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Phytochemical profiling of fruit pulp from *Tamarindus indica* L. (red tamarind)

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ABSTRACT

Tamarindus indica L. (red tamarind), belongs to the family Fabaceae, is lesser-known variety of the tamarind tree which is characterized by its unique red color which is due to the presence of anthocyanins. This study methodically analyzes the phytochemical profile of red tamarind fruit pulp to assess its possible uses as a natural source of bioactive compounds and environmentally friendly dye. The screening revealed the presence of secondary metabolites such as flavonoids, phenols, tannins, alkaloids, terpenoids, and anthocyanins. Significantly, the anthocyanins were found to possess antioxidants, anti-inflammatory and hypolipidemic activities. It was found through analysis that phenolic compounds (7.04 ± 0.03 GAE/g), flavonoids (13.02 ± 0.34 quercetin equivalents/g), and anthocyanins (15.06 ± 0.06 mg/g FW) had the highest values in methanol and aqueous extracts. Methanol was greatest in extracting anthocyanins through solvent extraction followed by ethyl acetate. The phytochemical richness of red tamarind highlights its potential applications in the food, pharmaceutical, and cosmetic industries as a sustainable and natural alternative to synthetic additives. Taken together, these results demonstrate the validity of red tamarind as a potential functional food and as a source of bioactive compounds, which underlines the need for further research on this natural product. Future investigations will need to focus on the bioavailability, stability, and practical uses of the bioactive components.

Keywords: *Tamarindus indica, Anthocyanins, Phytochemical screening, Secondary metabolites, Bioactive compounds*

Introduction

Tamarindus indica (red tamarind) is a rare tropical tree belonging to the family Fabaceae. This unique variant of tamarind is distinguished by its rosy-red pigmentation and has diverse applications in the food, pharmaceutical, cosmetic and textile industries due to its rich bioactive compound profile and natural colorant properties [1,2]. India, the largest producer of tamarind, extensively utilizes it in culinary preparations and traditional medicine. Historically, tamarind has been employed for its therapeutic benefits in managing fever, dysentery, jaundice, gonorrhea and gastrointestinal disorders [3-5].

The distinctive red pigment of red tamarind, primarily attributed to anthocyanins, has garnered interest as a natural red food colorant [6]. Anthocyanins, which are polyphenolic compounds responsible for red, blue and purple pigmentation in plants, exhibit antioxidant, anti-inflammatory and antimicrobial properties [7-9]. Despite extensive research on tamarinds regular variants, studies on this red variety remain limited, with its distribution confined to southern India [10].

Recent phytochemical investigations have identified red tamarind as a valuable source of secondary metabolites, including phenols, tannins, flavonoids, alkaloids, terpenoids and anthocyanins [11,12]. Among these, anthocyanins and tannins are particularly abundant in methanol and ethanol extracts, with phenols dominating in methanol extracts and flavonoids in ethanol extracts [13]. The potential of red tamarind as a natural, sustainable source of bioactive compounds for diverse industrial applications.

Anthocyanins, water-soluble polyphenolic flavonoids are for the pigmentation of fruits, flowers, vegetables and have demonstrated significant health benefits, like antioxidant, hypolipidemic and anti-inflammatory applications [14,15].

The use of anthocyanin-rich extracts from red tamarind has also been proposed as an eco-friendly alternative to synthetic colorants in the food and cosmetics industries [16]. Furthermore, red tamarind extracts have shown therapeutic potential, including antimicrobial activity, modulation of the complement system and alleviation of gastrointestinal disorders [17].

The promising applications, research on red tamarind remains sparse. This purpose of this work is to address this gap by analyzing the phytochemical composition of red tamarind, emphasizing its potential as a source of natural colorants and bioactive compounds with applications in the food, pharmaceutical, and cosmetic industries. By exploring its unique phytochemical profile, this study underscores the potential of *T. indica* for natural product development and sustainable industrial applications.

Materials and Methods

Collection and Authentication

The leaves of *Tamarindus indica* L. (Red tamarind) were collected from the Nalgonda district, Telangana State, India. The plant was authenticated by the Department of Botany, UCS, Osmania University. The collected leaves were cleaned thoroughly to remove unwanted materials, washed, shade-dried and then pulverized into a fine powder for further analysis.

