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Influence of Seasonal Variation and Solvent Polarity on Bioactive Potential of Selected *Terminalia Species*

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ABSTRACT

This study evaluates the effect of seasonal variation and solvent polarity of Terminalia arjuna and Terminalia catappa leaf extracts on antioxidant and antidiabetic potential. The leaf samples were collected across three seasons and extracted with ethanol and acetone solvents. Antioxidant activity was assessed using DPPH free radical scavenging assay and ABTS radical cation scavenging assay, while antidiabetic potential was evaluated through α-amylase and α-glucosidase inhibition. In the DPPH assay, T. arjuna ethanol showed lower IC_{50} values of 0.225–0.280 mg/ml and acetone of 0.229–0.303 mg/ml, compared to ascorbic acid (0.1749 mg/ml). T. catappa ethanol exhibited higher IC_{50} values of 0.514–0.719 mg/ml, and acetone 0.514–0.906 mg/ml. In the ABTS assay, T. arjuna ethanol showed lower IC_{50} values of 2.096–2.558 mg/ml and acetone 2.202–2.743 mg/ml, comparable to Trolox (2.088 mg/ml), while T. catappa ethanol ranged from 3.875–5.293 mg/ml and acetone 3.881–5.413 mg/ml. For antidiabetic activity, T. arjuna ethanol exhibited IC_{50} values of 2.309–2.369 mg/ml and acetone 2.310–2.371 mg/ml for α-amylase, and 2.017–2.124 mg/ml (ethanol) and 2.063–2.136 mg/ml (acetone) for α-glucosidase, compared to acarbose (1.310 mg/ml and 1.127 mg/ml). T. catappa ethanol showed comparatively higher IC_{50} values, ranging from 2.448–2.466 mg/ml and acetone 2.457–2.478 mg/ml for α-amylase, and ethanol 2.194–2.207 mg/ml and acetone 2.197–2.209 mg/ml for α-glucosidase. GC–MS analysis of both species revealed bioactive compounds, including fatty acids (n-hexadecenoic acid, linoleic acid), phytosterols (γ-sitosterol, stigmasterol), and terpenoids (phytol), which contribute to free radical scavenging and enzyme-inhibitory activities. These findings highlight the significant influence of seasonal variation and solvent polarity on the therapeutic potential of Terminalia species.

Keywords: *Terminalia arjuna, Terminalia catappa, α-amylase inhibition, α-glucosidase inhibition, DPPH assay, ABTS assay, antidiabetic, antioxidant, GC-MS analysis.*

Introduction

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) generation and antioxidant defense mechanisms, plays a crucial role in the pathogenesis of numerous chronic disorders, including diabetes mellitus, cardiovascular diseases, neurodegenerative conditions, and cancer [23;41] Excessive production of free radicals such as superoxide anions, hydroxyl radicals, and peroxyl radicals can induce lipid peroxidation, protein oxidation, and DNA damage, ultimately leading to cellular dysfunction [23].

Consequently, the search for natural antioxidants capable of scavenging free radicals and mitigating oxidative stress has gained considerable scientific attention [49]. Medicinal plants are recognized as rich sources of bioactive secondary metabolites, particularly phenolic compounds, flavonoids, tannins, and terpenoids, which exhibit potent antioxidant properties [46].

Among these, species belonging to the genus Terminalia (Family: Combretaceae) have been extensively used in traditional systems of medicine for their therapeutic benefits. Terminalia arjuna and Terminalia catappa are especially valued for their cardioprotective, antidiabetic, anti-inflammatory, and hepatoprotective activities [3;16].

These pharmacological properties are largely attributed to their diverse phytochemical profiles, including polyphenols, glycosides, triterpenoids, and fatty acid derivatives.

Although several studies have reported the antioxidant potential of *Terminalia species*, limited information is available regarding the influence of seasonal variation and extraction solvent on their radical scavenging efficiency. Environmental factors such as temperature, light intensity, and rainfall can significantly affect the biosynthesis and accumulation of secondary metabolites in plants [54]. Furthermore, the extraction efficiency of antioxidant compounds is strongly dependent on solvent polarity, which determines the yield and composition of phytoconstituent [6].

The present study was therefore designed to comparatively evaluate the antioxidant potential of acetone and ethanol leaf extracts of *T. arjuna* and *T. catappa* collected across three seasonal periods (March–June, July–October, and November–February). Antioxidant activity was assessed using two widely accepted *in vitro* assays, namely DPPH and ABTS radical scavenging methods [10;31], to ensure methodological reliability and cross-validation of results.

Materials and Methods:

Collection of Plant Material: Fresh leaves of *Terminalia arjuna*, and *T. catappa* (Combretaceae family), were collected from Mahim Nature Park, Mumbai. The plant specimen was authenticated at the Blatter Herbarium, Xavier's College, Mumbai, and a voucher specimen (NYD-2744 of N.Y. Das) and (PD-2812 of Divakar) was submitted for reference.

Plant Extract Preparation: The leaves were washed, air-dried and pulverized into a fine powder. The powdered material was subjected to successive extractions using different organic solvents: ethanol (95%, 90% and 75%), acetone, hexane, and ethyl acetate by maceration for 72 hours. The extractive values were calculated for each solvent. Among the tested solvents, the maximum extractive value was obtained with ethanol (95%) and followed by acetone, and both organic solvents were selected for further analysis. For large-scale extraction, the powdered leaf material was macerated with 95% ethanol and acetone in a shaker with continuous agitation for 72 hours. The extract was concentrated using a rotary evaporator to obtain crude ethanol (TAE, TCE) and acetone extract (TAA, TCA). This extract was subsequently used for biological activity assays (ABTS and DPPH).

