Journal of Plant Biochemistry & Physiology
ISSN: 2329-9029

Lalrinzuali et al., J Plant Biochem Physiol 2015, 3:3
DOI: 10.4172/2329-9029.1000152

Research Article Open Access

Phytochemical and TLC Profiling of *Oroxylum indicum* and *Milletia pachycarpa*

Lalrinzuali K, Vabeiryureilai M and Jagetia GC*

Department of Zoology, Mizoram University, Aizawl, India

Abstract

The humans have used medicinal plants for healthcare since the time immemorial. The systematic phytochemical analysis of traditionally used medicinal plants is needed to establish their use as medicine. *Oroxylum indicum* and *Milletia pachycarpa* have been used in India and China to treat various health related disorders. Therefore it was decided to undertake phytochemical and Thin Layer Chromatography (TLC) profiling of different extracts of *Oroxylum indicum* and *Milletia pachycarpa* using standard procedures. The dried powder of stem bark of *Oroxylum indicum* and root bark of *Milletia pachycarpa* was sequentially extracted in chloroform, ethanol and water. The dried extract of each plant was phytochemically analyzed for the presence of alkaloids, flavonoids, cardiac glycosides, phytosterols, saponins, tannins steroids and phlobatannins. Each extract from both plants was processed for TLC profiling on silica gel using various solvent combinations as mobile phase. The phytochemical analysis showed the presence of alkaloids in chloroform and ethanol extracts, whereas alkaloids were absent in aqueous extract of both *Oroxylum indicum* and *Milletia pachycarpa.* The flavonoids were observed in all extracts of both plants. However, cardiac glycosides were absent in the aqueous extract of *Milletia pachycarpa*. The saponins were detected in all the extracts of both plants except the chloroform extract of *Oroxylum indicum*. The tannins could not be detected in the aqueous extract of *M. pachycarpa*. The phlobatannins were absent in all extracts of both plants. Steroids were present in the ethanol extract of both plants. The TLC profiling confirmed the presence of different phytochemicals as evidenced by different R*f* values. The present study indicates that the properties of both *O. indicum* and *M. pachycarpa* may be due to presence of alkaloids, flavonoids, cardiac glycosides, saponins, tannins and phytosterols.

Keywords: *O. indicum*; *M. pachycarpa*; Alkaloids; Flavonoids; Cardiac glycosides; Tannins

Introduction

The plants usually synthesize many chemicals, which are either product of metabolism or intentionally for nutrition, defence, pollination and against stress and predators [1]. The phytochemicals synthesized by plants can be mainly grouped into primary and secondary metabolites [2]. The primary metabolites include phytosterols, acyl lipids, amino acids and organic acids that have shared biological function across all plant species [3]. The primary metabolites are responsible mainly for growth, development and other metabolic activities of the plants [4]. The metabolism of primary metabolites generates secondary metabolites, which are not involved in any of the metabolic activity of plants [2]. The properties of these phytochemicals have been under investigation since the 1850s and they have been used as dyes, polymers, fibers, glues, oils, waxes, flavoring agents, perfumes, and even as drugs [4]. It is now fairly well established that the synthesis of secondary metabolites plays an important role in the survival of plants and other activities [5]. The plants usually synthesize these phytochemicals in specialized cells during particular developmental phase making their extraction and purification difficult [6]. The various phytochemicals synthesized by plants as secondary metabolites have been found to exert various physiological effects in mammals including humans and hence they are also called the active principle of that plant [6]. The phytochemicals produce various biological activities, and this has been the reason that plants have been used to treat several ailments in traditional medicine since the time immemorial. It is also known that almost 70% of the modern medicines have a direct or indirect origin in plants [7]. The phytochemicals derived from plants include antibiotic, antifungal and antiviral, antitumor and antigerminative compounds, which helps plants to protect from plant pathogens, insects and predators. The plants also synthesize important UV absorbing compounds, to safeguard the leaves against the damaging effect of UV light from sunlight [5,8]. The phytochemicals synthesized by plants are usually complex and it is sometimes difficult to synthesize them in the laboratory therefore phytochemicals will continue to play crucial role in the new drug discovery.*Milletia pachycarpa Benth* (family: Fabaceae) is a deciduous climbing shrub, which grows up to a height of 6 meters. It has a lilac coloured flower that forms in a large pea-shaped cluster. It usually flowers during July-August and has a brown or grey stem with dark brown seeds [9]. *M. pachycarpa* is used as blood tonic and to induce the growth of red blood cells in Chinese traditional medicine and the preparation is called as 'Jixueteng' [10]. *M. pachycarpa* has been found to have a significant cytotoxic effect in Brine shrimp assay [11] and is also known to have anti-inflammatory activity [12]. It is used as fish poison, pesticide, blood tonic and in the treatment of cancer and infertility traditionally in India and China [11,13,14]. Some of the compounds isolated from *M. pachycarpa* have been reported to be cytotoxic and induce apoptosis in HeLa cells [15]. *Oroxylum indicum* (family Bignoniaceae), sona patha is a deciduous tree distributed throughout Asia and grows at an altitude of 1200 m mainly in ravines, in damp region and moist places in the forests. In India, it is distributed in the Himalayan foothills, Eastern and Western Ghats and North East India [16] *O. indicum* lives in relationship with an actinomycete *Pseudonocardia oroxyli*, a gram positive bacterium [17] that has the capacity to produce many secondary metabolites

***Corresponding author:** Jagetia GC, Department of Zoology, Mizoram University, Aizawl, India, Tel: 91-0389-2330724; Fax: 2330227; E-mail: gc.jagetia@gmail.com

Received July 04, 2015; **Accepted** July 22, 2015; **Published** July 28, 2015

Citation: Lalrinzuali K, Vabeiryureilai M, Jagetia GC (2015) Phytochemical and TLC Profiling of *Oroxylum indicum* and *Milletia pachycarpa*. J Plant Biochem Physiol 3: 152. doi:10.4172/2329-9029.1000152

exhibiting a wide variety of biological activity [8]. Almost every part of this tree possesses medicinal properties and has been used in several traditional Ayurvedic and folk medicines [18]. *O. Indicum* has been reported to possess several medicinal properties including analgesic, antibacterial, anti-inflammatory, anticancer, antioxidant [19-23]. Therefore, an attempt has been made to study the phytochemical constituents of *Milletia pachycarpa* and *Oroxylum indicum.*

Materials and Methods

Preparation of the extract

The non-infected stem bark of *O. indicum* was collected from Champhai whereas root bark of *Milletia pachycarpa* was collected from Kolasib district of Mizoram, India during the dry season in the month of January. The identification of plant was done by the Department of Horticulture and Aromatic and Medicinal Plants, Mizoram University, Aizawl, India. The barks of both plants was washed thoroughly with clean water and allowed to shade dry at room temperature in the dark in clean and hygienic conditions. The dried barks of both plants were separately powdered using an electrical grinder at room temperature. The powdered bark of *O. indicum* stem or root bark of *M. pachycarpa* was sequentially extracted with petroleum ether, chloroform, ethanol and distilled water according to increase in polarity using a Soxhlet apparatus until the solvents became colourless [24]. The liquid extracts were concentrated by evaporating their liquid contents using rotary evaporator. Each extract, except petroleum ether was concentrated *in vacuo* and stored at -70°C until further use.