Sequential Extraction using a Soxhlet Apparatus

The extracts of *T. indica L.* (Red tamarind), fresh fruit pulp has been obtained and thoroughly disinfected with water running through it to eliminate any soil or contamination.

The fruit pulp was extracted using an assortment of solvents, includes petroleum ether, chloroform, ethyl acetate, methanol, aqueous extracts. Powder of dried fruit pulp was prepared through the use of a grinder and then sieved to ensure that powder had a consistent size, fruit pulp powder was successively extracted through the Soxhlet apparatus with the help of petroleum ether at a temperature of 60° C chloroform at 61° C, ethyl acetate at 77 ° C, methanol at 65° C and aqueous extract at100° C. Extraction temperature have been optimized to correspond with its boiling point of solvent, which allows more rapid cycling of fresh solvent. Each solvent has been allocated an operating time of 5 hours for hot successive extraction. The extracts have been filtered through Whatman filter paper to obtain an extract without any of particles. The remaining substance was extracted two more times using a solvent and then filtered. The extracts undergo filtration and subsequent evaporation using a rotary evaporator to accomplish the volume that was needed. The extracted sample was placed in a desiccator to analyze qualitative, quantitative phytochemicals [18].

Determination of Percentage Yield

The dried extracts obtained from each solvent were weighed, and the yield was calculated relative to the air-dried weight of the plant material.

$$\text{Percentage Yield} = \frac{\text{Weight of the crude(mg/g)}}{\text{Weight of the plant material}} \times 100$$

Qualitative phytochemical examination of *Pluchea ovalis*

A screening procedure was conducted on the leaf extract to determine the presence of any secondary metabolites. A variety of tests were conducted using standard procedures to assess the presence or absence of many different bioactive substances including alkaloids, flavonoids, saponins, steroids and terpenoids, phenolic compounds, tannins, glycosides, cardiac glycosides, coumarins, phytosterols, lecuco anthocyanins, resins, fixed oils, anthraquinones, and quinones. The tests were undertaken qualitatively to ascertain the presence or absence of each component [19-30].

Phytochemical analysis Methods

Phytochemical analysis of the test sample was performed following established protocols [18-20], to identify various secondary metabolites

Detection of Alkaloids: The extracts were dissolved in dilute hydrochloric acid and filtered.

Mayer's Test: To 2 mL of the filtrate, 2–3 drops of Mayer's reagent were added along the side of the test tube. The formation of a creamy white precipitate indicated the presence of alkaloids.

Test for Flavonoids: To the test solution, magnesium turnings and a few drops of concentrated hydrochloric acid were added, followed by boiling for five minutes. The appearance of a red color confirmed the presence of flavonoids.

Test for Saponins: About 0.5 g of the powdered sample was boiled gently with 20 mL of water for 2 minutes, filtered while hot, and allowed to cool. Five milliliters of the filtrate were diluted with water and shaken vigorously. The formation of stable froth indicated the presence of saponins.

Detection of Steroids and Terpenoids

Liebermann-Burchardt Test: To 1 mL of the extract, 1 mL of chloroform, 2–3 mL of acetic anhydride, and 1–2 drops of concentrated sulfuric acid were added. The appearance of a dark green color indicated the presence of steroids.

Test for Phenolic Compounds: A small quantity of the powdered sample was treated with specific reagents, and the resulting color changes confirmed the presence of phenolic compounds.

a. 5% Ferric chloride solution- Deep bluish-black colour.
b. Lead acetate solution-white precipitate.

Test for Tannins: A small quantity of the powdered sample was extracted with water. To the aqueous extract, a few drops of ferric chloride solution were added. The appearance of a bluish-black color indicated the presence of tannins.

Detection of Glycosides: The test solution was prepared by dissolving the extract in alcohol or by boiling it with a hydro-alcoholic solution.

a) Baljet's Test: The test solution was treated with 2% sodium picrate. The appearance of a yellow to orange color indicated the presence of glycosides.

b) Legal's Test: The test solution was treated with pyridine and made alkaline. The addition of 2% sodium nitroprusside resulted in a pink to red color, confirming the presence of glycosides.

c) Keller-Killiani Test: The extract (100 mg) was dissolved in 1 mL of glacial acetic acid containing one drop of ferric chloride solution. This mixture was then underlayered with 1 mL of concentrated sulfuric acid. The formation of a brown ring at the interface indicated the presence of glycosides.

