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Phytochemical analysis and antibacterial evaluation against selected gram strains by *Oroxylum indicum* (L.) Kurz stem bark extract, a folklore medicine of Sikkim Himalaya

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Abstract

The aim of the present research work was to study the phytochemical constituents and evaluation of antibacterial potency and to perform TLC of the different extracts of the plant *Oroxylum indicum* (L.) Kurz. The crude drug (bark part) was successively extracted by simple maceration process using various solvents. Alkaloids were detected in hexane, chloroform, ethyl acetate and ethanol extracts. Carbohydrates were found in hexane and water extracts. Flavonoids and tannins were common in ethyl acetate, ethanol and water extracts. Glycosides and saponins in water extract only. TLC of hexane extract was performed on the solvent system hexane: ethyl acetate 1:1. Detection of spots was done using dragendorff's reagent and two spots was observed at visible light. Rf value was calculated to be 0.7 and 0.86 respectively of both the spots. Antibacterial study was done using Hexane, Ethanol and Water extract dissolve in DMSO 3% by cup-plate method. It was performed against both gram negative (*Escherichia coli*) and gram positive (*Staphylococcus aureus*). The activity was found to be equivalent to that of standard reference (Streptomycine-2mg/ml). The Hexane extract was found to have higher inhibition than other extracts. The bark extracts exhibited marketed dose dependent antimicrobial activity *in-vitro* against both gram bacteria and can be used as a good therapeutic approach for infection disease management and therapy.

Keywords: *Oroxylum indicum*, phyto-chemicals screening, antibacterial, TLC thin layer chromatography, Sikkim Himalaya, a folklore medicine

Introduction

Oroxylum indicum (L.) Kurz is a species of flowering plant belonging to the monotypic genus *Oroxylum* (frequently spelled *Oroxylon*) and the family Bignoniaceae, commonly called midnight horror, *oroxyllum*, or Indian trumpet flower. It is a mesacole tree which can reach a height of 18 metres (59 ft) [2]. The large leaf stalks wither and fall off the tree and collect near the base of the trunk, appearing to look like a pile of broken limb bones. These twice pinnate leaves in life are up to 7' 10.5" (240 cm) in length and comparably wide, borne on petioles or stalks up to 6' 7" (2 meters) in length, making this the largest of all dicot tree leaves. According to Corner they are quadripinnate (leaflets display four orders of branching). The individual leaflets can be up to six inches (15 cm) long by 3.5 inches (9 cm) wide. The tree is a night-bloomer and flowers are adapted to natural pollination by bats. They form enormous seed pods up to five feet (1.5 meters) long and four inches (10 cm) in width that hang down from bare branches. Those long fruits curve downward and resemble the wings of a large bird or dangling sickles or swords in the night. The seeds are round with papery wings [1].

The plant is native to: the Indian subcontinent, the Himalayan foothills with a part extending to Bhutan and southern China, Indochina and the Malaysia regions. In Vietnam the tree is called núcnác (sometimes sòđo) and specimens can be found in Cat Tien National Park. It is visible in the forest biome of Manas National Park in Assam, India. It is found, raised and planted in large number in the forest areas of the Banswara district in the state of Rajasthan in India. It is reported in the list of rare, endangered and threatened plants of Kerala (South India). It is also reported from Sri Lanka (Ceylon). The large *Oroxylum indicum* pods sold at a market in downtown Bangkok, Thailand [2].

Though plant flower leaves and shoots are often used as vegetables and herbal medicines in Sikkim and Darjeeling hills and also in North-eastern states of India and in parts of Nepal Thailand Myanmar. We used powder of the bark of the tree to perform the phytochemical screening. The crude bark of the plant is also used in wounds [3-6].

