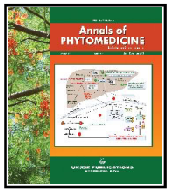
Annals of Phytomedicine 12(2): 866-873, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.2.102

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Quality control evaluation of *Tectona grandis* L. using phytochemical screening, TLC fingerprinting profiling and GC-MS analysis

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Article Info

Article history
Received 20 July 2023
Revised 4 September 2023
Accepted 5 September 2023
Published Online 30 December 2023

Keywords
Medicinal plants
Tectona grandis L.
Phytochemical screening
Quality control
TLC fingerprinting
GC-MS analysis

Abstract

Tectona grandis L. commonly known as Teak, is a widely utilized medicinal plant known for its diverse therapeutic properties. To ensure the safety, efficacy, and standardization of Teak-based products, a comprehensive quality control evaluation was conducted using phytochemical screening, TLC fingerprinting profiling, and GC-MS analysis. Phytochemical analysis showed various phytocompounds, including tannins, steroids, flavonoids, and alkaloids, confirming its medicinal significance. TLC fingerprinting profiling generated unique chemical patterns for Teak samples, facilitating the identification of authentic products and detection of adulteration. This aspect ensured consistent product quality and consumer safety. GC-MS analysis offered detailed information about volatile and semi-volatile compounds present in Teak, shedding light on its chemical composition and potential health benefits. The combined use of these analytical techniques provided a comprehensive insight into the phytochemistry of *T. grandis,* paving the way for its responsible use in various industries. This quality control evaluation has significant implications in the pharmaceutical, nutraceutical, and traditional medicine sectors, as it aids in developing standardized herbal products and quality standards. Furthermore, it enhances global trade and regulatory compliance, ensuring transparency and authenticity in the herbal medicine market. In conclusion, this integrated approach serves as a reliable and efficient method for evaluating the quality and authenticity of *T. grandis* based products. It establishes a solid foundation for further research and utilization of Teak's therapeutic potential, promoting its sustainable exploitation while preserving its natural resources. The findings contribute to the overall advancement of herbal medicine quality assurance and enrich our understanding of the medicinal properties of *T. grandis*.

1. Introduction

Diabetes is a metabolic illness that is defined by elevated blood sugar levels, or hyperglycemia, as a result of insufficient insulin synthesis, increased insulin sensitivity, increasing pancreatic beta cell degeneration, *etc*. Many national and international scientific meetings have placed a major emphasis on lowering the burden of diabetes and its rising prevalence as well as developing possible therapeutic regimens. Medicinal plant is the most of the essential therapeutic agents due to their multi-mechanistic and therapeutic actions against various disease. Since history, it cannot be denied that medicinal plants are playing an essential role in maintaining the healthcare system and targeted to play a vital role in different kind of acute and chronic ailments (Gaurav *et al*., 2020; Khan *et al*., 2022).

Quality control analysis of medicinal plants is a crucial step in ensuring the safety, efficacy, and consistency of herbal medicines. Modern analytical techniques have significantly improved the accuracy and efficiency of this process. Several steps are involved in quality control studies of herbal medicine using advanced analytical techniques (Dhama *et al*., 2022; Kiran *et al*., 2021; Mehrotra, 2020). Proper sampling of the medicinal plant is essential to obtain a representative sample. Samples should be collected from different batches and regions to account for natural variations. Once collected, the samples need to be properly cleaned, dried, and ground to obtain a homogeneous and finely powdered material (Gaurav, 2022; Khan *et al*., 2022).

Authentication of the plant material is vital to ensure that the correct plant species is being used. Modern analytical techniques can identify and quantify the chemical constituents present in the medicinal plant. High-performance thin layer chromatography (HPTLC), liquid chromatography-mass spectrometry (LC-MS), nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS) and other spectroscopic methods are employed for chemical profiling. Through these techniques, the concentration of active compounds or marker compounds is determined to assess the potency and consistency of the medicinal plant. This information is crucial for ensuring the desired therapeutic effect and batch-to-batch consistency (Ali *et al*., 2022; Amrutanand *et al*., 2021; Rana *et al*., 2021). Medicinal plants can be contaminated with heavy metals, pesticides, herbicides, mycotoxins, and other harmful substances. GC-MS is mostly used to detect and quantify contaminants. Residual solvents from extraction processes can be harmful, if present above acceptable limits. GC and headspace gas chromatography (HS-GC) are used to detect and quantify residual solvents (Gaurav *et al*., 2023b; Gautam *et al*., 2023).