Preliminary phytochemical analysis

The chloroform, alcoholic and aqueous extracts of *O. indicum* and *M. pachycarpa* were subjected to different phytochemical tests for the presence of tannins, alkaloids, steroids and flavonoids by using standard phytochemical procedures [25-27].

Alkaloids

The presence of alkaloids in *O. indicum* and *M. pachycarpa* was confirmed by employing the Dragendorff's test. Briefly, 0.1 g of different extracts of *O. indicum* or *M. pachycarpa* was mixed with 0.5 ml of Dragendorff's reagent. The development of reddish brown precipitate indicates the presence of alkaloids [25-27].

Flavonoids

The flavonoids were qualitatively estimated using alkaline reagent test, where 0.1 g of each extract of *O. indicum* and *M. pachycarpa* was dissolved in appropriate solvents and mixed with a few drops of sodium hydroxide solution. The formation of intense yellow colour, which turned colourless on addition of a few drops of dilute acid indicated the presence of flavonoids [25-28].

Cardiac glycosides (Keller-Killani test)

0.1 g of *O. indicum* or *M. pachycarpa* was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution with an under laying of 1 ml of concentrated sulphuric acid. The appearance of brown ring at the interface indicated the presence of deoxysugar, which is a characteristic of cardenolides [25,27].

Saponins

Usually 0.1 g of the extracts of *O. indicum* or *M. pachycarpa* was mixed with 3 drops of olive oil and shaken vigorously for a few minutes. The formation of a fairly stable emulsion indicated the presence of saponins [25,27,29].

Steroids

The presence of steroid in various extracts of *O. indicum* and *M. pachycarpa* was determined by Salkowski's test. Briefly 0.1 g of various extracts of *O. indicum* and *M. pachycarpa* dissolved in different solvents were mixed with a few drops of concentrated sulphuric acid. The development of red colour at lower layer indicated the presence of steroids, whereas the formation of yellow colour indicated the presence of triterpenoids [25-27].

Tannins

The presence of tannin was determined by Ferric chloride test. Usually 0.1 g of dried samples of each extract of *O. indicum* or *M. pachycarpa* was dissolved in their respective solvents and a few drops of 0.1% ferric chloride were added. The formation of brownish green or a blue-black colour indicated the presence of tannins [25-27].

Phlobatannins

The different extracts of *O. indicum* or *M. pachycarpa* were boiled in 1% aqueous hydrochloric acid and deposition of a red precipitate indicated the presence of phlobatannins [25,27].

Thin layer chromatography

Thin layer chromatography (TLC) was performed on the different extracts to visualize the separation of various phytochemical components as it is a simple, less cumbersome and rapid technique. The TLC can identify and separate a number of components present in any extract/organic mixtures and it also helps in finding a suitable solvent/s for separating the components by column chromatography as well as for monitoring reactions progress. Pre-coated TLC plates (Silica gel 60 F_{254}) procured from Merck India, Mumbai were used as an adsorbent. A small amount of each of the different extracts was applied as 1 mm diameter, 5 mm above the bottom of the plates. The TLC plates were transferred into the mobile phase consisting of numerous combinations of solvent systems of different polarity such as chloroform:methanol (9:1, 8:2) benzene:chloroform (1:1), pure chloroform, chloroform: ethyl acetate (1:1) and methanol:hydrochloric acid (9:1) and allowed to move on the adsorbent silica gel. The resultant spots were observed under visible and ultra-violet light, dilute acid (H_2SO_4), anisaldehyde, aluminium chloride and Dragendorff's stain. The measure of the distance a compound traveled is considered as the retention factor (R_f), which was calculated using the following formula:-

R_f=Distance travelled by solute/Distance travelled by solvent

Results

The results of phytochemical analysis are shown in Tables 1-3 and Figures 1-7.

Phytochemical screening

Qualitative analysis of chloroform, ethanol and aqueous extracts of *O. indicum* and *M. pachycarpa* showed the presence of different phytochemicals listed below.

Alkaloids

The chloroform and ethanol extracts of *O. indicum* and *M. pachycarpa* showed the presence of alkaloids, whereas alkaloids were not detected in their aqueous extract (Table 1).

Flavonoids

The analysis of flavonoid revealed that chloroform, ethanol

Extracts	Saponin	Tannin	Phlobatannin	Steroids	Cardiac glycoside	Alkaloid	Flavonoid
OIC	-	+	-	-	+	+	+
OIE	+	+	-	+	+	+	+
OIA	+	+	-	-	+	-	+
MPC	+	+	-	-	+	+	+
MPE	+	+	-	+	+	+	+
MPA	+	-	-	-	-	-	+

Legend: Present (+), Absent (-). OIC: *O. indicum* chloroform extract MPC: *M. pachycarpa* chloroform extract OIE: *O. indicum* ethanol extract MPE: *M. pachycarpa* ethanol extract OIA: *O. indicum* aqueous extract MPA: *M. pachycarpa* aqueous.

Table 1: Qualitative phytochemical analysis of various extracts of *O. indicum* and *M. pachycarpa*.