Detection of Cardio glycosides: The analysis of cardiac glycosides for extracts was performed with the Keller Killiani test. 1 mL of acetic acid and 2 drops of ferric chloride were added to 2 mL of extract, then 2 mL of sulfuric acid (concentrated) was added and the color change was observed. The reddish-brown color formation was deemed to be a positive test for cardiac glycosides.

Detection of Coumarins: Three milliliters of 10% sodium hydroxide (NaOH) were added to 2 mL of the aqueous extract. The appearance of a yellow coloration indicated the presence of coumarins.

Test for Phytosterols: A few drops of concentrated sulfuric acid were added to the extract solution, shaken well, and allowed to stand. The formation of a red color in the lower chloroform layer confirmed the presence of phytosterols.

Detection of Quinones: Dilute sodium hydroxide (NaOH) was added to 1 mL of the crude extract. The appearance of a blue-green or red coloration indicated the presence of quinones.

Detection of Resins: To 2 mL of the extract, 5–10 drops of acetic anhydride were added and dissolved by gentle heating. Subsequently, 0.5 mL of sulfuric acid was added. The formation of a bright purple color indicated the presence of resins.

Detection of leucoanthocyanins
1ml of crude extract was added to 1ml of isoamyl alcohol. Upper layer turned red in color indicating the presence of leucoanthocyanins.

Detection of Anthraquinones: One gram of the powdered plant material was mixed with chloroform and shaken for 5 minutes. The contents were filtered, and 5 mL of ammonia solution was added to the filtrate and gently agitated. The appearance of a bright pink color in the upper aqueous layer indicated the presence of anthraquinones.

Detection of Fixed Oils: A small quantity of the extract was pressed between two filter papers. The presence of an oil stain on the paper confirmed the presence of fixed oils.

Detection of Gums and Mucilages: One hundred milligrams of the extract were dissolved in 10 mL of distilled water, followed by the addition of 25 mL of absolute alcohol with constant stirring. The formation of a white or cloudy precipitate indicated the presence of gums and mucilages.

Preparation of Extracts
The shade-dried leaves were finely powdered using a mechanical blender. The powdered material was subjected to Soxhlet extraction with three different solvents: petroleum ether, ethyl acetate, and methanol. The extraction process was conducted at temperatures ranging from 60 to 65°C for 5 to 8 hours. Following extraction, the solvent mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure at 40°C using a rotary evaporator. The resulting concentrated extracts were dried, transferred to sterile vials, and stored at 4°C for subsequent experimental analyses.

Estimation of Total Phenolic Content (TPC)
The total phenolic content of the red tamarind medicinal plant was quantified spectrophotometrically using the Folin-Ciocalteu method. A standard curve was generated using gallic acid, and the phenolic content of the plant extracts was expressed as gallic acid equivalents (GAE) in mg per gram of dry weight. The analysis was performed for the petroleum ether, ethyl acetate, and methanol extracts. Each experiment was conducted in triplicate to ensure reproducibility and accuracy.

Estimation of Total Flavonoid Content
The $AlCl_3$ colorimetric method described [32] was employed to estimate the total flavonoid content. A 20 µl aliquot of the plant extract was mixed with a 2% solution of $AlCl_3 \cdot 6H_2O$, followed by vigorous shaking. The final volume of the reaction mixture was adjusted to 10 ml using double-distilled water. The mixture was incubated for 10 minutes, and the absorbance measured at 440 nm using a UV-Vis spectrophotometer. Quercetin was used for construct of calibration curve, results expressed as quercetin equivalents of mg/gram of dry material. The analyses were carried out in triplicates to ensure precision. Correlation coefficients (R2) between the results of reactive species scavenging assays (RSA) and the contents of phenolic and flavonoid compounds were calculated using MS office Excel software of Windows.

Estimation of Total Anthocyanin Content (TAC)
The anthocyanin content was quantified using pH differential method [33]. A Hitachi U-2000 UV spectrophotometer (Hitachi, Nagoya, Japan) calibrated at 510 and 700 nm was used. Cyanidin3-glucoside (cyd-3-glc) was calculated, using an extinction coefficient of 26,000 L cm-1mg-1 (Molecular Weight of 448.8).

Results

An analysis of phytochemical compounds in *T. indica* (red tamarind) required petroleum ether and chloroform and ethyl acetate and methanol and aqueous solution extracts. The comprehensive screening analysis revealed various bioactive substances dispersed through different extracts and this variability shows both plant phytochemical richness and solvent dependence of chemical compounds (Table 1 and Figure 1).

