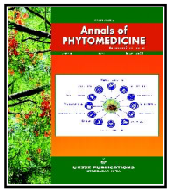
Annals of Phytomedicine 10(1): 215-221, 2021

DOI: http://dx.doi.org/10.21276/ap.2021.10.1.23

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

Phytochemical screening and GC-MS analysis of *Tamarindus indica* L. (Angiosperms: Fabaceae)

Bhawana Sharma, Shiv Charan Sharma and Afroz Alam◆

Department of Bioscience and Biotechnology, Banasthali Vidyapith, Banasthali-304022, Rajasthan, India

Article Info

Article history
Received 13 May 2021
Revised 29 June 2021
Accepted 30 June 2021
Published online 30 June 2021

Keywords
GC-MS
Fabaceae
Medicinal plants
Phytochemical
Tamarindus indica L.

Abstract

Plants have been utilized as preventive and curative medicines as age-old practice. The ethnobotanical data is full of such plants that have medicinal properties and on this basis, the contemporary researchers are now conducting various tests to validate their efficacy. According to the World Health Organization (WHO), around 21,000 plants have been mentioned with therapeutic values worldwide. Among those, *Tamarindus indica* L. (family Fabaceae) is a multipurpose tropical and subtropical tree that is primarily used for its edible fruits and leaves. But now, it is emerging an important plants due to its medicinal uses among many tribes. The present study has been conducted to determine the phytochemical composition and GC-MS analysis of this plant. The separate extracts of *T. indica* leaves and tender shoots were prepared in different solvents, *viz*., methanol, chloroform, and petroleum ether and analyzed comparatively. Since, the aqueous and ethanolic extracts were already studied earlier, therefore not repeated in this study. Finally, the best results were obtained in the methanol extract of the plant parts taken. The outcome of phytochemical screening indicated the presence of phytochemicals like alkaloids, amino acids, phenols, and flavonoids. The methanol extract of leaves was found superior over the extracts prepared with chloroform and petroleum ether.

1. Introduction

Plants are the precious gift of the creator to mankind that provides almost everything needed for the survival of human beings on this planet, including food, fiber, shelter and especially the remedies to treat an array of ailments. Numerous plants are being used as medicines by humans to combat various diseases since their inception on this earth (Alam *et al*., 2019). The database of this ancient knowledge of medicinal plants is huge as local information but the documentation of these is still very scarce. At present time, people are again interested in plant-based medicines consequently, many attempts have been made in the recent past to validate the claims about medicinal plants and then to check the possibility their use in the drug development to provide an easier and economical mode of treatment to the populace (Sofowora *et al*., 2013). With increasing knowledge regarding the valuable flora now many conservation programs and strategies have been developed, especially to stop the habitat loss and pollution. A huge amount of analysis has been done in recent time which is validating the therapeutic value of plants which lies in the bioactive phytochemical constituents that can modify the metabolism and can produce specific physiological effects on the human body to rectify the ailments (Nonita and Mylene, 2010).

India is rich in its floral diversity and contains two hot spots of biodiversity due to a great range of climatic zones that include tropical, subtropical, semi-arid, arid zones, *etc*. Similar to its floral diversity, the tribes of India also have amazing diversity which is region specific. Among the tribes of India, many plants have been reported with their ethnomedicinal importance. Some plants are very specific to their habitats and tribes, while numerous plants are common in their ethnobotanical uses among most of the tribes and frequently used in the treatment of many diseases of humans and their live stocks. These plants have great potential to provide new remedies to the mankind (Roy *et al*., 2020). Therefore, these plants of the most prevalent families in India like Asteraceae and Fabaceae have to be analyzed more and more to get novel phytochemicals. The family Fabaceae is widely distributed in India and especially in the harsh climatic zone such as Rajasthan due its potential to combat biotic and abiotic stresses by producing the secondary metabolites. The phytochemical defense system is very effective in the members of this family to protect them and synthesize very complex molecules with specific stereochemistry with new modes of action (Ugoh *et al*., 2013). These phytochemicals or secondary metabolites are usually large groups of chemicals that are not essential for the plant growth but important in providing defense response against predators through their unpalatably due to particular flavors and aromas (Barbieri *et al*., 2017). They also produce highly reactive oxygen species (ROS) that damage DNA, proteins, lipids, and carbohydrates in the cell during the defense response (MacAlister *et al*., 2018).

