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Original Article

Phytochemical Profile and Bioactivity of *Dioscorea transversa* R. Br: Antioxidant and Anti-Inflammatory Potential of Leaf and Tuber

K G Geetha[1], Mohind C Mohan[2], V Manju[1]*

ABSTRACT

Various species of *Dioscorea*, commonly referred to as wild yams, are employed as food and used globally for medicinal purposes. *Dioscorea transversa*, also known as the long or pencil yam, is a native to northern and eastern Australia and is also found in the open forests of southern India. The tubers of this plant are consumed by local tribes in northern Kerala during times of famine, and they are believed to enhance bone and muscle strength. Furthermore, it is incorporated with other ingredients in their traditional postnatal rejuvenation preparations. However, this plant which is infrequently examined for its biochemical impacts on humans. This research aimed to identify the phytochemicals present in the tuber and leaf of *Dioscorea transversa* and to assess solvent extracts for their antioxidant and anti-inflammatory properties. The leaf sample demonstrated a notable concentration of phenolic compounds, tannins, flavonoids, and terpenoids, while the tuber was rich in saponins, proteins, and steroids. It was noted that the aqueous methanolic extract of the leaves (AqML) exhibited superior antioxidant and anti-inflammatory activity in comparison to the other five extracts explored. This study underscores *D. transversa* as a significant source of natural bioactive compounds and advocates for further research to delve deeper into the pharmacological potential of this plant.

Keywords: *Dioscorea transversa*, Phytochemicals, Inflammation, Oxidative stress, COX-2, TNF-α.

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INTRODUCTION

Body cells are continuously exposed to oxidative stimuli and under conditions of homeostasis, the pool of exogenous or endogenous free radicals is inactivated by the action of antioxidants in the body. But frequently, there may be a rise in the generation of free radicals or a fall in antioxidant activity, leading to the development of oxidative stress. The most often seen free radicals are the hydroxyl radical, the superoxide radical, the nitric oxide radical, and the lipid peroxyl radical; non-free radical species are primarily H2O2 and molecular oxygen[1]. Such a state is assumed to lead to the process of ageing and pathogenicity of various inflammatory disease. It is believed that oxidative stress causes aging and increases the risk of developing long lasting inflammatory conditions resulting to cancer, diabetics, cardiovascular, neurologic and pulmonic diseases[1,2]. Inflammation is a multifaceted biological reaction of body tissue to detrimental stimuli, including pathogens, damaged cells, cell death, malignancy, and ischemia. Given its clinical manifestations and diverse classifications, it is crucial for the accurate diagnosis and management of numerous inflammatory disorders. Inflammation, whether acute, chronic, sub-acute, or of a specialized nature, is integral to the body's defense mechanisms; but when dysregulated, it can also exacerbate the pathology of numerous diseases. Pathological disorders are associated with the over expression of tumor necrosis factor alpha (TNF-α) and cyclooxygenase-2 (COX-2)[3]. The dual inhibition of COX-2 and TNF-α may serve as a novel strategy for the development of analgesics and cancer treatments[4].

Since antiquity, a diverse array of plants and their extracts have been utilized for prophylactic and therapeutic applications. Phytochemical and ethnobotanical investigations are conducted to elucidate the mechanisms of action of diverse natural chemicals found in plant extracts, representing a significant advancement in the development of innovative pharmaceuticals. Researchers worldwide examine phytochemicals such as polyphenols, terpenoids, alkaloids, carotenoids, flavonoids, saponins, and phytosterols for their health effects[5]. *Dioscorea* spp., commonly known as yam, encompasses over 600 species that are widely distributed across the globe. In tropical nations, yam serves as a fundamental source of sustenance for the populace. A multitude of species are both cultivated and thrive in their natural habitats. The indigenous populations utilize the wild species in times of scarcity. *Dioscorea* species hold a significant role in traditional medicine for the treatment of multiple ailments[6]. Numerous reports exist detailing its ethno-medicinal applications across the globe[7,8]. According to a thorough review of various Dioscorea species in India[9], there are eleven species for which the plant's tuber is used medicinally for conditions like gonorrhoea, leprosy, piles, dysentery, asthma, skin disorders, arthritis, rheumatism, and female hormone regulation. Two species leaves, tubers, and stems have been used to treat uterine sedatives, coughs, asthma, skin conditions, and pain in the abdomen, among other ailments. On the other hand, two species leaves and tubers have been used to treat jaundice, malaria, diarrhea, mumps, toothaches, and other ailments. A comprehensive analysis regarding *D. bulbifera* discusses the global utilization of its tubers for treating leprosy and tumors in Bangladesh, in addition to their role in relieving sore throats within traditional Chinese medicine[10]. It is beneficial for wound treatment in Zimbabwe, while in Cameroon and Madagascar, a formulation