The testing for alkaloids in both methanol and aqueous extracts resulted in positive findings because these solvents contained significant amounts of such compounds. The alkaloid delectables were absent in petroleum ether, chloroform and ethyl acetate extract samples. The presence of flavonoids was powerful in all four extracts and fluids including chlorine-form, ethyl acetate and methanol with aqueous solution. The analysis resulted in negative detection of flavonoids in the petroleum ether extract. Although saponins were found in methanol and aqueous extracts they were the only solvent systems where these compounds were detected. The petroleum ether and chloroform and ethyl acetate extracts failed to display any saponins in their chemical composition. The ethyl acetate extract contained the only identifiable quantities of steroids and terpenoids since it reacted positively to detection tests. Every extract except the petroleum ether solution failed to detect these compounds. All phenolic compounds found in chloroform extract and methanol extract as well as aqueous extract yielded prominent positive detection results. The phenolic compounds could not be detected in extracts obtained through petroleum ether and ethyl acetate. All three extracts of chloroform, methanol and water obtained positive results for tannins detection. The tannin content could not be measured in the petroleum ether extract and the ethyl acetate extract. Tests confirmed that glycosides exist in all tested solutions including chloroform, ethyl acetate, methanol and aqueous extracts. The positive reactions from chloroform, methanol and aqueous extracts were strong but ethyl acetate extract displayed a moderate level of positivity. The petroleum ether extract produced no reaction for glycosides. The chemical analysis results revealed coumarins only in ethyl acetate along with methanol and aqueous solutions where ethyl acetate yielded moderate results and methanol and aqueous solutions produced strong reactions. Positive reactions for detectable coumarin compounds were not identified either in petroleum ether or chloroform extracts. A positive reaction occurred in both chloroform and ethyl acetate extracts as they contained phytosterols. The phytosterol content evaluation of petroleum ether, methanol and aqueous extracts showed negative results. A positive reaction for quinones came from chloroform-based and methanol-based and aqueous-based extracts. Strong positive reactions were observed for coumarins when testing the methanol and aqueous extracts although chloroform extract produced a weak positive reaction. Research findings demonstrated that extract solutions made with petroleum ether and ethyl acetate did not contain any detectable quinone

compounds. The study identified the presence of resins in all three tested extracts including chloroform, methanol and aqueous while the reaction was strong in each case. Tests for detecting resins produced no identification from the petroleum ether and ethyl acetate extracts. The evaluations of cardiac glycosides on ethyl acetate and methanol and aqueous extracts revealed moderate positive test results. The petroleum ether and chloroform extracts produced no detectable results for anthocyanins and resins and cardiac glycosides. The analysis showed that both methanol and aqueous extracts contained Anthocyanins because they both displayed positive reactions. The petroleum ether along with chloroform and ethyl acetate extracts failed to detect anthocyanins within them. All anthraquinones identified from the study appeared only in the ethyl acetate extract and displayed a strong positive reaction result. The remaining tested solutions from various extracts showed no signs of these compounds.

Petroleum ether and chloroform extracts contained fixed oils as revealed by their strong affirmative results for these compounds. The fixed oil content remained undetected across all samples using extracts of ethyl acetate and methanol and aqueous solution.

The investigation has revealed that various extraction solvents achieve maximum extraction of particular phytochemicals. Methanol and aqueous extracts contained multiple bioactive compounds such as alkaloids, flavonoids, saponins, phenols and tannins together with glycosides, coumarins, quinones, resins and anthocyanins. The extracting power of fixed oils through petroleum ether and chloroform surpassed the extraction ability achieved by petroleum ether and chloroform. The extract of ethyl acetate displayed high effectiveness in separating steroids and terpenoids along with anthraquinones.

The extraction process yields 8.0% petroleum ether extract and 20.10% chloroform extract and 26.41% ethyl acetate extract and 33.22% methanol extract and 44.14% aqueous extract.

