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Research Article

Phytochemical and anti-microbial investigations on Oroxylum indicum

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ABSTRACT

Oroxylum indicum also known as 'Sonapatha' is an important herb in Ayurvedic medicine and indigenous medical system for over thousands of years. Oroxylum indicum, Sonapatha is traditionally used to treat asthma, biliousness, bronchitis, diarrhoea, dysentery, fevers, vomiting, inflammation, leukoderma, skin diseases, rheumatoid arthritis, wound injury, and deworm intestine. Oroxylum indicum has been collected from the premises of IMMT(Formerly known as RRL,Bhubaneswar and authenticated by Dr N.K.Dhal,Senior principal scientist,IMMT,Bhubaneswar.The extraction process for Oroxylum indicum has been done by using solvent of gradient polarity. The extractive value was in order of water> ethanol> chloroform> benzene> pet.ether. Column chromatographic techniques for different extract by using gradient elution process has been followed and the eluents are being subjected for antimicrobial activity by using different microbes such as Acineovacter, E.Coli, S.aureus, M.luteus and R.palanticola. All the bacterial strains were obtained from microbial type culture collection and gene-bank, International depository authority, IMTECH (Chandigarh), India maintained in nutrient broth at -20˚C. All the extracts were subjected for the presence of different type of secondary metabolites such as alkaloids, flavonoids, tannins, anthraquinones, saponins, carbohydrates, sugar, steroid, glycoside, amino acid.

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Introduction

Oroxylum indicum also known as 'Sonapatha' is an important herb in Ayurvedic medicine and indigenous medical system for over thousands of years. It is active ingredient of well-known Ayurvedic formulations like Chyavanprash, Dashmularistha etc. The root bark and stem bark possess anti-allergic properties and are used in treating allergic disease, urticaria, jaundice, asthma, sore throat, laryngitis, hoarseness, gastralgia, diarrhoea, dysentery, infantile, erythema and measles (1) The seeds are active in chronic cough and gastralgia, An alcoholic maceration of fresh bark is applied externally for lacquer allergic dermatitis. The fruits of Oroxylum indicum are acrid, sweet, stomachic, anthelmintic, and good in diseases of the heart and the throat, piles, bronchitis, used as an expectorant, improves the appetite, useful in leucoderma. *Oroxylum* species showed much has been worked out with regards to the various parts of the plants such as bark, heart wood, flowers pods, sap wood. Leaves /pods being available in abundantly throughout the year as compared to all other parts of the plant can be a good source of phytoconstituents even if the amount is less as compared to other parts. Leaves of *Oroxylum indicum* (Family: Bignoneaceae) have not been explored and has not gained attention regarding both phyto-chemical and pharmacological aspects so it was planned to evaluate the leaves/pods of *Oroxylum indicum* (family: Bignoneaceae) for its phyto constituents and pharmacological activity in view of literature reported so far (2,3).

In India, roots are used in Ayurvedic preparation called "Dasamoola" i.e., used as an astringent, anti-inflammatory, anti-helminthic, anti-bronchitic, anti-leucodermatic, anti-rheumatic, anti-anorexic and for treatment of leprosy and tuberculosis (4) . ***Oroxylum*** root bark is the part used in Ayurvedic medicine, administered as an astringent, bitter tonic, stomachic, and anodyne. It is included in famous tonic formulations, such as Chyawanprash. *O. indicum* bark can be used as a potent anticancer medicine, especially against nasopharyngeal cancer (Mao, 2002). It was also reported to possess anticancer properties. The plant was reported to possess various pharmacological activities, which may be due to its antioxidant potential (5).

a. Oroxylum Indicum Chemical Composition

The seeds of *Oroxylum* have a high content of flavonoids, including baicalein and chrysin. From the stem bark of *Oroxylum indicum,* three flavones namely

(1) baicalein (6), **(2)** oroxylin (5) and (**3)** pinostrobin (7) along with one sterol, Stigmast-7-en-3-ol **(**4)**,(7)** were isolated

and their structures were established by the use of spectroscopic techniques. Baicalein and oroxylin were found to be active against brine shrimp with LC_{50} value 10.0 µg/ml and 36.0 µg/ ml and also exhibited the antimicrobial activity on both Gram-positive and Gram-negative bacteria with MIC value 4.0 mg/ml and 8.0 mg/ml respectively.

The root bark is a well-known drug in Ayurvedic system and is prescribed fresh. The root bark is tonic and astringent and useful in diarrhoea and dysentery; it is diaphoretic and is used in rheumatism. Boiled in sesamum oil, it has been recommended for otorrhoea. Also tender fruits are refreshing and stomachic and the seeds purgative. In Malaya, a decoction of the leaves is given in stomach ache and rheumatism; the leaves are used externally or enlarged spleen, headache and ulcers. Seeds of this plant are reported to contain ellagic acid (5,8).

