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Phytochemical and bio–efficacy studies on methanolic flower extracts of *Peltophorum pterocarpum* (DC.) Baker ex Heyne.

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ABSTRACT

Objective: The present study was aimed to investigate the preliminary phytochemical analysis and HPTLC profiling and the antibacterial activity of *P. pterocarpum* methanolic flower extracts against the bacteria isolated from human infections. **Methods:** The preliminary phytochemical screening was performed according to the Harborne method. HPTLC studies were carried out using Harborne and Wagner et al method. The methanolic flower extracts of *P. pterocarpum* were tested against *Salmonella typhi* (MTCC 733), *Staphylococcus aureus* (MTCC 96), *Proteus mirabilis* (MTCC 742), *Bacillus subtilis* (MTCC 441) and *Escherichia coli* (MTCC 443). The antimicrobial activity was tested through well diffusion method. **Results:** The phytochemical studies on methanolic flower extract of *Peltophorum pterocarpum* (DC.) Baker ex Heyne. revealed the presence of glycosides, flavonoids, phenolics, saponins, catechin and alkaloids. The HPTLC separation was achieved using ethyl acetate–methanol–ethanol–water (8.1: 1.1: 0.4: 0.8) as the mobile phase. The methanolic extract of *P. pterocarpum* showed four different R_f values 0.16, 0.31, 0.77 and 0.82 which indicated various glycosides present in the flower extract. The methanolic extract of *P. pterocarpum* showed the maximum zone of inhibition against *Proteus mirabilis* followed by *Salmonella typhi*. **Conclusion:** Bio–assay revealed the presence of specific and selective antimicrobial compounds in the fractions. Broad range activity of plant extracts as per observations in this study was due to presence of multiple antimicrobial compounds or synergic effects of these compounds. Therefore, standardization of active fractions and study for in vivo efficacy may result in development of better antimicrobial drugs.

1. Introduction

Medicinal plants are an important source of inexpensive and practical drugs for people throughout the world. Medicinal plants are plants which contain thousands of substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs[1]. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavouring

and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is decreasing and people are returning to the naturals with hope of safety and security[2–5]. Over three–quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. As a result of indiscriminate use of antimicrobial drugs in the treatment of infectious diseases, microorganisms have developed resistance to many antibiotics[6]. There is an urge to develop alternative antimicrobial drugs to fulfill the present day requirement. One approach is to screen local medicinal plants, which represent a rich source of novel antimicrobial agents. The deficiency of the drugs available today, propels the discovery of new pharmacotherapeutic agents from medicinal plants[7]. Recent studies are involved in the identification and isolation

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of new therapeutic compounds of medicinal importance from higher plants for specific diseases^[8–11]. With the advances in experimental methods in phytochemistry and pharmacology, several medicinal plants were screened for active principles and biological activities^[12–14]. In recent years, pharmaceutical companies have spent considerable time and money in developing therapeutics based upon natural products extracted from plants^[15,16].

Peltophorum pterocarpum (DC.) Baker ex Heyne (Caesalpiniaceae) is a deciduous tree commonly used for ornamental purpose and as an avenue tree. Different parts of this tree are used to treat many diseases like stomatitis, insomnia, skin troubles, constipation and its flower extract is known to be a good sleep inducer and used in insomnia treatment. Its bark is used as medicine for dysentery, as eye lotion, embrocation for pains and sores. The traditional healers use the leaves in the form of decoction for treating skin disorders. There are many reports on the medicinal and other applications of various parts of *P. pterocarpum*^[7,17,18] except the flower extracts. Because of its abundance and widespread availability, this study was set out to investigate the antibacterial activity of *P. pterocarpum* flower extracts against the Gram positive and Gram negative bacteria isolated from human infections. In addition, the preliminary phytochemical analysis and HPTLC profiling was carried out to confirm the presence of various secondary metabolites constituents and trace the probable compounds responsible for anti-microbial activity.