Extractive Value (%): Weight of residue - Weight of sample / Weight of sample * 100

Antidiabetic assessment

α-Amylase Inhibition: The α-amylase inhibitory activity of the extracts was evaluated following the method with slight modifications, which is widely used for screening plant-derived antidiabetic agents [32;36]. Briefly, varying concentrations of the extracts (0.1–3.0 mg/mL) were prepared, and 500 µl of each concentration was mixed with 500 µl of 0.02 M sodium phosphate buffer (pH 7.0) containing α-amylase enzyme solution. The reaction mixture was incubated at 25°C for 10 minutes to allow enzyme–extract interaction.

After pre-incubation, 500 µl of 1% soluble starch solution prepared in 0.02 M sodium phosphate buffer (pH 7.0) was added to each test tube at different time intervals to initiate the enzymatic reaction. The reaction mixtures were further incubated at 25 °C for 10 minutes. The enzymatic reaction was terminated by the addition of 1.0 mL of dinitrosalicylic acid (DNS) color reagent, followed by incubation in a boiling water bath for 5 minutes to develop the colored complex. The tubes were then cooled to room temperature.

Subsequently, the reaction mixtures were diluted with 15 ml of distilled water, and the absorbance was measured at 504 nm using a UV–Vis double beam spectrophotometer (LMSP-UV1900). A blank was prepared containing a 500 µl buffer. Acarbose, a standard antidiabetic drug known as α-amylase inhibitor, was used as a positive control at the same concentrations as the plant extracts. All experiments were performed in triplicate to ensure reproducibility of the results.

The percentage inhibition of α-amylase activity was calculated using the following formula:

% of inhibition = (Acontrol – Asample) / Acontrol × 100

α-Glucosidase Inhibition:

The α-glucosidase inhibitory activity of the plant extracts was evaluated following the method described by [14] with slight modifications, which is commonly used for assessing the antidiabetic potential of plant-derived compounds [32;5].

Briefly, different concentrations of the extract (0.1–0.3 mg/mL) were prepared in distilled water. A volume of 500 µl of each extract concentration was mixed with 500 µl of

0.02 M sodium phosphate buffer (pH 7.0) containing α-glucosidase enzyme solution, and the reaction mixture was incubated at 25 °C for 10 minutes to allow enzyme–extract interaction.

Following the pre-incubation period, 500 µl of p-Nitrophenyl-β-D-glucopyranoside substrate prepared in 0.02 M sodium phosphate buffer (pH 7.0) was added to each tube at different time intervals to initiate the enzymatic reaction. The reaction mixtures were then incubated at 25°C for 5 minutes. The enzymatic reaction was terminated by the addition of 1.0 mL of dinitrosalicylic acid (DNS) color reagent, followed by incubation in a boiling water bath for 5 minutes to develop the colored complex. The tubes were subsequently cooled to room temperature.

The reaction mixtures were diluted with 15 mL of distilled water, and the absorbance was measured at 405 nm using a UV–Vis double beam spectrophotometer (LMSP-UV1900). A blank was prepared containing 500 µl of sodium phosphate buffer instead of the plant extract. Acarbose, a well-known α-glucosidase inhibitor used clinically for diabetes management, was used as the positive control at the same concentrations as the extracts. All experiments were carried out in triplicate to ensure accuracy and reproducibility.

The percentage inhibition of α-glucosidase activity was calculated using the formula mentioned for α-amylase inhibition.

Antioxidant Activity

ABTS (2,2'-Azino-bis- (3-ethyl) benzothiazoline)-6-sulfonic acid) radical cation scavenging assay: The antioxidant activity of the leaf extracts was evaluated using the ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging assay, following the method described by [45] with slight modifications, which is widely used for assessing the antioxidant potential of plant extracts [43;52].

The $ABTS^+$ radical cation was generated by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate in a ratio of 1:1 (v/v) and allowing the mixture to stand in the dark at room temperature for 12 hours to ensure complete formation of the radical cation. The resulting $ABTS^+$ stock solution was subsequently diluted with ethanol (1:10, v/v) to obtain the working solution suitable for the assay.

For the antioxidant assay, 1000 µl of the leaf extract was mixed with the $ABTS^+$ working solution and incubated at room temperature for 10 minutes to allow the reaction between antioxidants present in the extract and the ABTS radicals. The decrease in absorbance, indicating radical scavenging activity, was measured at 734 nm using a UV–Vis double beam spectrophotometer (LMSP-UV1900).

Trolox, a water-soluble vitamin E analogue, was used as the standard antioxidant reference compound for comparison. All experiments were performed in triplicate to ensure reproducibility and accuracy of the results.

The percentage of ABTS radical scavenging activity was calculated using the following formula:

% of radical scavenging activity: Acontrol – Asample / Acontrol x 100

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay

The free radical scavenging activity of the leaf extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, following the method described by [10] with slight modifications. This assay is widely used for assessing the antioxidant capacity of plant extracts due to its simplicity and reliability [43;52].

Briefly, a 0.1 mM DPPH solution was prepared in methanol, and 1000 µL of the extract at different concentrations was mixed with the DPPH solution. The reaction mixture was then incubated in the dark at room temperature for 30 minutes to allow the antioxidants present in the extract to react with the DPPH free radicals. After incubation, the decrease in absorbance was measured at 517 nm using a UV–Vis double beam spectrophotometer (LMSP-UV1900). Ascorbic acid was used as the positive control standard for comparison of antioxidant activity. All experiments were performed in triplicate to ensure reproducibility and accuracy of the results.

