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RESEARCH PAPER

Antidermatophytic active furanoflavonol compound from *Pongamia pinneta* bark

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Abstract

Researchers and medical professionals frequently employ the furanoflavonol that plants produce. Natural biodesiel plant is a Leguminaceae family member and a well-known economically productive plant in India. In the current study, bark was used to extract a novel antidermatophytic polyphenolic potential phytochemical from 10 solvent ratios during column chromatography. The isolated furanoflavonol compound was found to be effective against *M. gypsum* and *T. rubrum*. MIC values for *T. rubrum* and *M. gypseum* against the widely prescribed antifungal ketoconazole are 0.1 mg ml-1 and 0.12 mg ml-1, respectively, for the isolated molecules. It was possible to identify Furanoflavonol's structure by analysing 13C-NMR spectrum data.

Keywords: Antidermatophytic, furanoflavonol polyphenolic molecule, MIC.

Introduction

Pongamia pinnata L., a member of the Leguminaceae family, is recognised as one of India's cool, luxuriant trees. It goes by the names "Karanj," "Papar," and "Kanji" frequently. Both "Karum Tree" and "Poonga Oil Tree" are the names of this plant in English. India, Australia, Florida, Hawaii, Malaysia, Oceania, the Philippines, and Seychelles are the current home countries for it. It belongs to the Indo-Malay genus (Edward *et al.* 2004) One of the most cherished urban trees is this one (Duke 1994)

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Treatments for cancer using ethnotherapy comprised fruits and sprouts of *P. pinnata* L. (Hartwell 1971). Herbal medicines have been suggested for the treatment of a variety of disorders in a number of medical books. Different traditional medical systems have authorized of using it to treat a number of illnesses (Ghani 1998). To treat hypotensive symptoms and induce uterine contractions, extract from bark s is employed. Bark power can be used to cure a wide range of illnesses, including bronchitis, chronic skin disorders, whooping cough, persistent fever, and painful rheumatic joints (Kirtikar and Basu 1994) With bark oil, you can treat scabies, piles, ulcers, chronic fever, leprosy, lever pain, and lumbago.

Its oil serves as efficient biodiesel and as a fuel for cooking and lighting (Ashford 2006). Due to its purity, safety, and renewable nature, it is regarded as an alternative energy source. Illnesses, coughs, dyspepsia, flatulence, gonorrhoea, diarrhoea, and leprosy are all treated with the bark juice. The bark work well to combat Micrococcus. You can clean ulcers, gum disease, and teeth with roots. Bark is taken internally for bleeding piles. The anti-inflammatory, antiplasmodial, anti-nociceptive, anti-hyperglycemic, anti-lipidoxidative, anti-diarrheal, anti-ulcer, anti-hyperammonic, CNS depressing action, and antioxidant activities were found, according to the standard system (ShamimaJahan 2021).

Flavonoids and a vast selection of fixed oils as phytoconstituents. In addition to the eight fatty acids, the bark s of *P.pinnata* L. comprises six unique substances,

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including two sterols, three sterol derivatives, and one disaccharide (three saturated and five unsaturated). Their structures were clearly stated by using spectroscopic and physiochemical approaches. The metabolites stigma sterol, galactoside, and sucrose are being reported for the first time together with sitosteryl acetate and galactoside.

The quantity of saturated and unsaturated fatty acids was as follows (two monoenoic, one dienoic, and two trienoic). Karangin, pongamol, pongagalabrone, pongapin, pinnatin, and kanjone have all been isolated from bark s. In young bark s, there is a flavone derivative known as "pongol." The other flavonoid that has been taken out of the bark s is made up of glabrachalcone and isopongachromene. Among the flavone and chalcone substances discovered in the plant's bark and stem are Pongone, Galbone, Pongalabol, and Pongagallone A and B. Chemical investigation of the mangrove plant *P. pinnata* L. resulted in the isolation and identification of five structurally distinct flavonoid metabolites. Nine new flavonoid compounds, totaling 18 total, were isolated from Japan (Srinivasan *et al.* 2001)

Prabha et al. (2003) reported anti-ulcer efficacy that significantly protected against aspirin-induced ulceration but not ethanol-induced ulceration using methanolic extract of *P. pinnata* L. roots. It demonstrated a tendency to diminish acetic acid-induced (Meera *et al.* 2003).

