



Phytochemical screening and TLC studies of leaves and petioles of *Oroxylum indicum* (L.) Kurz an endangered ethno medicinal tree

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Abstract

Screening of phytochemicals was performed on leaves and petioles of *Oroxylum indicum*. In order to analyse the various biologically active compounds. Standard procedures developed by Trease & Evans (1989) and Harborne (1998) were followed for the analysis. Analysis of phyto constituents on various extracts of different parts of the plant revealed the presence of flavonoids, alkaloids, saponins, tannins, glycosides, sterols, fats and oils in high, moderate and low concentrations. These biologically active compounds can be used in the treatment of various diseases.

Key-Words: *Oroxylum indicum*, Phytochemical analysis, Phytoconstituents, TLC analysis, Fluorescence analysis

Introduction

The medicinal plants and their derivatives have long been recognized as an important source of therapeutically effective medicines as they contain secondary metabolites which are potential sources of drugs. Plant based products are healthier, safer and more reliable than synthetic products (Benli et al 2008). It has been estimated by WHO that approximately 80% of the world's inhabitants rely on traditional medicine for their primary health care (Farnsworth et al., 1985). Furthermore, increasing reliance of the medicinal plants in the industrialized countries has been traced to the extraction and development of several drugs and chemotherapeutic from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998). During the past 20 years, at least one novel compound from higher plants has been marketed every 2.5 years (Deans and Sovoboda, 1990).

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The species of *Oroxylum indicum* is an endangered and ethnomedicinally important tree. It is found in ravine and moist places of forests of India, Srilanka, Philippines and Indonesia (Anonymous 1972, Bennet et al., 1992). It grows upto a height of 12mts bearing large pinnately compound leaves, reddish purple flowers and elongated capsules.

The different parts such as leaves, seeds, roots, bark of stem of *O. indicum* have been reported to possess anti-rheumatic, anti-bronchitic, anti-leucodermatic, anti-helminthic, anti-anorexic and also in the treatment of leprosy and snakebite (Manonmani et al., 1995). Extracts of seeds known to possess anti-inflammatory, anti-tussive, anti-microbial, analgesic, anti-allergic (Vasanth 1991, Rasadah 1998, Zaveri et al., 2010), anti-bacterial, anti-viral (Tahara et al 1987, Kujumgier et al., 1999), anti-arthritis and diuretic properties (Warrier et al., 1995) and it is also a potential anti-cancer medicinal plant (Mao AA 2002), hence widely employed in tribal, herbal and folk medicine. In ayurvedic system of medicine it is the main ingredient of many therapeutic preparations such as *Dasamoola*, *Chyawanaprasha*, *Brahma rasayana*, *Dhanawantara ghrita*, *Awalwha*, *Narayana taila* and *Dantyardarishta* (Anonymous 1998).

The present investigation is aimed at screening seed, stem bark and root extracts of *Oroxylum indicum* for their biologically active components that can be lead to their potential applications for the treatment of various diseases and sicknesses.

Material and Methods

Collection of Plant material

Fresh leaves with petioles were collected from Mallur forest region, Warangal district of Andhra Pradesh, India, in the month of July, 2011. The plant material was authenticated and identified in the department of Botany, Kakatiya University, Warangal, AP, India.

Preparation of plant materials

The freshly collected samples were washed thoroughly with distilled water and air-dried under shade at room temperature for 30-45 days. Upon drying, the samples were grounded into fine powder mechanically using an electric blender then sieved using a muslin cloth. Finely powdered samples were then stored in air tight containers at ambient temperature until required.

Preparation of plant extracts

For the preparation of extracts the method developed by Odebiyi and Sofowora (1978) was followed. The air-dried finely powdered plant samples (5.0 g of each) were soaked in 50 ml each of chloroform, benzene, acetone, methanol, petroleum ether and distilled water contained in separate 100ml sterile conical flasks. The flasks were covered with sterile cotton plugs followed by wrapping with aluminium foil and shaken at 4h intervals for 24h at room temperature. These crude extracts were then filtered through Whatman No.1 filter paper. The supernatants were collected, covered, labeled and used for the phytochemical screening on leaves and petioles of *O.indicum* for the presence of biologically active constituents alkaloids, flavonoids, tannins, saponins, phenols, glycosides, lignins, phlobatannins, anthraquinones and carbohydrates as described by Trease and Evans (1989) and Harborne (1998).

