



**PHYTOCHEMICAL ANALYSIS OF SEEDS, STEM BARK AND ROOT OF AN  
ENDANGERED MEDICINAL FOREST TREE *OROXYLUM INDICUM*(L)KURZ**

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**ABSTRACT**

The species of *Oroxylum indicum* is an endangered forest tree known to possess flavonoids, alkaloids, saponins and tannins with significant biological activity. To screen the various medicinally important secondary metabolites and for the identification of the presence of biologically active compounds, standard procedures as described by Brindha *et al.*, (1977), Trease and Evans (1989), Sofowora (1993) and Harborne (1998) were followed. The presence of flavonoids, alkaloids, tannins, sterols, saponins, glycosides, phenols and quinones have been detected. The results of the phytochemical screening of crude extracts from seeds, stem bark and root of *Oroxylum indicum* showed the presence of bioactive substances that can be used in prevention of major diseases.

**KEY WORDS :** *Oroxylum indicum*, Phytochemical analysis, Bioactive compounds, Fluorescence analysis and TLC.

**ABBREVIATIONS :** TLC-Thin layer Chromatography, IUCN (International Union for Conservation of Nature)



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## INTRODUCTION

The usage of herbs and medicinal plants to cure various diseases was in practice from the time immemorial. They have been used since ancient days as the plants and plant products provide a useful source of medicine and pharmaceuticals, that can be used to treat not only human diseases but can also be used to enhance the animal production and health, food safety and quality, whilst conserving environment<sup>13</sup>. Plants have long been and continue to be the basis of many traditional medicines worldwide. Asian traditional medicinal systems such as traditional Chinese medicine, Korean Chinese medicine, Japanese Chinese medicine, Ayurveda from India, Jamu from Indonesia are well known<sup>15</sup>. Generally, woody plants are versatile plant materials having a wide range of local therapeutic applications, the leaves, roots, bark and seeds are found to be antipyretic, laxative, analgesic, antifungal, antibacterial<sup>5</sup>.

For present study, to screen the various phytochemicals, the medicinally important forest tree species *Oroxylum indicum* (Bignoniaceae) is selected. It is commonly called as *Sonapatha*, *Syonaka*, *Midnight horror* and *Indian trumpet tree*. Plants of *O.indicum* have been used as traditional medicine known to possess unique medicinal properties such as anti-inflammatory, diuretic, anti-arthritic, anti-bacterial, anti-tussive and anti-fungal properties<sup>23</sup>. The roots of *O.indicum* are known for anti-helminthic, anti-bronchitic, anti-luecodermatic, anti-rheumatic, anti-anorexic. The leaves and bark of stem are reported to possess flavonoids baicalein, chrysin, oroxylin-A and scutellarin. The flavonoid baicalein is reported to have an anti-oxidant<sup>18</sup>, hepato protective<sup>19</sup>, anti-ulcer<sup>9</sup> and immunomodulatory activity<sup>13</sup>. This plant is used as an astringent, carminative, diuretic, stomachic, aphrodisiac and high potential for stimulating digestion, curing fevers, coughs and preventing other respiratory disorders<sup>7</sup>. Bark of *O.indicum* is reported to be used to

cure fevers, gastritis, hypertension, liver disorders, cancer killing maggots, purgative, headache<sup>8</sup> epilepsy, muscular sprain and general weakness<sup>10</sup>; control hypertension<sup>16</sup>.

Four flavonoid constituents from the seeds of *O. indicum* have been isolated and identified as chrysin, baicalein, baicalein-7-O-glucoside and baicalein-7-diglucoside (Oroxilin- B)<sup>3</sup>. Baicalein was used to check proliferation of human breast cancer cell line MDA-MB-435<sup>12</sup> and also observed via induction of apoptosis<sup>11</sup>.

In spite of its extensive use in ayurvedic, herbal, folk and tribal medicine the phytochemical analysis on seeds, bark of stem and root of *O. indicum* was not studied. Hence the present investigation has been under taken the phytochemical analysis to screen the different types of biologically active compounds in *O.indicum*.

## MATERIALS AND METHODS

### *Plant Material*

The seeds, stem bark and root of *Oroxylum indicum*(Fig.1) were collected from Mallur forest region, Warangal district of AndhraPradesh, India in the month of July, 2011. The plant material was washed thoroughly with distilled water and was shade dried for two months. Each sample of the material was ground separately into fine powder and stored in air tight containers at ambient temperature.