Plant profile**Kingdom:** Plantae**Division:** Magnoliophyta**Class:** Lamiales**Family:** Bignoniaceae**Genus:** *Oroxylum***Species:** *indicum***Fig 1:** Tree of *Oroxylum indicum* (L.) Kurz with annual seeds cover**Fig 2:** Seeds of *Oroxylum indicum***Fig 3:** Roots and stem of *Oroxylum indicum***Vernacular Names**

There are many vernacular names of *Oroxylum indicum* in different languages according to distribution of ecozone (Ayurvedic Pharmacopoeia of India; Nadkarni, 1982).

Assamese: Bhatghila,**English:** Broken bones plant, Indian calosantes, Indian Trumpet, Indian trumpet flower, Midnight horror, *Oroxylum*, Tree of Damocles;**Chinese:** Hanyu pinyin: mǔhúdié, butterfly tree,**Nepalese:** Totola,**Bengali:** Tona,**Sanskrit:** Bhut-vriksha, Dirghavrinta, Kutannat, Manduk (the flower) patrona, Putivriksha, Shallaka, Shuran or Son, Vatuk,**Kannada:** Tattuna,**Konkani:** Davamadak,**Malayalam:** Palaqapayyani, ashrrpathiri, Vellappathiri,**Marathi:** Tayitu, Tetu,**Hindi:** Aralu, Shyonaka,**Sinhala (Sri Lanka):** Totila, Thotila,**Tamil:** Cori-konnai, Palai-y-utaicci, Putapusam (the flower),**Telugu:** Manduka-parnamu, Pampena, Suka-nasamu.**In Sikkim, Lepcha & Bhutia:** Phago Rip, Phapni & Kokomando (seeds) [7-8]**Material and Methods**

The raw bark of *Oroxylum indicum* (L.) Kurz was collected from the high altitude from Singtam area East Sikkim. The bark was shade dried and powdered and further stored in an air tight container for further studies. The choice of plant materials in the present study was based on their prospective folk medicine uses.

Preparation of plant extracts

Simple Maceration: 175g shade dried ground plant material for each sample was extracted with each of the solvents: [9-10] Hexane (1000ml), Chloroform (1000ml), Ethyl acetate (1000ml), Ethanol (1000 ml), Water (1000ml)

Note: For 72hrs at room temperature in a 5ltr round bottom flask with occasional shaking.

Filtration: The extracts were separated from residue by filtering 1st through several layers of muslin cloth for coarse filtration and then through watmann filter paper no.1

Evaporation: The filtered extracts were concentrated and the solvents were evaporated under reduced pressure at 40 degree centigrade using water bath [11].

Chromatographic studies

Thin layer Chromatography of Hexane was done using Hexane: Ethyl acetate (1:1) as mobile phase and the Rf value was calculated. The image of the TLC Plate is shown in the image below (Fig. 1). Sample for TLC was dissolved in small quantity of hexane. TLC plate pre coated with silica gel 60 thickness 0.2mm was used as the stationary phase. The mobile phase that is hexane: ethyl acetate (1:1) was saturated for 30 minutes in talc chamber. After activation of tlc plates sample was spotted using capillary tube and the plate was dried for few minutes. Then the plates was kept in the TLC chamber containing saturated mobile phase and allowed to run up to 3/4th of the plate. After development, the plate was removed and air dried. The distance travelled by mobile phase and stationary phase was measured and Rf value was calculated [12-13].

Preliminary phytochemical evaluation

Preliminary Phytochemical examinations were carried out for all the extracts as per the standard method [14-18].

Detection of alkaloid: Extracts were dissolved individually in dil. HCL and filtered:-

- **Mayer's Test:** Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

- **Dragendroff's Test:** Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

- **Molisch's Test:** Filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.
- **Benedict's test:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.
- **Fehling's Test:** Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

- **Modified Borntrager's Test:** Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.
- **Leal's Test:** Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of sponing

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins. **Foam Test:** 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phytosterols

- **Salkowski's Test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.
- **Liebermann Burchard's test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour

indicates the presence of phenols.

Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids

- **Alkaline Reagent Test:** Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
- **Lead acetate Test:** Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.
- **Detection of proteins and amino acids:**
- **Xanthoproteic Test:** The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.
- **Ninhydrin Test:** To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Detection of diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Antibacterial studies

Preliminary screening was carried out for antibacterial activity using different concentration of water and ethanol extracts. The extracts of ethanol and water was dissolved in DMSO 3% solution to check antibacterial activity. The petri Dishes were thoroughly washed and sterilized in hot air oven at 160 degree Celsius for 1 hour. 30ml of sterile nutrient agar medium was poured into sterile petri dish for solidifying. The culture was inoculated with e.coli and strephylococcus. Bores were made on the medium using sterile borer. The test solution (0.1ml) of concentration 0.1, 0.2, 0.3 and 0.4 was added to the respective bores. Streptomycin was used as the standard antibacterial reference of the same concentration as those of test samples. The petri dish was kept in incubator at 37 degree Celsius for 48 hours. The zone of inhibition was observed and measured using a scale and compared with the standard. Antibacterial activity of ethanol and water was carried out for both gram positive and gram negative bacteria in the same culture. The same media was used for sub culturing and antibacterial activity^[19-23].

Result

Extraction

The plant material (175g) was exhaustively extracted with 1000 ml of Hexane, Chloroform, Ethyl Acetate, ethanol and Water respectively using simple maceration process continuously for 15 days. The final extracts were concentrated and dried whose colour nature and yield and % yield is given in table1.

Table 1: Color, nature, yield and percentage yield of bark powder of *Oroxylum indicum* (L.) Kurz

Serial no.	Extraction	Solvent	Colour	Nature	Yield (in grams)	% yield
1	Sequential	Hexane	Yellow	Solid	0.651	0.372%
2	Sequential	Chloroform	Dark Brown	Solid	0.21	0.12%
3	Sequential	Ethyl acetate	Brownish black	Semi Solid	0.395	0.22%
4	Sequential	Ethanol	Brown	Solid	1.59	0.90%
5	Sequential	Water	Brown	Solid	3.39	1.93%

Phytochemical Investigation

Phytochemical investigation covers the identification and characterization of crude drugs with respect to phytochemical

constituents. The plant was evaluated for their chemical constituents. The results for the different types of phytochemicals are shown in table2.

Table 2: Qualitative Chemical Analysis of Extracts.

Serial No.	Chemical Constituents	Tests	Hexane Extract	Chloroform Extract	EtOAc Extract	Ethanol Extract	Water Extract
1	Alkaloids	1. Mayer's test	+	+	+	+	-
		2. Dragendorff's test	+	+	+	+	-
2	Carbohydrates	1. Molisch's test	+	-	+	-	+
		2. Benedict's test	+	-	-	-	-
		3. Fehling's Test	+	-	-	-	+
3	Glycosides	1. Modified borntrager's test	+	-	-	-	+
		2. Legal's test	-	-	-	-	+
4	Saponins	1. Froth Test	-	-	-	-	+
		2. Foam Test	-	-	-	-	+
5	Poytosterol	1. Salkowski's Test	-	+	+	-	-
6	Phenols	1. Ferric chloride Test	-	-	-	+	+
7	Tannins	1. Gelatin Test	-	-	+	+	+
		2. Ferric Chloride Test	-	-	+	+	+
8	Flavonoids	1. Alkaline reagent Test	-	+	+	+	+
		2. Lead Acetate Test	-	+	+	+	+
9	Protein and Amino acids	1. Xanthoprotectic Test	+	+	+	-	+
		2. Ninhydrin Test	+	+	+	-	+
10	Diterpenes	1. Copper Acetate Test	+	+	+	-	+