The percentage of DPPH radical scavenging activity was calculated using the same formula as ABTS activity.

GC-MS Analysis

Leaf extracts were analyzed using a Shimadzu GCMS-QP2010 Ultra system (Shimadzu Corporation, Japan) following previously reported procedures with minor modifications [21;28]. The sample was injected in split mode with an injection temperature maintained at 250°C. The oven temperature program was set as follows: an initial temperature of 80°C held for 2 minutes, followed by a temperature ramp of 5°C per minute up to 280°C, and a final isothermal hold at 280°C for 5 minutes, resulting in a total chromatographic run time of approximately 30 minutes.

Mass spectral data were acquired in full scan mode using a total ion chromatogram (TIC) to detect the chemical constituents present in the extracts. The total analytical run lasted approximately 35 minutes. Identification of the detected compounds was performed by comparing the obtained mass spectra with those available in the National Institute of Standards and Technology (NIST) Mass Spectral Library, which provides extensive spectral data for compound identification [28;22]. The identified compounds were further confirmed by comparing their retention times and mass fragmentation patterns with previously reported literature data [2].

Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM) from three independent replicates. Statistical significance was determined using one-way analysis of variance (ANOVA), with differences considered significant at $p < 0.05$.

Results

Extractive value determination:

Among the solvents tested, ethanol showed the highest extractive yield, ranging from 57% to 75%, with the maximum value observed in TAE (75%), followed closely by acetone (60–71%). Acetone also demonstrated consistently high extractive efficiency across all samples.

Ethanol exhibited relatively high extractive values (57–75%), comparable to methanol and acetone in most samples, indicating good solubility of phytoconstituents in this solvent.

Ethyl acetate showed moderate extractive values (35–52%), whereas hexane displayed the lowest yields (30–35%), suggesting limited extraction of polar phytoconstituents.

Among all plant extracts, TAE showed the highest extractive potential across all solvents, indicating a higher concentration of solvent-soluble phytoconstituents compared to other samples.

Table 1: Determination of extractive values

S.No	Plant extract	Ethyl acetate	Hexane	Acetone	Ethanol	Methanol
1	TAA	45%	34%	65%	65%	62%
2	TCA	35%	30%	60%	57%	55%
3	TAE	52%	35%	71%	75%	70%
4	TCE	36%	33%	61%	59%	59%

TAA=Terminalia arjuna acetone extract, TCA= Terminalia catappa acetone, TAE= Terminalia arjuna ethanolic extract, TCE= Terminalia catappa ethanolic extract

α-Amylase Inhibition

The α-amylase inhibitory activity of *Terminalia arjuna* and *Terminalia catappa* leaf extracts obtained using acetone and ethanol was evaluated across three seasonal periods (March–June, July–October, and November–February). The inhibitory activity was expressed as IC_{50} values (mg/mL) and compared with the standard inhibitor Acarbose (IC_{50} = 1.310 mg/mL).

All extracts exhibited moderate α-amylase inhibitory activity with IC_{50} values ranging from

2.309 to 2.478 mg/mL. Among the tested samples, *Terminalia arjuna* extracts showed a comparatively stronger inhibition than *Terminalia catappa*. The ethanol extract of T. arjuna collected during March–June showed the lowest IC_{50} value (2.309 mg/mL), indicating the highest inhibitory activity among the plant extracts. In contrast, the acetone extract of *T. catappa* collected during November–February showed the weakest inhibition (2.478 mg/mL).

Seasonal variation slightly influenced the activity, with extracts collected during March–June generally exhibiting stronger inhibition. Overall, the results suggest that *Terminalia species* possess moderate α-amylase inhibitory potential, which may be attributed to the presence of bioactive phytochemicals identified in the GC–MS analysis.

Table 6: Inhibitory activity of TAA, TAE, TCA, and TCE extracts on α-amylase activity

Plant Sample/Control	IC50 value (mg/ml)	Plant Sample/Control	IC50 value (mg/ml)
TAA (M-J)	2.31	TAE (M-J)	2.309
TAA (J-O)	2.368	TAE (J-O)	2.317
TAA (N-F)	2.371	TAE (N-F)	2.369
TCA (M-J)	2.457	TCE (M-J)	2.448
TCA (J-O)	2.464	TCE (J-O)	2.46
TCA (N-F)	2.478	TCE (N-F)	2.466
Acarbose	1.31	Acarbose	1.31

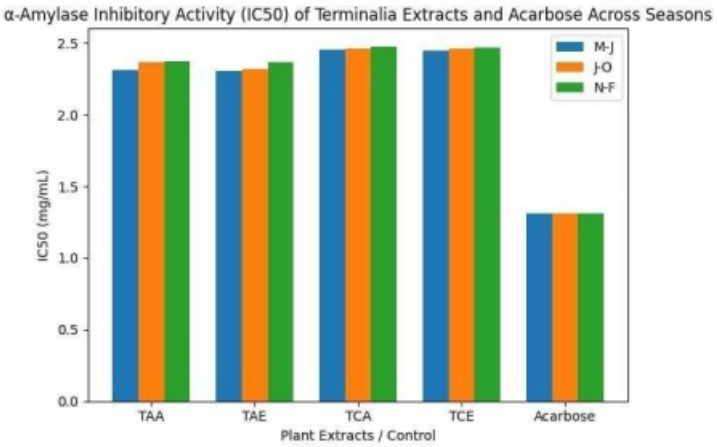

Figure 5: Inhibitory activity of TAA, TAE, TCA and TCE extracts on α-amylase activity

α-Glucosidase Inhibition

The α-glucosidase inhibitory activity of *Terminalia arjuna* and *Terminalia catappa* leaf extracts collected across three seasonal periods (March–June, July–October, and November–February) was evaluated and expressed as IC_{50} values (mg/mL). Lower IC_{50} values indicate stronger inhibitory activity.