### *Preparation of Crude Extracts*

5gms of each sample was soaked in conical flask containing 50ml petroleum ether, methanol, benzene, chloroform and water separately for 1hour. The extracts were filtered through Whatman No.1 filter paper. The supernatants were collected, covered, labelled and used for the screening of various phytochemicals.

### **Phytochemical analysis**

The phytochemical analysis of the dry seeds, bark of stem and root was carried out to determine the presence of following bioactive compounds using the standard qualitative procedures<sup>2,22,21,6</sup>.

### **REAGENTS USED FOR THE IDENTIFICATION OF PHYTOCONSTITUENTS**

#### **1. Test for Alkaloids**

Potassium mercuric iodide solution, potassium bismuth iodide solution, solution of iodine in potassium iodide, saturated solution of picric acid, 10% tannic acid solution.

#### **2. Test for Glycosides**

Dinitro-benzene in hot methanolic alkali, pyridine and alkaline sodium nitroprusside solution, bromine water, glacial acetic acid with ferric chloride and concentrated sulphuric acid.

#### **3. Test for Tannins & Phenolic Compounds**

Gelatin solution with sodium chloride, ferric chloride, sodium hydroxide solution, iron and ammonium citrate or iron and sodium tartarate. glacial acetic acid, potassium nitrite.

#### **4. Test for Flavonoids**

Magnesium ribbon and concentrated hydrochloric acid, Zinc dust and concentrated hydrochloric acid, sodium hydroxide.

#### **5. Test for Protein & Amino Acids**

Mercuric nitrate in nitric acid with nitrous acid, ninhydrin (Indane 1,2,3 trione hydrate),

#### **6. Test for Sterols & Triterpenoids**

Acetic anhydride, conc. sulfuric acid, chloroform with conc. Sulfuric acid.

#### **7. Test for Carbohydrates**

Alcoholic alpha naphthol, conc. sulfuric acid, alkaline cupric citrate complex

#### **8. Test for Quinones**

Alcoholic potassium hydroxide,

#### **9. Test for Lignins**

Gallic acid, furfuraldehyde

#### **10. Test for Fats & Oils**

Alcoholic potassium hydroxide, phenolphthalein

#### **11. Test for Saponins**

Olive oil

#### **12. Test for Pholbatannins**

Hydrochloric acid

### **TESTS PERFORMED FOR THE PRESENCE OF PHYTOCONSTITUENTS**

#### **a)Tests for Alkaloids**

- 1) Dragendorff's test: To 1 ml of each of the sample solution taken in a test tube few drops of Dragendorff's reagent (potassium bismuth iodide solution) was added. A reddish brown precipitate was observed indicating the presence of alkaloids.
- 2) Meyer's test: To 1ml of each of the sample solution few drops of Meyer's reagent (potassium mercuric chloride solution) was added. A creamish white precipitate was formed indicating the presence of alkaloids.
- 3) Wagner's test: To few ml of each of the sample solution, Wagner's reagent(Iodine in potassium iodide) was added, which resulted in the formation of reddish brown precipitate indicating the presence of alkaloids.
- 4) Hager's test: To 1 ml of each of the sample few drops of Hager's reagent (Picric acid) was added. Yellow precipitate was formed reacting positively for alkaloids.

- 5) Tannic acid test: When few ml of 10% Tannic acid was added to 1ml of each sample, a buff colour precipitate was formed giving positive result for alkaloids.
- 6)  $\text{FeCl}_3$  test: One drop of  $\text{FeCl}_3$  solution was added to each of the test sample, formation of yellow precipitate was resulted reacting positively for alkaloids.

#### **b) Tests for Glycosides**

1. Raymond's test: Test solution when treated with dinitrobenzene in hot methanolic alkali giving a violet colour
2. Legal's test: When the test samples were treated with pyridine and sodium nitroprusside solution blood red colour appears
3. Bromine water test: When treated with bromine water test solution gives yellow precipitate.
4. Kellar Kiliani test: 1ml of concentrated sulphuric acid was taken in a test tube then 5ml of extract and 2ml of glacial acetic acid with one drop of ferric chloride were added, formation of a blue colour.
5. Concentrated Sulphuric acid test: Conc.  $\text{H}_2\text{SO}_4$  was added to test sample which resulted in appearance of reddish colour.
6. Molisch test: When alpha naphthol and concentrated  $\text{H}_2\text{SO}_4$  were added to test samples reddish violet ring at junction of two layer was resulted.

