerchirin is a medicinally important xanthone/ bitter compound which have hypoglycemic activity ([9,10]. Bellidifolin is a xanthone obtained from Swertia species which possesses hypoglycemic and hypolipidemic [11] properties. Phoboo et al [12] showed that crude extract of *Swertia chirayita* contained three main phytochemicals mangiferin, swertiamarin and amarogentin. Among these amarogentin is reported to be antidiabetic.

Conclusion: Medicinal plants have been used successfully in Ayurvedic medicine for centuries.

Secondary metabolites are responsible for medicinal activity of these plants. More clinical trials should be conducted to support its therapeutic use, on the basis of presence of secondary metabolites. The alcoholic extracts *Pterocarpus marsupium* and *Swertia chirayita* were found to be positive for Anthracene derivatives, Bitter principles, Flavonoids, Coumarines and Glycosides and negative for Alkaloids and Saponins.

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