Extracts	Solvent system	NORMAL	R_f	UV 254	R_f	UV 365	R_f	DIL. H_2SO_4	R_f	ANISAL DEHYDE	RF	$AICL_3$ UV	R_f	DRAGEN DORFF	R_f
OIC	$CHCl_3$:CH_3OH (9:1)	3 light yellow	0.369, 0.492, 0.861	4black	0.329 0.487 0.682 0.878	3black, 1 uv active	0.543 0.574 0.659 0.851	1 purple, 3 yellow	0.347 0.495 0.661 0.826	1red 2yellow 3blue green	0.263 0.354 0.463 0.636 0.818	1yellow 2black	0.475 0.655 0.852	2 reddish brown, 2blue green	0.495 0.371 0.666 0 .886
OIE		2 light yellow	0.365, 0.486	4black	0.325 0.486 0.681 0.876	5 black 1 uv active	0.234 0.34 0.543 0.574 0.659 0.851	3Yellow	0.345 0.495 0.661	3 yellow	0.236 0.445 0.472	4 black 1yellow	0.229 0.327 0.459 0.655 0.851	2 reddish brown, 1 blue green	0.486 0.370 0.643
OIA		not visible	0	not visible	0	not visible	0	not visible	0	not visible	0	not visible	0	not visible	0
OIC	$CHCl_3$:CH_3OH (8:2)	2 yellow	0.639, 0.819	4black	0.59 0.77 0.836 0.934	4 black	0.591 0.771 0.836 0.934	3 yellow	0.591 0.773 0.835	2 purple 2 yellow	0.592 0.778 0.836 0.935	4 light yellow, 1brown, 1yellow 1black	0.158 0.301 0.396 0.666 0.73 0.825 0.968	3 black	0.687 0.734 0.812
IOIE		2 yellow	0.635, 0.815	4black	0.59 0.77 0.836 0.935	4 black	0.589 0.769 0.835 0.932	3 yellow	0.59 0.771 0.832 0.931	2 yellow	0.590 0.771	4yellow	0.666 0.73 0.825 0.968	1 reddish brown, 1 black	0.684 0.731
OIA		not visible	0	not visible	0	not visible	0	not visible	0	not visible	0	not visible	not visible	not visible	0
OIC	C_6H_6:$CHCl_3$ (1:1)	not visible	0	1black	0.109	1 deep blue, 3 uv active	0.115 0.393 0.571 0.964	1yellow, 2 black	0.098 0.215 0.980	2 purple, 1 yellow	0.04 0.12 0.96	1 black, 2yellow	0.104 0.541 0.916	1 black	0.123
OIE		not visible	0	1black	0.108	1 blue, 1 uv active	0.178 0.39	not visible	0	not visible	0	1 black	0.104	1 black	0.12
OIA		not visible	0	not visible	0	not visible	0	not visible	0	not visible	0	not visible		not visible	0
OIC	$ChCl_3$	not visible	0	1black	0.219	4 uv active	0.200 0.342 0.742 0.914	3yellow	0.200 0.342 0.742 0.914	1 yellow	0.218	2yellow	0.816 0.915	blue green	0.218
OIE		not visible	0	1black	0.218	not visible	0	not visible	0	not visible	0	not visible	0	blue green	0.216
OIA		not visible	0	not visible	0	not visible	0	not visible	0	not visible	0	not visible	0	not visible	
OIC	$ChCl_3$:$C_4H_8O_2$ (1:1)	2 yellow	0.596 0.865	2black	0.594 0.878	2black, 1active	0.638 0.833 0.888	yellow	0.842	yellow	0.842	3yellows	0.666 0.861 0.972	deep black	0.846
OIE		2 yellow	0.594 0.864	2black	0.592 0.876	2 black	0.631 0.829 0.885	yellow	0.842	yellow	0.842	3yellows	0.270 0.666 0.861	black	0.846
OIA		not visible	0	not visible		not visible	0	not visible	0	not visible	0	not visible	0	not visible	0
OIC	CH_3OH:HCl (9:1)	Green and brown mixed	0.694	black	0.846	2 black	0.846 0.641	1Yellow, 1 light brown	0.833 0.694	yellow	0.861	yellow UV active	0.861	blue green, dark brown	0.857, 0.628
OIE		Green and brown mixed	0.694	black	0.846	light green, black	0.820 0.641	2 light brown	0.833 0.556	light brown	0.833	yellow UV active	0.833	light brown, orange	0.8 0.6
OIA		Green and brown mixed	0.722	black	0.82	light green	0.82	2 dark brown	0.639	no proper spots	0	yellow UV active	.833	light brown	0.571

Table 2: TLC of different extracts of *O. indicum* and *M. pachycarpa*.

Extracts	Solvent system	NORMAL	R_f	UV 254	R_f	UV 365	R_f	DIL. H_2SO_4	R_f	ANISAL DEHYDE	RF	$AICL_3$ UV	R_f	DRAGEN DORFF	R_f
MPC	$CHCl_3$:CH_3OH (9:1)	1 light yellow	0.953	1black	0.953	3 uv active	0.531 0.659 0.787	1 purple 3yellow	0.157 0.297 0.347 0.512 0.917	5 purple	0.145 0.263 0.472 0.627 0.927	3yellow	0.472 0.62 0.927	2 reddish brown, 1 black	0.452 0.745 0.975
MPE		not visible	0	not visible	0	not visible	0	not visible	0	not visible	0	not visible	0	not visible	0
MPA		not visible	0	not visible	0	not visible	0	not visible	0	not visible	0	not visible	0	not visible	0
MPC	$CHCl_3$:CH_3OH-(8:2)	not visible	0	2black	0.786 0.983	2 black	0.786 0.983	4 purple	0.347 0.512 0.59 0.771	5 purple	0.512 0.590 0.771 0.786 0.983	yellow	0.656 0.733 0.821 0.888 0.967	1 reddish brown, 1 black	0.637 0.921
MPE		not visible	0	not visible	0	not visible	0	not visible	0	1 brown		not visible	not visible	1 reddish brown	0.657
MPA		not visible	0	1black	0.196	1black	0.196	not visible	0	not visible	0	not visible	not visible	not visible	0
MPC	C_6H_6:$CHCl_3$ (1:1)	not visible	0	2black	0.229 0.937	3 uv active	0.225 0.500 0.571 0.941	3 black	0.372 0.705 0.985	3 purple	0.24 0.70 0.96	4yellow	0.416 0.541 0.645 0.916	1 reddish brown 1 blue green	0.136 0.26
MPE		not visible	0	not visible	0	1 uv active	0.51	not visible	0	not visible	0	2yellow	0.395 0.520	not visible	0
MPA		not visible	0	not visible	0	not visible	0	not visible	0	not visible	0	not visible	0	not visible	0
MPC	$ChCl_3$	not visible	0	3black	0.097 0.218 0.390	4 uv active	0.342 0.571 0.742 0.914	5 yellow	0.097 0.218 0.390 0.495 0.818	2 yellow, 3 light blue	0.097 0.218 0.390 0.495 0.818	4yellow	0.218 0.390 0.495 0.818	3 reddish brown, 1blue green	0.074 0.121 0.243 0.485
MPE		not visible	0	not visible	0	1 uv active	0.572	1 yellow	0.572	1 yellow	0.572	1yellow	0.572	not visible	
MPA		not visible	0	not visible	0	not visible	0	not visible	0	not visible	0	not visible	0	not visible	
MPC	$ChCl_3$:$C_4H_8O_2$ (1:1)	not visible	0	1black	0.966	3 UV active	0.361 0.722 0.833	brown	0.926	2 deep blue	0.169 0.924	3yellows	0.722 0.833 0.972	black	0.927
MPE		not visible	0	not visible	0	not visible	0	not visible	0	not visible		not visible	0	not visible	0
MPA		not visible	0	not visible	0	not visible	0	not visible	0	not visible		not visible	0	not visible	0
MPC	CH_3OH:HCl (9:1)	light brown	0.694	black	0.82	light green	0.82	1 light purple	0.833	no proper spots	0	yellow UV active	0.778	not visible	0
MPE		light brown	0.833	black	0.82	not visible	0	2 purple	0.833 0.611	red	0.861	no UV active	0	light orange	0.8
MPA		light brown	0.75	not visible	0	not visible	0	1light purple	0.556	no proper spots	0	no UV active	0	not visible	0

Table 3: TLC profile of different extracts of *M. pachycarpa*.

and aqueous extracts of *O. indicum* and *M. pachycarpa* contained flavonoids (Table 1).