#### **c) Tests for Tannins and Phenolic Compounds**

1. Ferric chloride test: When few drops of ferric chloride were added to sample solution a blackish precipitate appears.
2. Gelatin test: When gelatin and water were added to test samples formation of white precipitate was resulted.
3. Lead acetate: Few ml of test samples were taken in different test tubes followed by the addition of aqueous basic lead acetate. It

results in the formation of reddish brown bulky preceipitate.

4. Alkaline reagent: When sodium hydroxide solution was added to the sample solution results in the formation of yellow to red precipitate.
5. Mitchell's test: Tannins give a water soluble iron-tannin complex with iron and ammonium citrate or iron and sodium tartarate.
6. Ellagic acid test: When 5% glacial acetic acid and 5% sodium nitrite were added to test samples a muddy niger brown colour appears, which is a positive result for phenols.

#### **d) Tests for Flavonoids**

1. Zinc Hydrochloride reduction test: To test the sample solution for the flavonoids added a mixture of zinc dust and concentrated hydrochloric acid results in red colour.
2. Lead acetate test: When aqueous basic lead acetate was added to test sample produces reddish brown precipitate.
3. Ferric chloride test: To few ml of test samples taken separately, few drops of ferric chloride were added which resulted in the formation of blackish red precipitate.
4. Shinoda test (Magnesium hydrochloride reduction test): To the test solution few fragementes of magnesium ribbon and concentrated hydrochloric acid were added drop wise and reddish to pink colour was resulted.
5. Alkaline reagent test: When sodium hydroxide solution was added to the test samples formation of intense yellow colour, which turns to colour less on addition of few drops of dilute acid indicates the presence of flavonoids.

#### **e) Tests for Sterols**

1. Libermann-Buchard test: When samples were treated with few drops of acetic anhydride, boiled and few drops of concentrated sulphuric acid from the sides of the test tube were added, shows a

brown ring at the junction of two layers and the upper layer turns green which shows the presence of steroids.

2. Salkowski test: Few drops of concentrated sulphuric acid were added to the test samples in chloroform, a red colour appears at the lower layer indicates the presence of sterols

**f) Tests for Fats and Oils**

1. Stain test: Press the small quantity of each extract between two filter papers, the stain on filter papers indicates the presence of the oils.
2. Saponification test: Added a few drops of 0.5N alcoholic potassium hydroxide to various extracts with a drop of phenolphthalein separately and heat on water bath for 1-2hours, formation of soap or partial neutralization of alkali indicates the presence of oils and fat

**g) Tests for Lignins**

1. Labat test: When gallic acid is added to the test sample, it results in the formation of olive green colour.
2. Furfuraldehyde test: When furfuraldehyde is added to the test sample a red colour appears indicating the presence of lignin.

**h) Tests for Quinones**

Alcoholic KOH test: When alcoholic KOH was added to the test samples red to blue colour appears reacting positively for quinones.

**i) Tests for Saponins**

1. Foam test: 5ml of extract was shaken vigorously to obtain a stable persistent froth. The froth was then mixed with three drops of olive oil and observed for the

formation of an emulsion, which indicated the presence of saponins.

**THIN LAYER CHROMATOGRAPHY**

The chromatograms of various solvent extracts of seed, bark of stem and root of *O.indicum* were developed by using commercially available aluminium sheets of Silica gel 60 F<sub>254</sub> (Merck) with methanol:ethylacetate:water in the ratio 36:36:28 as the solvent system. Spots of each solvent were carefully spotted using a capillary tube at about 3-4 cms from the bottom of the TLC plate and were carefully placed in the solvent system at an angle of 45° taking care that the solvent system is not coming in contact with the spots. The chromatogram was allowed to develop and after development the plates were taken out, allowed to dry and visualized by spraying Dragendorff's reagent which acts as a visualizing agent followed by fumigation in the Iodine chamber which further aids in clear visualization of the spots.

