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Analysis of phytochemical composition, antioxidant activity, and β-glucuronidase inhibition potential of *Arisaema tortuosum* leaf extract

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Summary

Introduction: Medicinal plants contain essential phytochemicals and are used in traditional herbal therapy for ages. These phytochemicals are bioactive compounds and possess significant antibacterial and antifungal properties in addition to more biologically important potentials. The important bioactive phytochemicals include polyphenolic compounds and flavonoids. *Arisaema tortuosum* has been reported as a medicinal plant in literature and is used in treating many health issues. **Aim:** To investigate the phytochemical composition, total polyphenolic content (TPC), total flavonoid content (TFC), antioxidant capacity, and β-glucuronidase inhibition potential of the extract obtained from leaves of A. tortuosum. **Methods:** The phytochemical composition, TPC, TFC, and antioxidant capacity of the extract was analysed. β-glucuronidase inhibition assay was used to determine β-glucuronidase inhibition potential. The dose-dependent data was used to determine IC_{50} value of the extract. Results: The gas chromatography-mass spectroscopy (GC-MS) analysis identified the fifty-three components from the leaf extract that was observed to possess a significant volume of TPC and TFC. 1,1-diphenyl-2-picryl-hydrazyl

(DPPH) assay revealed a IC_{50} value of 936 μg/mL indicating a high antioxidant activity of the extract. **Conclusion:** The study revealed the presence of many biologically important phytochemicals and a rich number of total polyphenols and flavonoids in leaf extract of *A. tortuosum* signifying the potential biomedical applications.

Keywords: *Arisaema tortuosum*, antioxidant activity, anti-inflammatory potential, total flavonoid content, total polyphenolic content.

Resumen

Análisis de la composición fitoquímica, actividad antioxidante y potencial de inhibición de la β-glucuronidasa del extracto de hoja de *Arisaema tortuosum*

Introducción: las plantas medicinales contienen compuestos fitoquímicos esenciales y se utilizan en la terapia herbolaria tradicional desde hace siglos. Estos fitoquímicos son compuestos bioactivos y poseen importantes propiedades antibacterianas y antifúngicas, además de potenciales efectos biológicamente más importantes. Los fitoquímicos bioactivos importantes incluyen compuestos polifenólicos y flavonoides. En la literatura se ha reportado a la *Arisaema tortuosum* como una planta medicinal y que se usa para tratar muchos problemas de salud. **Objetivo:** investigar la composición fitoquímica, el contenido polifenólico total (TPC), el contenido total de flavonoides (TFC), la capacidad antioxidante y el potencial de inhibición de la β-glucuronidasa del extracto obtenido de las hojas de *A. tortu*osum. **Métodos:** se analizó la composición fitoquímica, TPC, TFC y la capacidad antioxidante del extracto. Se utilizó el ensayo de inhibición de β-glucuronidasa para determinar el potencial de inhibición de este agente. Los datos dependientes de la dosis se usaron para determinar el valor IC_{50} del extracto. **Resultados:** el análisis de cromatografía de gases-espectroscopía de masas (GC-MS) permitió identificar cincuenta y tres componentes del extracto de hojas, que se observó que poseían un volumen significativo de TPC y TFC. El ensayo de 1,1-difenil-2-picril-hidrazilo (DPPH) reveló un valor IC_{50} de 936 μg/mL, lo que indica una alta actividad antioxidante del extracto. **Conclusión:** este estudio reveló la presencia de muchos compuestos fitoquímicos biológicamente importantes y una gran cantidad de polifenoles totales y flavonoides en el extracto de hoja de *A. tortuosum*, lo que significa aplicaciones biomédicas potenciales.

Palabras clave: *Arisaema tortuosum*, actividad antioxidante, potencial antiinflamatorio, contenido total de flavonoides, contenido de polifenoles totales.