Stability testing is conducted to assess the shelf life of the medicinal plant product. It involves subjecting the product to different storage

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conditions and monitoring its stability over time. Medicinal plants can interact with conventional drugs, affecting their efficacy and safety. Modern analytical techniques, along with *in vitro* and *in vivo* studies, can help assess potential herb-drug interactions. Network pharmacology is a computational technique used to determine the multitargeted and biological response of the medicinal plants and thus validating the biomechanical or molecular approaches in treatment of the diseased. This technique is well explored in different recent techniques and acts *via* determining the interaction of different ligands or molecules with the targeted proteins that have been involved in the disease (Gaurav *et al*., 2022; Hao *et al*., 2021).

T. grandis which also determined as Saka (family Verbenaceae). It is also known as Saka, Sagwan, Sag, *etc*. It is a large deciduous tree mostly occurs in the peninsular region and the states such as Madhya Pradesh that extends to Southern Uttar Pradesh, Rajasthan and Orissa, *etc*. The traditional Indian scriptures such as Ayurvedic Pharmacopeia of India claim *T. grandis* to possess its role as antidiabetic, in the purification of blood, urinary disorders, inflammation, *etc*. The constituents of *T. grandis* belongs to the category of terpenes, polyphenols, alkaloids, glycosides, *etc*. Due to lack of chemistry and pharmacology-based evidence on its quality, safety and efficacy are of critical need to generate scientific assets for its regulatory aspects. Considering the reasons, the present study is aimed to determine the protective effect of *T. grandis* against diabetic complications in streptozotocin (STZ) induced rat model. The study will explore several *in vitro* and *in vivo* approaches to investigate the effect of *T. grandis* against diabetic complication (Ogunmefun *et al*., 2017; Vyas *et al*., 2019).

Although, numerous reports published based on its biological effect against several acute and chronic biological dysfunctions. In a study, it has been reported that the constituents such as lapachol, tectoquinone, deoxylapachol, *etc*., are responsible for pharmacological action. Anthraquinones, naphthoquinones and isoprenoid quinones are exist in the high extent in the *T. grandis*. However, the constituents such as steroids, triterpenoids, lignans, phenolic, fatty esters compounds*, etc.,* also present in *T. grandis*. Pharmacologically, the plant has been investigated for antioxidant, antipyretic, anti-inflammatory, cytotoxic, hypoglycemic, analgesic, wound healing activities, *etc*. (Vyas *et al*., 2019).

Based on these factors, the present study is associated to explore the therapeutic potential and molecular mechanism of *T. grandis* in treatment of diabetes. GC-MS analysis and Network pharmacology studies has been performed to generates the scientific evidences of *T. grandis* in development of therapeutic targets or active principles of *T. grandis* in treatment of diabetes.

2. Materials and Methods

2.1 Instrumentation and reagents

GC-MS (Agilent Technologies, United States), Folin-Ciocalteu reagent was purchased from Sigma Aldrich sulfuric acid, sodium hydroxide, liquid ammonia $FeCl_3$, ethanol, distilled water, and all other chemicals acquired from Sisco Research Laboratories Pvt. Ltd. (SRL), India.

2.2 Collection, authentication and preparation of extract

Two hundred grams (200 g) of the dried heartwood of *T. grandis* was procured from the institutional region and authenticated through an expert and the Authentication No. SDMCAH-DG/2022/26. The crude material was powdered coarsely and proceed for the extraction process through the Soxhlet/reflux method to obtain the alcoholic (1.5 l, ethanol), hydroalcoholic (ethanol: water; 5: 5, v/v; 1.5 l) and aqueous (1.5 l distilled water) extract. The obtained content was filtered and concentrated on high steamed water bath to acquire dried residue of the extract. The extract yield was determined and placed in the air-tight container for further study.

2.3 Preliminary study for selection of best active extract based on phytochemicals analysis

Preliminary studies were carried out to screen the best extract based on phytochemicals test, and TLC fingerprinting. The analysis and observations was determined as per reported literature (Gaurav *et al*., 2020; Ibrahim *et al*., 2021; Godghate and Sawant, 2014).