2. Materials and methods

Fresh flowers of *P. pterocarpum* were plucked from the wild tree and shade dried for a week at room temperature. 50 g of dried flowers were extracted using 300 ml methanol as solvent in a soxhlet apparatus for 8h. The extract obtained was concentrated and stored at 4°C. The preliminary phytochemical screening was performed according to the Brindha et al^[19] method and the results are tabulated in Table 1. HPTLC studies were carried out following Harborne^[20] and Wagner et al^[21]. For the present study CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12bit CCD camera for photo documentation, controlled by WinCATS-4 software were used. All the solvents used for HPTLC analysis were obtained from MERCK. The 100 mg extract was dissolved in 5ml of Methanol (96%) and the solution was centrifuged at 3000 rpm for 5 min and used for HPTLC analysis as test solution. The samples (5 µl) were spotted in the form of bands of width 5mm with a Camag microlitre syringe on precoated silica gel glass plate 60F-254 (20cm × 10cm with 250 µm thickness (E. Merck, Darmstadt, Germany) using a Camag Linomat IV (Switzerland). The plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography. The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapor) with respective mobile phase and the plate was developed in the respective mobile phase up to 90 mm. The Ethyl acetate–Methanol–Ethanol–Water was employed as mobile phase. Linear

ascending development was carried out in 20 cm × 10cm twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with the mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical contents. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25±2 °C). The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo–documentation chamber (CAMAG REPROSTAR 3) and captured the images under White light, UV 254 nm and UV 366 nm. The developed plate was sprayed with Antimony (III) chloride as spray reagent and dried at 100 – 120 °C in hot air oven for 5 – 10 min. The plate was photo–documented at UV 366 nm and daylight using Photo–documentation (CAMAG REPROSTAR 3) chamber. Finally, the plate was fixed in scanner stage and scanning was done at 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag). The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm.

The methanolic extracts of *P. pterocarpum* were tested against a panel of microorganisms including *Salmonella typhi* (MTCC 733), *Staphylococcus aureus* (MTCC 96), *Proteus mirabilis* (MTCC 742), *Bacillus subtilis* (MTCC 441) and *Escherichia coli* (MTCC 443) obtained from MTCC, Chandigarh. The antimicrobial activity was tested through agar well diffusion method. Muller– Hinton agar was used as the standard test medium for the present study. Fresh cultures were prepared and used to inoculate 50 ml of Muller– Hinton broth that was incubated at 35 °C for 18 h. Overnight broth cultures were prepared, adjusted in peptone–physiological salt solution (1 g peptone and 8.5 g/l NaCl) to yield approximately 106 bacteria/ml. The agar plates were prepared in 90 mm Petri dishes with 22 ml of agar medium giving a final depth of 3 mm. Cylinders (diameter 5.5 mm) were placed on the inoculated agar surfaces and filled with 50, 75, 100 and 125 µg/ml of methanol extract. All plates were aerobically incubated at 37 °C for 18–24 h. The antimicrobial activity was estimated by measuring the radius of the zone of inhibition (mm). Each test was performed in triplicate and repeated thrice. Chloramphenicol (30 µg) and Tetracycline (30 µg) were used as positive controls. Methanol was used as a negative control.

3. Results

Preliminary phytochemical analysis revealed that the presence of secondary metabolites like phenolic group, catechin, saponin, flavonoids, sugar, and glycoside in the flowers of *P. pterocarpum* (Table 1). Methanolic flower extracts of *P. pterocarpum* were examined for the antibacterial activity against the isolated human pathogens and the results are depicted in the Table 2. The antibacterial activity of the methanolic flower extracts of *P. pterocarpum* was observed in all the tested bacteria with varied degree activity. The maximum zone of inhibition 15 mm for *Salmonella typhi*, 14 mm for *Bacillus subtilis*, *Proteus mirabilis* and *Staphylococcus aureus* were observed (Table