Cardiac glycosides

The phytochemical analysis of chloroform, ethanol and aqueous extracts of *O. indicum* showed the presence of cardiac glycosides. The cardiac glycosides were also present in the chloroform and ethanol extracts of *M. pachycarpa* however, these phytochemicals were completely absent in its aqueous extract (Table 1).

Saponins

Saponins were absent in the chloroform extract of *O. indicum*, whereas they were present in its ethanol and aqueous extracts. The analysis of chloroform, ethanol and aqueous extracts of *M. pachycarpa* showed the presence of saponins (Table 1).

Tannins

Analysis of tannins showed that these phytochemicals were present in all the extracts of *O. indicum, whereas M. pachycarpa* showed the presence of tannins in both the chloroform and ethanol extracts. Tannins were completely absent in the aqueous extract of *M. pachycarpa* (Table 1).

Steroids

Test for steroids showed the ethanol extract of both the *O. indicum* and *M. pachycarpa* contained steroids, however the other extracts of *O. indicum* and *M. pachycarpa* did not show any trace of steroids (Table 1).

Pholobatannins

Analysis for phlobatannins for both the *O. indicum* and *M. pachycarpa* revealed that these plants did not contain phlobatannins (Table 1).

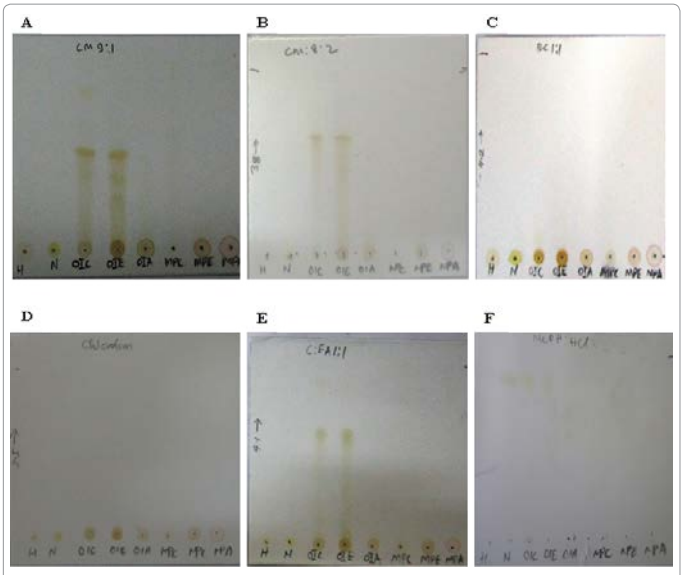

Figure 1: TLC profile of *O. indicum* and *M. pachycarpa*. Different solvent systems observed under normal light to detect phytochemicals present in the extracts. (A) $CHCl_3$:CH_3OH (9:1) (B) $CHCl_3$:CH_3OH (8:2) (C) C_6H_6:$CHCl_3$ (1:1) (D) $CHCl_3$ (E) $CHCl_3$:$C_4H_8O_2$ (1:1) (F) CH_3OH:HCl (9:1) H: Hesperidin, N: Naringin both are flavonoid used as standard. OIC: *O. indicum* chloroform extract MPC: *M. pachycarpa* chloroform extract OIE: *O. indicum* ethanol extract MPE: *M. pachycarpa* ethanol extract OIA: *O. indicum* aqueous extract MPA: *M. pachycarpa* aqueous.

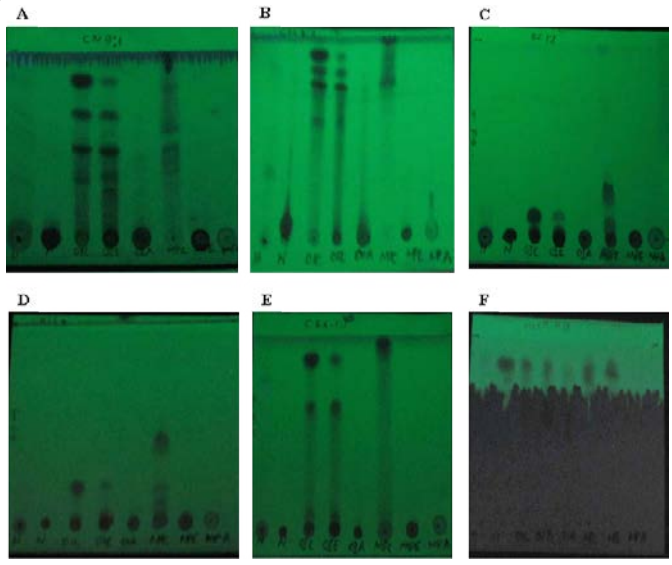

Figure 2: TLC profile of *O. indicum* and *M. pachycarpa*. Different solvent system observed under UV 254 nm to detect phytochemicals present in the extracts. (A) $CHCl_3$:CH_3OH (9:1) (B) $CHCl_3$:CH_3OH (8:2) (C) C_6H_6:$CHCl_3$ (1:1) (D) $CHCl_3$ (E) $CHCl_3$:$C_4H_8O_2$ (1:1) (F) CH_3OH:HCl (9:1) H: Hesperidin, N: Naringin both are flavonoid used as standard. OIC: *O. indicum* chloroform extract MPC: *M. pachycarpa* chloroform extract. OIE: *O. indicum* ethanol extract MPE: *M. pachycarpa* ethanol extract. OIA: *O. indicum* aqueous extract MPA: *M. pachycarpa* aqueous.

TLC profiling

The chloroform, ethanol and aqueous extracts were subjected to TLC profiling using different solvent systems as mobile phase. The different solvent systems provided different R*f* values for various spots under UV and day light or with anisaldehyde or aluminum chloride indicating the presence of a number of phytochemicals in the *O. indicum* and *M. pachycarpa* (Tables 2 and 3). The TLC plates of different solvent systems are shown in Figures 1-7.

Discussion

The plants have attracted the attention of men since its evolution and with the elapse of time humans have discovered the medicinal value of several natural products including plants for their healthcare. Several older systems of medicine including Ayurveda, Chinese and others are principally based on the plants/natural products. The advent of allopathic system of medicine reduced the dependence of humans