2.3.1 Phytochemical studies

Phytochemical tests were performed based on the reference protocol after doing some modifications (Godghate and Sawant, 2014). In phytochemical analysis, tannins, steroids, saponins, anthocyanin, coumarin, alkaloids, phenols, flavonoids, proteins, amino acids and carbohydrates were detected.

2.3.1.1 Estimation of tannins

For determination of tannin, 4 ml of sample was mixed with $FeCl_3$ solution. The green color of the obtained solution represents the presence of tannins.

2.3.1.2 Estimation of steroid

1 ml of the material was dissolved in 10 ml of strong sulfuric acid and chloroform to determine the presence of steroids. The resulting solution has two layers, with the innermost part being yellow with green fluorescence, indicating the presence of steroids, and the layer on top displaying red.

2.3.1.3 Estimation of saponin

20 ml of water that had been distilled was included in 5 ml of the plant extract in order to determine the amount of saponin. For 15 min, a vortex was used to mix the prepared solution. For 5 min, the solution was left alone, and the persistent presence of foams indicates the existence of saponins.

2.3.1.4 Estimation of anthocyanin

For the estimation of anthocyanin, 2 ml of the plant extract was mixed with 2 ml of 2NHCl and NH_3. The pink red appeared in the sample turns blue violet that indicates the occurrence of anthocyanin.

2.3.1.5 Estimation of coumarin

For determination of coumarin, 3 ml of 10% NaOH solution was added to 2 ml of sample in the different test tube. The development of yellow color that give the presence of coumarins.

2.3.1.6 Estimation of alkaloids

A test tube containing 3 ml of the sample and 1 ml of HCl was used to estimate the amount of alkaloids. After the mixture of ingredients was prepared, it was gently heated for 20 min, cooled, and screened. Wagner's reagent was then applied to the filtrate, and the detection of an orange-brown precipitate indicated the occurrence of alkaloids.

2.3.1.7 Estimation of phenol

1 ml of the sample, 1 ml of Folin-Ciocalteu reagent (1:10, v/v), that was mixed with 1 ml of sodium bicarbonate solution (7.5%) in a test tube to determine the presence of phenols. The mixture was vortexed for 2 minutes. The presence of phenols is indicated by the dark blue color.

2.3.1.8 Estimation of flavonoid

For determination of flavonoid, 1 ml of the sample was mixed with the solution of 10% of aluminum chloride (0.1 ml), followed by addition of 0.1 ml sodium acetate (1M), together. The green color of sample solution represents flavonoids presence.

2.3.1.9 Estimation of proteins

For determination of protein, 1 ml of the sample was mixed with some drops of highly concentrated HNO_3. The formation of yellow color of sample solution represents proteins presence.

2.3.1.10 Estimation of amino acids

For the estimation of amino acids, the ninhydrin test was used. Briefly, 1 ml extract and 1 ml of ninhydrin reagent was mixed through vortex and the obtained solution was boiled for few minutes. The formation of blue color of sample solution represents amino acid presence.

2.3.1.11 Estimation of carbohydrates

For determination of carbohydrates, the sample was treated with few drops of reagent (solution of alcoholic α-naphthol). The formation of a violet ring in the sample solution at the junction of the test tube indicates carbohydrates presence.

2.3.2 TLC fingerprinting analysis

30 mg of each extract will be dissolved in ethanol to make a solution and the resulting solution was applied to the TLC plate using HPTLC applicator. Toluene, ethyl acetate and formic acid (6: 3: 1, v/v/v) were used as solvent system. The TLC plate was advanced in a pre-saturated TLC chamber for development (mobile phase saturation time: 30 min). After development of TLC, the plate was observed and scanned under 254 nm UV light and R_f values of each separated constituent was recorded for record purpose (Gahlot and Yadav, 2021).

2.3.3 GC-MS analysis

GC-MS equipped with a CTC-PAL autosampler merged with a MS detector (Agilent 5975C inert XL EI/CI MSD, United States) was used to identify phytochemicals in *T. grandis* best extract screened from the phytochemical and TLC fingerprinting analysis. The equipment and chromatographic conditions kept same as described in the protocol (Gaurav, 2022). In brief, 1mg of the sample was directly partitioned with 1 ml of n-hexane, filtered with 0.22 µ syringe filter and proceed for GC-MS analysis. The data was screened based on the majority of the phytochemicals present in *T. grandis*. Area and peak retention time were considered as the main parameters for the screening of the compounds.

