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ISOLATION & STRUCTURAL CHARACTERIZATION OF BIOACTIVE COMPOUND IN HERBAL MEDICINAL PLANT *PTEROCARPUS MARSUPIUM*

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ABSTRACT

The study aimed to isolate and structurally characterize bioactive compounds from the herbal medicinal plant *Pterocarpus marsupium*. The extraction process utilized solvents of varying polarities, resulting in a petroleum ether extract (yielding 1.2% w/w) and a hydroalcoholic extract (yielding 8.5% w/w). Phytochemical screening of the hydroalcoholic extract indicated the presence of alkaloids, flavonoids, phenols, carbohydrates, saponins, and diterpenes, while glycosides, proteins, and tannins were absent. Quantitative analysis revealed total flavonoid and phenol contents of 0.852 mg and 0.523 mg per 100 mg of dried extract, respectively. Structural characterization through IR spectroscopy, 1H NMR spectroscopy, and mass spectrometry identified the isolated compound as 3,5,7-trihydroxy-2-(4-hydroxyphenyl) chromen-4-one, a flavonoid. This study highlights the significant presence of bioactive flavonoids in *Pterocarpus marsupium*, suggesting its potential therapeutic applications. Further investigations are warranted to explore the bioactivity and pharmacological properties of the identified compound.

Keywords: *Pterocarpus marsupium,* Bioactive compounds, Solvent extraction, Chromatography, Spectroscopic analysis, Mass spectrometry, NMR spectroscopy

INTRODUCTION

The exploration of bioactive compounds in herbal medicinal plants remains a crucial area of natural product research, offering potential insights into novel therapeutic agents. *Pterocarpus marsupium*, commonly known as Indian Kino or Malabar Kino, is a medicinal plant with a rich history in Ayurvedic medicine, recognized for its diverse pharmacological properties. These include anti-diabetic, anti-inflammatory, antioxidant, and hepatoprotective activities, making it a significant subject of scientific inquiry.

Pterocarpus marsupium has been utilized for centuries in traditional medicine. Its heartwood, bark, and leaves are integral components of various therapeutic formulations aimed at treating conditions such as diabetes, wounds, and other ailments. The heartwood, in particular, is notable for its potent anti-diabetic properties, attributed to its ability to regenerate pancreatic beta cells (Grover & Vats, 2001). Traditional uses of *Pterocarpus marsupium* underscore its importance in managing diabetes and related metabolic disorders, highlighting the need for

scientific validation of its medicinal potential (Nadkarni, 1976).

The therapeutic potential of *Pterocarpus marsupium* is largely due to its rich phytochemical composition. Key bioactive compounds isolated from this plant include pterostilbene, marsupsin, epicatechin, and liquiritigenin (Shanmugasundaram & Panneerselvam, 1987). These compounds exhibit a wide range of biological activities, such as antioxidant, anti-inflammatory, and antidiabetic effects. For instance, pterostilbene has been shown to possess significant anti-diabetic and antioxidant properties, which contribute to the plant's overall pharmacological profile (Ahmad & Pillai, 1999).

Isolating and structurally characterizing these bioactive compounds is essential for understanding their mechanisms of action and potential therapeutic applications. Techniques such as chromatography, nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS) are commonly employed to elucidate the structures of these compounds (Silverstein & Webster, 2005). Such studies not only validate the traditional uses of the plant but also pave the way for the development of novel drugs. Advanced analytical techniques facilitate the precise identification and structural characterization of the active constituents, providing a foundation for further pharmacological research (Wolfender *et al.,* 2015).The primary objective of this study is to isolate and structurally characterize the bioactive compounds from the heartwood of *Pterocarpus marsupium*. By employing advanced chromatographic and spectroscopic techniques, this study aims to identify the compounds responsible for the plant's pharmacological activities and explore their potential therapeutic applications. Understanding the structural intricacies of these compounds will enhance our knowledge of their bioactivity and therapeutic potential.

MATERIALS AND METHODS

Extraction by maceration process

68 gram shade dried fruits was coarsely powdered and subjected to extraction with petroleum ether by maceration process. The extraction was continued till the defatting of the material had taken place. Defatted powdered of *Pterocarpus marsupium*has been extracted with hydroalcoholic solvent (ethanol: water; 80:20v/v) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Farnsworth, 1996).