Brijesh *et al*. (2006) evaluated its impact on enteropathogenic E. coli adhesion and invasion, as well as Shigella flexneri and E. coli invasion of epithelial cells, in in addition to evaluating its impact on the production and action of enterotoxins (cholera toxin, Escherichia coli labile toxin, and E. coli stable toxin). Nonetheless, the decoction lacked antibacterial, anti-giardia, and anti-rotaviral qualities. It also decreased the synthesis of cholera toxin and bacterial invasion of epithelial cells (Goel 1985).

Mathias and Majeed (2001) examined the effect of *P. pinnata* L. bark extract on circulatory lipid peroxidation and antioxidant status in ammonium chloride-induced hyperammonium rats. It increased lipid peroxidation in the blood of rats receiving treatment with ammonium chloride (Majeed *et al.* 2005). *Plasmodium falciparum* was resistant to the antiplasmodial effect, per Simonsen *et al.* (Essa 2006).

P. Pinnata L. flower extract significantly lowers hyperglycemia in alloxan-induced diabetics, lowers lipid peroxidation, and boosts the antioxidant system (Simonsen *et al.* 2001). Patients with diabetes may administer these extracts in place of risky antihyperglycemic medications (Punitha *et al.* 2006).

70% ethanol bark extract from *P. pinnata* L. exhibits powerful anti properties against acute, subacute, and chronic inflammation without affecting the gastric mucosa (Kirtikar & Basu 1993) It also has antipyretic capabilities *P. pinnata* L. bark extract's in vitro antiviral activity was evaluated against HSV-1 and HSV-2. HSV-1 and HSV-2 were severely suppressed at doses of 1 mg/ml and 20 mg/ml w/v, acute and chronic toxicological studies showed safer (Ahmad G 2004, Carcache Blanco EJ 2003)

Antimicrobial effects of *P. pinnata* L. bark were shown to have potential. Antibacterial medications are used to treat intestinal infections, as according Ahmad et al [22]. This plant can be utilised to discover bioactive natural compounds that could act as a starting point for the creation of innovative pharmaceuticals that address unmet therapeutic needs at this time (Annie S 2004). Punita R *et al.* (2006). all showed that *P. pinnata* L. bark extracts were excellent against lice.

Materials and methods

Plant material collection

The plant components were collected from several locations in the Hyderabad, Karnataka region and placed in new bags before being transported to the lab. The materials were initially cleaned with condensed water to remove pollutants and dried on sheets in a laboratory at 37 °C for a week before the plant was verified and placed at the herbarium centre of Gulbarga University in Karnataka (Seetharam 2000).

Soxhlet apparatus extraction (Horborne 1998)

In a bowl, the dehydrated plant material is converted to coarse powder. A sieve was used to remove the fine dust or powder, and 25g of the powder was weighed and moved sequentially from a low-polar to a high-polar method in a soxhlet apparatus over the course of 48 hours. The methanol extortion was strong with less force and sealed in a hermetic bottle for secondary use.

Preparation of extract dilution series

Liquefy 400 mg of extract in 10 ml DMSO for the various concentrations of extract stock preparations, then eddy to regiment and prepare two old sequential intensities. As a precautionary measure, each extract was fitted with a reasonably high concentration of 0.62 to 40 mg per ml in order to be active without overlooking the trace amount of antifungal used for preliminary screening.

Microbes used in the studies

The current study examined the efficacy of dermatophytes including Microsporum gypseum, Trichophyton rubrum, Trichophyton tonsurans, Aspergillus flavus, and Candida albicans. The Department of Microbiology of Maha Devappa Rampure Therapeutic College in Karnataka, MTCC of Chandigar, India, is where all of the test strains were purchased. As usual, fungal strains were grown in PDA at 28oC and retained on PDA slants at 4oC, while bacterial cultures were cultured in NB at 37oC and retained on NA incline.