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derived from bulbils is used topically to treat boils and wounds. Diosgenin is recognized as the most significant steroidal sapogenin, which accounts for the extensive range of medicinal properties associated with the genus. Approximately 50% of the global steroid drug production is attributed to species within the *Dioscorea* genus[11]. Numerous scholars have explored the biochemical activities of various species within the *Dioscorea* genus[12,13], yet the specific biochemical effects *of Dioscorea transversa (D.transversa*), commonly known as long yam, remain under-researched in relation to human health. This species is observed in the northern and eastern regions of Australia, as well as in southern India. The tuber serves as a sustenance option during times of scarcity for the indigenous communities in northern Kerala. They believe that it contributes to the strengthening of their bones and muscles, thereby promoting an overall enhancement of their health. The tubers are utilized in conjunction with various herbs for the formulation of medicinal preparations following delivery. This study sought to broadly screen the phytochemicals found in both the leaf and tuber of *D. transversa*, examine the solvent extractions for antioxidant and anti-inflammatory properties.

MATERIALS AND METHODS

Chemicals and Reagents

The reagents utilized comprised analytical grade of methanol, ethanol, chloroform, ethyl acetate, acetone, ferric chloride, hydrochloric acid, sulfuric acid, aluminium chloride, Dragendorff's reagent, Wagner's reagent, Mayer's reagent, Benedict's reagent, Million's reagent, α-naphthol, potassium phosphate buffer, 1,1-diphenyl-2-picryl hydrazyl radical, fluorescein dye, 2,2'-azobis (2-amidinopropane) dihydrochloride, trolox, hydrogen peroxide, cobalt (II) fluoride tetrahydrate, picolinic acid, gallic acid (Sigma-Aldrich, MA, USA) COX (ovine/human) inhibitory screening assay kit (Cayman Chemical, MI, USA), lipopolysaccharide, RPMI 1640 medium, and TNF-α kit (R and D system, MN, USA).

Sample Preparation

Fresh leaves and tubers (Figure 1) of *D. transversa*, as identified from the 8th edition of the Australian rainforest plants database and the edible plants of the globe database, were collected in February and March from Kanayi, northern Kerala, India. *D. transversa* is alternatively referred to as *D. punctata* or *D. sativa var. elongata*. The herbarium specimen of the plant was submitted to the Department of Botany at Periyar University. The harvested leaves and tubers of the plant were meticulously washed to eliminate soil and muck, subsequently dried in the shade. The desiccated substances were pounded in a blender to form very fine particles.

Preparation of extractants

250 g of finely powdered leaf (L) and tuber (T) samples were subjected to extraction with double the volume of aqueous methanol (AqM-1:1-methanol: water), methanol (M), and water/aqueous (Aq) solvents utilizing the indirect ultrasonic-assisted extraction method[14]. The ultrasonic water bath was maintained at a temperature of 50°C for three hours. Two further cycles of extraction were conducted, and all six extracts were individually concentrated and evaporated utilizing a rotary evaporator. The percentage (%) yield of six extracts, specifically aqueous methanolic leaf (AqML), methanolic leaf (ML), aqueous leaf (AqL), aqueous methanolic tuber (AqMT), methanolic tuber (MT), and aqueous tuber (AqT), was determined.