3. Results

The extraction method of *T. grandis* for the preparation of alcoholic, hydroalcoholic and aqueous extract was completed successfully. The extractive yields of alcoholic, hydroalcoholic and aqueous extract were found as 14.287 ± 0.384, 7.834 ± 0.856 and 5.364 ± 0.669 %, respectively. The different extracts of *T. grandis* have been depicted in Figure 2. Thereafter, preliminary studies such as phytochemicals assay, TLC fingerprinting analysis and GC-MS analysis were performed.

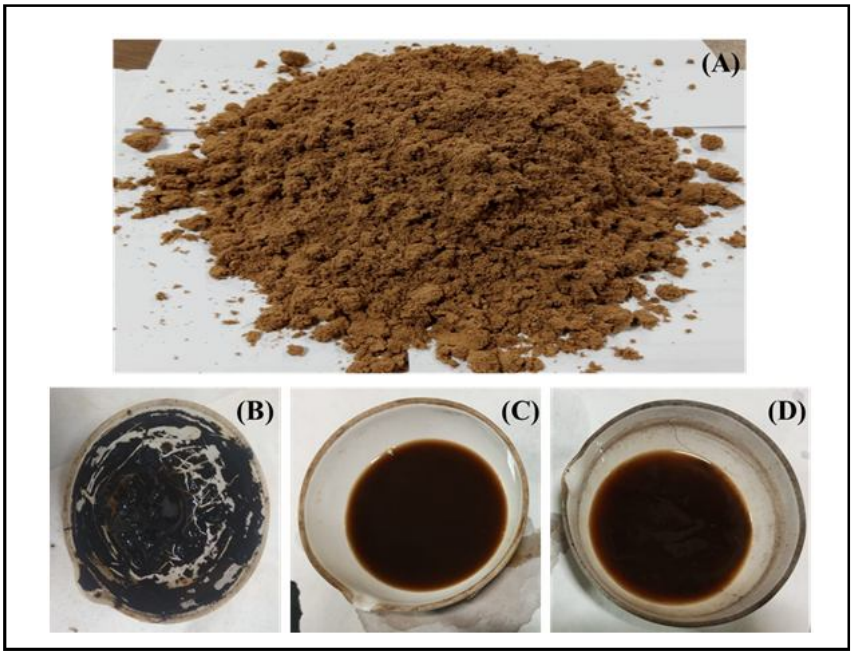

Figure 1: Figure (A) represents the crude extract of *T. grandis* heart wood, Figures (B, C and D) represents the alcoholic, hydroalcoholic and aqueous extract of *T. grandis*.

3.1 Preliminary study for selection of best active extract based on phytochemicals analysis

3.1.1 Phytochemical studies

In the phytochemical screening of *T. grandis,* several categories of phytochemicals were qualitatively identified using different chemical tests. In phytochemical analysis, tannins, steroids, saponins, anthocyanin, coumarin, alkaloids, phenols, flavonoids, proteins, amino acids and carbohydrates were detected. The results have been described in Table 2.

Table 1: Phytochemical screening of *T. grandis*

S. No.	Chemical test	Observations or inference		
		Alcoholic extract	Hydroalcoholic extract	Aqueous extract
1.	Tannin	+	+	+
2.	Steroid	+	+	-
3.	Saponin	+	-	-
4.	Anthocyanin	+	+	-
5.	Coumarin	+	+	-
6.	Alkaloids	++	-	-
7.	Phenol	+++	-	-
8.	Flavonoid	++	+	+
9.	Protein	++	+	+
10.	Amino acid	++	+	-
11.	Carbohydrates	+	+	++

(+) represents the least significant of the chemicals, (++) represents the moderate or least high presence of the chemicals while (+++) represents a significantly high concentration of the chemicals. The outcome of the study was matched with the previous studies, which supports the present findings (Godghate *et al*., 2014; Ogunmefun *et al*., 2017).

3.1.2 TLC fingerprinting analysis

TLC analysis was conducted to examine the number of phytochemicals in each extract and based on the majority of number of metabolites each extract was screened. The results of the study showed that out of three extracts, alcoholic extract contains several major and minor compounds. The results of the TLC fingerprinting have been summarized in Table 3.