Among the tested extracts, *Terminalia arjuna* showed a comparatively stronger inhibition than *Terminalia catappa*. The ethanol extract of *T. arjuna* collected during March–June (TAE M–J) exhibited the lowest IC_{50} value (2.017 mg/mL) among all plant extracts, followed by TAA (M–J) with an IC_{50} of 2.063 mg/mL. A slight increase in IC_{50} values was observed in the July–October and November–February seasons, indicating a small seasonal variation in enzyme inhibitory activity.

In comparison, *T. catappa* extracts demonstrated relatively higher IC_{50} values ranging from 2.194 to 2.209 mg/mL, suggesting comparatively weaker inhibition of the α-glucosidase enzyme. The standard antidiabetic drug acarbose showed the strongest inhibition with an IC_{50} value of 1.127 mg/mL, which was significantly lower than all plant extracts. Nevertheless, the results indicate that *Terminalia species* possess moderate α-glucosidase inhibitory potential, supporting their possible role in the management of postprandial hyperglycemia.

Table 7: Inhibitory activity of TAA, TAE, TCA and TCE extracts on α-glucosidase activity

Plant Sample/Control	IC50 value (mg/ml)	Plant Sample/Control	IC50 value (mg/ml)
TAA (M-J)	2.063	TAE (M-J)	2.017
TAA (J-O)	2.104	TAE (J-O)	2.089
TAA (N-F)	2.136	TAE (N-F)	2.124
TCA (M-J)	2.197	TCE (M-J)	2.194
TCA (J-O)	2.206	TCE (J-O)	2.204
TCA (N-F)	2.209	TCE (N-F)	2.207
Acarbose	1.127	Acarbose	1.127

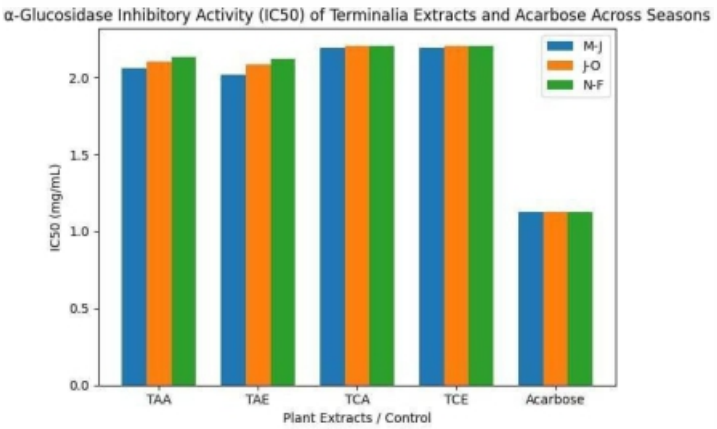

Figure 6: Inhibitory activity of TAA, TAE, TCA and TCE extracts on α-glucosidase activity

Antioxidant activity

ABTS (2,2'-Azino-bis- (3-ethyl) benzothiazoline)-6-sulfonic acid) radical cation scavenging assay

The ABTS radical scavenging activity of acetone and ethanol leaf extracts of *Terminalia arjuna,* and *Terminalia catappa* was determined and expressed as IC_{50} (mg/ml).

Terminalia arjuna exhibited the highest ABTS scavenging activity among the tested species, with IC_{50} values ranging from 2.096-2.743 mg/ml. The lowest IC_{50} (2.096 mg/ml) was recorded in the March to June ethanol extract, which was comparable to the standard Trolox (2.088 mg/ml).

Terminalia catappa demonstrated comparatively lower activity, with IC_{50} values ranging from 3.875-5.413 mg/ml. The highest IC_{50} (5.413 mg/ml) was observed in the November to February acetone extract of *T. catappa*.

Across all species, antioxidant activity followed the trend: March–June > July–October > November–February, and ethanol extracts consistently showed slightly lower IC_{50} values than acetone extracts. Overall activity followed the order: Trolox ≥ *T. arjuna* > *T. catappa*.

Table 8: ABTS activity of TAA, TAE, TCA and TCE extracts

Plant Sample/Control	IC50 value (mg/ml)	Plant Sample/Control	IC50 value (mg/ml)
TAA (M-J)	2.202	TAE (M-J)	2.096
TAA (J-O)	2.402	TAE (J-O)	2.351
TAA (N-F)	2.743	TAE (N-F)	2.558
TCA (M-J)	3.881	TCE (M-J)	3.875
TCA (J-O)	4.68	TCE (J-O)	4.13
TCA (N-F)	5.413	TCE (N-F)	5.293
Trolox	2.088	Trolox	2.088