**RESULTS AND DISCUSSION**

The preliminary phytochemical screening of seeds, stem bark and root extracts of *O.indicum* was done to identify bioactive compounds by using standard procedures<sup>2,22,21,6</sup>. Fluorescence analysis of all the extracts under UV and normal light are given in Table-1. The analysis showed that the extracts in different organic solvents and water are presented in tables 2-4. The phytochemical analysis performed for the presence of various bioactive compounds revealed the high concentrations of flavonoids, alkaloids, tannins, saponins, phenols, fats&oils and lower concentrations of glycosides, quinones, lignins and sterols.

**Table-I**  
**Showing the fluorescence analysis of various extracts of *Oroxylum indicum* under normal and UV light**

Name of the extract	Colour of the extract under normal light			Colour of the extract under UV light		
	Seed	Bark	Root	Seed	Bark	Root
Acetone	Muddy brown	Muddy brown	Pale green	Yellowish green	Yellowish green	Dark Green
Benzene	Muddy brown	Yellowish brown	Pale green	Yellowish green	Yellowish green	Dark Green
Chloroform	Muddy brown	Muddy brown	Pale green	Yellowish green	Yellowish green	Dark Green
Methanol	Muddy brown	Golden brown	Dark green	Yellowish green	Yellowish green	Dark Green
Petroleum ether	Muddy brown	Muddy brown	Pale green	Yellowish green	Yellowish green	Dark Green
Water	Muddy brown	Muddy brown	Pale green	Yellowish green	Yellowish green	Dark Green

**Table-2**  
**Analysis of Phytochemicals on Seed extracts of *Oroxylum indicum***

Phytochemical test	Methanol Extract	Petroleum ether Extract	Benzene Extract	Chloroform Extract	Aqueous Extract	
ALKALOID S	Dragendorff's test	+	+	+	-	+
	Mayer's test	+	-	-	-	+
	Wagner's test	+	+	+	-	+
	Hager's test	+	+	+	-	+
	Tanicacid test	+	+	+	-	+
GLYCOSIDES	Raymond's test	+	+	+	-	+
	Legal's test	+	+	+	-	+
	Bromine water test	+	+	+	-	+
	Kellar Kiliani test	+	+	+	-	+
	Conc. H <sub>2</sub> SO <sub>4</sub> test	+	+	+	-	+
	Molisch test	+	+	+	-	+
TANNINS	FeCl <sub>3</sub> test	+	+	+	+	+
	Gelatin test	+	+	+	+	+
	Lead acetate test	+	+	-	+	+
	Alkaline reagent test	+	+	+	+	+
	Mitchell's test	-	-	+	+	+

F L A V O N O I D S	Zn-HCl reduction test	+	-	-	-	+
	Lead acetate test	+	+	-	-	+
	FeCl <sub>3</sub> test	+	+	+	-	+
	Shinoda's test	-	+	-	-	-
	Alkaline reagent test	+	+	+	-	+
STEROLS	Liebermann Burchard test	+	+	+	+	-
	Salkowski test	+	-	+	+	-
FATS & OILS	Stain test	+	+	+	+	+
	Saponification test	+	+	+	+	+
PHENOLS	FeCl <sub>3</sub> test	-	-	-	-	+
	Elagic acid test	-	-	-	-	+
LIGNINS	Labat test	-	-	-	-	-
	Lignin(furfuraldehyde ) test	-	-	-	-	-
QUINONES	Alcoholic KOH test	-	-	-	-	-
SAPONINS	Foam test	+	+	+	+	+

+ = Present; - = Absent

**Table-3**  
**Analysis of Phytochemicals on Bark extracts of *Oroxylum indicum***

Phytochemical test		Methanol Extract	Petroleum ether Extract	Benzene Extract	Chloroform Extract	Aqueous Extract
A L K A L O I D S	Dragendorff's test	-	+	+	+	+
	Mayer's test	+	-	-	-	-
	Wagner's test	-	+	+	+	+
	Hager's test	-	+	+	-	+
	Tanicacid test	-	+	+	-	+
G L Y C O S I D	Raymond's test	-	-	-	-	-
	Legal's test	-	-	-	-	-
	Bromine water test	-	-	-	-	-
	Kellar Kiliani test	-	-	-	-	-
	Conc. H <sub>2</sub> SO <sub>4</sub> test	-	-	-	-	-
	Molisch test	-	-	-	-	-

