

Phytochemical profiling and GC-MS analysis of *Pterocarpus indicus* willd. Using ethanolic stem bark and leaves extract

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Abstract

Nature has blessed us with enormous wealth of herbal plants which are widely distributed all over the world. *Pterocarpus indicus* belongs to the family Fabaceae is well known in Indian system for its medicinal uses. The present investigation was carried out to determine the possible bioactive components from the ethanolic stem bark and leaves extract of *Pterocarpus indicus* using preliminary phytochemical screening and GC-MS analysis, while the mass spectra of the compounds found in the extract was matched with the WILEY and National Institute of Standards and Technology (NIST Library). The results of the GC-MS analysis provide different peaks determining the presence of 20 phytocomponents in stem bark and 10 phytocomponents in leaves. The presence of these bioactive compounds in *Pterocarpus indicus* is an excellent source to treat various diseases and health complications in humans.

Keywords: *Pterocarpus indicus*, GC-MS analysis, ethanolic extract, WILEY, NIST library

Introduction

Pterocarpus indicus is an important therapeutic and medicinal plant belonging to family Fabaceae [1]. The treasure of India is stored in the vast natural flora, which has been boon to mankind [2]. Various plants available in the nature are still unexplored for their medicinal potential. Among them, *Pterocarpus indicus* is one of the most valuable multipurpose forest trees. It is a large deciduous tree widely distributed in the central, western and southern regions of India, having a long history of numerous traditional and ethnobotanical applications in diverse cultures [3]. All parts of *Pterocarpus indicus* is used as a primitive medicine for domestic remedy against several human diseases. It has been used in Homeopathic, Ayurvedic and Unani systems of medicine [4-5]. The medicinal value of the plants lies in the bioactive phytochemical constituents that produce definite physiological effect on human body [6]. Phytochemicals derived from various parts of the plants are the important components with numerous applications in pharmaceutical industry [7]. The aim of this work was to investigate the bioactive constituents of *Pterocarpus indicus*, which may provide an insight about the use of traditional medicine.

Materials and methods

Collection of Plant Material

The *Pterocarpus indicus* bark and leaves were collected in the month of December from the Senthankudi Village, Pudukkottai, Tamil Nadu, India. The plant was identified and authenticated by Dr. S. John Britto, Director, Rapinat herbarium, St. Joseph College, Tiruchirappalli, Tamil Nadu for identifying the plants.

Preparation of Plant Extract

Pterocarpus indicus stem bark and leaves were separately washed in running water, cut into small pieces and then shade dried for a week at 35-40°C, after which it was grinded to a uniform powder of 40 mesh size. The extracts were prepared by soaking 100 g each of the dried powder plant materials in 1 L of ethanol using a soxhlet extractor continuously for 10 hrs. The extracts were filtered through whatman filter paper No.42 (125mm) to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using a rotary evaporator under reduced pressure. The final dried samples were stored in labeled sterile bottles and kept at -40°C. The filtrate obtained was used as sample solution for the further isolation [8-9].

Preliminary Phytochemical Screening

Phytochemical analysis of ethanolic stem bark and leaves extract of *Pterocarpus indicus* was carried out as described by (Harborne 1988). By this analysis, presence of several phytochemicals like tannin, phlobatannin, saponin, flavonoids, steroids, terpenoids, cardiac glycosides, leucoanthocyanin, anthocyanin, anthroquinone, coumarin, glycosides, phenol, xanthoproteins, alkaloids, emodine, carbohydrate and proteins were tested [10-11].

Identification of Components

Gas chromatography-mass spectrometry (GC-MS) interpretation was conducted using the database of WILEY and NIST. Spectrum of the unknown component was compared with the spectrum of known components stored in the WILEY and NIST Library.

GC-MS Analysis

GC-MS QP2010 Plus (Shimadzu, Kyoto, Japan) system was utilized. The system was equipped with an auto injector (AOC-20i), head space sampler (AOC-20s), a mass selective detector with an iron source (220 °C) and an interface (260 °C). Rtx-5 MS capillary column having 30 mm X 0.25 mm of length X diameter and 0.25 µm of film thickness was used for MS analyses. The mass range of 40-650 m/z with 1,000 ev of the threshold was purposed. The injector was set in the split injection mode having 250 °C of temperature. The ratio applied for split mode was 10.0. The starting temperature was adjusted to 80 °C (3 min), which afterwards increased to 280 °C with a ramp rate of 10 °C/min. Helium (>99.99 %) with 40.5 cm/s of linear velocity was employed as a carrier gas. The system was programmed with 16.3 ml/min of total flow rate and 1.21 ml/min of column flow. Components were recognized by their retention time (RT) and elucidation of mass spectra. The spectral fragmentation of unknown components was compared with the known and standard components provided by the databases of WILEY and NIST Library.