Resumo

Análise da composição fitoquímica, atividade antioxidante e potencial de inibição da β-glucuronidase do extrato da folha de *Arisaema tortuosum*

Introdução: as plantas medicinais contêm compostos fitoquímicos essenciais e têm sido utilizadas na terapia tradicional à base de plantas há séculos. Esses fitoquímicos são compostos bioativos e possuem importantes propriedades antibacterianas e antifúngicas, além de efeitos potencialmente mais importantes biologicamente. Fitoquímicos bioativos importantes incluem compostos polifenólicos e flavonóides. Arisaema tortuosum tem sido relatada na literatura como planta medicinal e utilizada para o tratamento de diversos problemas de saúde. **Objetivo:** investigar a composição fitoquímica, teor de polifenóis totais (TPC), teor de flavonoides totais (TFC), capacidade antioxidante e potencial de inibição da β-glucuronidase do extrato obtido das folhas de *A. tortuosum*. **Métodos:** foram analisados a composição fitoquímica, TPC, TFC e a capacidade antioxidante do extrato. O ensaio de inibição da β-glucuronidase foi utilizado para determinar o potencial de inibição deste agente. Os dados dependentes da dose foram usados para determinar o valor IC_{50} do extrato. **Resultados:** a análise por cromatografia gasosa-espectroscopia de massa (GC-MS) permitiu a identificação de cinquenta e três componentes do extrato da folha, que apresentaram um volume significativo de TPC e TFC. O ensaio de 1,1-difenil-2-picril-hidrazil (DPPH) revelou um valor de IC_{50} de 936 μg/mL, indicando uma alta atividade antioxidante do extrato. **Conclusão:** este estudo revelou a presença de muitos compostos fitoquímicos biologicamente importantes e uma grande quantidade de polifenóis totais e flavonóides no extrato da folha de *A. tortuosum*, significando potenciais aplicações biomédicas.

Palavras-chave: *Arisaema tortuosum*, atividade antioxidante, potencial anti-inflamatório, teor total de flavonoides, teor total de polifenóis.

Introduction

Medicinal plants have remarkable importance in traditional Ayurveda and Unani treatments because of their significant biological activities including antibacterial, antioxidant, and anti-cancer potential [1]. Scientists have analyzed various phytochemicals present in various parts of medicinal plants including leaves, stem, root, and fruits for their antioxidant and antibacterial potential [2]. Many phytochemicals such as phe-

nolic compounds, proteins, flavonoids, and terpenoids have been found to possess significant biological potential and hence these can be used as therapeutic agents in health care [3]. Along with their potent antioxidant action, flavonoids and phenolic compounds have also been identified as anti-inflammatory agents [4]. The different inflammation-related enzymes may be targeted by a collection of phenolic compounds that includes proteolytic enzymes of neutrophils [5]. Additionally, the effects of anti-inflammation may come from a synergistic action of scavenging radicals and interactions with enzymes of inflammation [6]. Hence, there is a continuous search to explore phytochemicals in medicinally important plants [7].

Arisaema tortuosum belonging to subfamily Aroideae relates to the herbaceous Araceae family and is popularly referred to as "Cobra or Whipcord lily". Various species of genus *Arisaema* are widely distributed throughout the world especially in the Himalayan region and have been explored for their biological potential [8]. The essential oil obtained from *A. fargesii* has been found to have a great inhibitory impact on the development of *Aedes* mosquito larvae and hence it is utilized to control *Aedes* mosquito hatchlings to forestall the spread of dengue fever [9]. The methanolic extract of the *A. utile* rhizomes has been found to exhibit significant cytotoxic activity against cancer and antioxidant activity [10]. The essential oil obtained from *A. lobatum and A. franchetianum* has been found to have the anthelmintic effect against *Haemonchus contortus* [11]. The Methanolic root extract of *A. jacquemontii* displayed antibacterial action against *Fusarium oxysporum* [12].

A. tortuosum is another medicinally important species of genus *Arisaema* as its various parts are utilized for the treatment of many contagious and bacterial diseases [5]. It is also utilized to check the noxious effect of snakebite and hair follicle infection in addition to its use for recuperation of cracked bones and as antinematodal [13]. The leaves of *A. tortuosum* are utilized to treat stomachache and rheumatism [14]. It has also been found to exhibit cyto-toxic activity against human cancer cells [15]. These biological activities like antifungal, antimicrobial, anti-inflammatory, and anticancer properties have been attributed to the presence of various metabolites and phytochemical compounds like alkaloids, polyphenols, terpenoids, glycosides, saponins, amino acids, lactones, and flavonoids, etc. [10]. Thus, it is very important to ascertain the phytochemical composition and recognize the medicinally important plants.