Microbial inoculums preparation (Santos 2006)

Dermatophytes had been growing on SDA for a week before spores were produced by flooding with 0.85% briny.

After the larger particles had settled in the test tube, the supernatant was taken out, and the conidia present were counted using a hemocytometer. A tenfold dilution was carried out. Nine millilitres of ordinary saline solution were placed in each of five test tubes. One cc of spore suspension was mixed into the first test tube while maintaining aseptic conditions. To the second test tube, which contained 9 ml of ordinary saline solution, homogenised pieces of the first test tube's solution were added.

The procedure was repeated for the sixth test tube. In each case, pipettes were used that had been sterilised. 0.1 ml of suspension from each test tube was added to SDA petri plates that had been cleaned for each dilution. The three duplicates of each dilution were stored. The SDA-bark ed petri plates were subjected to hemocytometer counts. An average of 3 petri plates were collected for each case. The tested inoculum range of 1.5x105 spores/ml was modified.

Anti-dermatophytic activity

Using the agar well diffusion method to determine the MIC (Magaldi, 2004)

The experiment was carried out using the cup well diffusion technique. PDA medium was placed in clean petri dishes, which were then placed behind you to harden. A fungal lawn was produced using a five-day-old culture strain. The different fungal strains had a turbidity threshold of 0.5 Mac. Farland standards are 108 CFU/ml, suspended in a 0.85% NaCl saline solution. One cc of the test strains was spread over the medium using a sterilised glass spreader. Using a flamed sterile borer, 4 mm-diameter wells were pierced in the culture medium.

The required concentrations of serially diluted extracts (0.6, 1.2, 2.5, 5, 10, 20 and 40mg/ml) were applied to the wells. Before being incubated at 37°C, the plates were then placed in the refrigerator for an hour to allow extracts to mix with the media. After 48 hours, the plates were checked for inhibitory zones. The diameter of the inhibitory zone was measured and expressed in millimetres. It was used as a negative control similar to DMSO. Identical to positive control, whereas ketoconazole (500 g/ml) was used. Each experiment was conducted three times. The nutritional agar medium, which was incubated at 37°C for 18 hours, was also tested for its antibacterial effectiveness using the same approach.

Broth Dilution Assay for MIC Determination (Magaldi 2004)

The minimal inhibitory concentration of a crude extract was determined using a broth dilution test. The medium, which was produced through serial dilution, comprised varying concentrations of plant extract, ranging from 100 mg to 1 mg per ml (10-1 dilution). After culture inoculation, the test tubes were supposed to incubate for 72 hours at 280°C. The MIC of each sample was calculated after measuring the absorbance primarily with a spectro-photometer at 520 nm and comparing the results to those of the non-inoculated broth used as a blank. A control was created using medium and inoculums devoid of plant extract. The experiment was carried out in compliance with NCCLS guidelines, which are now known as CLSI guidelines Secondary metabolite preliminary screening tests:

All plant extracts from the selected species underwent preliminary testing using standard techniques for the detection of secondary metabolites [28]. The test solution was created by combining the appropriate solvent with 500 mg of each extract in 100 ml, which was then filtered through Whattman filter paper No. 1. The filtrates were consequently utilised as test solutions for the subsequent screening tests.

Separation of active fraction from selected plant solvent extract using column Chromatography (CC) (Horborne 1998)

- *Extraction*

The successive selected solvent crude Soxhlet extract of selected plants were extracted as mentioned earlier.

Column Chromatography (CC)

In to prepare a column, a 45 cm portion of a clean, dry, 500 ml volume column (made by Vinsel) is filled with a slurry of silica gel-H with a mesh size of 60–120 (hi-media, Mumbai). Hexane is then used to fill the remaining space. The stationary phase was making it possible into the column to minimize air bubbles. It was then cycled through twice with a solvent system that contains analytical grade hexane to generate an airtight and compact column (Sd-fine Chem., India).