In the present study, a very common plant of the family Fabaceae has been taken to find out its phytoconstituents and GC-MS analysis.

2. Description and distribution of *T. indica*

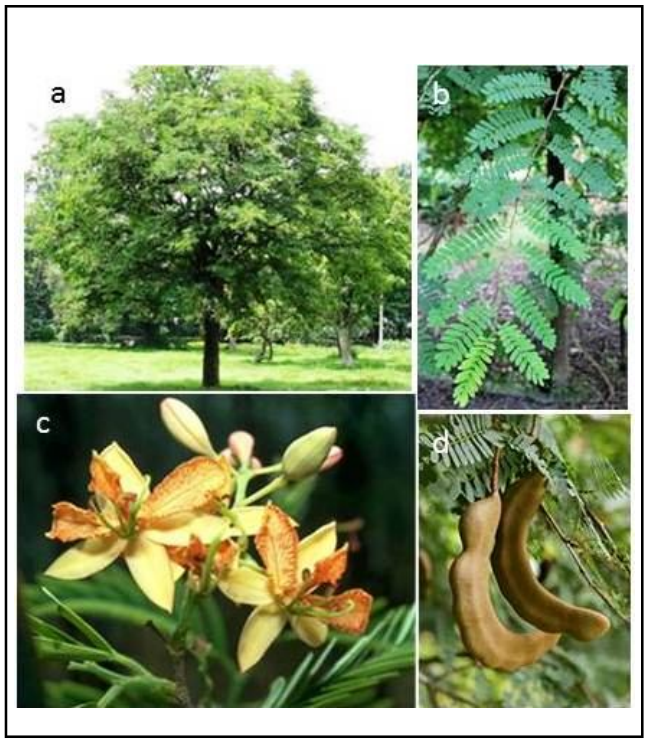
Tamarindus indica L. is known by a variety of names in India, *viz*., Imli (Hindi and Punjabi), Ambala (Gujarati), Chinta (Telugu), Puli (Malayalam), and Tetuli (Assamese).

Corresponding author: Dr. Afroz Alam
Associate Professor, Department of Bioscience and Biotechnology, Banasthali Vidyapith,Vanasthali-304022, Rajasthan, India
E-mail: afrozalamsafvi@gmail.com
Tel.: +91-9415596994

T. indica belongs to the Fabaceae family, the third-largest family of flowering plants and recognized well as an effective and safe medicine to treat many ailments due to lack of essential oils in it (Khanzada *et al*., 2018).

This tree is large, robustly branched and long-lived and well known for its edible fruits and leaves (Plate 1). The flowers of this plant range from pale, yellow, or pinkish and clearly visible. Fruit is an indehiscent pod about 10-18 × 4 cm in size, either linear or coiled. The fruit pulp is primarily utilized for several industrial and domestic purposes (Adeniyi *et al*., 2017). The leaves are thin longitudinal in shape, green in color, alternate, compound with 10-18 pairs of opposite leaflets. The bark is brownish-gray, rough, and scaly. Leaves and flowers are used to make salads, curries, and soups in many countries especially in times of scarcity (Escalona-Arranz *et al*., 2010).

It is native to equatorial Africa; however, many researchers believed that it was evolved in North and South America ranging from Florida to Brazil. It is often cultivated in subtropical countries such as Pakistan, India, Indonesia, Spain and China (Idu and Osadolor, 2020).



Figures 1: (a) *Tamarindus indica* tree, (b) Leaf, (c) Flowers, (d) Fruits.

2.1 Therapeutic uses

T. indica is well known for its therapeutic effects because of the occurrence of several bioactive constituents such as alkaloids, steroids, phenolic compounds, amino acids, flavonoids (Khanzada *et al*., 2008).