Table 1. Preliminary phytochemical analysis of T. indica (red tamarind)

S. No	Phyto. Name	Pet. ether	Chloroform	Ethyl acetate	Methanol	Aqueous
1.	Alkaloids Mayer's	-	-	-	+++	+++
2.	Flavonoids Lead acetate test	-	+++	+++	+++	+++
3.	Saponins Foam test	-	-	-	+++	+++
4.	Steroids & Terpenoids Salkowki's test	-	-	+++	-	-
5.	Phenols Ferric chloride test	-	+++	-	+++	+++
6.	Tannins Gelatin test	-	+++	-	+++	+++
7.	Glycosides Borntrager's Test (Modified)	-	+++	++	+++	+++
8.	Coumarins NaOH test	-	++	++	+++	+++
9.	Phytosterols Salkowski's test	-	+++	+++	-	-
10.	Quinones Precipitate test	-	+	-	+++	+++
11.	Resins Acetic-anhydride test	-	+++	-	+++	+++
12.	Cardiac Glycosides Kellar – Kiliani	-	-	++	++	++
13.	Anthocyanins Iso-amyl-alcohol test	-	-	-	+++	+++
14.	Anthraquinones Borntrager's	-	-	+++	-	-
15.	Fixed oils Spot test/ Stain test	+++	+++	-	-	-

(-ve = Absent; +ve= Present)

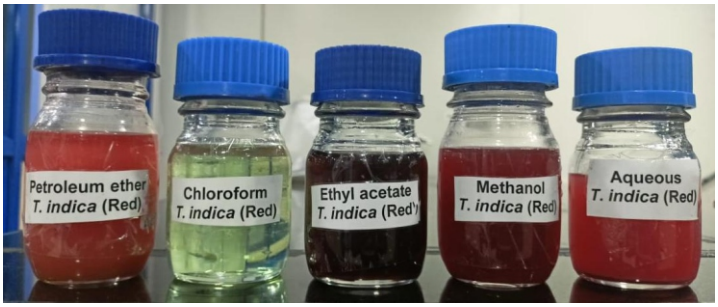

Fig:1. Phytochemical extractions of T. indica (red tamarind)

Quantitative analysis of *T. indica* (red tamarind)

The experimental outcomes reveal variations in phytochemical concentrations across different solvent extracts, including petroleum ether, chloroform, ethyl acetate, methanol, and aqueous extracts.

Total Phenolic Content (GAE/g)

The aqueous extract overtook other tested solvents by producing the highest phenolic content measurement of 7.04 ± 0.03 GAE/g. The phenolic amount in methanol reached (6.84 ± 0.36 GAE/g).

Solvents ethyl acetate, petroleum ether and chloroform produced results for phenolic content measurements which were similar to one another with values at (4.58 ± 0.44, 3.08 ± 0.14, and 2.78 ± 0.34 GAE/g). Methanol demonstrates effective extraction potential for phenolic compounds but the aqueous extraction method remains superior based on the results shown in Table 2 and Fig. 2.

Total Flavonoid Content (Quercetin Equivalents/g)

The aqueous extract produced the highest total flavonoid level of 13.02 ± 0.34 quercetin equivalents/g which corresponded to the phenolic content measurements. Of the tested solvents methanol and petroleum ether showed the second and third highest flavonoid concentrations at 12.10 ± 0.74 quercetin equivalents/g and 4.13 ± 0.25 quercetin equivalents/g respectively. The flavonoid amounts in extracts from chloroform (3.04 ± 0.18) and ethyl acetate (3.84 ± 0.48) remained lower compared to other extracts. Aqueous extraction proved most effective for producing maximum flavonoid yields according to the data in Table 2 and Fig. 2.

Anthocyanin Content (Cyanidin-3-Glucoside mg/g FW)

The aqueous extraction method produced extract with the most abundant anthocyanin content at 15.06 ± 0.06 mg/g FW followed by methanol at 14.07 ± 0.26 mg/g FW then by ethyl acetate at 9.31 ± 0.26 mg/g FW. The anthocyanin content measured at 5.64 ± 0.16 mg/g FW in chloroform extract matched closely to petroleum ether extract at 5.22 ± 0.33 mg/g FW. The results indicate aqueous extraction yields the highest amount of anthocyanin in produced extracts according to measurements presented in Table 2 and Figure 2.