However, there is no study available in the literature on the phytochemical analysis of leaf extract of *A. tortuosum*, to the best of our knowledge. Consequently, the purpose of this research was to offer a scientific knowledge of its phytochemical composition, as well as support for its ethnomedicinal use in the treatment of different illnesses. The study has been directed to analyze the different volatile organic components of the

extract obtained from leaves of *A. tortuosum*. The study also explores the antioxidant as well as the β-glucuronidase inhibition potential of the extract of this medicinally important plant.

Materials and methods

Preparation and Characterization of Extract

The collection of the plant was carried out from the Himalayan region of Dharamshala (Naddi), Himachal Pradesh, India and identification was carried out at the University school of Agricultural Sciences (RBU, Chandigarh) under voucher with Ref. No. RBU/USAS/HOD/22/190. Young and healthy leaves of *A. tortuosum* were selected and firstly cleaned by washing with tap water and afterward with demineralized water to expel the residue and dust particles over their surface. After draining free water, the material was dried under the shade at the room temperature for 30 days and ground to obtain the powdered form. 100 g of the powdered sample was processed for 12 hrs. By the Soxhlet extraction process utilizing 300 mL of methanol as a solvent at its boiling point [16]. The solvent was removed from the extract by using a rotary vacuum evaporator. The obtained final product was characterized by gas chromatography-mass spectroscopy (GC-MS) analysis (Model THERMO Scientific Trace 1300GC) using the capillary column (Model, Restek USA RTx-5Sil MS) with dimensions of 30 m × 0.25 mm × 0.25 mm. Helium was delivered at a flow rate of 1 mL/min. as a carrier at a split ratio of 1:5. The mass spectra were acquired utilising the electron ionisation (EI) mode with an energy of 70 electron volts (eV) at a spectral range (m/z) of 40-700. The experimental setup involved setting the interface temperature to 280 °C and the ion source temperature to 200 °C. The identification of different constituents was conducted through the comparison of their retention time and mass spectral data with the Wiley8 and NIST11 library spectral database. The quantification of the compounds was performed by analysing the area under the peak, and the resulting data was expressed as a percentage. The analysis of each sample was conducted in triplicate.

Total Phenolic Content

The Folin-Ciocalteu spectrophotometric method was used for the quantitative determination of the total phenolic content of the extract as described by Rebaya *et al.* [17]. 1 mL of extract (1 mg/mL) or standard gallic acid solution (30-150 mg/L) was diluted up to 10 mL by adding deionized water followed by addition of Folin-Ciocalteu reagent solution (1 mL). After the addition of 2 mL of sodium carbonate solution, 20% (w/v), the mixture was kept in dark for 60 min. and the absorbance was measured

at 750 nm (UV-Visible spectrophotometer, Agilent Cary 60). The calibration curve provided the estimate of the total phenolic content (mg of gallic acid equivalent per g of dry extract weight).

Total Flavonoid Content

Aluminum chloride colorimetric technique was used to assess the TFC present in the extract as outlined by Stoyanova *et al.* [18]. 1 mL of extract (1 mg/mL) or standard Rutin trihydrate solution (60-300 mg/L) was diluted up to 5 mL by adding deionized water. After adding 0.3 mL of NaNO2 solution, 5% (w/v), the mixture was left untouched for 5 min. In this mixture, 0.3 mL of AlCl3 solution, 10% (w/v) and 2 mL of 1 M NaOH were added followed by dilution to 10 mL with deionized water. The mixture was again kept in dark for 30 minutes at 25 °C and the absorbance at 510 nm was recorded (UV-Visible spectrophotometer, Agilent Cary 60). The total flavonoid content (mg of rutin trihydride equivalent per g of dry extract weight) was estimated from the calibration curve.