Phytochemical analysis

The phytochemical analysis of the dry leaf, fresh leaf juice and petiole was carried out to determine the presence of following bioactive compounds using the standard qualitative procedures (Trease and Evans, 1989; Sofowora, 1993 ; Harbone 1998).

Experimental Reagents

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.

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.

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.

Test for Flavonoids

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

Test for Protein & Amino Acids

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

Test for Sterols & Triterpenoids

Acetic anhydride, conc. Sulfuric acid, Chloroform with conc. Sulfuric acid

Test for Carbohydrates

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

Test for Quinones

Alcoholic potassium hydroxide,

Test for Lignins

Gallic acid, furfuraldehyde

Test for Fats & Oils

Alcoholic potassium hydroxide, phenolphthalein

Test for Saponins: Olive oil

Test for Phlobatannins: Hydrochloric acid

Tests for Alkaloids

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.

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.

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.

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.

Tannic acid test: When few ml of 10% Tannic acid were added to 1ml of each sample, a buff colour precipitate was formed giving positive result for alkaloids.

FeCl₃ test: One drop of FeCl₃ solution was added to each of the test sample, formation of yellow

precipitate was resulted reacting positively for alkaloids.

Tests for Glycosides

Raymond's test: Test solution when treated with dinitrobenzene in hot methanolic alkali giving a violet colour

Legal's test: When the test samples were treated with pyridine and sodium nitroprusside solution blood red colour appears

Bromine water test: When treated with bromine water test solution gives yellow precipitate.

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.

Concentrated Sulphuric acid test: Conc.H₂SO₄ was added to test sample which resulted in appearance of reddish colour.

Molisch test: When alpha naphthol and concentrated H₂SO₄ were added to test samples reddish violet ring at junction of two layers was resulted.

Tests for Tannins and Phenolic Compounds

Ferric chloride test: When few drops of ferric chloride were added to sample solution a blackish precipitate appears.

Gelatin test: When gelatin and water were added to test samples formation of white precipitate was resulted.

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 preprecipitate.

Alkaline reagent: When sodium hydroxide solution was added to the sample solution results in the formation of yellow to red precipitate.

Mitchell's test: Tannins give a water soluble iron-tannin complex with iron and ammonium citrate or iron and sodium tartarate.

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.

Tests for Flavonoids

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.

Lead acetate test: When aqueous basic lead acetate was added to test sample produces reddish brown precipitate.

Ferric chloride test: To few ml of test samples taken separately, few drops of ferric chloride were added

which resulted in the formation blackish red precipitate.

Shinoda test (Magnesium hydrochloride reduction test): To the test solution few fragments of magnesium ribbon and concentrated hydrochloric acid were added drop wise reddish to pink colour was resulted.

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.

Tests for Sterols

Liebermann-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.

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.

Tests for Fats and Oils

Stain test: Press the small quantity of each extract between two filter papers, the stain on filter papers indicates the presence of the oils.

Saponification test: Added a few drops of 0.5N alcoholic potassium hydroxide to various extracts with a drop of phenolphthalein separately end heat on water both for 1-2hours formation of soap or partial neutralization of alkali indicates the presence of oils and fats.

Tests for Lignins

Labat test: When gallic acid is added to the test sample, it results in the formation of olive green colour.

Furfuraldehyde test: When furfuraldehyde is added to the test sample a red colour appears indicating the presence of lignin.

Tests for Quinones

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

Tests for Saponins

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.

Flourescence analysis of extracts

Flourescence analysis of various organic solvent extracts of leaves and petioles of *O. indicum* was

studied and the observations are made according to their difference in the colour.