2. Description and Distribution

It is a tree which can reach a height of 12 m (40 ft). It is appearing to look like a pile of broken limb bones. Oroxylum Indicum is native to the Indian subcontinent, in the Himalayan foothills with a part extending to Bhutan and southern china, in Indo-China and the malasia ecozone. It is visible in the forest biome of manas National park in Assam (2).

a. Taxonomical classification:

Kingdom: Plantae

Family: Bignoniaceae

Division: Magnoliophyta

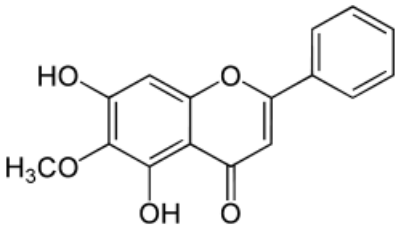
Genus: Oroxylum

Class: Magnoliopsida

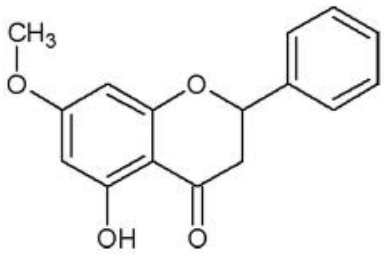
Species: indicum

Order: Lamiales

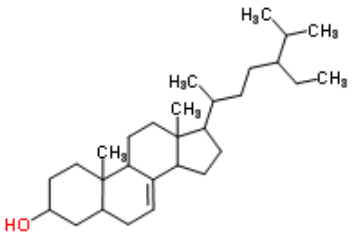
b. Structures of compounds isolated from O. *INDICUM*



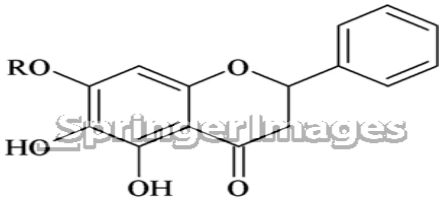
OROXYLIN



PINOSTROBIN



SIGMANT 7-EN-3OL



Baicalein: R = H
Baicalin: R = beta-glucopyranurosyl

BAICALEIN

3. Material and Methods

3.1. Solvents Used For Purification

a) Petroleum ether

b) Chloroform

c) Methanol

d) Ethyl acetate

e) Acetone

3.2. Apparatus Used For Purification

a) Round bottom flask

b) Condenser

c) Conical Flask

d) Glass rod

3.3. Reagents

a) Libermann-Burchard Reagent

b) Mayer's reagent

c) Dragendroff's Reagent

d) Wagner's reagent

e) Hager's reagent

f) Dilute ammonium solution

g) Fehling's reagent

h) Benedict's reagent

i) Molish's reagent

j) Alcohol Ferric chloride solution

k) Kedde's reagent

Distillation of the filtrate

Figure 1. Distillation of Oroxylum indicum

3.4. Thin layer chromatography

One or more compounds are spotted on a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flows through because of capillary action against gravitational force. The components move according to their affinities towards the adsorbent. The component with more affinity towards stationary phase travels slower. The component with lesser affinity towards the stationary phase travels faster. Thus the components are separated on a thin layer chromatographic plate based on the affinity of the components towards the stationary phase (9, 10).

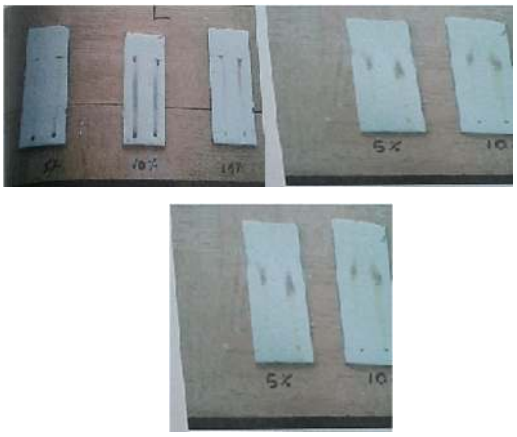


Figure. 2. TLC of Extract (leaves)

3.5. Column chromatography

In this method, the mixture to be separated is dissolved in a suitable solvent and allowed to pass through a tube containing adsorbent. The component, which has greater adsorbing power, is adsorbed in the upper part of column. The next component is adsorbed in the lower portion column, which has lesser adsorbing power than the first component. The process is continued. As a result, the materials are partially separated and adsorbed in various parts of the column. The banded column of adsorbent is termed a chromatogram and the operation is spoken of as the development of chromatogram. The portion of column, which is occupied by a particular substance, is the zone. The suitable solvent used for developing the chromatogram is known as developing solvent (11).

Figure. 3 Column chromatographic elution

3.6. Estimation of the Anti-microbial activity

50 micro liter of sample of 1/1000000 dilutions was poured on the agar media on Petri dish and inoculated at 37 Celsius. Colonies were obtained from surface of Petri dish. Antimicrobial activity of the extracts was screening against 5 bacterial pathogens,

Pathogens are: *Acinetobacter, M.luteus, S.aureus, R.palanticola* and *E.Coli*

Extract concentration: All extracts was taken 20mg/ml (sterile DMSO)

Antimicrobial screening was done by pour plate method by taking 10^{-5} cells/ml of pathogen (by taking OD at 600nm. From the stock solution 50µl of extract was taken for study. After 24 hours of incubation result was taken (4).