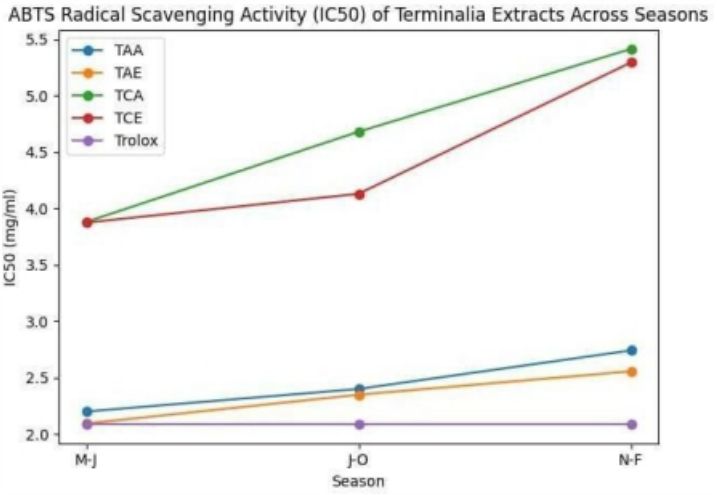

Figure 7: ABTS activity of TAA, TAE, TCA and TCE extracts

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay

The DPPH radical scavenging activity of acetone and ethanol leaf extracts of *Terminalia arjuna,* and *Terminalia catappa* was determined and expressed as IC_{50} (mg/ml). Among the tested species, *T. arjuna* exhibited the strongest antioxidant activity in all seasons, with IC_{50} values ranging from 0.225 to 0.303 mg/ml. The lowest IC_{50} (0.225 mg/ml) was observed in the ethanol extract collected during March–June.

T. catappa demonstrated comparatively lower antioxidant potential, with IC_{50} values ranging from 0.5144 to 0.906 mg/ml. The maximum IC_{50} (0.906 mg/ml) was recorded in the November–February acetone extract.

Across all species, extracts collected during March–June exhibited the lowest IC_{50} values, followed by July–October and November–February.

Ethanol extracts consistently showed slightly better radical scavenging activity than acetone extracts. The standard antioxidant, ascorbic acid, showed an IC_{50} value of 0.1749 mg/ml, indicating higher activity compared to all plant extracts evaluated in the present study.

Table 9: DPPH activity of TAA, TAE, TCA and TCE extracts

Plant Sample/Control	IC50 value (mg/ml)	Plant Sample/Control	IC50 value (mg/ml)
TAA (M-J)	0.229	TAE (M-J)	0.225
TAA (J-O)	0.249	TAE (J-O)	0.245
TAA (N-F)	0.303	TAE (N-F)	0.28
TCA (M-J)	0.5148	TCE (M-J)	0.5144
TCA (J-O)	0.715	TCE (J-O)	0.579
TCA (N-F)	0.906	TCE (N-F)	0.719
Ascorbic acid	0.1749	Ascorbic acid	0.1749

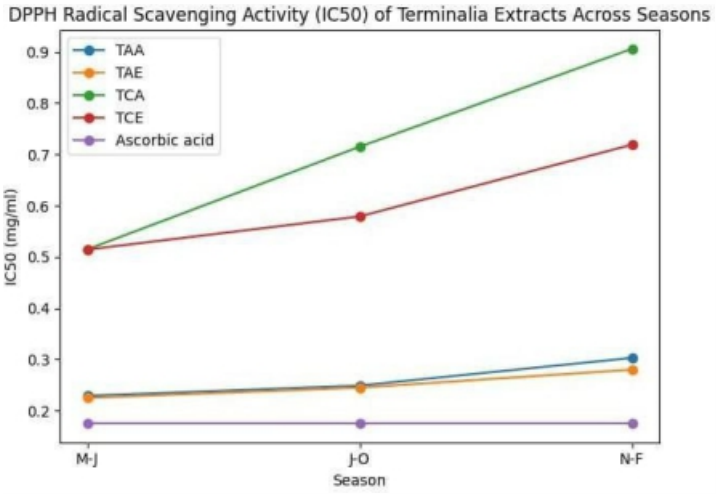

Figure 8: DPPH activity of TAA, TAE, TCA and TCE extracts

GC-MS Analysis of plant extracts:

Based on the results obtained from the enzyme inhibitory and antioxidant assays, the extracts collected during the March to June season demonstrated comparatively superior biological activity than those collected during July to October and November to February. In general, ethanol extracts showed slightly better activity than acetone extracts, while *T. arjuna* demonstrated greater bioactivity compared to *T. catappa*. Seasonal variation also influenced the biological potential, with extracts collected during March–June consistently showing lower IC_{50} values, indicating enhanced antioxidant and antidiabetic activities. The improved activity observed during this season may be associated with the increased accumulation of bioactive phytoconstituents influenced by environmental and physiological factors. Therefore, considering the comparatively higher biological efficacy of the March–June extracts, further GC–MS analysis was carried out for the extracts collected during this season in order to identify the phytochemical constituents responsible for the observed antioxidant and enzyme inhibitory activities.

TAA extract: GC–MS analysis of the *Terminalia arjuna* leaf extract revealed several bioactive compounds belonging to different chemical classes such as phenolics, fatty acids, terpenoids, phytosterols, and vitamins, many of which are reported to possess antioxidant and antidiabetic activities. Based on peak area percentage, the compounds were categorized into major, moderate, and minor constituents. The major compounds included n-hexadecenoic acid (6.21%), γ-sitosterol (4.11%), and octadecanoic acid (2.12%), which are known to exhibit antioxidant activity and hypoglycemic effects by regulating lipid metabolism and improving insulin sensitivity (Kumar et al.; Ostlund; Ghosh et al.). Moderately abundant compounds such as phytol acetate (1.96%) and linoleic acid (1.31%) also contribute to biological activity through free-radical scavenging and regulation of glucose metabolism (Santos et al., 2013; Calder, 2015).