Results

Preliminary Phytochemical Screening

Qualitative analysis of ethanolic stem bark and leaves extract of *Pterocarpus indicus* showed positive response for tannin, phlobatannin, saponin, flavonoids, steroids, terpenoids, cardiac glycosides, leuco anthocyanin, anthocyanin, anthroquinone, coumarin, glycosides, phenol, xanthoproteins, alkaloids, emodine and carbohydrates (Table:1). Quantitative analysis of important phytochemicals in *Pterocarpus indicus* contains phytochemicals in varying amounts in the bark. The phytochemicals with the highest quantity was tannin (0.053mg/g) followed by flavonoids (0.018mg/g), saponin (0.02mg/g), alkaloids (0.025mg/g), phenols (0.019mg/g), terpenoids (0.012mg/g) respectively and leaves also revealed flavonoids (0.036mg/g), tannin (0.083mg/g), saponin (0.051mg/g), alkaloids (0.057mg/g), phenols (0.010mg/g), terpenoids (0.028mg/g) respectively (Table: 2).

Table 1: Qualitative Analysis of Ethanolic Stem Bark and Leaves Extract of *Pterocarpus indicus*

S. No	Phytochemical Constituents	Observation	Stem Bark	Leaves
1	Tannin	Brownish green	+++	+++
2	Phlobatannin	Red precipitate	+++	+
3	Saponin	Foam	+++	+++
4	Flavonoids	Yellow colour	+++	+++
5	Steroids	Blue colour	++	+++
6	Terpenoids	Reddish brown precipitate	+++	+++
7	Cardiac glycosides	Brown ring	+++	+++
8	Leuco anthocyanin	Red layer	+	+
9	Anthocyanin	Bluish violet	++	+
10	Anthroquinone	Red colour	+++	+
11	Coumarin	Yellow colour	+++	+++
12	Glycosides	Green colour	++	+++
13	Phenol	Blue black	+++	+++
14	Xanthoprotein	Reddish orange precipitate	++	+
15	Alkaloids	Yellow colour	+++	+++
16	Emodine	Red colour	+++	++
17	Carbohydrate	Reddish violet ring	+++	++

(+ = Slightly Present, ++ = Moderately Present, +++ = Strongly Present)

Table 2: Quantitative Analysis of Ethanolic Stem Bark and Leaves Extract of *Pterocarpus indicus*

S. No	Phytochemical Constituents	Bark (mg/g)	Leaves (mg/g)
1.	Flavonoids	0.018	0.036
2.	Tannin	0.053	0.083
3.	Saponins	0.020	0.051
4.	Alkaloid	0.025	0.057
5.	Phenol	0.010	0.024
6.	Terpenoids	0.012	0.028

GC-MS Analysis

Gas Chromatography–Mass Spectrometry (GC-MS) is a valuable tool for reliable identification of bioactive compounds. In the present study, 20 bioactive compounds have been identified from the ethanolic extract of

Pterocarpus indicus stem bark and 10 bioactive compounds have been identified in leaves by GC - MS analysis. The active principles with their Retention Time (RT), molecular formula, molecular weight and peak area in percentage are presented in Table: 3 & 4.

Table 4: Bioactive compounds detected in ethanolic leaves extract of *Pterocarpus indicus*

S. No	R. Time	Area%	Height%	Compound Name	Molecular Formula	Molecular Weight
1	14.031	3.31	8.16	Docosane	C ₂₂ H ₄₆	310
2	15.609	48.92	33.61	Phthalic acid, di-(1-hexen-5-yl) ester	C ₂₀ H ₂₆ O ₄	330
3	15.842	5.36	13.35	Propanoyl chloride	C ₃ H ₅ ClO	92
4	15.967	9.47	8.19	Methane, Thiobis-	C ₂ H ₆ S	62
5	16.092	7.72	7.63	1,3-dioxolane, 2-(phenylmethyl)-	C ₁₀ H ₁₂ O ₂	164
6	16.283	3.45	2.51	n- Allyloxymethyl acrylamide	C ₇ H ₁₁ N O ₂	141
7	21.084	3.59	7.53	Silane, [1-(5-hexenyl)-2-methylenecyclopropy	C ₁₃ H ₂₄ Si	208
8	21.236	11.29	10.13	14-Heptadecenal	C ₁₇ H ₃₂ O	252
9	23.518	3.44	3.28	4,6-Decadiyn-3-ol, 3-isopropyl-9- (methoxyeth	C ₁₇ H ₂₈ O ₄	296
10	25.058	3.44	5.62	Silicate anion tetramer	C ₂₄ H ₇₂ O ₁₂ Si ₁₂	888
		100.00	100.00			

