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Research Article

Anti bacterial activity of *Derris indica* leaf extracts

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ABSTRACT

Derris indica, family Fabaceae also known as Pongamia pinnata has various therapeutic properties. It shows activities like hepatoprotective, antirheumatic, hypoglycemic, anti bacterial etc. The plant leaves are is rich in flavanoids, alkaloids which are proved by phytochemical analysis. The aqueous, chloroform, methanolic and petroleum ether extracts were screened for anti bacterial activity using Bacillus subtilis and E. coli strain. The anti bacterial activity was performed using diffusion assay method using spread plate method. The study showed methanoilc and chloroform extracts have potent antibacterial activity. Thus Derris indica have abti bacterial activity along with other therapeutic activities.

Key word: Antibacterial, *Derris indica*

INTRODUCTION

The plant kingdom represents an enormous reservoir of biologically active compounds with protective, disease preventive properties. The role of traditional medicines in the solution of health problems is invaluable on a global level. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine [1]. With the associated side effects of the modern medicine, traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions [2]. The diverse approaches to herbal drugs have led to interesting hits and novel activities, which need further in depth drug development efforts, both as herbal as well as new single molecule drugs.

A bacterial disease can be initiated only when pathogenic bacteria comes in contact with a susceptible host in a disease favoring environment. The environment plays a crucial role in modulating the virulence of the pathogens as well as reducing the host defense and thus increases the susceptibility of the host towards various pathogens. A pathogenic agent can enter the animal body via., various possible routes of exposure but the immune system of host can certainly phagocytes the pathogen by secreting chemical factors and thus checks the disease progress. Moreover, alterations in the microenvironment such as abrasions, wound, malnutrition, pathophysiological conditions may further facilitate the disease development.

For development of a disease, immune status of the animals and human population is an important issue. A susceptible host population can favor even a mild pathogen to produce an epidemic form of disease

while immune competent population can resist even highly pathogenic microbial strains. The immune status of the animals depends on a number of environmental variables for its fluctuations [3].

DERRIS INDICA

General description

It is a medium sized glabrous tree. Although it grows widely throughout the tropics, it can be found at higher elevations. The tree is frequently found in pastures, waste lands, cultivated lands, roadsides, lawns and in planted forests. It is adaptable tree for tropical and sub-tropical regions which requires excellent drainage and a sunny location. It grows easily from seed. Historically, this plant has long been used in India and neighboring regions as a source of traditional medicines, animal fodder, green manure, timber, fish poison and fuel.

Family

Fabaceae

Derris indica plant

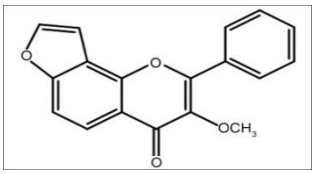
Taxonomy

Kingdom -Plantae
Subkingdom -Tracheobionta
Super division -Spermatophyte
Division -Magnoliophyta
Class -Magnoliopsida
Subclass -Rosidae
Order -Fabales
Family -Fabaceae
Genus -Derris
Species -indica

CHEMICAL CONSTITUENTS

Leaves

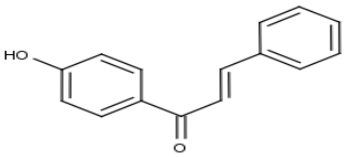
Leaves contain furanoflavone-3′-methoxypongapin in addition to karanjin, kanjone and its two isomers 7-methoxyfurano-(4″, 5″6, 5) -flavone and 8-methoxyfurano-(4″, 5″-6, 7)-flavones.



Karanjin

Bark

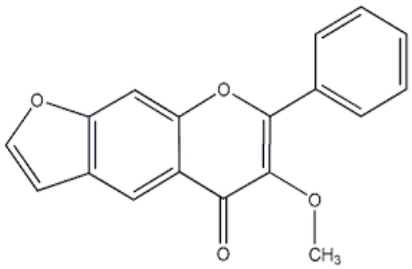
Bark contains a hydroxychalcone, pongapinone A, which is useful for treatment of autoimmune and inflammatory diseases.



Pongachalone

Stem bark also contain chromenoflavone, pongachromene, glabra-I, glabra-II, kuranjin, pongapin, kanugin and de-methoxykanugin.

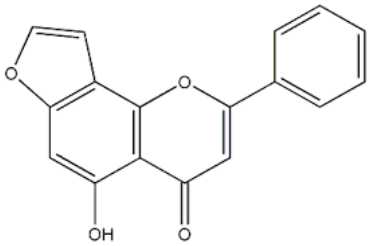
Root bark contains a large number of the flavonoid compounds, ponganones, four furoflavones, keranjin, pongapin, pinnatin and gamatin and tetra-methoxyfisetin.