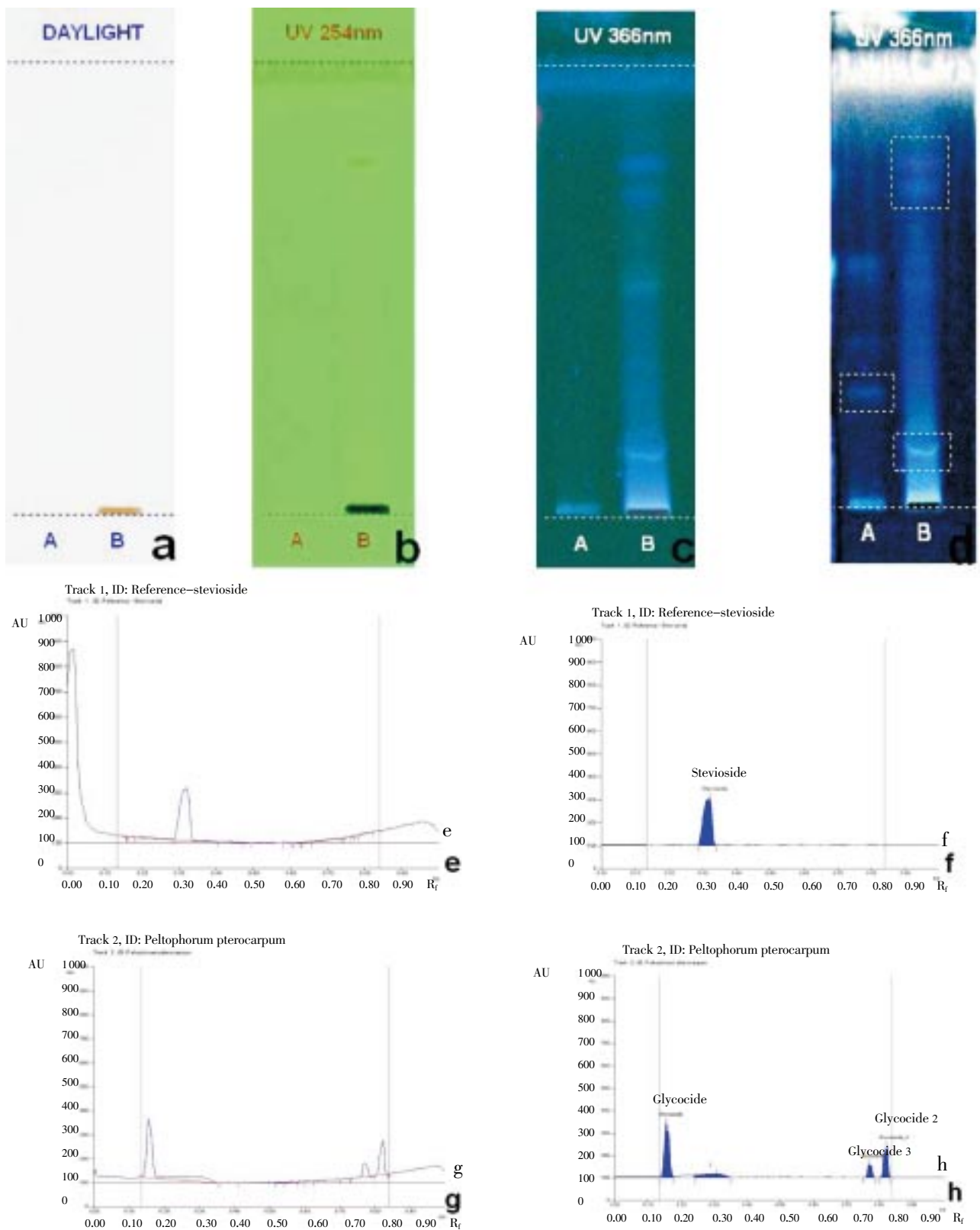


Figure 1. HPTLC analysis of methanol flower extract of *P. pterocarpum*
 a – c. Chromatograms of Glycoside analysis of methanolic flower extract of *P. pterocarpum* (before derivation) A– reference sample and B– extract,
 d. Chromatograms of Glycoside analysis of methanolic flower extract of *P. pterocarpum* (after derivation) A– reference sample and B– extract,
 e. Peak densitogram display of Reference stevioside for Glycoside (Scanned at 366nm),
 f. Baseline display of methanolic flower extract of *P. pterocarpum* – Glycoside Scanned at 366nm
 g. Peak densitogram display of methanol flower extract of *P. pterocarpum* for Glycoside (Scanned at 366nm).

2).

Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The desired aim was achieved using Ethyl acetate–Methanol–Ethanol–Water (8.1: 1.1: 0.4: 0.8) as the mobile phase (Table 3; Figure 1 a–d). The methanolic extract of *P. pterocarpum* showed four different Rf values 0.16, 0.31, 0.77 and 0.82 which indicated various glycosides present in the flower extract (Figure 2.c and d). Stevioside was used as reference for glycoside profile (Figure 1.e). Blue colored fluorescent zone at UV 366 nm appeared in both tracks flower extracts (A) and reference (B) (Figure 2.c and d). It confirms the presence of glycosides in the flower extracts of *P. pterocarpum*. The reference samples were run parallel to the sample for easy and accurate detection of the components (Figure 1. a–e). Fig.1. e–g shows the Peak densitogram display of reference stevioside (Sigma–Aldrich) for Glycoside (Scanned at 366nm), baseline display of methanolic flower extract of *P. pterocarpum* for glycoside and peak densitogram display of methanolic flower extract of *P. pterocarpum* for Glycoside (Scanned at 366nm).

