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

M. Bharani

Abstract

The leaves and petioles of Oroxylum indicum were subjected to a phytochemical screening. To evaluate the numerous bioactive substances. Trease and Evans (1989) and Harborne (1998) methodology standards were used for the analysis. Flavonoids, alkaloids, saponins, tannins, glycosides, sterols, fats, and oils were found in high, moderate, and low amounts, respectively, when phyto component analysis was performed on extracts from different regions of the plant. It is possible to cure a wide range of illnesses with the help of these biologically active chemicals.

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

INTRODUCTION:

Because they contain secondary metabolites that might be used to create pharmaceuticals, medicinal plants and their derivatives have long been acknowledged as an essential source of therapeutically effective medicines. Healthier, safer, and more dependable are plant-based goods as opposed to synthetic ones (Benli et al., 2008). According to the World Health Organization (Farnsworth et al., 1985), over 80% of the world's population uses traditional medicine as their primary health care. In addition, the extraction and production of various medicines and treatments from these plants, as well as from traditionally used rural herbal remedies, has led to an increased dependence on medicinal plants in industrialized nations (UNESCO, 1998). Oroxylum indicum is a critically endangered plant with significant traditional medicinal uses. It is native to India, Sri Lanka, the Philippines, and Indonesia, where you may find it in forested ravines and damp areas (Anonymous 1972, Bennet et al., 1992). Large, pinnately complex leaves, reddish purple blooms, and long, slender capsules characterize this tall (12 m) plant.Anti-rheumatic, anti-bronchitic, anti-leucodermatic, anti-helminthic, anti-anorexic, and even leprosy and snakebite healing properties have been attributed to O. indicum's various leaves, seeds, roots, bark, and stem (Manonmani et al., 1995).

CMR INSTITUTE OF TECHNOLOGY, KANDLAKOYA VILLAGE, MEDCHAL RD, HYDERABAD, TELANGANA,501401R.

It is widely used in tribal, herbal, and folk medicine because extracts of the seeds have been shown to have anti-inflammatory, anti-tussive, anti-microbial, analgesic, anti-allergic (Vasanth 1991, Rasadah 1998, Zaveri et al.,2010), anti-bacterial, anti-viral, anti-arthritic, and diuretic properties (Warrier et al.,1995). Dasamoola, Chyawanaprasha, Brahma rasayana, Dhanawantara ghritha, Awalwha, Narayana taila, and Dantyadarishta are only few of the various medicinal formulations that use it as their primary constituent (Anonymous 1998).

The current study's goal is to identify physiologically active components in Oroxylum indicum seed, stem bark, and root extracts that may one day be used to treat a wide range of medical conditions.

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 thoroughlywith distilled water and air-dried under shade at roomtemperature for 30-45 days. Upon drying, the samples were grounded into fine poweder mechanically usingan electric blender then sieved using a muslin cloth.Finely powedered samples were then stored in airtight 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 phytochemica 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 cirtrate 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 withconc. Sulfuric acid

Test for Carbohydrates

Alcoholic alpha napthol, 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, phenopthalein

Test for Saponins: Olive oil

Test for Pholbatannins: 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'sreagent (potassium bismuth iodide solution) was added. A reddish brown precipitate was observed indicatingthe presence of alkaloids.

Meyer's test: To 1ml of each of the sample

solutionfew 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 ofalkaloids.

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.

$FeCl_3$ test: One drop of $FeCl_3$solution was added to each of the test sample, formation of yel low

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 extractand 2ml of glacial acetic acid with one drop of ferric chloride were added, formation of a blue colour.

Concentrated Sulphuric acid test: Conc.H_2SO_4 was added to test sample which resulted in appearance of reddish colour.

Molisch test: When alpha naphthol and concentrated H_2SO_4 were added to test samples reddish violet ring atjunction 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 aqueousbasic lead acetate. It results in the formation of reddishbrown bulky preceipitate.

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 ironand 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 redprecipitate.

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

Alkaline reagent test: When sodium hydroxide solutionwas added to the test samples formation of intenseyellow colour , which turns to colour less on addition of few drops of dilute acid indicates the presence of flavonoids.

Tests for Sterols

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.

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 witha 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 appearsreacting 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
examined, and findings drawn, based on how their colors vary.

Thin-Layer Chromatography

Using commercially available aluminium sheets of Silica gel 60 F254 (Merck) with a methanol:ethylacetate:water ratio of 36:36:28 as the solvent system, chromatograms of different solvent extracts of seed, leaf, and bark of stem of O.indicum were generated. Careful placement of solvent spots in the solvent system at an angle of 45 degrees, ensuring that the solvent system does not come into contact with the spots, was accomplished using a capillary tube. After the chromatogram had developed, the plates were removed, allowed to dry, and then visualized by spraying a visualizing agent called Dragendorff's reagent on them, followed by fumigation in an iodine chamber, which let the spots stand out more clearly.

Discussion and Results

Table-1 displays the results of fluorescence tests performed on all extracts using both UV and normal illumination. The results of the investigation revealed that there was a negligible change in coloration between UV light and regular light when viewing the extracts.Leaves and petioles of o.indicum were extracted using a variety of organic solvents, and the resulting phytochemical analysis indicated the presence of flavonoids, tannins, saponins, alkaloids, phenols, sterols, glycosides, lipids, and oils. Radhika L.G. 2011 and Zaveri et al. 2010 both found similar findings.Tables 2 and 3 show the results of a phytochemical screening conducted using extracts of fresh and dried leaves and petioles utilizing several organic solvents in addition to water.Flavonoids, alkaloids, tannins, saponins, phenols, lipids, and oils were found in abundance during the phytochemical examination, whereas glycosides, quinones, lignans, and sterols were found in lesser amounts.Flavonoids, alkaloids, glycosides, tannins, sterols, phenols, saponins, lipids, and oils were found in both fresh and dried O. indicum leaf extracts, as were fatty acids. All solvent tests for identifying these beneficial chemicals on fresh leaves came back positively, however the phytochemical detection was lower and glycosides were totally missing from the dried leaf extracts. Both extracts were negative for phlobatannins. Based on their polarity, various phytoconstituents are soluble in a variety of solvents to varying degrees (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 were 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 beenused 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 thestudy of effects of these compounds.

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