Thin Layer Chromatography

The chromatograms of various solvent extracts of seed, leaf and bark of stem of *O.indicum* were developed by using commercially available aluminium sheets of Silica gel 60 F₂₅₄ (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

Fluorescence analysis of all the extracts under UV and Normal light are given in Table-1. The analysis showed that the extracts showed a slight variation in their colours under UV light compared to normal light.

Phytochemical analysis of the various organic solvents and extracts of the leaves and petioles of *o.indicum* revealed the presence of flavonoids, tannins, saponins, alkaloids, phenols, sterols, glycosides, fats and oils. Similar results were reported by Radhika L.G.2011 and Zaveri et al 2010. The results on phytochemical screening of the fresh and dry leaf and petiole extracts using different organic solvents and water are presented in tables 2 and 3.

The phytochemical analysis performed for the presence of various bioactive compounds revealed the high concentrations of flavonoids, alkaloids, tannins, saponins, phenols, fats and oils and lower concentrations of glycosides, quinones, lignins and sterols.

Fresh and dry leaf extracts

Analysis of phytochemicals in fresh and dry leaf extracts of *O. indicum* showed the presence of flavonoids, alkaloids, glycosides, tannins, sterols, phenols, saponins, fats and oils. In fresh leaf extracts of all the solvents performed to identify these bioactive compounds were strongly positive, where as in the dry leaf extracts the phytochemical detection was weaker than the fresh extract and glycosides were completely absent. The tests for phlobatannins were negative in both the extracts. It has been reported that different phytoconstituents have different degree of solubility in different type of solvents depending on their polarity (Mahmood and Doughari, 2008).

Petiole extracts

Phytochemical screening of petiole extracts resulted in the presence of alkaloids, glycosides, tannins, flavonoids, sterols, phenols, lignins, and saponins while quinones were absent in all the extracts.

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 where they were reported to be strongly present. Sterols, saponins and Lignins were positive in all the extracts while quinones were negative in all the extracts.

TLC Analysis

The extracts of leaf of each solvent were subjected to TLC. Chloroform, methanol and petroleum ether showed one similar spot with R_f value 0.610. Methanolic extract showed four spots of light green, dark green, blackish green and dark green with R_f values 0.288, 0.440, 0.508 and 0.644 respectively where as chloroform extract showed 8 spots of blackish green, yellowish green and yellow with R_f values 0.254, 0.389, 0.610, 0.728, 0.762, 0.847, 0.881 and 0.915. TLC of petroleum ether extract resulted in 4 spots with R_f values 0.389, 0.610, 0.762 and 0.915.

TLC analysis of petiole extract 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 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.

Conclusion

From the above results it is evident that not only the phytochemical screening but also TLC studies of various solvent extracts of leaves and petiole revealed the presence of different phytoconstituents as evidenced by separated compounds with different R_f values. The plant species of *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 leaf and petiole of *O. indicum* revealed the presence of many bioactive substances hence can be used in preventing many major diseases as the results show therapeutic

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

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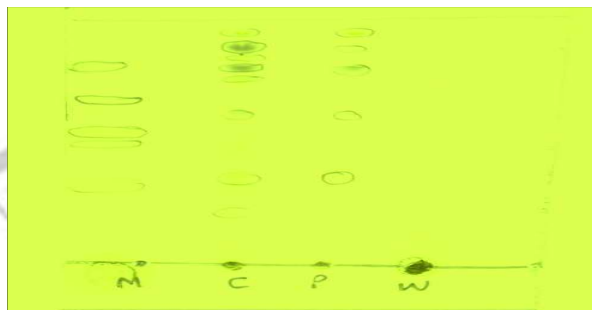


Fig. 1: TLC –Plate showing spots of different solvent extracts of leaf of *Oroxyllum indicum*

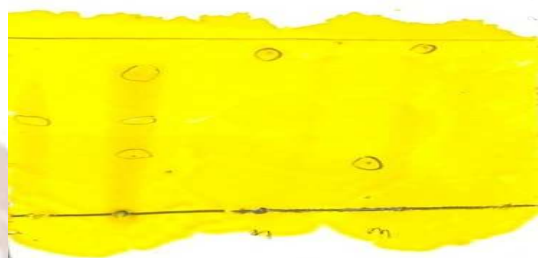


Fig. 2: TLC Plate showing spots of different solvent extracts of petiole of *Oroxyllum indicum*