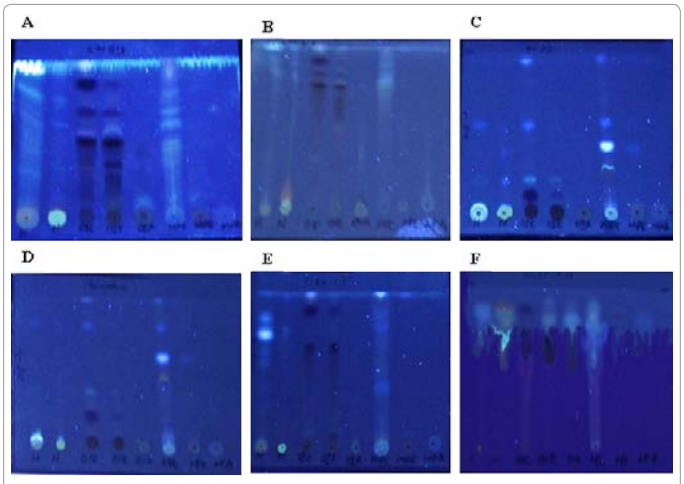

Figure 3: TLC profile of *O. indicum* and *M. Pachycarpa*. Different solvent systems observed under UV 365 nm to detect phytochemicals present in the extracts. A) $CHCl_3$:CH_3OH (9:1) B) $CHCl_3$:CH_3OH (8:2) C) C_6H_6:$CHCl_3$ (1:1) D) $CHCl_3$ E) $CHCl_3$:$C_4H_8O_2$ (1:1) F) CH_3OH:HCl (9:1) H: Hesperidin, N: Naringin both are flavonoid used as standard. OIC: *O. indicum* chloroform extract MPC: *M. pachycarpa* chloroform extract OIE: *O. indicum* ethanol extract MPE: *M. pachycarpa* ethanol extract OIA: *O. indicum* aqueous extract MPA: *M. pachycarpa* aqueous.

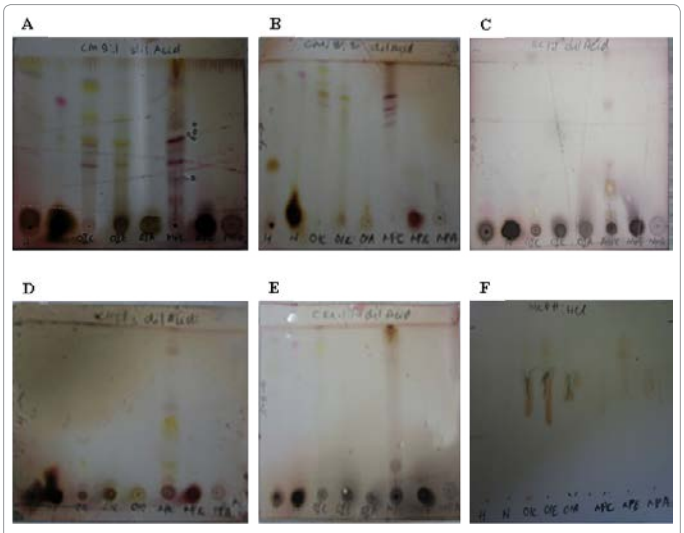

Figure 4: TLC profile of *O. indicum* and *M. Pachycarpa*. Different solvent systems sprayed with dil H_2SO_4 to detect phytochemicals present in the extracts. A) $CHCl_3$:CH_3OH (9:1) B) $CHCl_3$:CH_3OH (8:2) C) C_6H_6:$CHCl_3$ (1:1) D) $CHCl_3$ E) $CHCl_3$:$C_4H_8O_2$ (1:1) F) CH_3OH:HCl (9:1) H: Hesperidin, N: Naringin both are flavonoid used as standard. OIC: *O. indicum* chloroform extract MPC: *M. pachycarpa* chloroform extract OIE: *O. indicum* ethanol extract MPE: *M. pachycarpa* ethanol extract OIA: *O. indicum* aqueous extract MPA: *M. pachycarpa* aqueous.

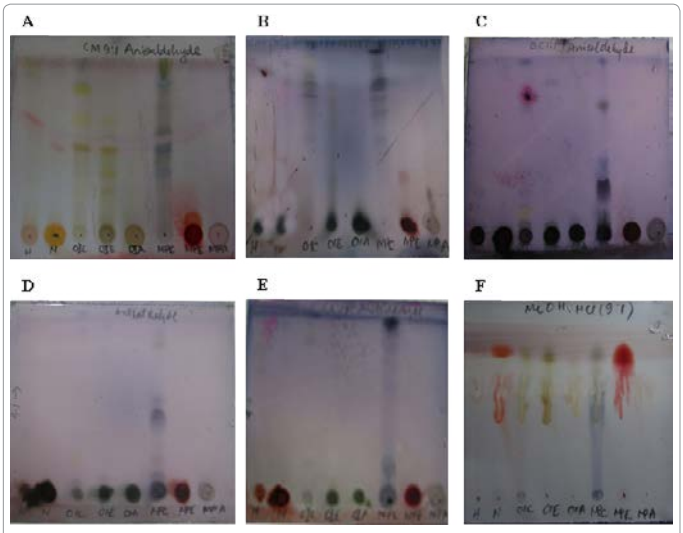

Figure 5: TLC profile of *O. indicum* and *M. pachycarpa*. Different solvent systems sprayed with anisaldehyde to detect phytochemicals present in the extracts. A) $CHCl_3$:CH_3OH (9:1) B) $CHCl_3$:CH_3OH (8:2) C) C_6H_6:$CHCl_3$ (1:1) D) $CHCl_3$ E) $CHCl_3$:$C_4H_8O_2$ (1:1) F) CH_3OH:HCl (9:1) H: Hesperidin, N: Naringin both are flavonoid used as standard. OIC: *O. indicum* chloroform extract MPC: *M. pachycarpa* chloroform extract OIE: *O. indicum* ethanol extract MPE: *M. pachycarpa* ethanol extract OIA: *O. indicum* aqueous extract MPA: *M. pachycarpa* aqueous.

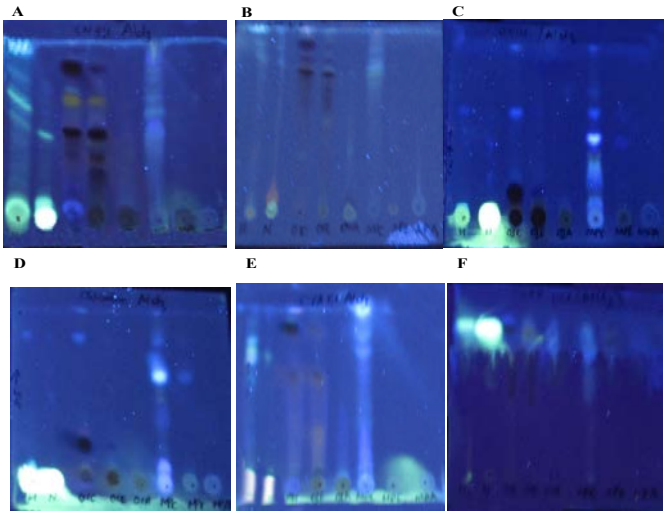

Figure 6: TLC profile of *O. indicum* and *M. pachycarpa* of different extracts. Different solvent systems sprayed with Aluminium chloride and observed under UV 365 nm to detect phytochemicals present in the extracts. A). $CHCl_3$:CH_3OH (9:1) B) $CHCl_3$:CH_3OH (8:2) C) C_6H_6:$CHCl_3$ (1:1) D) $CHCl_3$ E) $CHCl_3$:$C_4H_8O_2$ (1:1) F) CH_3OH:HCl (9:1) H: Hesperidin, N: Naringin both are flavonoid used as standard. OIC: *O. indicum* chloroform extract MPC: *M. pachycarpa* chloroform extract OIE: *O. indicum* ethanol extract MPE: *M. pachycarpa* ethanol extract OIA: *O. indicum* aqueous extract MPA: *M. pachycarpa* aqueous.