Determination of percentage yield

The extraction yield is an assessment of the efficiency of the solvent in extracting bioactive components from the selected natural plant samples and was defined as the quantity of plant extracts recovered after solvent extraction compared to the original quantity of plant samples. The yield of the collected plant extracts was measured in grams after extraction, and then converted into percentage. For calculating the percentage yield of selected plant products, formula following was introduced. By using the following formula the percentage yield of extract was calculated:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

Phytochemical screening

Medicinal plants are traditional pharmaceutical commodities and many of the

current medicinal drugs are derived indirectly from plants. Phytochemical materials consist of two main bioactive components (chlorophyll, vitamins, amino acids, sugar etc.) and secondary bioactive components; (Alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical analyses were performed according to the normal protocols for extract. Phytochemical examinations were carried out for all the extracts as per the standard methods (Le *et al.,* 2022).

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Mishra *et al.,* 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm (Lin *et al.,* 2007).

Estimation of total phenolic content

The total phenolic content of the extract was determined by the modified folin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer ((Lin *et al.,* 2007).

Isolation of compound from hydroalcoholic extract of *Pterocarpus marsupium*

Coloum size: Glass Coloum, 100 x 3 Cms

Stationary Phase: Silica gel (60 to 120 mesh size)

Elution mode: Isocratic elution

Mobile Phase: Toluene: Ethyl Acetate (7:3)

Extract*:* Hydroalcoholic extract of *Pterocarpus marsupium*

Visualized by*:* Short UV (254nm), long UV (365nm) and Normal light

Identification of similar fractions: By UV vis. spectroscopy and Visualized by Short UV (254nm), long UV (365nm) and Normal light

Identification of similar fractions

The different fractions of column chromatographic elution were monitored by TLC Toluene: Ethyl Acetate (7:3); using UV chamber and derivatization with specific reagent for identification of single isolated compound with comparison of reference compounds. The fractions which show similar fingerprinting profile on TLC were collected and mixed. Fraction showed single compound and have similar R_f value as compared to reference compound were dried, compound were purified by recrystalization procedure.

Characterization of Isolated compound

70 fraction, each 10ml were collected and isolated compound was characterized by UV-absorption spectra, IR, NMR and Mass Spectra studies.

Spectroscopic Analysis- The UV absorption spectrum of compound was recorded in the range of 200-400nm on (LABINDIA 3000 +) UV spectrophotometer at 1 cm path length.The compounds obtained showed the U.V. absorption maxima (max.) in mm

observed in Lab India Spectrophotometer. Spectroscopic

I.R. Analysis- The IR spectrum of compounds was recorded on (Bruker Alpha) using solid plate technique with KBr.

NMR Analysis- H^1NMR was recorded on Bruker DRX -300 (300 MHz FT-NMR) in $CDCL_3$ using TMS as internal standard.

RESULTS AND DISCUSSION

The isolation of bioactive compounds from *Pterocarpus marsupium* involved using solvents with different polarities, resulting in various extracts. The petroleum ether extract, characterized by a dark black semi-solid texture, yielded 1.2% (w/w). This low yield suggests that non-polar compounds are present in minor quantities within the plant. In contrast, the hydroalcoholic extract, a brown solid, yielded a significantly higher 8.5% (w/w), indicating a higher content of polar compounds.

Phytochemical screening of the hydroalcoholic extract revealed the presence of various bioactive constituents. Alkaloids were detected using Dragendroff's test, which showed a red precipitate. Flavonoids were confirmed by both the Lead acetate and Alkaline tests, which resulted in a yellow precipitate and a colorless solution, respectively. Phenolic compounds were indicated by a positive Ferric chloride test, which produced a black color. Proteins were absent, as shown by the negative Xanthoproteic test, which resulted in a brown color without precipitation. Carbohydrates were present, confirmed by a red precipitate in the Fehling's test. Saponins were identified by the Foam test, which produced a layer of foam. Diterpenes were detected by the Copper acetate test, which showed an emerald green color.

Quantification of total flavonoids and phenol content in the hydroalcoholic extract showed significant amounts of these compounds. The extract contained 0.852 mg of flavonoids and 0.523 mg of phenols per 100 mg of dried extract. These values highlight the substantial presence of flavonoids and phenols, which are likely responsible for the extract's bioactivity.

The structural characterization of the isolated compound involved IR spectroscopy, 1H NMR spectroscopy, and mass spectrometry. The IR spectrum revealed the presence of hydroxyl (O-H), carbon-carbon double bonds (C=C), carbonyl (C=O), and ether (C-O) functionalities. The 1H NMR spectrum showed characteristic proton signals consistent with aromatic and hydroxyl protons. The mass spectrum revealed a molecular ion peak at m/z 286.12 and significant fragment ions at m/z 240.63, 202.84, and 185.23, supporting the molecular formula $C_{15}H_{10}O_6$.