Table 1: Showing the fluorescence analysis of various extracts under normal and UV light

Name of the extract	Colour of the extract under normal light		Colour of the extract under UV light	
	Leaf	Petiole	Leaf	Petiole
Acetone	Bluish green	Light green	Dark Green	Dark Green
Benzene	Light green	Light green	Dark Green	Dark Green
Chloroform	Light green	Light green	Dark Green	Dark Green
Methanol	Light green	Light green	Dark Green	Dark Green
Petroleum ether	Light green	Light green	Dark Green	Dark Green
Water	Muddy brown	Light green	Dark brown	Dark Green

Table 2: Analysis of Phytochemicals on Leaf extracts of *Oroxyllum indicum*

Phytochemical test		Methanol		Pet. Ether		Benzene		Chloroform		Water	
		F	D	F	D	F	D	F	D	F	D
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	Raymond's test	+	-	+	-	+	-	+	-	+	-
	Legal's test	-	-	-	-	-	-	-	-	-	-
	Bromine water test	+	-	+	-	+	-	+	-	+	-

Y C O S I D E S	Kellar Kiliani test	+	-	+	-	+	+	+	-	+	-
	Conc. H ₂ SO ₄ test	-	-	-	-	-	-	-	-	-	-
	Molisch test	+	-	+	-	+	-	+	-	+	-
T A N N I N S	FeCl ₃ 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 ₃ test	-	+	-	+	-	+	-	+	-	+
	Shinoda's test	-	-	-	+	-	-	-	-	-	-
	Alkaline reagent test	+	+	+	+	+	+	+	+	+	+
S T E R O L S	Liebermann Burchard test	+	+	+	-	+	+	+	-	+	-
	Salkowski test	-	+	-	-	-	+	-	+	-	-
F A T S & O I L S	Stain test	-	+	-	+	-	+	-	+	-	+
	Saponification test	+	+	+	+	+	+	+	+	+	+
P H E N O L S	FeCl ₃ test	-	-	-	+	-	+	-	+	-	-
	Elagic acid test	+	-	+	+	+	+	+	+	+	-
L I G N I N S	Labat test	+	-	+	-	+	-	+	-	+	-
	Lignin(furfuraldehyde) test	-	-	-	-	-	-	-	-	-	-
Q U I N O N E S	Alcoholic KOH test	-	-	-	-	-	+	-	-	-	+
S A P O N I N S	Foam test	+	+	+	+	+	+	+	+	+	+

F= Fresh leaf extract; D= Dry leaf extract; + = Present; - = Absent

Table 3: Analysis of Phytochemicals on petiole extracts of *Oroxylum indicum*

Phytochemical test		Methanol Extract	Petroleum ether Extract	Benzene Extract	Chloroform Extract	Aqueous Extract
A L K A	Dragendorff's test	+	+	+	+	+
	Mayer's test	+	-	+	+	-
	Wagner's test	+	+	+	+	+

L O I D S	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 ₂ SO ₄ test	-	-	-	-	-
	Molisch test	+	+	+	+	-
T A N N I N S	FeCl ₃ 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 ₃ test	+	-	-	-	+
	Shinoda's test	+	-	-	-	-
	Alkaline reagent test	+	+	+	+	+
STEROLS	Libermann Burchard test	+	-	+	-	-
	Salkowski test	+	-	-	+	+
FATS & OILS	Stain test	+	+	+	+	+
	Saponification test	-	-	+	-	+
PHENOLS	FeCl ₃ test	+	+	+	+	+
	Elagic acid test	+	-	-	+	+
LIGNINS	Labat test	-	-	-	-	-
	Lignin(furfuraldehyde) test	+	+	+	+	+
QUINONES	Alcoholic KOH test	-	-	-	-	-
SAPONINS	Foam test	+	+	+	+	+

+ = Present; - = Absent