The present study was aimed to determine the phytochemicals composition and GC-MS analysis of different extracts of leaves and tender shoots of *T. indica*, separately.

2.2 Phytochemistry

Humans have relied on nature for his basic needs since antiquity and plants are one of the most valuable gifts for them, as they fulfill all of their requirements that are necessary for their good life (Caluwe *et al*., 2010). The chemical composition of amino acids, fatty acids, and minerals in the tamarind plant part has been studied. *T. indica* leaves and stems contain many bioactive constituents such as stilibenes, flavones, coumarins which possess therapeutic application to treat malaria, diabetes, hyperglycemia, *etc*. *T. indica* is a medicinal plant and a phytochemical study on *T. indica* indicates the presence of bioactive constituents like phenolic acids, organic acids, flavonoids, triterpenes, and sterols (Kapoor, 2015).

3. Material and Methods

3.1 Collection of plant material

The plant was identified and plants parts were collected from the Banasthali Vidyapith Campus (Tonk), Rajasthan. The reference specimen was deposited to the Banasthali University Rajasthan India (BURI) Herbarium with taxonomical and ethnobotanical details.

3.2 Extraction

The fresh leaves and tender shoots were washed thoroughly initially with tap water followed by distilled water and then dried at room temperature for 10 days before being pulverized.

Then 20 grams of leaf and shoot powder was transferred in a thimble and extracted with methanol, chloroform, and petroleum ether using the Soxhlet apparatus for 12 h. The extracts were concentrated using a rotatory flash evaporator under reduced pressure at temperatures below 35°C (Zawawi *et al*., 2020). Since, the aqueous and ethanolic extracts were already studied by Bhadoriya *et al*. (2011), therefore not repeated in this study.

Subsequently, the standard estimations were performed to identify and determine the existing secondary metabolites in the extracts. Both qualitative (alkaloids, amino acids, fixed oils and fats, carbohydrates, flavonoids) and quantitative (total phenolic and flavonoid contents) determination were done (Tables 1, 2, 3). The total phenolic content was calculated using the Folin-Ciocalteau method (Vats, 2014) and expressed as gallic acid standards (Mean ± S.D). While, the total flavonoid content was determined using the aluminum chloride colorimetric method (Chandra *et al*., 2014). The stock quercetin solution was prepared by dissolving 5.0 mg quercetin in 1.0 ml methanol and extract was separately mixed with 0.6 ml of 2% aluminum chloride. Both the extract and stock were prepared separately by serial dilutions using methanol. After incubation at room temperature for 60 min, the absorbance of the reaction mixture was measured against blank at 420 nm.

Among all the solvents used for extract, the methanolic extract gave the best result (Figures 1, 2, 3) and hence the GC-MS analysis was done to get the clearer information about the phytoconstituents.

3.3 GC-MS analysis

GC-MS analysis of the methanol extracts of *T. indica* leaves and tender shoots was performed using Thermo Scientific Triple quadruple GC-MS (trace 1300 GC, Tsq 8000 triple quadrupole MS) equipped with TG 5 MS (30 m × 0.25 mm, 0.25 µm) column

(Figure 4; Table 4). Helium was employed as a carrier gas with a flow rate of 1 µl/min and a volume of 1.0 µl. The injector temperature was kept at 25°C while the ion source temperature was fixed at 230°C. The oven temperature was held at 50°C (Mehdi *et al*., 2020).

3.4 Statistical analysis

The outcomes are given as means (n = 3) of triplicates. All obtained data were evaluated by IBM SPSS Statistics 20 software. Three way interactions were executed between the chosen variable. For each and every output variables multiple-comparison Turkey's $p \leq 0.05$ post test was performed to compare the variance of data. All data are offered as means ± standard error.