E S  T A N N I N S	FeCl <sub>3</sub> test	+	+	+	+	+
	Gelatin test	+	+	+	+	+
	Lead acetate test	+	+	-	+	+
	Alkaline reagent test	+	+	+	+	+
	Mitchell's test	-	-	+	+	+
F L A V O N O I D S	Zn-HCl reduction test	+	-	-	-	+
	Lead acetate test	+	+	-	-	+
	FeCl <sub>3</sub> test	+	+	+	-	+
	Shinoda's test	-	+	-	-	-
	Alkaline reagent test	+	+	+	+	+
STEROLS	Libermann Burchard test	+	-	+	-	-
	Salkowski test	+	-	+	+	-
FATS & OILS	Stain test	+	+	+	+	+
	Saponification test	+	+	+	+	+
PHENOLS	FeCl <sub>3</sub> test	+	+	+	+	+
	Elagic acid test	+	+	+	+	+
LIGNINS	Labat test	-	-	-	-	-
	Lignin(furfuraldehyde) test	-	-	-	-	-
QUINONES	Alcoholic KOH test	-	-	-	-	-
SAPONINS	Foam test	+	+	+	+	+

+ = Present; - = Absent

**Table-4**  
**Analysis of Phytochemicals on root extracts of *Oroxylum indicum***

Phytochemical test	Methanol Extract	Petroleum ether Extract	Benzene Extract	Chloroform Extract	Aqueous Extract
A L K A L O I D S	Dragendorff's test	+	+	+	+
	Mayer's test	+	+	+	+
	Wagner's test	+	+	+	+
	Hager's test	+	+	+	+
	Tanicacid test	+	+	+	+



G L Y C O S I D E S	Raymond's test	-	-	-	-	-
	Legal's test	+	+	+	+	+
	Bromine water test	+	+	+	+	+
	Kellar Kiliani test	-	-	-	-	-
	Conc. H <sub>2</sub> SO <sub>4</sub> test	-	-	+	+	-
	Molisch test	-	-	-	-	-
T A N N I N S	FeCl <sub>3</sub> test	+	+	+	+	+
	Gelatin test	-	+	+	+	-
	Lead acetate test	-	-	-	-	-
	Alkaline reagent test	+	+	+	+	+
	Mitchell's test	-	-	-	-	-
F L A V O N O I D S	Zn-HCl reduction test	+	+	+	+	+
	Lead acetate test	-	-	-	-	-
	FeCl <sub>3</sub> test	+	+	+	+	+
	Shinoda's test	+	+	+	+	+
	Alkaline reagent test	+	+	+	+	+
STEROLS	Liebermann Burchard test	+	+	+	+	+
	Salkowski test	+	+	+	+	+
FATS & OILS	Stain test	+	+	+	+	+
	Saponification test	+	-	-	-	+
PHENOLS	FeCl <sub>3</sub> test	+	+	+	+	+
	Elagic acid test	+	+	+	+	+
LIGNINS	Labat test	-	-	-	-	-
	Lignin(furfuraldehyde) test	+	+	+	+	+
QUINONES	Alcoholic KOH test	-	-	-	-	-
SAPONINS	Foam test	+	+	+	+	+