- *Loading*

A modest amount of silica gel (60-120 mash) was loaded into the 45 cm-high silica gel column after already being mixed with 10 g of a selected plant section's methanol extract. solvent polarity ranges between low to high).

- *Fractions were gathered*

100ml at a time, in a succession of conical flasks as they emerged from the column (Borosil, India). Thin layer chromatography was performed using these fractions. Based on the TLC results, similar fractions were gathered and vacuum-condensed to isolate the active ingredient. All of the fractions that were collected underwent testing for dermatophytic fungus. The active fraction is utilized for subsequent separation and purification procedures.

Isolation purification of partially purified compounds by using Preparative Thin Layer Chromatography (PTLC) (Horborne 1998)

The active compound has been separated, isolated, and refined using the cc fraction's colour intensity band width as determined by PTLC or a re-column.

- *Preparation of PTLC plates*

As stated earlier, 20x10 cm glass plates containing 1mm thick neutral silica gel were prepared and used for compound separation.

- *Development*

A capillary tube was loaded with 500 l of the active cc collected fraction, and it was let to dry. These plates were placed in a saturated, developing solvent-mixture-filled chromatographic chamber.

- *Detection and Scraping*

The developed chromatograms were meticulously marked with a needle while being examined under wave length (UV254 and UV365 nm). The indicated area was then scraped off and inserted in glass vials after being collected. Testing was done on all of the fractions collected for dermatophytic fungus. Re-column chromatography must be carried out in order to harvest more compound.

- *Purification of compounds*

The combination containing the now-obtained Silica gel-G powder was thoroughly blended after being thoroughly dissolved in a mixture of its relevant solvents, such as chloroform and methanol (90:10). 5000 rpm centrifuged for 10 minutes to get the purified compound-containing supernatant. The pellet was centrifuged after being once more re-suspended in the solvent combination. The combined supernatants were vacuum-condensed to dryness at 40°C, and the compound was then retained at 4°C in a refrigerated vial with a tight screw closure of a 5 ml capacity (Hi-Media Lab., Mumbai).

Results and Discussion

Several primary and secondary metabolites with therapeutic significances were identified, isolated from bark , and some of them were purified using biochemical and other hyphenated analytical chromatographic and spectrophotometric techniques. The findings are discussed with respect to previous literatures.

Antidermatophytic activity and minimum inhibitory concentrations

In the proposed investigation, five fungus species and six bacterial species were examined to determine the antifungal and antibacterial activity of pet-ether bark extract of *P. Pinnata* L. Tables 1 and 2 show the average of the three observations as represented by the figures. Candida albicans was suppressed by Pet-ether bark extract to a maximum of 16. 66 ± 0.57 mm at a concentration of 40 mg/ml, followed by *Microsporum gypseum* (14. 66 ± 1.15 mm), Trichophyton rubrum (13. 00 ± 0.00 mm), Aspergillus flavus (11. 00 ± 0.00 mm), and Trichophyton tonsurans (09. 00 ± 0.00 mm). Figure 1 shows the values of the test fungus's minimum inhibitory conc. *M. gypseum* and *C. albicans* have MICs of 0.62 mg/ml conc, while *T. rubrum, A. flavus*, and *T. tonsurans* have readings of 1.25 mg/ml conc, 2.5 mg/ml conc, and 5 mg/ml conc, accordingly.

66 x 0.57 mm, evaluated versus *Psudomonas aeruginosa* 14, the Pet-ether bark extract inhibited Escherichia coli to a maximum of 20.00 ± 0.00 mm, followed by Serratia marcescens (19.66 ± 1.15 mm), Bacillus subtilis, Staphylococcus aureus, and Brevibacillus brevis (16.66 ± 0.57 mm), with the least activity. Figure 1 displays the data for the minimal inhibitory concentration for the test bacteria. The MIC was established to be 0.6 mg/ml conc for *S. marcescens*, *B. subtilis*, *E. coli*, and *S. aureus*. Following *P. aeruginosa*, at 1.25 mg/ml conc.