Table 2: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of red tamarind plant extracts

Solvents	(Total phenolic) (GAE)/g	(Total flavonoid) quercetin equivalents/g	Anthocyanin (Cyanidin3-glucoside mg/g FW)
Pet. ether	3.08±0.14	4.13±0.25	5.22±0.33
Chloroform	2.78±0.34	3.04±0.18	5.64±0.16
Ethyl acetate	4.58±0.44	3.84±0.48	9.31±0.26
Methanol	6.84±0.36	12.10±0.74	14.07 ± 0.26
Aqueous	7.04±0.03	13.02±0.34	15.06 ± 0.06

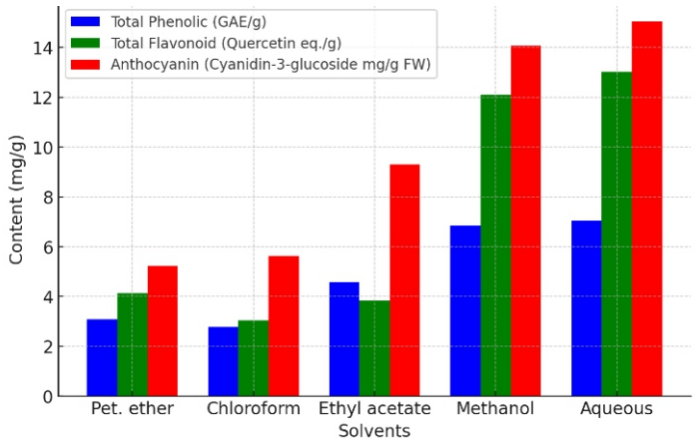

Fig 2: Total Phenolic Content, Total Flavonoid Content and Total Anthocyanin content of red tamarind plant extracts

Studies show methanol to be the suitable and efficient solvent for extracting plant compounds including phenolic and flavonoid and anthocyanin components which leads to its evaluation as a significant solvent for phytochemical research.

Discussion

Phytochemical analysis of *T. indicia* (red tamarind) showed that bioactive compounds have different solubility levels based on the solvent selection for targeted extractions. Methanol proved to be the most potent solvent for extracting polar plant compounds including alkaloids together with flavonoids, saponins, phenols, tannins, glycosides, coumarins, quinones and anthocyanins. The research results from [34] supported earlier findings which showed polar solvents work best for dye-yielding plants. Research shows that phenolic and flavonoid compounds selectively dissolve in methanol extraction solutions in agreement with [31] who demonstrated their influence on sensory qualities and antioxidant properties of foods. The research findings confirm results presented in [35] which states anthocyanins function as water-soluble pigments that polar solvents can extract efficiently.

The anthocyanins found in this study demonstrated the highest phytochemical concentration level within methanol extract at 14.07 ± 0.26 mg/g FW and ethyl acetate extract contained 9.31 ± 0.26 mg/g FW of anthocyanins while chloroform extract had 5.64±0.16 mg/g FW and petroleum ether extract contained 5.22 ± 0.33 mg/g FW of anthocyanins. Polar solvents successfully extract bioactive pigments as supported by findings from [36] and [37] which showed that polar solvents produce maximum pigment yield. Research indicates that anthocyanins both determine plant pigmentation patterns and exhibit antioxidant properties and anti-inflammatory functions and anticancer abilities [38]. Ethyl acetate extracts contained only steroids and terpenoids according to studies [39] whom showed mid-polarity solvents work best for extracting such compounds. The absence of saponins and alkaloids in petroleum ether tests confirms that these compounds rely on specific solvent conditions based on the research in [40].

The analyses of petroleum ether and chloroform extracts showed the presence of fixed oils which confirms that non-polar solvents work effectively for lipid-based compounds according to [41-42]. The analysis of ethyl acetate extracts revealed strong anthraquinone content which could not be detected in either polar or non-polar solvents thus demonstrating the selective solubility patterns of plant compounds.

Conclusion

Through this study scientists found that *Tamarindus indica* L. (red tamarind) possesses multiple phytochemical elements which solubilize differently based on different extraction solvents. Methanol proved best for extracting polar compounds including anthocyanins and phenols as well as flavonoids and saponins from the plant but ethyl acetate efficiently extracted both polar and non-polar compounds such as steroids and terpenoids. Petroleum ether extracted non-polar fixed oils together with other non-polar compounds from the sample. *T. indica* bioactive potential now has a foundation established through the understanding of selecting proper solvents for

efficient phytochemical extraction. The plant's potential as a natural antioxidant source emerges through the identification of anthocyanins which strengthens its application in functional food development together with pharmaceutical usage.

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Declarations
Conflict of interest
The authors declare that there are no conflicts of interest.

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