Table 1: % Yield of *Pterocarpus marsupium*

S. No.	Extracts	Colure/ Texture	% Yield (w/w)
1.	Pet. ether	Dark black semi solid	1.2%
2.	Hydroalcoholic	Brown solid	8.5%

Table 2: Phytochemical screening of extract of *Pterocarpus marsupium*

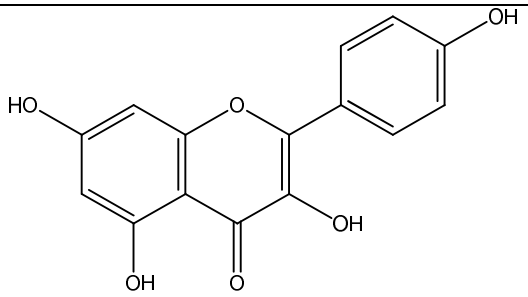
S. No.	Constituents	Hydroalcoholic extract	Observation
1.	**Alkaloids** Dragendroff's test Hager's test	 +ve -ve	 Red precipitated Yellow but no precipitated
2.	**Glycosides** Legal's test	 -ve	 Dark brown coloured
3.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve	 Yellow precipitate Colourless
4.	**Phenol** Ferric chloride test	 +ve	 Black coloured
5.	**Proteins** Xanthoproteic test	 -ve	 Brown coloured
6.	**Carbohydrates** Fehling's test	 +ve	 Red colour precipitate
7.	**Saponins** Foam test	 +ve	 Layer of foam
8.	**Diterpenes** Copper acetate test	 +ve	 Emerald green colour
9.	**Tannins** Gelatin Test	 -ve	 Brown coloured

Table 3: Estimation of total flavonoids and phenol content of extract of *Pterocarpus marsupium*

S. No.	Extract	Total flavonoids content	Total phenol content
		(mg/ 100 mg of dried extract)	
1.	Hydroalcoholic	0.852	0.523

Table 4: Interpretation of spectra

IR	O-H 3500-3000, C=C- 2907.1644, C=O- 1572.3103, C-O- 1444.5606,
^{1}H NMR ($CDCl_3$, 500 MHz)	δ, 15.83 (s, 1H, OH), 7.59 (d, 2H, J= 7.65, CH), 6.65(d, 2H, J = 10.7, CH), 6.25(s, 1H, CH), 5.94(s, 1H, CH), 5.35(s, 3H, OH)
Mass (m/z)	286.12, 240.63, 202.84, 185.23
Molecular formula	$C_{15}H_{10}O_6$
IUPAC Name	3,5,7-trihydroxy-2-(4-hydroxyphenyl) chromen-4-one

Structure of compound	

CONCLUSION

The isolation and characterization of the bioactive compound from *Pterocarpus marsupium* have been successfully achieved. The high yield of the hydroalcoholic extract, along with positive phytochemical screenings for flavonoids and phenols, highlights the potential medicinal value of this plant. The structural elucidation confirms the presence of a significant flavonoid, 3,5,7-trihydroxy-2-(4-hydroxyphenyl) chromen-4-one, which likely contributes to the therapeutic properties of Pterocarpus marsupium. Further studies on the bioactivity and pharmacological properties of this compound are warranted to fully explore its medicinal potential.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Grover, J.K. & Vats, V. (2001) Shifting paradigm from conventional to alternative medicine: An introduction on traditional Indian medicine. *Asia Pacific Biotech. News*, 5, 701–705.
- Nadkarni, K.M. (1976). *Indian Materia Medica*. Popular Press Prakashan Pvt, Ltd: Mumbai.
- Shanmugasundaram, K.R. & Panneerselvam, C. (1987) Enzyme changes and glucose utilisation in diabetic rabbits: The effect of Gymnema sylvestre, R. Br. *Journal of Ethnopharmacology*, 20, 323–327.
- Ahmad, A. & Pillai, K.K. (1999) Hypoglycaemic activity of Pterocarpus marsupium in rats. *Fitoterapia*, 70, 265–267.
- Silverstein, R.M. & Webster, F.X. (2005). *Spectrometric Identification of Organic Compounds*. John Wiley & Sons: New York, USA.
- Wolfender, J.L., Marti, G., Thomas, A. & Bertrand, S. (2015) Current approaches and challenges for the metabolite profiling of complex natural extracts. *Journal of Chromatography. A*, 1382, 136–164.