on plants and natural products for human healthcare since most of the drugs are chemically synthesized. Despite this, it is well known that many of the modern drugs are directly or indirectly derived from plants or natural products until their chemical synthesis began [7]. Further, many of the molecules synthesized by plants are very complex and difficult to synthesize in the laboratory therefore we have to still depend on nature for them. There has been a recent spurt in research in the plant/natural products as medicine since it is believed that they are either non-toxic or less toxic than the synthetic drugs, which is to some extent may be true due to their biologic origin. Since vast array of plants are used for human healthcare their systematic scientific evaluation is required. Therefore, the present study was undertaken to evaluate the phytochemical constituents of *O. indicum* and *M. pachycarpa* that are used as a traditional medicine in India and China.Out of several phytochemicals synthesized by plants alkaloids are essential for plant defense against stimulation, protection, flavouring, pigmentation, microbe infection, insects and herbivory [30,31]. The alkaloids are more commonly synthesized by angiosperms than the other plants. The alkaloids are nitrogen containing organic molecules and more than 12,000 alkaloids have been isolated from plants and many more will be extracted from plants in the years to come. Their structure is very complex and laboratory synthesis has always been challenging. The alkaloids are toxic and usually this property has been ingeniously used by humans as a medicine or poisons since time immemorial [32]. The alkaloids have been used as stimulants [33-35]. Several drugs used for treatment of cancer, neurological, cardiovascular and several other health related disorders in human are alkaloids [36]. We have detected presence of alkaloids in both *O. indicum* and *M. pachycarpa* and their medicinal use may lie in these phytochemicals. The *O. indicum* has shown the presence of alkaloids in the chloroform and aqueous extracts but not in the methanol extract [37]. However we have not observed the alkaloids in the aqueous extract, which may be due the nonpolar nature of the alkaloids, which may not be soluble in water. A similar observation has been made earlier [38].The flavonoids are polyphenolic compounds and they protect plants against pathogenesis, stress and the adverse effect of UV light and also provide multitude of colours to flowers that help in pollination [39-41]. No wonder that plants produce a wide array of more than 8000 different flavonoids. Many of the medicinal activities of both *O. indicum* and *M. pachycarpa* may be attributed to the presence of polyphenolic flavonoids. Earlier *O. indicum* has been found to contain flavonoids in chloroform, alcohol and water extracts [42]. However, systematic report regarding the presence of flavonoids in *M. pachycarpa* is lacking. Flavonoid have been found to be of great medicinal value in humans as they have been found to act as antiallergic, antiatherosclerotic, antioxidants, antifungal, antimutagenic, antithrombogenic, anti-inflammatory, antiviral antiosteoporotic, cardioprotective and radioprotective in several studies [43-55]. The flavonoids also stimulate signaling pathways required for various activities in the cells [56]. They have also been reported to modulate various transcription factors in different study systems [57].The organisms do not waste their energy in futile exercises and plants synthesize cardiac glycosides for defence since some of them are poisonous [58]. Digitalis is a cardiac glycoside that has been used to protect heart [58]. However, cardiac glycosides possess numerous other activities including diuretic, expectorant cytotoxic and anticancer (as early as 1967). The cardiac glycosides have been found to be active against numerous cancers like breast, prostate, melanoma, pancreatic and lung cancers, and leukaemia, neuroblastoma and renal adenocarcinoma [58-60]. The cardiac glycosides are helpful in treating cardiac disorders like heart failure and atrial arrhythmia [59]. The use of cardiac glycosides in clinical trials has shown that digoxin administration with chemotherapy increased the overall survival in patients suffering from breast, colorectal, head and neck, and hepatocellular carcinoma [60]. The cardiac glycosides have also been reported to induce apoptosis [61]. The cardiac glycosides have been detected in all the extracts of *O. indicum* and *M. pachycarpa,* except the aqueous extract of the latter. The presence of cardiac glycosides reaffirms their use a traditional medicine.Saponins are produced by plants to protect them against pathogens and herbivory. They have been found to kill fungus, insects and molluscs that attack plants and also act as allelopathic [62-64].

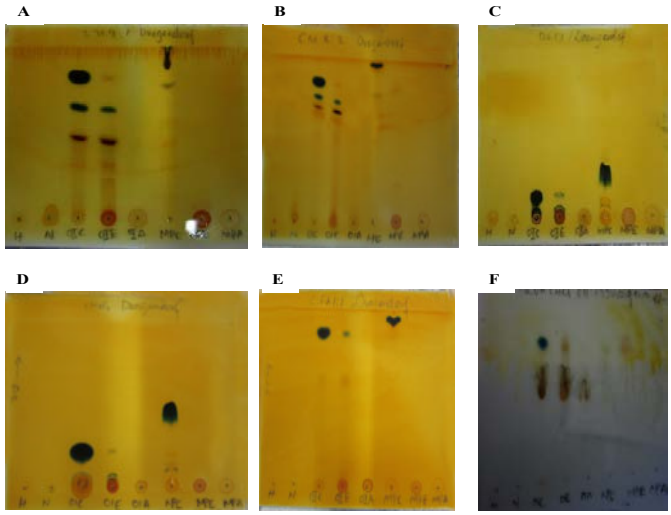

Figure 7: TLC profile of *O. indicum* and *M. pachycarpa* of different extracts. Different solvent systems sprayed with Dragendorff's reagent to detect phytochemicals present in the extracts. A). $CHCl_3$:CH_3OH (9:1) B) $CHCl_3$:CH_3OH (8:2) C) C_6H_6:$CHCl_3$ (1:1) D) $CHCl_3$ E) $CHCl_3$:$C_4H_8O_2$ (1:1) F) CH_3OH:HCl (9:1) H: Hesperidin, N: Naringin both are flavonoid used as standard. OIC: *O. indicum* chloroform extract MPC: *M. pachycarpa* chloroform extract; OIE: *O. indicum* ethanol extract; MPE: *M. pachycarpa* ethanol extract; OIA: *O. indicum* aqueous extract MPA: *M. pachycarpa* aqueous.