4. Results and Conclusion

Test for presence of various phyto-constituents:

Phyto-constituents	Result
Alkaloids	-ve
Flavonoids	+ve
Tannins	-ve
Anthraquinone	+ve
Carbohydrates	-ve
Saponins	-ve
Sugar	-ve
Steroid	-ve
Glycoside	+ve
Amino acid	-ve

Qualitative phytochemical analysis of various extracts obtained from O. *indicum*

Test for phyto compound	Name of The Test	Name of the extract				
		PE	BZ	CF	EA	AQ
Alkaloids	Wagner	-	++	+	+	-
Saponins	Foam	-	-	-	-	+
Phenolic & Flavonoids	Ferric chloride	+	+	+++	+++	+
	Gelatin	-	-	-	+	+
	Lead acetate	-	-	-	+	+

PE-Petroleum ether
BZ-Benzene
CF-Chloroform
EA-Ethanol
AQ-Water

(+) ⟶ Presences
(++) ⟶ Significantly present
(+++) ⟶ Present in more quantity

4.1. TLC ANALYSIS OF EXTRACTS

(By methanolic extraction)

SOLVENT SYSTEM	NUMBER OF SPOTS	DETECTORS
Methanol: chloroform (95:5)	1 (brick red colour)	Alcoholic sulphuric acid
Methanol: chloroform (90:10)	1 (brick red colour)	Alcoholic sulphuric acid
Methanol: chloroform (85:15)	1 (brick red colour)	Alcoholic sulphuric acid
Methanol: chloroform (80:20)	No spots	Alcoholic sulphuric acid

TLC OF ELUENT (5% FRATION):

MOBILE PHASE	PERCENTAGE	NO.OF SPOTS	DETECTORS
Chloroform:methnol	2% mobile phase	1(green to violet)	Alc.sulphuric acid
	4% mobile phase	1(green to violet)	Alc.sulphuric acid
	6% mobile phase	1(green to violet)	Alc.sulphuric acid
Chloroform:ethyl acetate	2% mobile phase	1(green to red)	Ferric chloride
	4% mobile phase	1(green to red)	Ferric chloride
	6% mobile phase	1(green to red)	Ferric chloride

TLC OF ELUENT (10% FRATION):

MOBILE PHASE	PERCENTAGE	NO.OF SPOTS	DETECTORS
Chloroform:methnol	8% mobile phase	1(green to violet)	Alc.sulphuric acid
	10% mobile phase	1(green to violet)	Alc.sulphuric acid
	12% mobile phase	1(green to violet)	Alc.sulphuric acid
Chloroform:ethyl acetate	8% mobile phase	No spots	Ferric chloride
	10% mobile phase	No spots	Ferric chloride
	12% mobile phase	No spots	Ferric chloride

ELUENTS COLLECTED BY COLUMN CHROMATOGRAPHY:

Fractions	Eluent	Eluate	Remarks
1-6	Methanol: chloroform (95:5)	Dark colour viscous liquid	Mixture of waxes, terpines, flavonoids, oily substances.
7-11	Methanol: chloroform (90:10)	Yellow viscous liquid	Mixture of fatty acid, waxes. Not further examined
12-14	Methanol: chloroform (85:15)	Pale yellow oil	Mixture of fatty acids
15-17	Methanol: chloroform (80:20)	Colourless liquid	Mixture of fatty acids
18-20	Methanol: chloroform (80:20)	Brownish colour oil	Mixture of fatty acids
20-22	Methanol: chloroform (80:20)	Colourless liquid	

4.2.ESTIMATION OF ANTI-MICROBIAL ACTIVITY

Extract Name	Pathogen(zone size in mm)				
	Acinetobacter	***E.Coli***	***S.aureus***	***M.luteus***	***R.palanticola***
Leaf 5%	6	6	-	12	-
10%	6	6	-	6	-
15%	6	6	-	6	-
20%	8	7	-	6	-
25%	-	5	-	6	-

Well size-4mm

5. CONCLUSION:

Based on the results obtained from the above table it has been concluded that , 5% leaves shows maximum effect on M.luteus>Acinetobacter=E.Coli., 10% and 15% leaves extract shows equivalent effect in all microorganisms, 20% leaves extract shows maximum effect in Acinetobacter>E.Coli>M.luteus, 25% leaves extract shows effect only in M.luteus>E.Coli.

All the leaf extract does not showed any effect in the organisms S.aureus and R.palanticola. In order to identify the active constituents responsible for activity against different microbes, further isolation of different components by using chromatographic techniques and subsequent molecular characterization technique by using spectroscopic methods of analysis should be performed.

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