Table 5: Biological activity of phyto-components identified in the ethanolic stem bark and leaves extract of *Pterocarpus indicus*

Phytoconstituents	Biological Activity
Hexadecanoic acid (CAS) Palmitic acid	Antioxidant, Hypocholesterolemic, Flavor, Antiandrogenic Pesticide, Nematicide, Lubricant. [12]
Benzoldicarbonyls, di-(hex-1-en-	Antimicrobial, Antifouling [12]
Phthalic acid, butyl ester, ester with butyl glycol	Antimicrobial, Antifouling [12]
9,12,15-Octadecatrienoic acid, methyl ester	Anti-inflammatory, Insectifuge, Hepatoprotective, Cancer preventive, Antihistaminic. [12]
Zingiberene	Antimicrobial, Anti-inflammatory, Blood pressure-lowering, Cholesterol-lowering, Antiplatelet aggregation, Chemo preventive, Antioxidant, and Hypoglycemic properties. [13-14]
Dodecanoic acid (CAS) Lauric acid	Antibacterial
Nonadecanoic Acid	Nematicide, Lubricant, Antioxidant, Hypocholesterolemic, Cancer Preventive. [12]
Docosane	Antibacterial and Antioxidant [15]
1,3-dioxolane, 2-(phenylmethyl)-	Antimicrobial [16]
14-Heptadecenal	Antimicrobial [16]
9-Octadecenoic acid (Z)- (CAS) Oleic acid	Antioxidant, Cell Proliferation and Prevents the Pro-Apoptotic Effect [17-18]

Discussion

The present study shows that the qualitative analysis reveals flavonoids, tannins, saponins, alkaloid, phenol, terpenoids, Cardiac glycosides and Coumarin are strongly present in both stem bark and leaves extract, hence quantitative test were performed for flavonoids, tannins, saponins, alkaloid, phenol and terpenoids. The leaves extract shows the increased concentration of tannin (0.083mg/g), alkaloid (0.057mg/g) and saponins (0.051mg/g) and the stem bark shows the increased concentration of tannin (0.053mg/g), alkaloid (0.025mg/g) and saponins (0.020mg/g) respectively. These secondary metabolites serve as defence mechanism against different infectious diseases. The phytoconstituents such as, phenols and flavonoids were reported for enhancing the scavenging of free radicals [19]. Natural antioxidant compounds are considered to be an endogenous defence mechanism as a protection against oxidative stress due to extreme level of environmental conditions [20]. GC-MS analysis of ethanolic stem bark and leaves extract of *Pterocarpus indicus* revealed the presence of some important bioactive compounds. 5, 8, 11, 14-Icosatetraynoic Acid, Nonadecanoic Acid and Cytidine are present in the increased concentration in stem bark. Phthalic acid and 14-Heptadecenal are present in the higher concentration in leaves extract, which shows antioxidant, anticancer, antimicrobial, hypoglycemic hypo cholesterololemic and anti-inflammatory properties that has been already reported. According to the previous studies the main chemical constituent of *Pterocarpus indicus* are glucosidal tannin namely kinotannic acid. Several other chemical constituents like pterostilbene, (-)-epicatechin, pterosupin, marsupsin, etc. have been identified and isolated. It also shows promising results in treatment of cataract [21]. And hypertriglyceridaemia [22]. This plant also finds its use as cardiotoxic [23-24], hypoglycaemic [25], antihyperinsulinaemic

[26], antifungal [27], cox-2 inhibitor [28]. And hepatoprotective agent [29]. *Pterocarpus indicus* is widely used in 'Ayurveda' as 'Rasayana' for management of various metabolic disorders including hyperlipidemia [30-31]. Also used to improve eye sight [32]. In combination with other drugs the wood is used to treat snake bites and scorpion stings [33]. Its toxicity, wound healing potential also evaluated in animal studies [34] and also show antioxidant and antimicrobial activity [35].

Conclusion

The present study revealed that the ethanolic stem bark and leaves extract of *Pterocarpus indicus* found to be phyto pharmaceutical important plant, which is traditionally used for the treatment of various ailments and its medicinal application can be easily noticed by the presence of different bioactive molecules. In addition, further research is needed to identify and purify the active compounds responsible for therapeutic activity.

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