Not all of the examined bacterial and fungal species were able to be inhibited by the DMSO-based negative control. Inhibition is shown by streptomycin when used as a standard against bacteria, but antifungal activity is shown by ketoconazole when used as a standard at conc. 5mg/ml at 24. 66**±**1.15 mm.

Preliminary screening of secondary metabolites

Several secondary metabolites, including alkaloids, phenol, flavonoids, tannins, triterpenes, steroids, saponins, and glycosides, were qualitatively screened for in the crude sequential extract of bark , which included petroleum

Table 1: Pet-ether bark extract from Pongamia pinnata L. exhibits antidermatophytic action (Well diffusion technique).

Fungal strains	*Different conc. (mg/ml) of crude and inhibition zone in mm*								
	40	*20*	*10*	*5*	*2.5*	*1.25*	*0.62*	*Control (DMSO)*	*Standard (Ketoconazole)*
T. rubrum	13. 00±0.00	11. 66±0.57	10. 66±0.57	09.66±0.57	08. 66±0.57	06. 66±0.57	---	--	23. 00±0.00
M. gypseum	14. 66±1.15	10. 66±0.57	09. 66±0.57	08.00±0.00	07. 66±0.57	06. 66±0.57	05. 66±0.57	---	18. 66±0.57
C .albicans	16. 66±0.57	13. 66±1.15	12. 66±0.57	09.66±0.57	08. 66±0.57	06. 00±1.00	05. 66±1.15	---	24. 66±0.57
T.tonsurans	09. 00±0.00	08. 66±0.57	06. 66±1.15	05.00±0.00	--	---	---	---	17. 66±1.15
A. flavus	11. 00±0.00	09. 00±0.00	07. 66±1.15	06.00±0.00	05. 00±1.00	---	---	---	16. 00±0.00

T. rubrum: Trichophyton rubrum, M. gypseum: Microsporum gypseum, C .albicans: Candida albicans, T.tonsurans: Trichophyton tonsurans, A. flavus: Aspergillus flavus,
Negative control: DMSO N, N- Dimethyl Formamide, Standard: Ketoconazole (Positive control).

Table 2: Pet-ether bark extract from Pongamia pinnata L. exhibits antibacterial action (Well diffusion technique).

Bacterial strains	*Different conc. (mg/ml) of crude and inhibition zone in mm*								
	40	*20*	*10*	*05*	*2.5*	*1.25*	*0.62*	*Control (DMSO)*	*Standard (Streptomycin)*
E. coli	20. 00±0.00	18. 66±1.15	17. 66±0.57	14. 66±0.57	11. 00±1.00	10. 66±0.57	07. 66±0.57	-	24. 66±1.15
B. subtilis	16. 66±0.57	15. 66±0.57	14. 66±1.15	13. 00±1.00	10. 66±0.57	09. 00±0.00	06. 00±0.00	-	20. 66±0.57
S. marcescens	19. 66±1.15	18. 00±0.00	17. 00±1.00	16. 66±1.15	12. 00±1.00	10. 66±0.57	07. 33±1.52	-	24. 00±0.00
S. aureus	16. 66±0.57	12. 66±0.57	10. 00±1.00	08. 00±0.00	07. 33±1.52	06. 33±1.52	05. 00±0.00	-	19. 00±1.00
P. aeruginosa	14. 66±0.57	12. 66±0.57	11. 00±0.00	09. 33±1.52	07. 33±1.52	05. 33±1.52	-	-	18. 66±0.57
B. brevis	16. 00±0.00	14. 66±1.15	12. 66±1.15	11. 00±0.00	09. 00±0.00	07. 66±1.15	06. 00±0.00	-	24. 66±1.15

E. coli: Escherichia coli, B. subtilis: Bacillus subtilis, S.marcescens: Serratia marcescens, S. aureus: Staphylococcus aureus, P. aeruginosa: Psudomonas aeruginosa,
B. brevis: Brevibacillus brevis, Negative control: DMSO N, N- Dimethyl Formamide, Standard: Streptomycin sulphate (Positive control).