Several minor compounds, including β-amyrin, squalene, stigmasterol, phytol, tocopherols (vitamin E), oleamide, and phenolic derivatives, were detected in smaller amounts but are known to possess strong antioxidant properties and inhibitory effects on carbohydrate-digesting enzymes such as α-amylase and α-glucosidase (de Oliveira et al., 2009; Smith, 2000; Jiang et al., 2001).

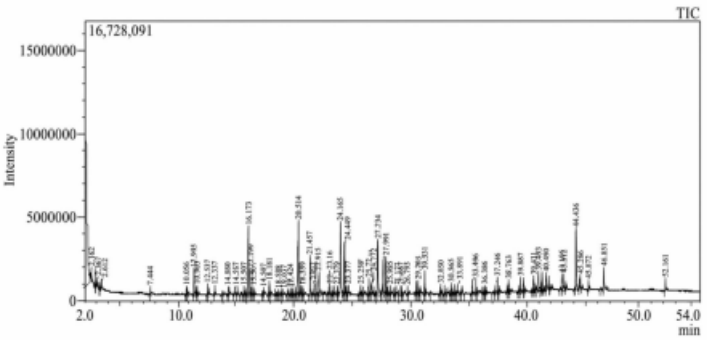

Figure 1: TIC of Terminalia arjuna leaf acetone extract

Table 2: GC-MS analysis of TAA extract showing antidiabetic and antioxidant activities

Peak No.	Peak Area (%)	Compound Name	Chemical Class
19	0.09	Phenol, 2,4-bis(1,1-dimethylethyl)-	Phenolic compound
59	1.96	Phytol acetate	Diterpene ester
63	0.21	Phytol	Diterpene alcohol
70	0.63	7,9-Di-tert-butyl-1-oxaspiro (4,5) dec a-6,9-diene-2,8-dione	Spiro-ketone
77	6.21	n-Hexadecanoic acid (Palmitic acid)	Saturated fatty acid
187	0.3	β-Amyrin	Triterpenoid
105	1.31	9,12- Octadecadienoic acid (Linoleic acid)	PUFA
106	0.09	Linoleic acid	PUFA
108	2.12	Octadecanoic acid (Stearic acid)	Fatty acid
119	0.67	9- Octadecenamide (Oleamide)	Fatty acid amide
158	0.39	Squalene	Triterpene
170	0.32	γ-Tocopherol	Vitamin E
178	0.41	Vitamin E (Tocopherol)	Vitamin
183	0.36	Stigmasterol	Phytostero l
185	4.11	γ-Sitosterol	Phytostero l

TAE extract:

GC–MS analysis revealed several bioactive compounds with different peak area percentages, indicating their relative abundance in the extract. Major constituents included cis-vaccenic acid (5.84%), n-hexadecanoic acid (4.77%), 2-Gala-1-ido-octose (3.93%), hexadecanoic acid methyl ester (3.09%), γ-sitosterol (2.7%), and α-tocopheryl acetate (2.56%). These compounds mainly belong to fatty acids, phytosterols, terpenoids, phenolic compounds, and vitamin derivatives.

Fatty acids such as linoleic acid, cis-vaccenic acid, and n-hexadecanoic acid are reported to exhibit antidiabetic and antioxidant activities, while phytosterols (γ-sitosterol and stigmasterol) contribute to glucose regulation and lipid metabolism. Phenolic compounds and vitamin E derivatives (γ-tocopherol and α-tocopheryl acetate) are known for strong free-radical scavenging activity. The presence of these bioactive constituents suggests that the extract may possess significant antioxidant and antidiabetic potential.

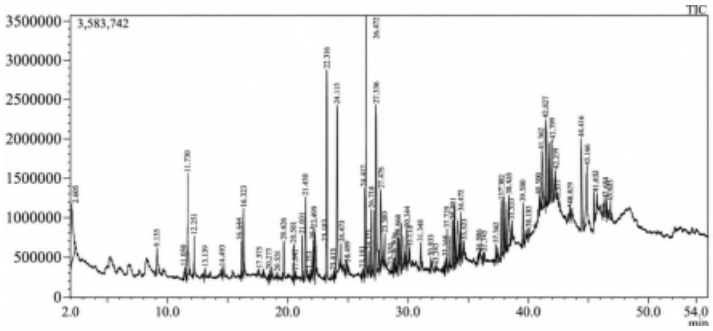

Figure 2: TIC of Terminalia arjuna ethanol extract

Table 3: GC-MS analysis of TAE extract showing biological activity potential

Peak No.	Pea k Are a (%)	Compound Name	Chemical Classification
1	3.93	2-Gala-1-ido-octose	Sugar alcohol / Polyol
8	0.1	2,4-Di-tert-butylphenol	Phenolic compound
22	1.38	Phytol acetate	Diterpene ester
24	0.5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	Diterpene alcohol
29	0.55	7,9-Di-tert-butyl-1-oxaspiro(4,5) dec a-6,9-diene-2,8-dione	Phenolic antioxidant
30	3.09	Hexadecanoic acid methyl ester	Fatty acid ester
33	4.77	n-Hexadecanoic acid (Palmitic acid)	Saturated fatty acid
39	1.35	Linoleic acid methyl ester	PUFA ester
41	1.69	Linoleic acid (9,12-Octadecadienoic acid)	PUFA
45	5.84	cis-Vaccenic acid	MUFA
47	1.74	Octadecanoic acid (Stearic acid)	Saturated fatty acid
62	0.56	9-Octadecenamide (Oleamide)	Fatty acid amide
92	0.73	Stigmast-5-en-3-ol oleate	Phytosterol ester
96	0.34	γ-Tocopherol	Vitamin E
104	2.56	α-Tocopheryl acetate	Vitamin E ester
110	0.31	Stigmasterol	Phytosterol
112	2.7	γ-Sitosterol	Phytosterol