+ = *Present*; - = *Absent*

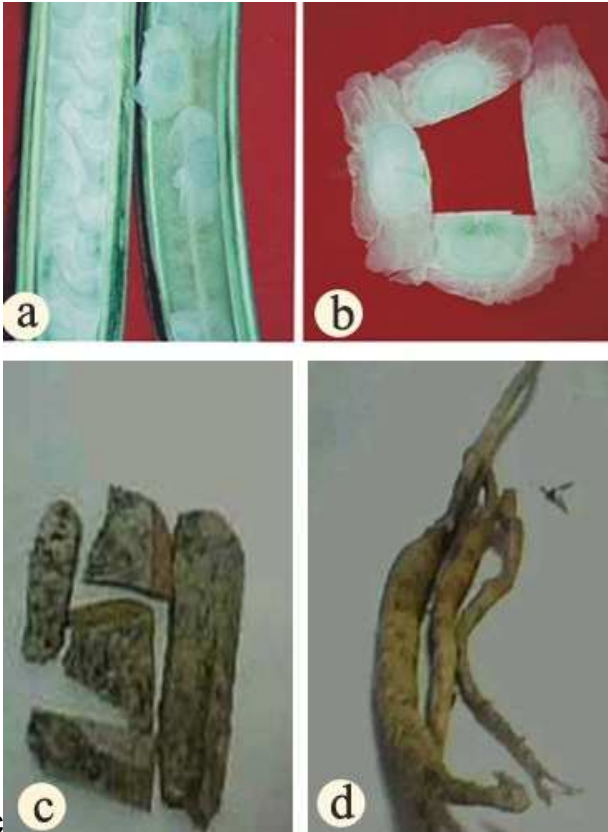


Figure 1: Showing a) fruit split open with seed, b) Fresh seeds, c) bark of stem and d) roots of *Oroxylum indicum*

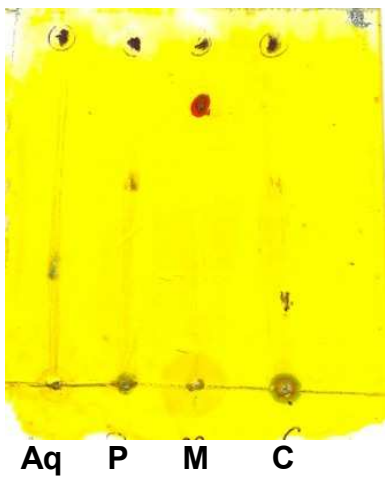


Figure 2:  
(Aq-aqueous, P-Petroleum ether, M-Methanol, C-Chloroform )  
TLC plate showing spots developed from various extracts of seed of *O. indicum*.

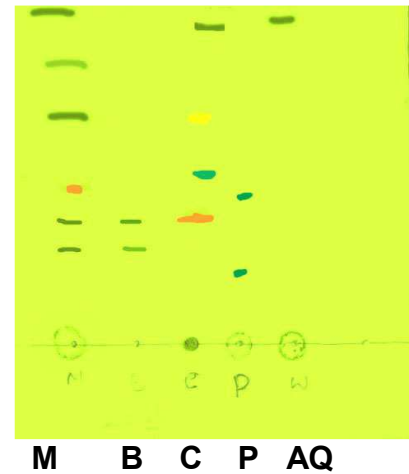


Figure 3  
(M-Methanol, B-Benzene, C-Chloroform, P-Petroleum ether, AQ-Aqueous)  
TLC plate showing spots developed from various extracts of stem bark of *O. indicum*.



Figure 4  
(B-Benzene, C-Chloroform, M-Methanol, Aq-aqueous )  
TLC plate showing spots developed from root extracts of *Oroxylum indicum* .

### **Seed extracts**

Out of the four organic solvents (methanol, benzene, petroleum ether, chloroform) of seed extracts except chloroform other three solvents showed positive results for the presence of alkaloids, flavonoids, tannins, glycosides, sterols, phenols, saponins, fats and oils. Whereas chloroform extract of seed showed negative results for alkaloids, glycosides, flavonoids, lignins and quinones.

Alkaloids were screened positive in all the extracts except in petroleum ether and water. Glycosides were absent in aqueous extracts and weakly detected in methanol, benzene and petroleum ether. Tannins were screened positive in all the extracts and flavonoids were weakly present in all the extracts except in methanol and they were reported to be strongly present. Sterols, saponins and Lignins were positive in all the extracts while quinones were negative.

### **Stem Bark extracts**

The screening for the phytochemicals present in the bark extract of *O. indicum* revealed the presence of low concentrations of alkaloids, saponins, lignins, fats and oils, moderate concentration of tannins, flavonoids, sterols and very low concentrations of phenols and complete absence of quinones. All the organic solvent extracts of bark were strongly positive for tannins, saponins, sterols, phenols, fats and oils. Alkaloids were weakly detected in methanolic extract and chloroform extracts of bark. Glycosides were almost absent in all the five extracts. Flavonoids were screened positive in all the extracts of bark except in chloroform extract. Petroleum ether and aqueous extracts of bark showed positive result for lignins while in methanol and chloroform extracts they were absent. Quinones were absent in all the solvent extracts of stem bark tested.