Saponins also act as anticancerous, and antiangiogenic agents and have been reported to inhibit the progression of the cell cycle and induce apoptosis [65]. The other activities attributed to saponins include antioxidant, anticarcinogenic, immunostimulatory, antibacterial, antifungal, antiviral, antiprotozoal, hypoglycemic, hemolytic, immune adjuvant and membrane permeabilizing [62,63,64]. The saponins were present in all the extracts of *O. indicum*, and *M. pachycarpa*, except the chloroform extract of the former. The presence of saponins in these plants may have also contributed to their medicinal properties.The presence of phytosterols in both *O. indicum* and *M. pachycarpa* has been confirmed by test for sterols. The plant sterols are responsible for their growth and maintenance of temperature [66]. The sterols have been found to possess a variety of activities in humans. Steroids are anticancerous, antiinflammatory, antiatherosclerotic, and antiobese, and pain relieving. They also act as hypoglycemic, hypocholesterolemic, analgesic and hormones in humans [67-69]. The phytochemical analysis of both *O. indicum* and *M. pachycarpa* showed the presence of alkaloids, flavonoids, cardiac glycosides, saponins, phytosterol, taninns and triterpenoids. Their presence was further confirmed by TLC profiling. The medicinal properties of both *O. indicum* and *M. pachycarpa* may be due to the presence of these phytochemicals which have been individually reported to possess a diverse array of activities. Elders may not be aware of their chemical constituents but they certainly knew well the medicinal applications of *O. indicum* and *M. pachycarpa* for the human healthcare.

Acknowledgements

The authors are thankful to the UGC-Rajiv Gandhi National Fellowship-ST for providing financial assistance to Miss K. Lalrinzuali and the Mizoram University-UGC Fellowship to Mr. M. Vabeiryureilai. The financial assistance from DBT and UGC to Prof. G. C. Jagetia is also thankfully acknowledged.

References

1. Cseke LJ, Kirakosya A, Kaufman PB, Warber SL, Duke JA, et al. (2006) Natural products from plants (2nd edn) Taylor and Francis CRC Press.
2. Irchhaiya R, Kumar A, Yadav A, Gupta N, Kumar S, et al. (2015) Metabolites in plants and its classification. World J Pharm Pharma Sc 4: 287-305.
3. Waterman PG (1992) Roles for secondary metabolites in plants. Ciba Found Symp 269-275.
4. Croteau R, Kutchan TM, Lewis NG (2000) Natural Products (Secondary Metabolites) In: Buchanan B, Gruissem W, Jones R (eds) Biochemistry & Molecular Biology of Plants. American Society of Plant Physiologists. Chapter 24: 1251-1318.
5. Li J, Ou-Lee TM, Raba R, Amundson GG, Last RL (1993) Arabidopsis mutants are hypersensitive to UV-B radiation. Plant Cell 5: 171-179.
6. Shula YM, Jitendra J, Dhruve, Patel NJ, Bhatnagar R, et al. (2009) Plant secondary metabolites (1st edn) New India publishing agency, New Delhi, India p. 4.
7. Newman, Cragg (2014) Marine-Sourced Anti-Cancer and Cancer Pain Control Agents in Clinical and Late Preclinical Development. Mar Drugs 12: 255-278.
8. Qin S, Xing K, Jiang JH, Xu LH, Li WJ, et al. (2011) Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. Appl Microbiol Biotechnol 89: 457-473.
9. Miquel FAW (1852) Millettia pachycarpa Bentham. Pl jungh 250.
10. Haifan, Zhang (1996) Observation on curative effect of Huteng Tang (Huzhang and Millettia Combination) in treating side effects caused by cancer chemotherapy". Pract J Integ Chin West Med 9: 137.
11. Jainul MA, Azam S, Chowdhury A (2013) In vitro cytotoxic activity of methanolic extract of M. pachycarpa (Benth) leaves. Pharm Innov J 2: 10-13.
12. Chowdhury A, Mamun AA, Rahman S, Azam S, Shams K, et al. (2013) Human red blood cell membrane stability testing for the estimation of anti-inflammatory activity of methanolic extract of Millettia pachycarpa Benth leaves. Int J Pharm Sci Res 4: 4587-4590.
13. Agarwal VS (2003) Directory of Indian Economic Plants. In: Bishen Singh Mahendra Pal Singh, Dehradun, India p: 335.
14. Chopra RW, Badwar RL, Ghosh S (1949) Poisonous Plants in India. Calcutta: Government of India Press. 1: 391-393.
15. Ye H, Fu A, Wu W, Li Y, Wang G, et al. (2012) Cytotoxic and apoptotic effects of constituents from Millettia pachycarpa Benth. Fitoterapia 83: 1402-1408.
16. Kirtikar KR, Basu BD (2001) Indian Medicinal Plants. Dehradun: Oriental Enterprises.
17. Gu Q, Luo H, Zheng W, Liu Z, Huang Y (2006) Pseudonocardia oroxyli sp. nov., a novel actinomycete isolated from surface-sterilized Oroxylum indicum root. Int J Syst Evol Microbiol 56: 2193-2197.
18. Sastry AVS, Sastry VG, Mallikarjun P, Srinivas K (2011) Chemical and pharmacological evaluation of aqueous extract of root bark of Oroxylum indicum Vent. Int J Pharm Technol 3: 1796-1806.
19. Hosen SMZ, Das R, Rahim ZB, Chowdhury N, Paul L, et al. (2011) Study of analgesic activity of the methanolic extract of Acorus calamus L. and Oroxylum indicum Vent by acetic acid induced writhing method. Bull Pharm Res Inst 3: 63-7.
20. Talari S, Sampath A, Sujatha K, Nanna RS (2013) Antibacterial activity of stem bark extracts of Oroxylum indicum an endangered ethnomedicinal forest tree. IOSR J Pharm Biol Sci 7: 24-28.
21. Rasadah AM, Houghton JP, Amala R, Hoult JRS (1998) Antimicrobial and anti-inflammatory activities of extract and constituent of Oroxylum indicum (L) vent. Phytomedicine 5: 375-381.
22. Ong CY, Ling SK, Ali RM, Chee CF, Samah ZA, et al (2009) Systemic analysis of in vitro photo-cytotoxic activity in extracts from terrestrial plants in Peninsula Malaysia for photodynamic therapy. J Photochem Photobiol B 96: 216-22.
23. Kumar AR, Rajkumar V, Guha G, Mathew L (2010) Therapeutic potentials of Oroxylum indicum bark extracts. Chin J Nat Med 8: 121-126.
24. Suffness M, Douros J (1979) Drugs of plant origin. Methods in Can Res 26: 73-126.
25. Harborne JB (1998) Phytochemical methods. A guide to modern techniques of plant analysis (3rdedn).
26. Kokate CK, Purohit AP, Gokhale SB (2006) Pharmacognosy (35thedn) Mirali Publisher, Pune, India: 593-597.
27. Doughari JH (2012) Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents, phytochemicals - A global perspective of their role in nutrition and health. In: Venketeshwer Rao (eds), InTech, Rijeka, Croatia.

28. Sofowara A (1993) Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria: 289.

29. Trease GE, Evans WC (1989) Pharmacognosy (11thedn) Brailliar Tiridel Can. McMillian publishers.

30. Ghasemzadeh A and Ghasemzadeh N (2011) Flavonoids and phenolic acids: Role and biochemical activity in plants and human. J Med Plants Res 5: 6697-6703.

31. Pedras MS, Yaya EE (2015) Plant chemical defenses: are all constitutive antimicrobial metabolites phytoanticipins? Nat Prod Commun 10: 209-218.

32. De Luca V, St Pierre B (2000) The cell and developmental biology of alkaloid biosynthesis. Trends Plant Sci 5: 168-173.