Table 1

Phytochemical screening of methanolic flower extracts of *Peltophorum pterocarpum*.

Tests	Flower extracts of <i>Peltophorum pterocarpum</i>
Flavonoids	+
Glycosides	+
Sugars	–
Alkaloids	+
Catechin	+
Phenols	+
Tannins	–
Saponins	+

Table 2

Antibacterial activity of Methanolic Flower extracts of *P. pterocarpum*.

Methanolic Extracts in μ g/mL	Zone of Inhibition (in mm)					
	50	75	100	125	Chloramphenicol	Tetracycline
<i>S. typhi</i>	5	11	13	15	5	4
<i>B. subtilis</i>	9	11	13	14	4	5
<i>P. mirabilis</i>	5	11	13	14	3	5
<i>S. aureus</i>	7	10	11	14	5	6

Table 3

HPTLC Analysis of methanolic flower extracts of *Peltophorum pterocarpum* (DC.) Baker ex Heyne.

Track	Peak	Rf	Height	Area	Assigned substance
A	1	0.16	243.4	3376.6	Glycoside 1
A	2	0.31	17.1	1156.7	Unknown
A	3	0.77	58.3	730.8	Glycoside 2
A	4	0.82	140.8	1627.0	Glycoside 3
B	1	0.32	208.1	4826.0	Stevioside

Track A– Samples; B– Stevioside (Reference).

4. Discussion

Phytochemical constituents such as tannins, saponins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and other herbivores[22]. The zone of inhibition for every bacterial strain was found to be increased as the extract concentrations were increased. The demonstration of antimicrobial activity against both Gram–positive and Gram–negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds and coincided with Cichewicz and Thorpe[24] and Srinivasan et al[25]. The secondary metabolites presence confirms the demonstration of antimicrobial activity by the flower extracts of *P. pterocarpum*. The HPTLC method was validated by determining linearity, peak purity, limit of detection, repeatability, and percentage recovery of glycosides from flower extracts of *P. pterocarpum*. Similar to the present study Tiwari et al[12] observed six spots in the alcohol extract of *Helicteres isora* root at 366 nm. Yamunadevi et al[26] reported glycosides presence in different parts of *A. lanata*. The HPTLC profile can be used as a tool for the taxonomical identification. Yamunadevi et al[26] also used the HPTLC profile for the identification of *A. lanata* from the adulterant.

Natural product substances have historically served as the most significant source of new leads for pharmaceutical development[27]. These products as drugs play a significant role in the pharmaceutical care. The terrestrial plants have played a vital therapeutic role in the treatment of human ailments from time immemorial. Various active compounds from bark, leaves, root, flower and other parts of plants are found in a new guise in the existing treatments or may be used as basis for design of novel medicinal agents[26]. In the present study also we observed the different types of glycosides presence in the methanolic flower extracts of *P. pterocarpum*. Glycosides comprise a very wide range of compounds that are of common and ubiquitous occurrence in almost all plants. Glycosides play important roles in our lives. Many plants store medicinally important chemicals in the form of inactive glycosides. The non–sugar portion contains the biochemically active properties of medical interest. Once the glycoside is split into its two components (sugar and non–sugar parts), the non–sugar component is now free to exert its chemical effects on the body. For example, digitalis is a glycoside that when ingested, causes the heart to contract (pump) more forcefully. This is useful in medicine, where heart failure is present. A considerable number of glycosides are of great medicinal value, all of them are of natural origin. These pharmaceutically valuable glycosides contribute to almost every therapeutic class, cardiac drugs, laxatives, counter irritants, analgesics, renal disinfectants, anti–rheumatics, anti–inflammatory, anti–tuberculosis, expectorant and antispasmodic action[28]. In the present study we observed a number of glycoside from the flowers of *P. pterocarpum*, thus the present study authenticated the traditional medical practice and previous pharmacological observations and supplement to treat other health problems such as cardiac disorder, rheumatism, tuberculosis etc. By isolating and identifying these bioactive compounds new drugs can be formulated to treat various diseases.

In conclusion, bio–assay revealed the presence of specific

and selective antimicrobial compounds in the fractions and extracts which may or may not have broad range activities. Broad range activity of plant extracts as per observations in this study was due to presence of multiple antimicrobial compounds or synergic effects of these compounds. Therefore, standardization of active fractions and study for in vivo efficacy may result in development of better antimicrobial drugs. It may provide nature friendly and cheap drugs accessible to all the people of world.

Conflict of interest statement

We declare that we have no conflict of interest.

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