TCA extract

The GC–MS analysis identified several bioactive compounds mainly belonging to fatty acids, fatty acid esters, aldehydes, and tocopherols. Among them, Oleic acid (Peak 13) showed the highest peak area (28.98%), indicating it as the major compound and suggesting significant antidiabetic and antioxidant potential. Other compounds such as n-Hexadecanoic acid, Octadecanoic acid, 9,12-Octadecadienoic acid methyl ester, and cis-9-Hexadecenal were detected in moderate to minor amounts and are also reported to possess antidiabetic and antioxidant activities. Additionally, Vitamin E (2.42%), a well-known tocopherol, contributes strong antioxidant activity. Overall, the predominance of fatty acids and their derivatives indicates the potential therapeutic value of the extract.

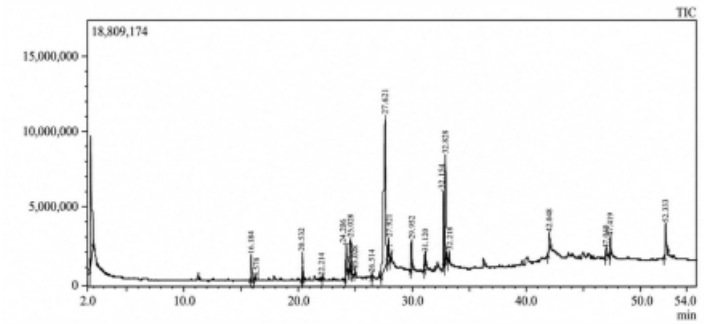

Figure 3: TIC of Terminalia catappa leaf acetone extract

Table 4: GC-MS analysis of TCA extract showing biological activity potential

Pea k No	Peak Area (%)	Compound Name	Chemical Class
8	2.17	n-Hexadecanoic acid	Saturated fatty acid
10	0.49	cis-9-Hexadecenal	Fatty aldehyde
12	0.19	9-Octadecenoic acid, methyl ester	MUFA
13	28.98	Oleic acid	MUFA
14	1.42	Octadecanoic acid	Saturated fatty acid
16	1.67	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	Fatty acid ester
17	0.56	9,12-Octadecadienoic acid, methyl ester	PUFA
18	0.93	9-Octadecenamid e (Z)	Fatty acid amide
20	2.64	9-Octadecenoic acid, 1,2,3-propanetriyl ester	Triglycerid e derivative
22	0.47	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	Fatty acid ester
23	2.42	Vitamin E	Tocopherol

TCE extract

GC–MS analysis revealed several bioactive constituents with notable peak areas that may contribute to the antidiabetic and antioxidant potential of the extract. Among the identified compounds, linoleic acid (19%) and oleic acid (16.46%) exhibited the highest peak areas, indicating that polyunsaturated and monounsaturated fatty acids are the predominant constituents. Other major compounds included linoleic acid methyl ester (8.8%), n-hexadecanoic acid (6.07%), and hexadecanoic acid methyl ester (4.09%), which belong mainly to the fatty acid and fatty acid ester classes known for their antioxidant and antidiabetic activities. Minor constituents such as phytol acetate (0.17%), phytol (0.33%), vitamin E (0.58%), γ-sitosterol (0.44%), and stigmast-4-en-3-one (0.42%) were also detected, representing diterpenes, tocopherols, and phytosterols with reported biological activities. These compounds have been documented to possess antioxidant properties and inhibitory effects on carbohydrate-digesting enzymes, which support their potential role in diabetes management. Overall, the predominance of fatty acids, sterols, and terpenoid derivatives in the extract suggests that these constituents may synergistically contribute to the observed antidiabetic and antioxidant activities.

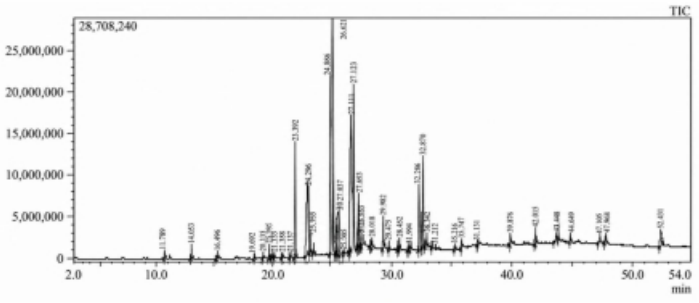

Figure 4: TIC of Terminalia catappa leaf ethanol extract

Table 5: GC-MS analysis of TCE extract showing biological activity potential

Peak No	Peak Area (%)	Compound Name	Chemical Class
9	0.17	Phytol acetate	Diterpene ester
20	0.33	Phytol	Diterpene alcohol
12	4.09	Hexadecanoic acid, methyl ester	Fatty acid ester
13	6.07	n-Hexadecanoic acid (Palmitic acid)	Saturated fatty acid
17	8.8	Linoleic acid, methyl ester	PUFA ester
18	12.54	Oleic acid, methyl ester	MUFA ester
23	19	Linoleic acid	PUFA
24	16.46	Oleic acid	MUFA
48	0.58	Vitamin E (α-Tocopherol)	Tocopherol (Phenolic)
51	0.44	γ-Sitosterol	Phytostero l
52	0.42	Stigmast-4-en-3-one	Plant steroid

Discussion

The present investigation evaluated the phytochemical composition and biological activities of *Terminalia arjuna*, and *Terminalia catappa* leaf extracts collected across different seasons (March–June, July–October, and November–February) using two solvents (acetone and ethanol). The study integrated extractive value determination, GC–MS profiling, antioxidant assays (DPPH and ABTS), and antidiabetic enzyme inhibition assays (α-amylase and α-glucosidase) to understand the influence of species, solvent polarity, and seasonal variation on the bioactivity of *Terminalia species*. Plants belonging to the genus Terminalia are known to contain diverse phytochemicals with significant pharmacological properties, including antioxidant and antidiabetic activities [12;3].