ether, chloroform, ethyl-acetae, and 98% methanol extracts. The reactions of these reagents revealed the presence of metabolites, and the outcomes are summarised in table 3

Alkaloids

The preliminary alkaloids tests, which used Wagner's, Dragendroff's, and Mayers' reagents, yielded positive results for the petroleum-ether and ethyalacetate extracts. With Mayer's reagent, these extracts produced a creamy white precipitate; with Dragendroff's reagent, an orange red precipitate; and with Wagner's reagent, a reddish brown precipitate. However, the chloroform and methanol extracts did not do well in any of the experiments with the exception of Dragendroff's test).

Phenols

The ferric chloride test, the ellagic acid test, and the hot water test all yielded positive results for the chloroform, ethyl acetate, and 98% methanolic bark extracts, indicating the presence of of phenols. The bark displayed a distinct brownish-black separation at the junction of the dipped and undipped portions in the hot water test. The aforementioned test produced negative results for all other extracts.

Flavonoids or Flavonoids Using ferric chloride, the ethyl

Table3: Preliminary screening of secondary metabolites in *Pongamia pinnata* L. bark.

Secondary metabolites	*Name of the test*	*PE*	*CHCL3*	*EtOH*	*98% Methanol*
Alkaloids	Mayers test	+	-	+	-
	Dragendoff's test	+	+	+	+
	Wagner's test	+	-	+	-
Phenol	Hot water test	-	-	-	-
	Ferric chloride test	-	+	+	+
	Ellagic acid test	-	+	+	+
Flavonoids	Ferric chloride test	-	+	+	+
	Lead acetate test	-	-	+	+
	Shinoda test	-	-	+	+
	Zinc/Hcl test	-	+	+	+
Tannins	Gelatin test	-	-	+	-
Triterpenoids	Salkowski's test	+	+	+	+
	Libermann-Burchard test	-	+	+	-
	Tschugajiu test				
Steroids	Salkowski's test	+	+	+	+
	Libermann-Burchard test	-	+	+	-
Saponins	Foam test	-	-	-	+
Steroidal glycosides	Keller-Killiani test	-	+	+	+
	Conc. H2So4 test	-	-	-	+
	Molisch's test	-	-	-	-
	Glycoside test	-	-	-	+

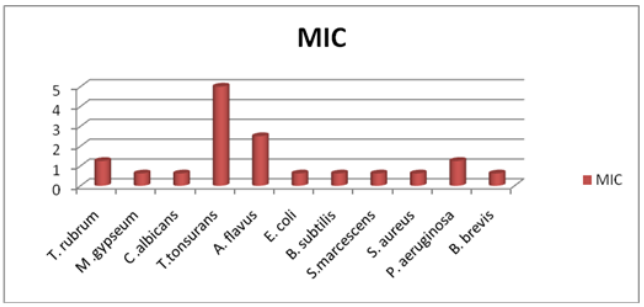

Figure 1: Minimum Inhibitory Concentrations of pet-ether bark extract of *Pongamia pinnata* L. against test strains.

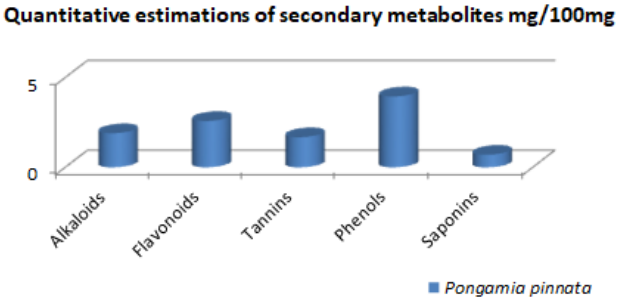

Figure 2: Quantitative estimations of secondary metabolites in bark s of *Pongamia pinnata* in mg/100mg.

acetate and 98% methanolic bark extracts were discovered to contain flavonoids. The presence of flavonoids is validated by tests employing lead acetate, shinoda, and NaOH. Unlike other pet.ether, chloroform, or extracts, it had no effect.