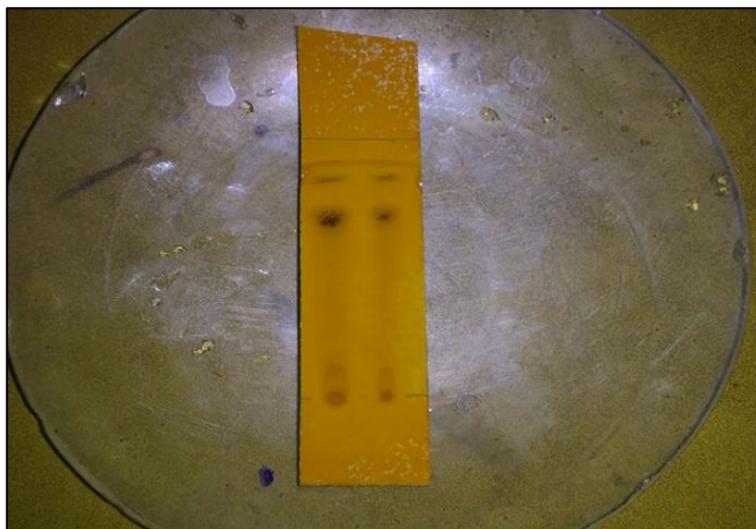
TLC of Hexane Extract

Thin layer chromatography of the hexane extract was done using hexane: ethyl acetate (1:1) as mobile phase and

dragondroff's reagent was sprayed. The rf value were recorded (plate 4.3 and table3).

Table 3: TLC of hexane extract.

Serial no.	Distance travelled by solvent (cm)	Distance travelled by solute (cm)	Rf Value	Colour of the spot
1	6	4.2	0.7	Dark brown
2	6	5.2	0.86	Dark Brown

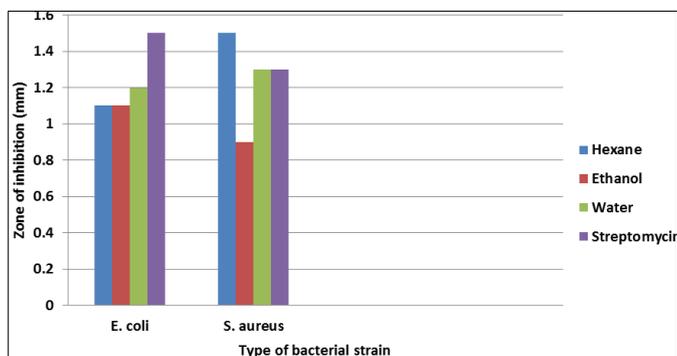
**Fig 4:** TLC of hexane extract**Antibacterial activity**

Antibacterial study was done using ethanol and water extract dissolve in DMSO by cup-plate. It was performed against

both gram negative (*Escherichia coli*) and gram positive (*Staphylococcus aureus*). The activity was found to be equivalent to that of standard reference.

Table 4: Evaluation of antibacterial activity of different extracts of *Oroxylum indicum* (L.) Kurz bark obtained from Sikkim Himalayan regions

Serial No.	Extracts	Dose (mg/ml)	Zone of inhibition. Gram -ve bacteria <i>Escherichia coli</i> .	Zone of inhibition. Gram +ve bacteria. <i>Staphylococcus aureus</i>
1	Hexane	4 mg/ml	1.1 cm	1.5 cm
2	Ethanol	4 mg/ml	1.1 cm	0.9 cm
3	Water	4 mg/ml	1.2 cm	1.3 cm
4	Streptomycin (standard)	2 mg/ml	1.5 cm	1.3 cm



Graph 1: Zone of Inhibition of different extracts against two gram strains bacteria (*E. coli* and *S. aureus*)