4. Results

4.1 Qualitative and quantitative analysis

The results of a qualitative phytochemical analysis of separate extracts of *T. indica* leaves and shoots, respectively, exhibited that the methanolic extracts had high levels of carbohydrates, flavonoids, phytosterols, and amino acids. While the extracts prepared in chloroform had the moderate presence of carbohydrates, flavonoids, and the lowest amount of alkaloids was also detected (Table 1). Both leaf and shoot extracts prepared in petroleum ether showed no traces of selected variables. Finally, the best result was obtained in the methanol extract of the plant parts. The total phenols and flavonoid content in leaf and shoot extracts of *T. indica* expressed as gallic acid and quercetin, respectively (Tables 2 and 3). The total phenolic content in extracts prepared in different solvents is shown in these tables.

4.2 GC-MS analysis of methanol leaf extract of *T. Indica*

The GC-MS study of methanolic extract of *T. indica* leaves showed the presence of eight distinct compounds in it. The prevailing compounds present in higher amounts were hexadecanoic acid (palmitic acid) saturated fatty acids, 1, 2-benzene dicarboxylic acid, phthalic acid. While the compound which was present in the least amount, identified as di-terta-butyl-phenol (acts as antioxidants for hydrogen-based products) (Figure 4 and Table 4).

Table 1: Qualitative analysis of *T. indica* leaves and tender shoots in three different solvents

Sl.No.	Phytoconstituents	Leaves extracts			Shoot extracts		
		Methanol	Chloroform	Petroleum ether	Methanol	Chloroform	Petroleum ether
1.	**Alkaloids**						
	Mayer's test	++	+	–	++	+	–
	Wagner test	+ +	–	–	+ +	–	–
2	**Flavonoids**						
	Alkaline reagent test	++ +	+ +	–	+++	–	–
3.	**Phytosterols**						
	Lebermannburchard test	++	+	–	+	+	–
	Salkalowski test	+++	+	–	++	+	–
4	**Amino acids**						
	Ninhydrin	+	–	–	+	–	–
5.	**Carbohydrates**						
	Benedict's test	+++	++	–	++	+	–
+++ (highly present), ++ (moderately present),+ (low present), - (Absent)							

Table 2: Quantitative analysis of *T. indica* fresh leaf extracts in different solvents

Variable	Methanol	Chloroform	Petroleum ether
Total Phenolic Content	41.5 ± 1.36 mg/g GAE	35.3 ± 1.26 mg/g GAE	Nil
Total Flavonoid Content	30.2 ± 0.21 mg/g QE	24.0 ± 0.02 mg/g QE	Nil
Data are the means and standard deviation of the mean for *n* = 3 independent experiments.			

Table 3: Quantitative analysis of *T. indica* shoot extracts in different solvents

Variables	Methanol	Chloroform	Petroleum ether
Total Phenolic content	37.9 ± 1.32 mg/g GAE	32.7 ± 1.26 mg/g GAE	Nil
Total Flavonoid content	23.6± 0.27 mg/g QE	19.2 ± 0.01 mg/g QE	Nil
Data are the means and standard deviation of the mean for *n* = 3 independent experiments			

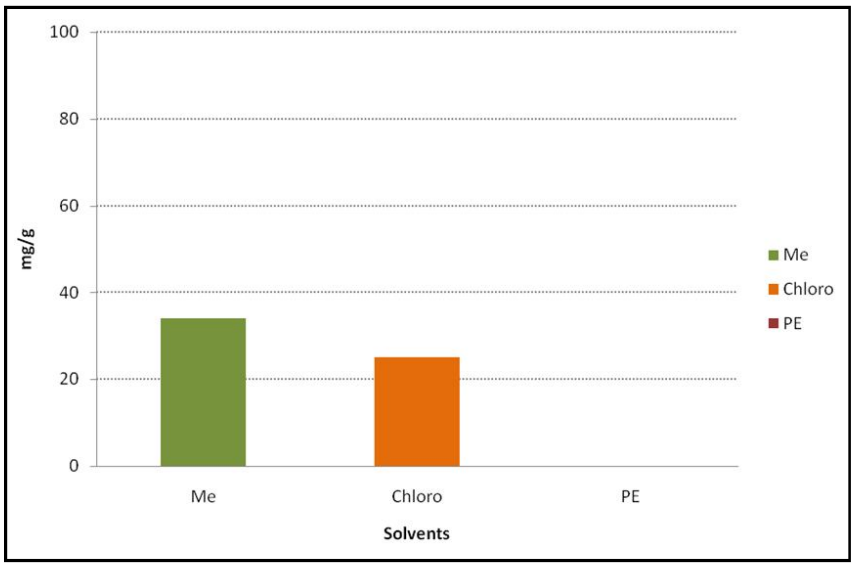


Figure 1: Total phenolic content in shoot extracts.