Tannins

A positive gelatin test result was revealed by the ethyl acetate extract. This demonstrates that tannin is contained in the ethyl acetate extract. All other extracts failed the gelatin test, which produced negative results..

Triterpenes

The libermann-addition Burchard's of triterpenes and the chloroform and ethyl acetate extracts both responded favourably to Salkowski's approach. However, extracts of petroleum ether and methanol were detected in Salkowski's test.

Steroids

Salkowski's extracts responded positively to Libermann-addition Burchard's of steroids to the chloroform and ethyl acetate extracts. However, extracts of petroleum ether and methanol were detected in Salkowski's test.

Glycosides

The 98% methanol extracts tested positive for glycosides by the Kellar-Kiliani, Glycoside, and sulphuric acid assays. The chloroform and ethyl acetate extracts both passed the Kellar-Kiliani test, nonetheless.

Saponins

Tests using foam saponins showed that the 98% methanol extract contained saponins when the extract successfully responded.Quantitative estimations of secondary metabolites

Five important secondary metabolites from the dried, powdered bark of Pongamia pinnata L. were isolated and then quantified using various methods (Figure 2). The highest estimated concentration was just for total phenol (3.95 mg/100 mg), followed by flavonoids (2.58 mg/100 mg), total alkaloids (1.9 mg/100 mg), total tannins (1.68 mg/100 mg), and total saponins (0.7 mg/100 mg).

TLC method for qualitatively separating secondary metabolites

Secondary metabolites can be identified qualitatively using the TLC method.

The following therapeutically important alternative chemicals were separated from bark utilize different solvent systems and thin layer chromatography. The hRf values and distinguishable colours of the bands were recorded.

Phenol separation, first

The bark 's pet-ether extract indicated four different, brownish-yellow bands with hRf values of 14.28, 21.42, 30.35, and 46.42, respectively. (Table-4).

Flavonol separation

The accompanying bark chromatogram showed four different bands with hRf values of 35.59, 49.15, 61.01, and 94.91, respectively, that were yellow, light yellow, and brownish (I and II band) (III-VI band). E. Alkaloids' Separation The bark chromatogram exhibited three distinct bands with the colours dark yellow (hRf value 13.18), light brown (hRf value 20.32), and yellow (hRf value 28.30).

Alkaloids have been discovered in the Asclepiadaceae family, according to various researchers. *Tylophora hirsuta*

Table 4: Qualitative separation of secondary metabolites from *Pongamia pinnata* bark

Secondary metabolites	*No of bands*	*hRf values*	*Colour of the bands*
Phenols	4	14.28	Brownish
		21.42	Light yellow
		30.35	Light brown
		46.42	Yellow
Flavonoids	4	35.59	Brownish
		49.15	Brownish
		61.01	Yellow
		94.91	Light brownish
Alkaloids	3	13.18	Light yellow
		20.32	Light brown
		28.30	Yellow

Table 5: Antidermatophytic activity & Minimum Inhibitory concentration of isolated compound P-1.

Compound code	*Test strain*	*Inhibition zone in different conc. of compound (in mm)*					
		01mg^{-1}	*0.5mg^{-1}*	*0.25mg^{-1}*	*0.12mg^{-1}*	*Control*	*Standard(K) 01mg^{-1}*
P-1	*T.rubrum*	16. 33±1.52	12. 66±0.57	08. 66±1.15	06. 00±0.00	_	19. 33±1.52
	M.gypseum	12. 66±1.15	09. 00±0.00	06. 66±0.57	05. 33±1.52	_	18. 66±1.15

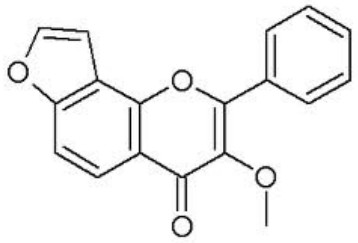

Figure 3: Structure of Karanjin (Furanoflavonol)

was the source of five unique phenanthroindolizidine alkaloids that were described by Bhutani et al. between 1983 and 1985. Similar to this, Ali and Bhutani (1989) separated two types of *Tylophora indica* in to the seven rare and four recognised alkaloids. Stepelia hirsute is said to contain new alkaloids, as according Shabana et al. (2005).