Antioxidant potential (DPPH Assay)

The antioxidant potential of the extract was quantitatively determined as free radical scavenging assay by using the DPPH method as given by Garcia *et al.* [19]. 3 mL of fresh DPPH solution (0.1 mM) was poured into 0.2 mL of extract (1 mg/mL) and kept in dark for 30 minutes. The absorbance was recorded by Agilent Cary 60 UV-Visible spectrophotometer at 517 nm. The scavenging impact of the extract against DPPH free radical was determined using Ascorbic acid as a standard on the basis of the equation given below.

$$\% \text{ scavenging effect} = \left[1 - \frac{A_{test}}{A_{control}}\right] \times 100$$

Where A_{test} and $A_{control}$ are the absorbances of test sample/ standard solution and control respectively.

Anti-inflammatory Potential (β-glucuronidase inhibition assay)

As per the method given by Otang-Mteng *et al.* [1], 0.1 mL of the extract, with varying dosage from 25-100 mg/mL, was preincubated for 5 min. in 0.1 M acetate buffer (pH 7.4) at 37 °C [1]. This mixture was further kept in dark for 30 min. after addition of 0.1 mL β-glucuronidase solution and 2 mL of NaOH (0.5 N) was added to terminate the reaction. A reference, Salicylic acid, was utilized with a concentration of 1 mM and the absorbance of the solution was recorded at 410 nm (UV-Visible spectrophotometer, Agilent Cary 60).

Results and discussion

GC-MS analysis

Figure 1 illustrates the existence of various secondary metabolites or phytochemicals in the GC-MS profile of the extract. A total of fifty-three phytochemicals were detected in the chromatograph of the methanolic leaf extract of *A. tortuosum* as shown in Table 1. Nonane was eluted first with a retention time of 4.064 min. in the leaf extract. The major peak area was obtained for 6, 10, 14-trimethyl-2-pentadecanone as 54.55% and phytol as 18.86% in the leaf extract. The other identified major phytochemicals included 2-Pentadecanone, 6,10,14-trimethyl-, Phytol, Benzene, 1,3-dimethyl-, 2-Pentanone, 4-hydroxy-4-methyl-, Cyclohexane, (2-methylpropyl)-, Cyclohexane, 1-methyl-2-propyl-, Cyclohexane, butyl-, Naphthalene, decahydro-, trans-, Undecane and hexadecamethyl cyclooctasiloxane. The results confirmed the presence of important bioactive compounds like terpenoids, polyphenols, hydrocarbons and organosiloxanes in the leaf extract of *A. tortuosum*. Some of these phytochemicals with significant biopotential have been listed in Table 2.

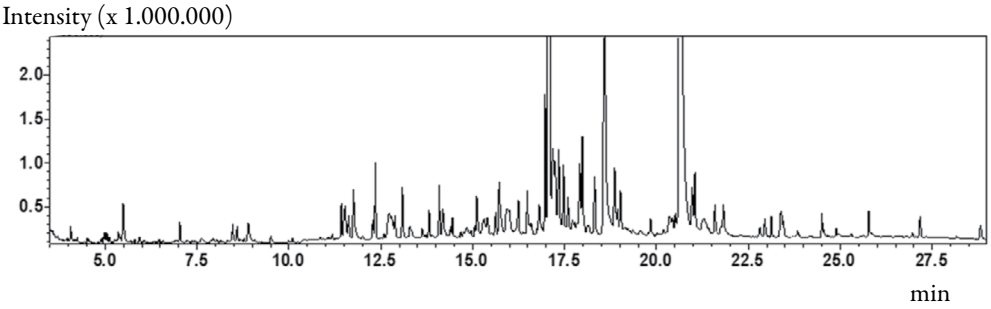


Figure 1. GC-MS analysis of leaf extract of *A. tortuosum.*

Total Phenolic Content (TPC)

The total phenolic content is the quantitative approach to determine the amount of polyphenols present in the plant. Phenols are one of the important plant constituents as the radical scavenging ability depends on the hydroxyl group [12]. The phenolic groups are secondary metabolites and have redox properties that allow them to function as a radical scavenger in various natural activities such as antioxidant, antibacterial and antifungal [20]. The total phenolic content also depends upon the nature of the extraction method and solvent used. The quantity of phenolic content available in methanolic leaf extract of *A. tortuosum* was calculated from a calibration curve. The

TPC for leaf extract of *A. tortuosum* was found as 171.05 gallic acid equivalents/g. The high phenolic content of the plant extract has been correlated with the high antioxidant activity of the medicinal plants in various reports [21, 22].