Table 2: TLC fingerprinting of *T. grandis* extracts

S. No.	R_f	Alcoholic extract	Hydroalcoholic extract	Aqueous extract
1.	0.09	-	-	+
2.	0.37	+	+	+
3.	0.40	-	-	+
4.	0.44	+	+	-
5.	0.52	+	+	-
6.	0.57	-	-	+
7.	0.61	+	+	-
8.	0.62	+	+	-
9.	0.64	+	+	-
10.	0.68	+	+	-
11.	0.72	+	-	+
12.	0.75	-	-	+
13.	0.78	+	-	+
14.	0.82	-	+	-
15.	0.85	-	-	+
16.	0.89	+	+	-
17.	0.98	+	-	-
Total metabolites		**11**	**10**	**08**

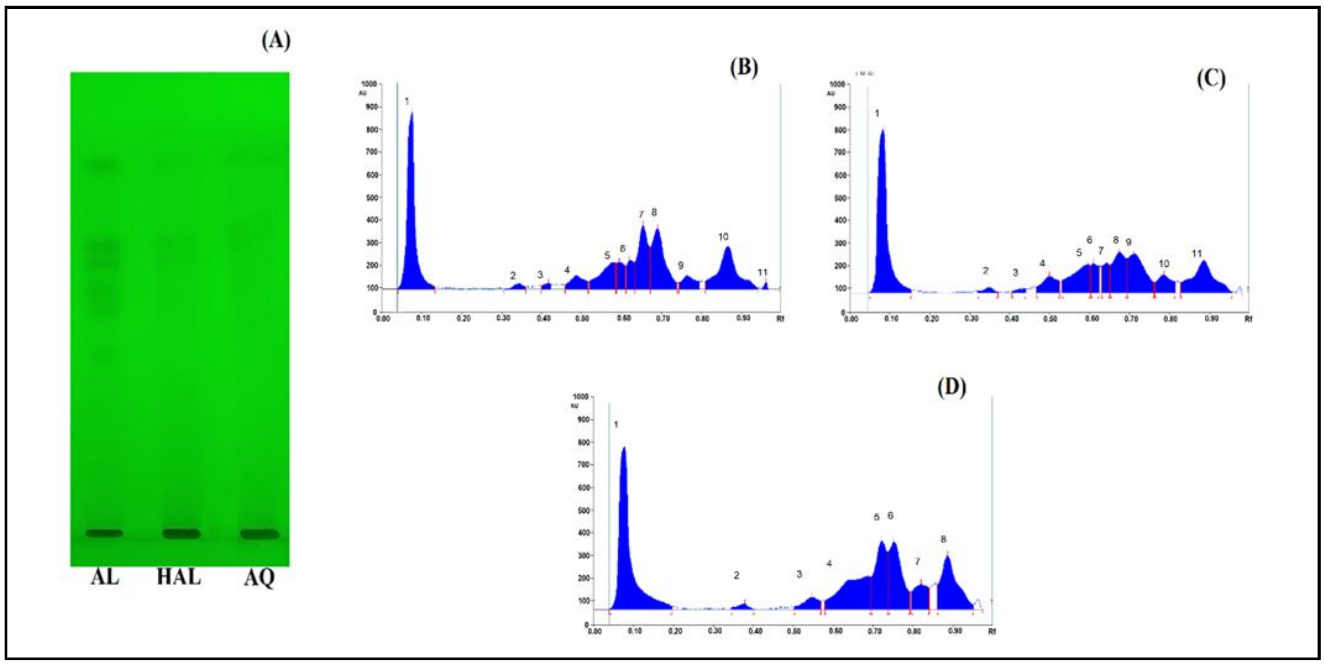

Figure 2: TLC fingerprinting analysis of *T. grandis* extracts, Figure (A) represents TLC plate at 254 nm, Figure (B) represents the chromatogram of alcoholic extract, Figure (C) represents the chromatogram of hydroalcoholic extract and Figure (D) represents the chromatogram of aqueous extract. **AL = Alcoholic extract, **HAL = Hydroalcoholic extract, **AQ = Aqueous extract.