Ponganone

Flowers

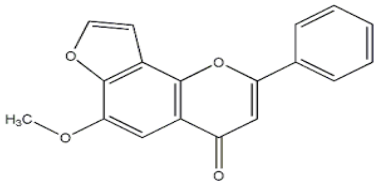
Flowers contain new OH-furanoflavone, pongaglabol, aurantiamide acetate, 4 known furano flavones, β-sitosterol and kaempferol.



Pongaglabol

Seeds

Seeds contain a bitter fatty oil, crystalline substances, β-diketone, karanjin, pongamol, glabrin, pongapin, lanceolatin-B, kanjone, isopongaflavone and pongapine and also traces of essential oil.

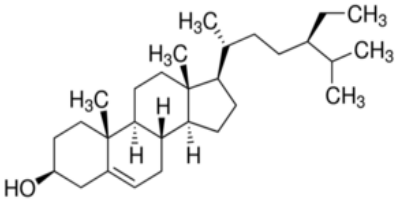


Kanjone

Seed oil contains di-methylchromeno flavanone, isolonchocarpin and demethoxy kanugin; oil from immature seed yields new OH-furanoflavone and pongol.

Wood

Heartwood contains pongachalcone, glabrachromen, de-methoxykanugin, kanugin, pongaglabrone, β -sitosterol.



B -sitosterol

USES

Fodder and Feed

The Derris indica leaves contain 43% dry matter, 18% crude protein, 62% neutral detergent fiber, and in vitro dry matter digestibility of 50% and are eaten by cattle and readily consumed by goats. The trees have a significant value in arid regions; however the use is not common. The deoiled cakes could be used as poultry feed and cattle feed [4].

Apiculture

D. indica flowers are considered a good source of pollen for honeybees in India and they yield adequate nectar.

Fuel

With a calorific value of 4 600 kcal/kg, it is commonly used as a fuel wood. The seed oil was formerly indispensable as an illuminant in lamps, but has been largely replaced by kerosene.

Timber

Wood varies from white to yellowish-grey with no distinct heartwood; beautifully grained and medium to coarse textured. Although it is a moderately strong timber that is relatively easy to saw, turn and finish, the wood is not considered a quality timber because it is not durable, tends to split and warp during seasoning and is susceptible to insect attack.

Wood is used for stove top fuels, poles and ornamental carvings [5], cabinet making, cart wheels, posts [6] agricultural implements, tool handles, and some usual activities [7]. The ash produced from burning wood is used for dyeing [8].

Tannin or dyestuff

Roots yield a natural pigment, pinnatin, which was synthesized in 1967. The wood ash is employed in dyeing. Oil from the seeds is used for leather dressing in tanning industries.

Lipids

Oil is the most important product of the pongam tree and vast amounts of seeds are collected in India for commercial processing of industrial uses.

It has been found that the seed contains 27-40% of thick, yellow or reddish-brown oil and that 2 kg of mature pods will yield about 1 kg of husked kernels. Extracted oil amounts to 13.4% of the whole seed pod; 26.97% of the kernels. The oil has a bitter taste, a disagreeable aroma and a specific

gravity of 0.9371 at 15 deg. C. It is used as a lubricant, varnish, water-paint binder and in soap making. It is one of the few nitrogen-fixing trees to produce seeds containing oil.

Poison

The press cake, when applied to the soil is valued as a pesticide, particularly against nematodes. In rural areas, dried leaves are stored with grain to repel insects.
Pounded and roasted seeds used to be utilized as fish poison.

Medicine

Even though all parts of the plant are noxious, the flowers and fruits along with the seeds are used in many traditional medicines.

Flowers are used to treat bleeding hemorrhoids whereas fruits aid treatment of abdominal tumors, ulcers, and hemorrhoids.

Seed powder reduces fever and helps in treating bronchitis and whooping cough. On the other hand, leaves juices aid in treatment of leprosy, gonorrhea, diarrhea, flatulence, coughs, and colds. Besides, bark relieves coughs and colds and mental disorder. Root is used as a toothbrush for oral hygiene while root juice is used to clean ulcers [9].

Pongamia pinnata oil is styptic, anthelminthic, and good in leprosy, piles, ulcers, chronic fever, liver pain [10], and rheumatism arthritis scabies [11].

Seed Cake as Fertilizer

Seed cake, a byproduct of oil extraction, is bitter and unfit for animal feed. It is rich in protein and nitrogen and is used as green manure to fertilize the land. It is also used as a pesticide, especially against nematodes. Besides, the seed cake can be used for biogas production.

Soil Erosion

Derris indica trees are usually planted along the highways, roads, and canals to stop soil erosion. The plants develop a lateral network of roots for controlling soil erosion and binding sand dunes [12]. Thus, karanja plantation can reduce soil erosion

MATERIALS

Collection of Plant materials

Leaves of Derris indica were collected from Holy Mary Institute of Technology & Sciences (College of Pharmacy), Bogaram.