Total Flavonoid Content (TFC)

Flavonoids such as flavanols and flavones are secondary plant metabolites that are more soluble in polar solvents as compared to the nonpolar solvent, due to this methanol extract has higher flavonoid content as compared to the other extract [17]. These metabolites are responsible for the color as well as antioxidant, anti-inflammatory and antimicrobial activity of plants. The antioxidant activity of the extract is also found to be dependent upon the quantity and position of hydroxyl groups in the flavonoids. The amount of flavonoid present in the leaf extract of *A. tortuosum* was also measured by the regression calibration curve. TFC for the leaf extract was found as 183.5 rutin trihydride equivalents/g. A similar result was reported previously in the case of tuber extract of *A. tortuosum* [5].

Antioxidant Assay

The polyphenolic compounds present in medicinal plants are referred to as antioxidant agents and used as radical scavengers or metal chelators [16]. The antioxidant effect of the plant depends on phytochemicals present as both major and minor constituents that play an effective role in DPPH radical activity. Literature reports different methods for evaluating the antioxidant activity dependent upon free radical scavenging activity having different mechanisms [23-25].

The general mechanism given for DPPH activity has been illustrated in Figure 2 [25]. In this examination, the leaf extract of *A. tortuosum* was found to exhibit significant DPPH scavenging activity with IC_{50} value as 936 μg/mL. The results have been found consistent with similar studies carried out on the tuber extract of *A. tortuosum* with scavenging activity against stable DPPH radical having IC_{50} value of 852 μg/mL [5].

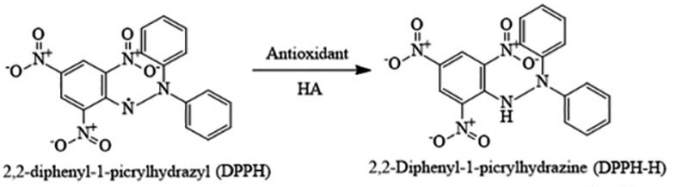


Figure 2. Illustrative mechanism for DPPH scavenging activity.

Table 1. GC-MS analysis of the leaf extract of *A. tortuosum*

Peak#	R. Time	Name	Mol. Formula	Mol. Weight	Compound Nature
1	4.064	Nonane	C_9H_20	128.259	Linear alkane hydrocarbon
2	17.341	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278.348	Phthalic anhydride
3	24.547	1,2-Propanediol, 3-benzyloxy-1,2-diacetyl-	$C_{14}H_{18}O_5$	266.293	Organic compound
4	8.894	1-Butanone, 1-cyclohexyl-	$C_{10}H_{18}O$	154.253	Organic compound
5	15.108	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$	242.447	Branched alcohol
6	19.02	1-Decanol, 2-octyl-	$C_{18}H_{38}O$	270.501	Organic compound
7	12.723	1-Dodecanol	$C_{12}H_{26}O$	186.34	Organic compound
8	15.934	1-Dodecanol, 3,7,11-trimethyl-	$C_{15}H_{32}O$	228.42	sesquiterpenoids
9	8.471	1-Dodecene	$C_{12}H_{24}$	168.319	Unsaturated aliphatic hydrocarbon
10	16.483	1-Heptadecene	$C_{13}H_{26}O$	198.35	Organic compound
11	17.983	1-Hexacosene	$C_{26}H_{52}$	364.702	Unsaturated aliphatic hydrocarbons
12	23.84	1-Nonadecene	$C_{19}H_{38}$	266.513	Unsaturated aliphatic hydrocarbon
13	14.088	1-Pentadecene	$C_{15}H_{30}$	210.405	Unsaturated aliphatic hydrocarbon
14	11.426	1-Tridecene	$C_{13}H_{26}$	182.351	Unsaturated aliphatic hydrocarbon
15	13.63	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4	$C_{11}H_{16}O_2$	180.247	Volatile terpenes
16	20.513	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl	$C_{10}H_{18}O$	154.2493	Organic compound
17	22.945	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-	$C_{13}H_{18}O_3$	222.28	Organic compound
18	15.398	2-methyltetracosane	$C_{15}H_{32}$	212.421	Acyclic branched hydrocarbon
19	17.072	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268.485	Saturated terpenoid alkane
20	11.626	2-Undecanone, 6,10-dimethyl-	$C_{13}H_{26}O$	198.35	Chemical compound