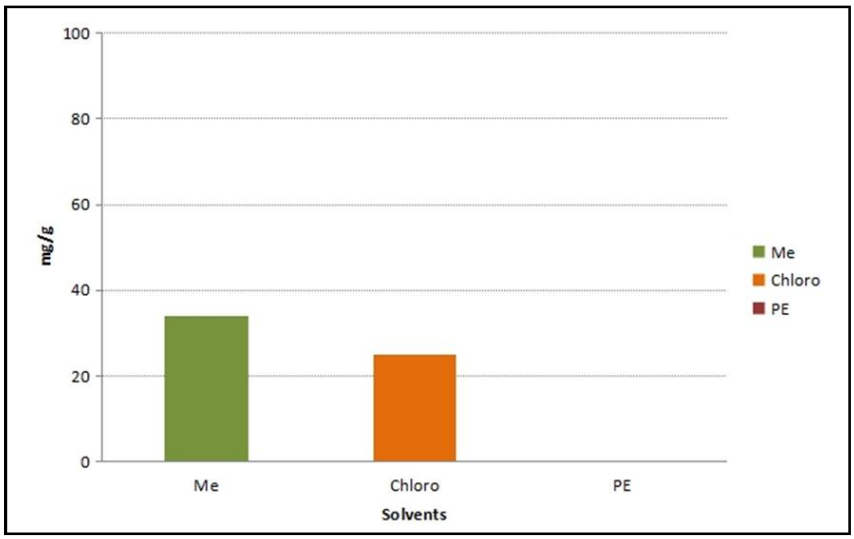


Figure 2: Total flavonoid content in shoot extracts.

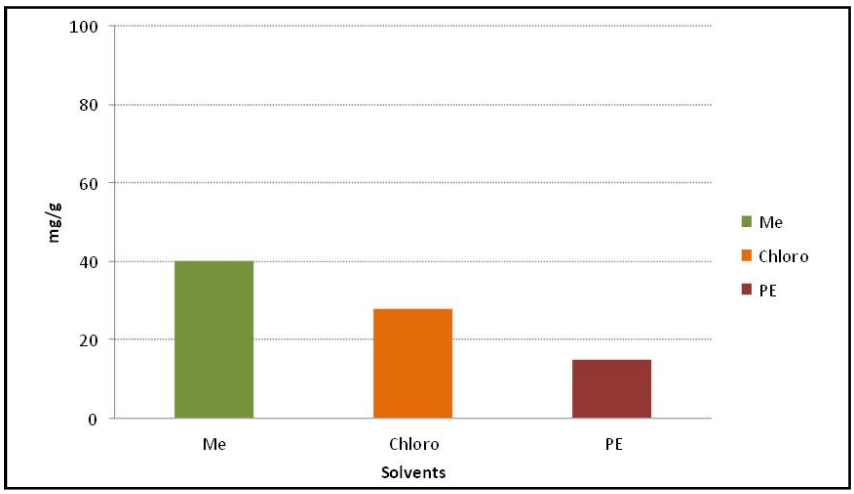


Figure 3: Total flavonoid content in leaf extracts.