Isolation of Pongamia pinnata L. bark furanoflavonol fractions by column chromatography and PTLC (Preparative thin layer chromatography).

Pongamia pinnata L. bark is a rich source of flavonols of pharmacological value, as evidenced by earlier findings of qualitative and quantitative research of flavonol. The research that is now accessible on Pongamia sps further reinforced this. In this study, an effort was made to purify some of these flavonol fractions from Pongamia pinnata L. bark using preparative thin layer chromatography and column chromatography (PTLC).

Column chromatography studies (CC)

At room temperature and pressure (26°C. 1 bar), 10 g of the crude effective extract of Pongamia pinnata L. bark was fractionated on a Silica gel-H (60-120Mesh) column. Total 21 fractions of 100 ml each were recovered after 200 ml of the column's dead volume (hexane) were removed.

The fractions 1 to 11 were obtained from the Hexane: methanol. (1) 100:00, 90:10 (2), 80:20 (3), 70:30 (4), 60:40 (5), 50:50 (6), 40:60 (7), 30:70 (8), 20:80 (9), 10:90 (10) and 00:100 (11).

However, owing to their similarity in colour, the 21 fractions that have been obtained were amalgamated into eleven major fractions. The waxy nature fractions of 1 to 11 are colourless, white, or yellow in the concentrated forms of these fractions.

Separation of flavonols fractions by PTLC

Of the four flavonol fractions, two fractions (P-1) were obtained through the preparative thin layer approach, which involved locating a suitable solvent, maximising their economics, and isolating the most compound possible quickly. Additionally, the TLC was used to verify the purity of these fractions using a variety of solvent systems, and the results showed that each effective fraction appeared as a single discrete spot. The purified substance that was isolated using a chromatographic technique from the effective fraction is then the subject of additional in-depth spectroscopic research crystalline in nature, yellowish in colour, with hRf values of 61.01, an n-hexane:chloroform (3:1) solvent system, and a melting point between 200 and 210 °C. Hexane, methanol, and water are all soluble. n-Hexane-chloroform, 70:30, semi-waxy, colourless, white, yellow; active fraction. The compound had no colour, and it had a melting point between 214 and 216 degrees Celsius. This is consistent with Karanjin's reported temperature of 195oC. It displayed hRf values of 61.01 in TLC using the n-hexane: chloroform solvent system (3:1). After five minutes of heating at 110°C and 1:1 H2SO4 spraying, the spot turned yellow. The substance was soluble in diluted acids, alkalies, and water. P-I is designated as furanoflavonol-Karnjin based on its physicochemical characteristics. UV: Spectrum of P-1 peak shows at 425nm

FTIR: 3053 (Aromatic –C-H Structure), 16136 (CO), 1227 (C-O-C), 1163 (C-O-C), 1132 cm-1 (C-O-C)..LCMS: peak 293 m/z

It was revealed that the isolated furanoflavonol compound (Figure 3) could be active against T. rubrum and M. gypsum. The MIC range of the separated components was 0.2 mg ml-1, while the MIC of the common antifungal drug ketoconazole against T. rubrum and M. gypseum was 0.3 mg mL^{-1}.

Conclusion

We came to the conclusion that *Pongamia pinneta* bark extract could be a source of biologically useful components based on the information gathered throughout the inquiry, and that the constituents of the investigated extract and fractions displayed significant antidermatophytic activity. While examined against *T. rubrum*, the active furanoflavonol showed astounding antidermatophytic efficacy. Finally, this study's results showed that *Pongamia pinneta* is skilled at handling remedial circumstances. New study will be needed to fully comprehend the mechanisms and factors generating these anti-dermatophytic effects.

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Conflicts of interest

There are no conflicts of interest.

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