3.1.3 GC-MS analysis

GC-MS study of *T. grandis* extract was conducted to explore the components pattern as well as the diversity of the metabolites present in the best active extract. The analysis was performed in the favored chromatographic condition and the developed total ions chromatogram was recorded for the record purpose. The outcome of the study showed several major and minor metabolites found at different retention times. Furthermore, it was found that among several constituents the components such as 1-ethyl-2-benzimidazolinone, methyl cis-isoeugenol, 9h-carbazole, 9,12-octadecadienoic, palmitic acid, acid and linoleic acid exhibits the highest concentration or present in high percentage in the sample. The enlisted metabolites have been summarized in Table 3.

Table 3: Metabolites identified in *T. grandis* extract using GC-MS analysis

S. No.	Rt	Metabolite	% age AUC
1.	6.513	Cyclohexanone, 2-butyl-	0.21
2.	6.865	Trans-2-hydroxycyclohexanyl acetate	0.11
3.	7.004	1-Octanol, 2-butyl-	0.16
4.	8.088	Alpha-chloro-beta-[4-imidazoly l]propionic acid	0.12
5.	8.520	Oxirane	0.03
6.	8.563	(-)-trans-verbenol	0.02
7.	8.974	2-Octanamine, N-(1-methylheptyl)-	0.30
8.	9.362	Tetradecanal	0.48
9.	10.116	Methyl 3,4-dimethyl-2(Z)-pentenoat	0.15
10.	10.482	Cis-2-nonene	0.14
11.	10.701	Z-citral	0.04
12.	10.994	3-Methoxy-4-methyl-2(5h)-furanone	0.45
13.	12.510	2-Methoxy-4-vinylphenol	0.77
14.	12.971	Syringol	0.74
15.	13.118	1-Ethyl-2-benzimidazolinone	1.25

16.	13.542	Hydroquinone	0.58
17.	14.677	Methyl cis-isoeugenol	1.97
18.	15.065	Phenol	2.72
19.	15.915	Guanine	0.64
20.	16.713	Cyromazine	0.24
21.	17.496	9H-carbazole	1.12
22.	18.302	Gamma-hydroxyisoeugenol	0.59
23.	20.257	Tetradecanoic acid	0.36
24.	21.780	Palmitic acid	5.46
25.	24.262	9,12-octadecadienoic acid	1.04
26.	26.048	Linoleic acid	51.98

4. Discussion

T. grandis phytochemicals analysis showed the presence of various chemical compounds. In phytochemical screening of *T. grandis,* polyphenolic compounds reported to have astringent properties. They are known for their antioxidant and anti-inflammatory effects. Steroids exhibits roles in numerous physiological processes in plants and animals. Saponins are glycosides that have foaming properties and are known for their potential health benefits, such as cholesterol-lowering effects and immunomodulatory activity. Anthocyanins are the colored components that are water-soluble found in many vegetables, fruits, and flowers. They are potent antioxidants (Fisseha *et al*., 2021; Mandal *et al*., 2018; Saleem *et al*., 2019). Coumarin is a natural compound with a distinct sweet aroma found in various plants. It is used in the perfume industry and has medicinal properties as well. Alkaloids are nitrogen-containing compounds that often have significant pharmacological effects. They are commonly found in medicinal plants. Phenols are a class of aromatic compounds with antioxidant properties, and they are involved in various metabolic processes in plants. Flavonoids are a diverse group of polyphenolic type of chemicals found in plants. They have antioxidant, anti-inflammatory, and anticancer properties (Sasidharan *et al*., 2011; Shamkuwar *et al*., 2012). Proteins, amino acids, carbohydrates, *etc*., are crucial for various biological processes and determined as the primary energy source for living organisms, including plants. The presence of these compounds indicates the potential medicinal and nutritional value of *T. grandis*. However, it is essential to determine that the targeted composition and quantities of these compounds may vary depending on the plant's geographical location, growth conditions, and other factors. Additionally, the presence of these compounds does not necessarily guarantee therapeutic effects, and further research is needed to understand their potential health benefits and applications (Abubakar and Haque, 2020; Gautam *et al*., 2023).