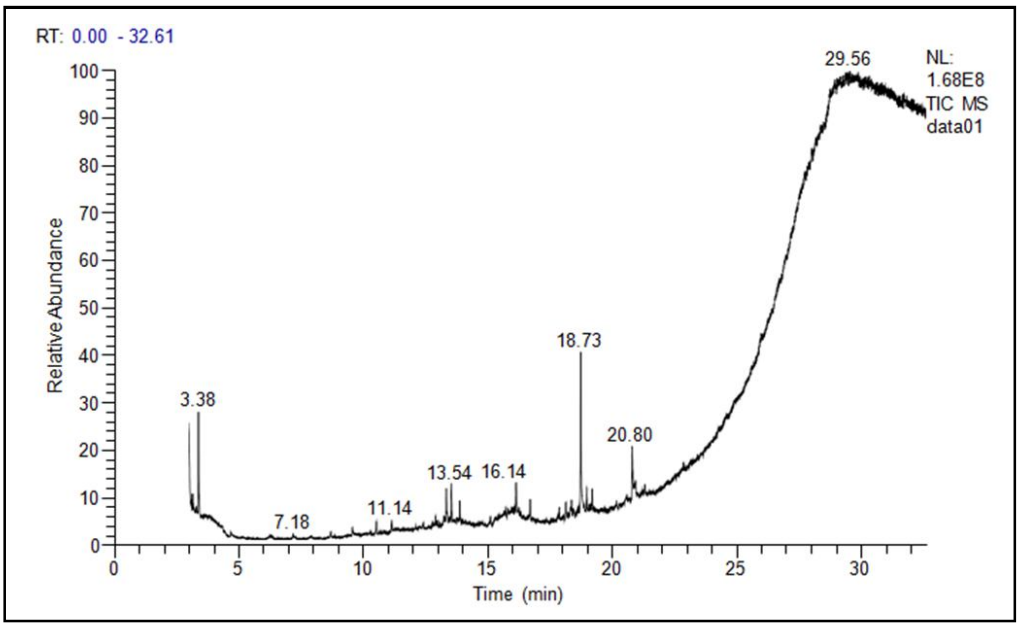


Figure 4: Determination of bioactive compounds through GC-MS.

Table 4: GC-MS analysis of bioactive compounds of methanolic extracts of *T. indica* leaves

Sl.No.	RT	Area	Names of the compound	Molecular formula
1	3.40	14.76	Boron,trihydro(pyridine)-, (T-4)-	C_5H_8BN
2.	13.34	5.48	octadecane,3-ethyl5- (2-ethylbutyl)-octadecane	$C_{26}H_{54}$
			1,1'-[1,3-propanediyl bis(oxy)] bis-heptacosane	$C_{39}H_{8002}$
3.	13.54	6.46	2,4-Diterbutylphenol	$C_{14}H_{220}$
			Phenol,2,6-bis(1,1-dimethylethyl)	$C_{14}H_{220}$
4	16.14	6.13	Heptacosane	$C_{27}H_{56}$
			Octadecane,3-ethyl-5(-2-ethylbutyl)	$C_{26}H_{54}$
			7,7 Diethylheptadecane	$C_{21}H_{44}$
5	18.13	4.27	1,2-Benzenedicarboxylic acid,	
			Butyloctylester	$C_{20}H_{3004}$
			Phthalic acid, isobutyl	
			Octadecyl	$C_{30}H_{50}O$
			Ester	
			Phthalic acid, butyl tetradecyl	
			Ester	$C_{26}H_{420}$
6.	18.73	31.81	Hexadecanoic acid, methyl	
			Ester	$C_{17}H_{34}O_2$
			Pentadecanoic acid,1-methyl-,	
			Methyl ester	$C_{17}H_{34}O_2$
7.	20.80	15.79	Nhexadecanoic acidhexadecanoic acid (palmitic Acid)	$C_{16}H_{32}O_2$
8.	27.49	6.03	Methyl glycocholate,	
			3TMS derivative	$C_{36}H_{69}NOSi_3$

5. Discussion

5.1 Qualitative and quantitative analysis

In leaf extracts, of *T. indica* the maximum TPC was recorded in polar solvent methanol followed by chloroform. However, the TPC of chloroform extract was much lesser than that of methanol extract. In the case of shoot extracts, the maximum phenol content was again observed in methanolic extract followed by chloroform extract. While extract prepared in petroleum ether showed insignificant results due to the polarity of the solvents.