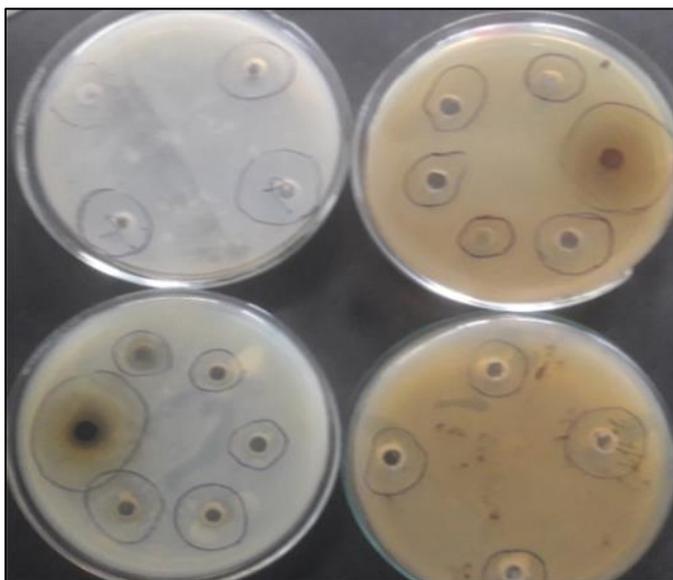


Fig 5: Antibacterial activity of different extracts (Hexane, ethanol and water) as compared to standard drug (Streptomycin-2mg/ml dose).

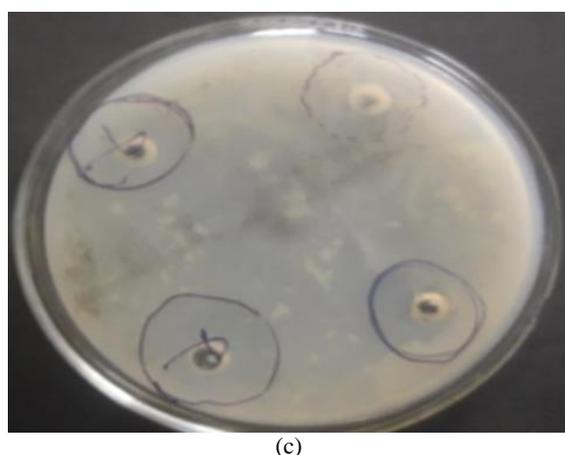
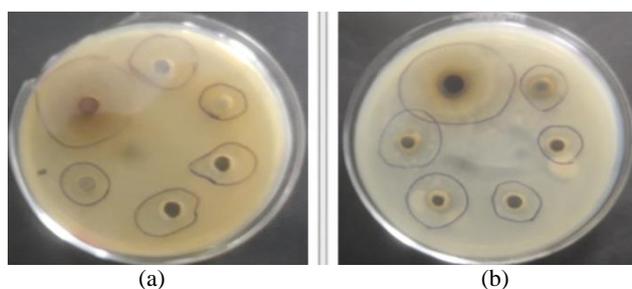


Fig 6: Inhibition zones of ethanol extract (a), streptomycin (b) and water extract (c).

Discussion and Conclusion

Previous paper on this bark reported that phytochemical tests show the presence of carbohydrates in alcohol and water extracts. Phenolic compounds and tannins in acetone, alcohol and water extracts. Flavanoids in chloroform, acetone, alcohol and water extracts were as saponins were observed in water extracts and steroids in petroleum ether extracts only. In our work alkaloids was detected in hexane, chloroform, ethyl acetate and ethanol extracts. Carbohydrates were found in hexane and water extracts. Flavonoids and tannins were common in ethyl acetate, ethanol and water extracts. Glycosides and saponins in water extract only. TLC of hexane extract was performed on the solvent system hexane: ethyl acetate 1:1. Detection of spots was done using dragondroff's reagent and two spots was observed at visible light. Rf value was calculated to be 0.7 and 0.86 respectively of both the spots. Antibacterial study was done using Hexane, Ethanol and Water extract dissolve in DMSO 3% by cup-plate method. It was performed against both gram negative (*Escherichia coli*) and gram positive (*Staphylococcus aureus*). The activity was found to be equivalent to that of standard reference (Streptomycin-2mg/ml). The Hexane extract was found to have higher inhibition than other extracts.

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Conflicts of Interest

Authors declared there are no conflicts of interest to disclose

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