Drying

The collected leaves were washed with water to remove dust and unwanted particles and cleaned. These leaves are dried under shade for 1 week.

Bacteria Collection

Strains of Escherichia coli and Bacillus subtilis were obtained from Department of Microbiology, K.P Labs, Kothapet, and Hyderabad.

Chemicals for extraction

For the leaf extraction by maceration distilled water was used as solvent. For extraction by soxhlation

- Chloroform (500ml)
- Methanol (500ml)
- Petroleum ether (500ml)

METHOD

Extraction by Maceration

The dried leaves were powdered and extracted initially by **simple maceration**. It is performed at room temperature.

The drug was mixed with solvent in the ratio of 1:10 and the mixture is left for several days with occasional shaking and stirring. The extract is then filtered by using muslin cloth. The extract is then collected and stored.

Extraction by Soxhlation

Soxhlet apparatus consists of a body of extractor attached with a side tube and siphon tube. The extractor from the lower side can be attached to distillation flask and the mouth of the extractor is fixed to a condenser by standard joints. The powdered crude plant materials packed in the soxhlet apparatus directly or in the thimble pack. Extraction assembly is set up by fixing condenser and distillation flask. Initially for setting the powder, is allowed to siphon once before heating. Fresh porcelain pieces are added to flask to avoid bumping of solvent.

The vapors pass through the side tube and the condensed liquid gradually increases the level of liquid in extractor and in the siphon tube. A siphon is set up and the liquid reaches the point of return and contents of the extraction chamber are transferred to the flask.

The cycle of solvent evaporation and siphoning back can be continued many times without changing the solvent, to get efficient extraction.

In the present study, dried leaf powder of Pongamia pinnata was extracted by soxhlation using chloroform, methanol and petroleum ether as solvents. After obtaining the extracts they were evaporated to dryness to room temperature. The extracts thus obtained were weighed and percentage yields were calculated.

EVALUATION OF ANTI BACTERIAL ACTIVITY

Test organisms

Gram Positive Bacteria: Bacillus subtilis
Gram Negative Bacteria: Escherichia coli

Nutrient medium for growth of micro organisms

Luria-Bertani (LB) broth [13, 14]:

Tryptone	10 g
Yeast extract	5 g
Sodium chloride	10 g
H_2O (dist)	1000 ml

• For LB plates: add 15 g/l of agar

Agar, tryptone, yeast extract, Sodium chloride were dissolved in distilled water by stirring and then kept in autoclave for 15 minutes at 121°C for sterilization. The p^H was adjusted to 7.2 .The agar medium was poured in petri plates that are previously sterilized then they were allowed to set at room temperature for 30min into uniform thickness.

Spread plate method

To the solidified petri plates the bacterial cultures were inoculated by spread plate method. In this method the bacterial culture is spread on the surface of agar medium using spreader. In an individual petri plate, single sample was taken at different concentrations by means of sterile micropipettes under sterile conditions.

The plates thus prepared were left for 2hours for diffusion and then kept for incubator at 37°C. After 24hours the plates were examined for zone of inhibition and zones were measured in millimeters.

Zone of inhibition

In a Kirby-Bauer test, the size of the zone of inhibition indicates the degree of sensitivity of bacteria to a drug. In general, a bigger area of bacteria-free media surrounding an antibiotic disk means the bacteria are more sensitive to the drug the disk contains.

RESULTS AND DISCUSSION

In the present study various bacteria like Bacillus subtilis and E.coli were used for the evaluation of activity. Zone of Inhibition was calculated and Methanolic activity showed better results than other extracts when Bacillus subtilis was used. When E.coli strains were used for evaluation chloroform extract showed better results. Aqueous extracts showed least activity for both the strains.

Activity against Bacillus subtilis

The zone of inhibition was calculated by using aqueous extract, chloroform, methanol and petroleum ether. Penicillin is used as standard.

Test organism	Sample	Zone of inhibition (in mm)
Bacillus subtilis	Aqueous extract	4
	Chloroform	12
	Methanol	18
	Petroleum ether	8
	Penicillin	21

Activity against E. coli

Various extracts of Pongamia pinnata showed following results against E. coli.

Test organism	Sample	Zone of inhibition (in mm)
E. coli	Aqueous extract	0
	Chloroform	11
	Methanol	9
	Petroleum ether	5
	Penicillin	16

CONCLUSION

Leaf extracts of Derris indica showed anti bacterial activity on both gram positive and gram negative bacteria. They showed more activity on gram positive compared to gram negative bacteria. All the activities were compared with standard drug Penicillin. The methanolic and chloroform extract showed potent anti bacterial activity than aqueous extract.

So from this we can conclude that Derris indica leaves have anti bacterial activity along with other therapeutic uses.

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