Overall, for both the leaf and shoot extracts, the maximum flavonoids content was observed in methanolic extracts. Hence, it can be said that the methanol plant extracts were the better extracts to be processed further. Moreover, the methanolic extract of leaves showed higher contents of phenol and flavonoid as compared to the methanolic extract of the shoots.

Based on the observation, the leaf extract of leaves prepared in methanol was processed further for GC-MS analysis to get a complete profile of phytochemicals (Table 4).

5.2 GC-MS analysis of methanol leaf extract of *T. Indica*

The bioactive compounds like methyl ester of hexadecanoic acid act as an antifungal agents (Beulah *et al.*, 2018), n-hexdecanoic acid acts as anti-inflammatory, antimicrobial, and antioxidant (Abdullah *et al.*, 2020). 1, 2-Benzenedicarboxylic acid possesses antifouling and antimicrobial properties (Abubacker and Deepalakshmi, 2013).

Consequently, every identified phytochemical of the methanolic extract of *T. indica* leaves has its bioactivity and is significant for therapeutic values which can be used in the future herbal formulations in a safe and cost-effective way.

6. Conclusion

T. indica is a valuable tree that could be evaluated more and more for its medicinal used besides its conventional use as food and fuel tree. Based on the present analysis and previous reports, it can be concluded that *T. indica* is a valued reservoir of useful phyto-constituents like, phytosterols, phenols, flavonoids, and alkaloids. The use of three different solvents also indicates the significance of methanol as a solvent to be used in future studies. The leaves appeared more valuable in terms of phytochemicals as compared to shoots. During this analysis, eight phytoconstituents with bioactivity were also identified, showing their potential as an antimicrobial agent against drug-resistant pathogens. Hence, this common tree would be a special candidate for the search for future pharmaceuticals and nutraceuticals.

Acknowledgements

The author would like to express their gratitude to Professor Ina Shastri, Vice-Chancellor, Banasthali Vidyapith, Rajasthan for her encouragement and assistance. We also thank DST for providing networking support through the FIST program at the Department of Bioscience and Biotechnology, Banasthali Vidyapith as well as the Bioinformatics Center, Banasthali Vidyapith, funded by DBT.

Conflict of interest

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

References

Abdullah, B.M.; Mehdi, M.A.H.; Khan, A.R. and Pathan, J.M. (2020). Gas chromatography-mass spectrometry (GC-MS) Analysis of Ajwain (*Trachyspermum ammi)* seed extract. International Journal of Pharmaceutical Quality Assurance, **11**(2):228-231.

Abubacker, M.N. and Deepalakshmi, T. (2013). *In vitro* antifungal potentials of bioactive compound methyl ester of hexadecanoic acid isolated from *Annona muricata* Linn. (Annonaceae) leaves. Biosciences Biotechnology Research Asia, **10**(2):879-884.

Adeniyi, O.V.; Olaifa, F.E.; Emikpe, B.O. and Ogunbanwo, S.T. (2017). Phytochemical components and antibacterial activity of *Tamarindus indica* L. extracts against some pathogens. Biotechnology Journal International, **17**(2):1-9.

Alam, A.; Kanchan and Iwuala E. (2019). Contemporary medicinal uses of ethnomedicinally important plant: Arjuna (*Terminalia arjuna* (Roxb.) Wight and Arn.). Ann. Phytomed., **8**(1):63-69.

Barbieri, R.; Coppo, E.; Marchese, A.; Daglia, M.; Sobarzo-Sanchez, E.; Nabavi, S.F. and Nabavi, S.M. (2017). Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. Microbiological research, **196**:44-68.

Beulah, G.G.P.; Soris, P.T. and Mohan, V.R. (2018). GC-MS determination of bioactive compounds of *Dendrophthoe falcata* (L.F) Ettingsh: An Epiphytic plant. International Journal of Health Sciences and Research, **8**:261-268.

Bhadoriya, S.S.; Ganeshpurkar, A.; Narwaria, J.; Rai, G. and Jain, A.P. (2011). *Tamarindus indica*: Extent of explored potential. Pharmacogonosy Reviews, **5**(9):73-81. doi:10.4103/0973-7847.79102.