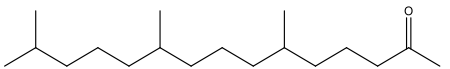
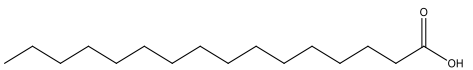
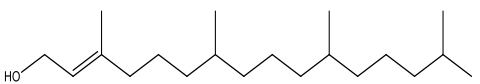
(Continued)

Peak#	R. Time	Name	Mol. Formula	Mol. Weight	Compound Nature
21	16.97	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	Terpene alcohol
22	23.39	3-methyl-5-(2,6-dimethylheptyl)-1,5-Pent-2-en	$C_{15}H_{26}O_2$	238.371	Organic compound
23	23.451	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324.5	Terpene
24	12.283	5,9-Undecadien-2-one, 6,10-dimethyl-	$C_{13}H_{22}O$	194.3132	Volatile flavor compound
25	17.591	Benzene, 1-(dodecyloxy)-2-nitro-	$C_{18}H_{29}NO_3$	307.434	Organic compound
26	18.862	Chlorpyrifos	$C_9H_{11}C_{l3}NO_3PS$	305.57	Organothiophosphate
27	16.245	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	444.924	Organo silicon compound
28	19.837	Cyclononasiloxane, octadecamethyl-	$C_{16}H_{54}O_9Si_9$	667.386	Organosiloxane compound
29	21.588	Benzene, 1,3-dimethyl-	C_8H_{10}	106.167	Aromatic compound
30	23.127	Naphthalene, decahydro-, trans-	$C_{10}H_{18}$	138.254	Aromatic compound
31	24.499	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	116.16	Beta-hydroxyketones
32	25.773	Cyclononasiloxane, octadecamethyl-	$C_{16}H_{54}O_9Si_9$	667.386	Organosiloxane compound
33	27.17	Cyclohexane, 1-methyl-2-propyl-	$C_{11}H_{22}$	154.297	Hydrocarbon compound
34	28.814	Cyclohexane, butyl-	$C_{10}H_{20}$	140.27	Monocyclic hydrocarbon
35	14.451	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	593.232	Macrocylicorganosiloxane
36	20.967	Cyclopentanone, 2-(5-oxohexyl)-	$C_{11}H_{18}O_2$	182.263	Organic compound
37	14.185	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.24	Benzoic acid ester
38	25.294	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390.564	Organic compound
39	11.755	Diphenyl ether	$C_{12}H_{10}O$	170.211	Organic compound
40	8.591	Dodecane	$C_{12}H_{24}$	168.319	Unsaturated aliphatic hydrocarbon
41	24.887	Eicosane	$C_{20}H_{42}$	282.556	Unbranched hydrocarbon
42	15.72	Ethanol, 2-(tetradecyloxy)-	$C_{16}H_{34}O_2$	258.446	Organic compound
43	22.815	Heneicosane	$C_{21}H_{44}$	296.583	Acyclic alkanes
44	12.352	Hexadecane	$C_{16}H_{34}$	226.448	Saturated aliphatic hydrocarbon
45	18.317	Isophytol	$C_{20}H_{40}O$	296.539	Diterpene alcohol

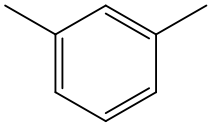
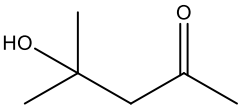
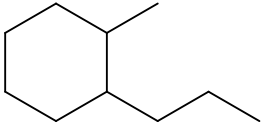
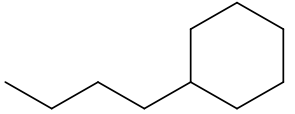
(Continued)