### **Root extracts**

All the solvent extracts of root were positively resulted for the presence of flavonoids, alkaloids, glycosides, tannins, sterols, phenols,

lignins, saponins, fats and oils while quinones were completely absent in all the solvent extracts of root. Alkaloids, flavonoids, tannins, sterols and phenols were found to be strongly positive while glycosides and saponins were weakly positive.

Secondary metabolites are chemicals produced by plants. Secondary metabolites can be classified on the basis of chemical structure, composition, their solubility in various solvents. A simple classification includes three main groups: terpenes, phenolics and nitrogen containing compounds. Tannins have antibacterial, antidote, haemostatic, mild diuretic, stomachic and insecticidal properties. Saponins are known to possess anti mutagenic, anti fungal, hypocholesterolic, anti cancerous, anti cytotoxic, zenotoxic and clastogenic, anti inflammatory, hemolytic, immunoadjuvent, anti protozoal, spermicidal activities. Tannins protect against microbiological degradation of dietary proteins in the semen<sup>1</sup>.

Alkaloids comprised the largest single class of secondary plant substances. They have a remarkable range of pharmacological activity. The pharmacological studies in alkaloids have been largely concerned with effect of alkaloids on physiological processes other than inflammation. Alkaloids have anti-tumor, anti-amoebic, anti plasmodial, anti fungal, anti-bacterial, anti-ulcer and anti-feedant activities.

Glycosides possess anti-HIV, anti-oxidant, anti-leukaemia, antidiabetic, cardiac, molluscidal, antibacterial, analgesic, antipyretic, aphrodisiac, laxative and anti-stress characters.

Saponins have been reported to control human cardiovascular diseases and can also decrease cholesterol. These are surface active agents which interfere with or alter the permeability of the cell wall whereas phenols have bactericidal, anti-oxidant, anti-microbial, anti-viral, anti-inflammatory and vasodilatory effects.

In view of the above medicinal properties of various bioactive compounds, due

to high demand and scarcity of genuine herbal drugs used in ayurveda, adulteration and substitution has become a common problem which is increasing very rapidly now a days. *O. indicum* is the one among the group of ten drugs named *Dasamoola*<sup>20</sup>. It has been reported that the presence of bioactive substances in plants play a role in preventing colorectal carcinoma, hypercholesterolcemia and renal calculi<sup>17</sup>.

### Results of TLC analysis

TLC analysis of seed extracts (Fig.2) was performed which resulted in a similar spot of blackish green in all the solvent extracts with  $R_f$  value 0.96 indicating the presence of same compound in all the extracts where as aqueous extract showed a spot of light green with  $R_f$  value 0.37. Petroleum ether extract showed a spot with  $R_f$  value 0.53, methanolic extract showed a yellow spot with  $R_f$  value 0.74 and chloroform extract showed two spots with  $R_f$  value 0.53 and 0.28.

### CONCLUSION

The species *Oroxylum indicum* has been used as a traditional medicine for many different purposes in various medicines such as ayurveda, herbal, tribal and folk. The preliminary phytochemical screening of crude extracts of seeds, bark of stem and root of *O. indicum* revealed the presence of many bioactive substances, hence can be used in preventing many major diseases as the results

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TLC analysis of stem bark extract (Fig.3) was also performed using the same solvent system. The analysis showed the presence of 3 spots in benzene with  $R_f$  values of 0.84, 0.54 and 0.33 and one each in chloroform, methanol, petroleum ether and aqueous extracts with  $R_f$  values 0.54, 0.90, 0.27 and 0.93 respectively.

The extracts of root (Fig.4) of each solvent were subjected to TLC. All the extracts showed two similar spots with  $R_f$  values 0.37 and 0.48, while chloroform, methanol and benzene showed an additional spot with same  $R_f$  value of 0.89 and different spots of dark green with  $R_f$  values 0.13, 0.10 and 0.06 respectively. TLC of aqueous extract showed two different spots with  $R_f$  values 0.68 and 0.86 when visualized with Dragendorff's reagent.  $R_f$  values represent relative migration only, whereas absolute values depend on various environmental parameters (e.g. temperature, humidity) which may vary depending on location.

show therapeutic compositions. Further research is in progress on the study of effects of these compounds.

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