Caluwe, E.De.; Halamova, K. and Damme, P.V. (2010). *Tamarindus indica* L.: A review of traditional uses, phytochemistry, and pharmacology. Afrika Focus, **23**:53-83.

Chandra, S.; Khan, S.; Avula, B.; Lata, H.; Yang, M.H.; Sohly, M.A.; and Khan, I.A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study." Evidence-based Complementary and Alternative Medicine: eCAM vol. 2014 (2014): 253875. doi:10.1155/2014/253875.

Escalona Arranz, J.C.; Peres Roses, R.; Urdaneta Laffita, I.; Camacho-Pozo, M.I.; Rodriguez Amado, J. and Licea Jimenez, I. (2010). Antimicrobial activity of extracts from *Tamarindus indica* L. leaves. Pharmacognosy Magazine, **6**:242-246.

Idu, M.D. and Osadolor. (2020). Phytochemical screening and antimicrobial properties of the ethanolic root extract of *Tamarindus indica* L. Chemistry Research Journal, **5**(3):14-23.

Kapoor, S. (2015). Bioactive and therapeutic potential of legumes: A review. International Journal of Pharmacy and Biological Sciences, **5**:65-74.

Khanzada, S.K.; Shaikh, W, Sofia, S.; Kazi, T.G.; Usmanghani, K.; Kabir, A. and Sheerazi, T.H. (2008). Chemical constituents of *Tamarindus indica* L. medicinal plant in Sindh. Pakistan Journal of Botany, **40**(6):2553-2559.

MacAlister, D.; Muasya, A.M. and Chimphango, S.B.M. (2018). Linking root traits to superior phosphorus uptake and utilization efficiency in three Fabales in the Core Cape Subregion, South Africa. Functional Plant Biology, **45**(7):760-770

Mehdi, M.A.H.; Alwai-AIA-H.M.A.; Thabet, A.Z.A.; Alarabi, F.Y.S.; Omar, G.M.N. and Pradhan V. (2020). Analysis of bioactive chemical compounds of leaves extracts from *Tamarindus indica* using FT-IR and GC-MS spectroscopy. Asian Journal of Research in Biochemistry, **8**(1): 22-34.

Nonita, P.P. and Mylene, M.U. (2010). Antioxidant and cytotoxic activities and phytochemical screening of four Philippine medicinal plants. Journal of Medicinal Plant Research, **4**:407-414.

Nooreen, Z.; Rai, V.K. and Yadav, N.P. (2018). Phytopharmaceuticals: A new class of drug in India. Ann. Phytomed., **7**(1):27-37.

Roy, M.; Roy, S.; Roopali, B.; Ottalwar; Singh, N. and Singh, J. (2020). Phytochemical characterization of different extracts isolated from *Tamarindus Indica* L. in and around Durg district of Chhattisgarh state, India. International Journal of Current Microbiology and Applied Sciences, **9**(3):2819-2824.

Sofowora, A.; Ogunbodede, E. and Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. African Journal of Traditional and Complementary and Alternative Medicines, **10**(5):20-229.

Ugoh; Haruna, S.C. and Mohammed, I. (2013). Phytochemical screening and antibacterial activity of the fruit and leaf extracts of *Tamarindus indica* Linn. Academia, **5**(8):18-27.

Vats, S. (2014). Antioxidant activity of *Clitoria ternatea* L. and *Origanum vulgare* L.: A comparative analysis. Researchers, **6**(11):56-58.

Zawawi, A.; Dabwa, A.H.A. and Hassan, L.S. (2020). Extraction of essential oils from *Tamarind* leaves and seeds using microwave extraction. Journal of Wastes and Biomass Management, **2**(2):49-52.

Citation **Bhawana Sharma, Shiv Charan Sharma and Afroz Alam (2021). Phytochemical screening and GC-MS analysis of *Tamarindus indica* L. (Angiosperms: Fabaceae). Ann. Phytomed., 10(1):215-221. http://dx.doi.org/10.21276/ap.2021.10.1.23**