TLC fingerprinting profiling is a valuable analytical technique widely used in quality control, authenticity assessment, and standardization of herbal medicines and natural products. Its significance lies in its ability to provide unique chemical fingerprints for complex mixtures of compounds present in botanicals and herbal products. TLC fingerprinting allows for rapid identification and comparison of samples, ensuring product consistency and authenticity. It serves as a valuable tool for botanical drug authentication, helping to detect adulteration or substitution, thus ensuring consumer safety. Additionally, TLC fingerprinting is an essential step in developing monographs and quality standards for herbal products, facilitating global trade and regulation compliance. In this study, TLC fingerprinting profile of *T. grandis* was determined and it was found that it has several major and minor phytochemicals of different category that play a multi-mechanistic and therapeutic role in reduction of ailments. Out of three extracts, it was determined that alcoholic extract of *T. grandis,* contains several phytochemicals then the others extracts (Gaurav *et al*., 2023a, 2020; Khan *et al*., 2021; Kumar *et al*., 2020).

GC-MS is a highly powerful and versatile analytical technique with significant importance in various fields. Its significance lies in its ability to separate, identify, and quantify complex mixtures of volatile and semi-volatile compounds with exceptional sensitivity and selectivity. GC-MS is extensively used in environmental, food, pharmaceutical, and forensic analyses, providing valuable insights into compound structures and characteristics. It aids in detecting trace-level pollutants, drug metabolites, and chemical residues, contributing to environmental protection and public health. In the pharmaceutical industry, GC-MS ensures drug quality and safety by detecting impurities and verifying drug formulations. Its reliability and wide applications have made it an indispensable tool for research, quality control, and safety assurance across multiple industries (Gaurav, 2022; Gautam *et al*., 2020; Rathi and Balasubramanian, 2018). In this study, GC-MS profile of *T. grandis* best extract was done (alcoholic) and the outcomes of the study showed several major and minor phytochemicals belongs to the non-polar and semi-polar category, were present in the *T. grandis* extract. of different category that play a multi-mechanistic and therapeutic role in reduction of ailments. Out of three extracts, it was determined that alcoholic extract of *T. grandis,* contains several phytochemicals then the others extract. Furthermore, it was found that among several phytochemicals the components such as 1-ethyl-2-benzimidazolinone, methyl cis-isoeugenol, 9h-carbazole, palmitic acid, 9,12-octadecadienoic acid and linoleic acid exhibits the highest concentration or present in high percentage in the sample.

Furthermore, from the present analysis, it was confirmed that the quality control evaluation of *T. grandis* using phytochemical screening, TLC fingerprinting profiling, and GC-MS analysis holds paramount importance in several aspects. Phytochemical screening helps identify the presence of numerous bioactive components such as flavonoids, tannins, steroids, and alkaloids responsible for its medicinal and nutritional properties. TLC fingerprinting profiling

provides unique chemical patterns, enabling the authentication, standardization, and detection of adulteration in *T. grandis*-based products, ensuring consumer safety and product consistency (Gaurav *et al*., 2020). GC-MS analysis offers detailed information about the volatile and semi-volatile compounds, giving insights into its chemical composition and potential health benefits. The combination of these analytical techniques ensures the quality, authenticity, and efficacy of *T. grandis*-based herbal products, as well as facilitating the development of quality standards and regulatory compliance. Ultimately, this comprehensive approach enhances our understanding of Teak's phytochemistry, supporting its applications in pharmaceutical, nutraceutical, and traditional medicine industries (Basist *et al*., 2022; Rathi and Balasubramanian, 2018).

5. Conclusion

In conclusion, the quality control evaluation of *T. grandis* using phytochemical screening, TLC fingerprinting profiling, and GC-MS analysis proves to be an indispensable approach for ensuring the safety, efficacy, and standardization of Teak-based products. Through phytochemical screening, the presence of vital bioactive compounds is identified, substantiating its medicinal significance. TLC fingerprinting profiling enables the reliable authentication and detection of adulteration, maintaining product consistency and consumer trust. GC-MS analysis provides in-depth insights into its chemical composition, further enhancing our understanding of its potential health benefits. This comprehensive evaluation contributes significantly to the pharmaceutical, nutraceutical, and traditional medicine industries, ultimately promoting the responsible use of *T. grandis* as a valuable natural resource.

Acknowledgements

The author would like to thank Parul Institute of Pharmacy, Parul University, Limda, Waghodiya Vadodara-391760 for providing assistance in completing of the study.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **H.T. Hemalatha and Komal Patel (2023). Quality control evaluation of *Tectona grandis* L. using phytochemical screening, TLC fingerprinting profiling and GC-MS analysis. Ann. Phytomed., 12(2):866-873. http://dx.doi.org/10.54085/ap.2023.12.2.102.**