33. Rothman RB, Baumann MH, Dersch CM, Romero DV, Rice KC, et al. (2001) Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. Synapse 39: 32-41.

34. Dani JA, Bertrand D (2007) Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu Rev Pharmacol Toxicol 47: 699-729.

35. Karamese M, Akdag O, Kara I, Yildiran GU, Tosun Z (2015) The comparison of intrathecal morphine and IV morphine PCA on pain control, patient satisfaction, morphine consumption, and adverse effects in patients undergoing reduction Mammoplasty. Plasty 15: e15.

36. Jiang QW, Chen MW, Cheng KJ, Yu PZ, Wei X, et al. (2015) Therapeutic Potential of Steroidal Alkaloids in Cancer and Other Diseases. Med Res Rev.

37. Samatha T, Srinivas P, Shyamsundarachary R, Rajinikanth M, Swamy NR (2012) Phytochemical analysis of seeds, stem bark and root of an endangered medicinal forest tree Oroxylum indicum (L) Kurz. Int J Pharm Bio Sci 3: (B) 1063-1075.

38. Ramaswamy N, Samatha T, Srinivas P, Chary RS (2013) Phytochemical screening and TLC studies of leaves and petioles of Oroxylum indicum (L.) Kurz. an endangered ethnomedicinal tree. Int J Pharm Life Sci (IJPLS) 4: 2306-2313.

39. Mouradov A, Spangenberg G (2014) Flavonoids: a metabolic network mediating plants adaptation to their real estate. Front Plant Sci 5: 620.

40. Carletti G, Nervo G, Cattivelli L (2014) Flavonoids and Melanins: a common strategy across two kingdoms. Int J Biol Sci 10: 1159-1170.

41. Iwashina T (2015) Contribution to flower colors of flavonoids including anthocyanins: a review. Nat Prod Commun 10: 529-544.

42. Tripathy BN, Panda SK, Sahoo S, Mishra SK, Nayak L (2011) Phytochemical analysis and hepatoprotective effect of stem bark of Oroxylum indicum (L) Vent. on carbon tetrachloride induced hepatotoxicity in rat. Int J Pharm Biol Sci Arch 2: 1714-1717.

43. Yanoshita R, Chang HW, Son KH, Kudo I, Samejima Y (1996) Inhibition of lyso PAF acetyl transferase activity by flavonoids. Inflamm Res 45: 546-549.

44. Lindahl M, Tagesson C (1997) Flavonoids as phospholipase A2 inhibitors: importance of their structure for selective inhibition of group II phospholipase A2. Inflammation 21: 347-356.

45. Bagchi M, Milnes M, Williams C, Balmoori J, Ye X, et al. (1999) Acute and chronic stress-induced oxidative gastrointestinal injury in rats, and the protective ability of a novel grape seed proanthocyanidin extract. Nutr Res 9: 1189-1199.

46. Manthey JA, Grohmann K, Guthrie N (2001) Biological properties of citrus flavonoids pertaining to cancer and inflammation. Curr Med Chem 8: 135-153.

47. Nijveldt RJ, van Nood E, van Hoorn DEC, Boelens PG, van Norren, et al. (2001) Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 74: 418-25.

48. Jagetia GC, Reddy TK (2002) The grapefruit flavanone naringin protects against the radiation-induced genomic instability in the mice bone marrow: a micronucleus study. Mutat Res 519: 37-48.

49. Jagetia GC, Reddy TK (2005) Modulation of radiation-induced alteration in the antioxidant status of mice by naringin. Life Sci 77: 780-794.

50. Jagetia GC, Reddy TK (2014) The grape fruit flavonone naringin protects mice against doxorubicin-induced cardiotoxicity. J Mol Biochem 3: 64-49.

51. Jagetia GC, Venkatesha VA, Reddy TK (2003) Naringin, a citrus flavonone, protects against radiation-induced chromosome damage in mouse bone marrow. Mutagenesis 18: 337-343.

52. Jagetia GC, Venkatesha VA (2005) Effect of mangiferin on radiation induced micronucleus formation in cultured human peripheral blood lymphocytes. Environ Mol Mutagen 46: 12-21.

53. Schuier M, Sies H, Illek B, Fischer H (2005) Cocoa-related flavonoids inhibit CFTR-mediated chloride transport across T84 human colon epithelia. J Nutr 135: 2320-2325.

54. Cushnie TP, Lamb AJ (2005) Antimicrobial activity of flavonoids. Int J Antimicrob Agents 26: 343-356.

55. Tanaka T (2013) Flavonoids as complementary medicine for allergic diseases: current evidence and future prospects. OA Alter Med 1: 11.

56. Grassi D, Desideri G, Mai F, Martella L, De Feo M, et al. (2015) Cocoa, Glucose Tolerance, and Insulin Signaling: Cardiometabolic Protection. J Agric Food Chem.

57. Mansuri ML, Parihar P, Solanki I, Parihar MS (2014) Flavonoids in modulation of cell survival signalling pathways. Genes Nutr 9: 400.

58. Hollman A (1985) Plants and cardiac glycosides. Br Heart J 54: 258-261.

59. Prassas I, Diamandis EP (2008) Novel therapeutic applications of cardiac glycosides. Nat Rev Drug Discov 7: 926-935.

60. Menger L,Vacchelli E, Adjemian S, Martins I, Ma Y, et al. (2012) Cardiac glycosides exert anticancer effects by inducing immunogenic cell death. Sci Transl Med 18: 4 (143).

61. Cerella C, Muller F, Gaigneaux A, Radogna F, Viry E (2015) Early down regulation of Mcl-1 regulates apoptosis triggered by cardiac glycoside UNBS1450. Cell Death Dis 6: e1782.

62. Aladesanmi AJ (2006) Tetrapleura tetraptera: molluscicidal activity and chemical constituents. Afr J Tradit Complement Altern Med 4: 23-36.

63. Sung WS, Lee DG (2008) In vitro candidacidal action of Korean red ginseng saponins against Candida albicans. Biol Pharm Bull 31: 139-142.

64. Nielsen JK, Nagao T, Okabe H, Shinoda T (2010) Resistance in the plant, Barbarea vulgaris, and counter-adaptions in ?ea beetles mediated by saponins. J Chem Ecol 36: 277-285.

65. Man S, Gao W, Zhang Y, Huang L, Liu C (2010) Chemical study and medical application of saponins as anti-cancer agents. Fitoterapia 81: 703-714.

66. Dufourc EJ (2008) The role of phytosterols in plant adaptation to temperature. Plant Signal Behav 3: 133-134.

67. Okwu DE (2001) Evaluation of the chemical composition of indigenous spices and flavouring agents. Global J Pure Appl Sci 7: 455-459.

68. Marangoni F, Poli A (2010) Phytosterols and cardiovascular health. Pharmacol Res 61: 193-199.

69. Schonfeld G (2010) Plant sterols in atherosclerosis prevention. Am J Clin Nutr 92: 3-4.