The determination of extractive values revealed that ethanol extracts yielded slightly higher extractive values than acetone extracts, indicating that ethanol efficiently extracts a wider range of polar phytoconstituents such as phenolics, flavonoids, glycosides and tannins. Previous studies have shown that solvent polarity plays a crucial role in extracting bioactive compounds from plant materials [6]. Higher extractive values generally suggest a greater concentration of bioactive compounds, which may contribute to enhanced biological activity. Seasonal variation also influenced extractive yields, with samples collected during March–June showing relatively higher extractive values, possibly due to increased metabolic activity and accumulation of secondary metabolites during the active growth period of plants [18;54].

GC–MS analysis of the extracts revealed the presence of several biologically active compounds, including n-hexadecanoic acid, cis-vaccenic acid, phytol, phytol acetate, octadecanoic acid, and linoleic acid derivatives. These compounds belong to chemical classes such as fatty acids, diterpenes, phenolics and esters, many of which have been reported to exhibit antioxidant, anti-inflammatory, and antidiabetic properties [34]. For instance, phytol and its derivatives are known to possess strong antioxidant and antimicrobial activities, while fatty acid derivatives such as hexadecanoic acid and octadecanoic acid have been reported to exhibit various biological activities, including enzyme inhibition and antioxidant effects [34]. The presence of such phytoconstituents supports the pharmacological potential of *Terminalia species* and may explain the observed antioxidant and enzyme inhibitory activities.

The antioxidant potential evaluated using DPPH and ABTS radical scavenging assays demonstrated that both *Terminalia species* possess notable antioxidant activity, although the potency varied with species, solvent, and season. These assays are widely used methods for evaluating the radical scavenging potential of plant extracts [10;45]. In general, extracts collected during March–June exhibited lower IC_{50} values, indicating stronger radical scavenging capacity. This may be attributed to higher levels of phenolic antioxidants synthesized during favorable environmental conditions [46]. Ethanol extracts typically showed slightly better antioxidant activity than acetone extracts, likely due to the efficient extraction of phenolic compounds. Among the species studied, *Terminalia arjuna* exhibited comparatively stronger antioxidant potential than *Terminalia catappa*, suggesting a higher abundance of antioxidant phytochemicals, which is consistent with earlier reports highlighting the medicinal importance of *T. arjuna* [3;12].

The antidiabetic potential of the extracts was evaluated through α-amylase and α-glucosidase inhibition assays, which are important therapeutic targets for controlling postprandial hyperglycemia in diabetes mellitus. All extracts exhibited inhibitory activity against both enzymes, although their potency was lower than the standard drug acarbose. Inhibition of carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase is an established strategy for the management of type 2 diabetes [33;36;]. Consistent with the antioxidant results, *Terminalia arjuna* extracts showed comparatively stronger inhibitory activity than *Terminalia catappa*. Seasonal variation also influenced enzyme inhibition, with extracts obtained during the March–June season generally showing stronger inhibitory effects. The inhibitory activity observed in this study may be attributed to the presence of bioactive compounds such as fatty acids, phenolic compounds and diterpenoids, which have been previously reported to inhibit carbohydrate-hydrolyzing enzymes [33;36]. These compounds may interact with the active sites of α-amylase and α-glucosidase, thereby slowing the breakdown of complex carbohydrates and reducing glucose absorption.

Overall, the results demonstrate that species type, seasonal variation, and extraction solvent significantly influence the phytochemical composition and biological activity of Terminalia leaf extracts. The strong correlation between GC–MS identified compounds and the observed antioxidant and enzyme inhibitory activities highlights the therapeutic potential of these plant species in the management of oxidative stress and diabetes.

Conclusion

The present study evaluated the phytochemical composition and biological activities of *Terminalia arjuna*, and *Terminalia catappa* leaf extracts collected across different seasons using acetone and ethanol as extraction solvents. The extractive value analysis indicated that ethanol extracts yielded higher extractive content than acetone extracts, suggesting more efficient extraction of polar bioactive compounds [6].

GC–MS analysis identified several pharmacologically important compounds such as phytol, n-hexadecanoic acid, cis-vaccenic acid, and octadecanoic acid, which are known to possess antioxidant and antidiabetic properties [34]. Antioxidant assays (DPPH and ABTS) confirmed that the extracts exhibited notable free radical scavenging activity [10;45]. The extracts also showed α-amylase and α-glucosidase inhibitory activities, suggesting their potential role in controlling postprandial hyperglycemia [33;36].

Seasonal variation influenced the biological activity of the extracts, with samples collected during March–June showing stronger antioxidant and enzyme inhibitory activities, possibly due to increased accumulation of secondary metabolites under favorable environmental conditions [18]. Among the studied species, *Terminalia arjuna* exhibited comparatively higher bioactivity than *Terminalia catappa*, highlighting its greater therapeutic potential [3;12].

Overall, the findings suggest that *Terminalia species* represent promising natural sources of antioxidant and antidiabetic compounds and emphasize the importance of seasonal harvesting and appropriate solvent selection to maximize the extraction of bioactive phytochemicals.

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