Peak#	R. Time	Name	Mol. Formula	Mol. Weight	Compound Nature
46	5.488	Mesitylene	C_9H_{12}	120.19	Benzene derivative
47	13.289	Methanone, dicyclohexyl-	$C_{13}H_{22}O$	194.3132	Volatile flavor compound
48	18.588	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Palmitic acid
49	13.086	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.32	Organic compound
50	13.814	Phytol	$C_{20}H_{40}O$	296.539	Diterpene alcohol
51	21.821	Phytol, acetate	$C_{22}H_{42}O_2$	338.576	Acyclic diterpenoids
52	15.626	Tetradecanal	$C_{14}H_{28}O$	212.377	Fatty aldehyde
53	7.033	Undecane	$C_{11}H_{24}$	156.313	Hydrocarbon compound

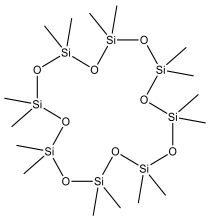
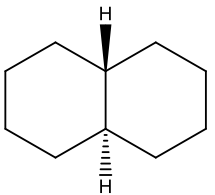
Table 2. Structure and activities of major phytocompounds reported in GC-MS analysis

S. No.	Compound Name	Structure	Area %	Activity
1	2-Pentadecanone, 6,10,14-trimethyl-		54.55	Antibacterial agent, cosmetics, antiperspirant
2	n-Hexadecanoic acid		5.44	Antifibrinolytic, Hemolytic, lubricant, Nematicide, and Antialopecic
3	Phytol		18.86	Anti-inflamatory, antioxidant activity, anticancer and antimicrobial agent

(Continued)

S. No.	Compound Name	Structure	Area %	Activity
1	Benzene, 1,3-dimethyl-		39.54	Antiabortive and antitussive agent
2	2-Pentanone, 4-hydroxy-4-methyl-		87.7	Corrosion inhibitor, anti-scaling, and plating agent
4	Cyclohexane, 1-methyl-2-propyl-		5.48	Antiviral, antiabortive, antipyretic, and anti-inflammatory
5	Cyclohexane, butyl-		3.11	Antiabortive agent, impotence, Antipsoriatic and Antipruritic agent

(Continued)

S. No.	Compound Name	Structure	Area %	Activity
6	Cyclooctasiloxane, hexadecamethyl-		2.28	Antibacterial agent
7	Naphthalene, decahydro-, trans-		5.11	Anti-spasmodic, antitussive, antiseborrheic, and antiasthmatic agent
8	Undecane		6.41	Antitussive, antiasthmatic, antipruritic and anti-acne agent

β-Glucuronidase inhibition potential

β-glucuronidase, an enzyme found in neutrophil lysosomal membranes, has been identified as one of the mediators of the inflammatory response [5]. Hence, the anti-inflammatory potential of leaf extract was determined in terms of β-glucuronidase inhibition. A substantial inhibitory action against the β-glucuronidase enzyme in a concnetration-dependent manner was observed, with the highest inhibition observed at a concentration of 100 mg/mL in comparison to the control (salicyclic acid). 82.36% inhibition was observed for the extract (IC_{50} value as 37.45 mg/mL) as compared to the 72.81% inhibition by the control. Thus, *A. tortuosum* leaf extract exhibited a greater ability to inhibit β-glucuronidase activity than an established anti-inflammatory agent, salicylic acid [26].

Conclusion

In this study, the analysis of GC-MS has identified fifty-three bioactive compounds including mainly phytol, terpenoids, and siloxanes, from the leaf parts of *A. tortuosum*. The presence of diverse bioactive compounds justifies that this plant can be used for treating various ailments. The extract exhibited significant availability of high content of total polyphenols and flavonoids and possessed significant antioxidant and β-glucuronidase inhibition activity. Therefore, this plant can be used for many biomedical applications. But, isolating specific phytochemical constituents and confining their biological activity will provide beneficial outcomes. Further studies are however needed to ensure its profile of bioactivity for potential biomedical applications.

Conflict of interests

The authors report no conflict of interest.

Author contributions

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