Qualitative assessment of phytochemicals

Analyses for tannins, saponins, resins, alkaloids, glycosides, flavonoids, steroids, terpenoids, proteins, and carbohydrates were conducted utilizing established methodologies[15,16] as detailed below:

Examination for Total Phenolics: Powdered samples of leaf, tuber and the six extracts were individually subjected to boiling with 20 ml of methanol and subsequently filtered. Incorporated 1 ml of the filtrate into a tube containing 1 ml of diluted Folin-Ciocalteau reagent and stirred thoroughly. Allowed the tube to rest for 5 minutes. Subsequently, 1.5 ml of a 5% w/v sodium carbonate aqueous solution was added to each tube, stirred thoroughly, and allowed to stand for 15 to 20 minutes. The blue hue indicated the presence of phenolic chemicals.

Tannin Test: The six extracts, powdered leaf and tuber samples were boiled in 20 ml of distilled water in individual test tubes and subsequently filtered using Whatman No. 1 filter paper. Incorporated 0.1% FeCl3 into the leaf and tuber filtrate respectively and monitored for a brownish-green or blue-black hue, indicated as tannin present.

Saponin Test: Each six extracts, powdered leaf and tuber samples was subjected to boiling with 20 ml of distilled water individually in a water bath and subsequently filtered. 10 ml of the individual filtered samples were combined with distilled water (5 ml) in a test tube, mixed thoroughly and forcefully to achieve a stable, persistent froth. The foam was combined with three drops of olive oil and monitored for emulsion formation, indicating the presence of saponins.

Resin Analysis: Each six extracts, powdered leaf and tuber samples were individually subjected to extraction using 15 ml of 95% ethanol. Each ethanol extract was thereafter added to 20 ml of distilled water in a beaker. The production of a resinous precipitate signified the presence of resins. Additionally, all the eight samples were individually extracted using chloroform, and the respective extracts were concentrated to dryness. The residues were re-dissolved in 3 ml of acetone, to which 3 ml of HCl was added. The solutions were subjected to heating in a water bath for 30 minutes. A pink hue transitioning to magenta red confirmed the presence of resins.

Alkaloid Test: 5 ml of 1% HCl was added to the six extracts, powdered leaf and tuber samples and all the eight samples were boiled for 5 minutes in a steam bath. Later this was cooled and filtered. The filtrates were treated with few drops of Dragendorff's reagent individually. A second 1 ml aliquot was subjected to treatment with Wagner's reagent, while a third aliquot was treated with Mayer's reagent. The occurrence of alkaloid was indicated by the emergence of cherry, reddish-brown, and white creamy precipitate.

Glycoside testing: 10 ml of distilled water was combined with the six extracts, powdered leaf and tuber samples, followed by a 5-minute boiling period. All the eight filtrate (2 ml) was hydrolyzed with a few drops of strong HCl individually. A small quantity of ammonia solution was incorporated into this. Five drops of this solution were combined with Benedict's reagent (2 ml) and subjected to boiling. A reddish-brown precipitate indicates the presence of glycosides.

Flavonoid Test: Each six extracts, powdered leaf and tuber samples were subjected to heating with 10 ml of ethyl acetate in boiling water for three minutes and filtered individually. Each filtrate (4 ml) was combined with 1 ml of 1% aluminum chloride solution and then observed. The yellowish hue in the individual ethyl acetate layer signifies the presence of flavonoids.

Steroid and Terpenoid Testing: The six extracts, powdered leaf and tuber samples was added with 10 ml of ethanol and refluxed for several minutes individually. The filtrate was concentrated to approximately 2.5 ml in a boiling water bath, followed by the addition of 5 ml of hot water. The combination was permitted to rest for one hour, after which the waxy substance was filtered away. The filtrate was extracted with 2.5 ml of chloroform utilizing a separating funnel individually. To 0.5 ml of the chloroform extract in a test tube, 1 ml of strong sulfuric acid was added to create a lower layer. A reddish-brown interface indicates the presence of steroids. An additional 0.5 ml of the chloroform extract was

evaporated to dryness on a water bath and subsequently heated with 3 ml of concentrated sulfuric acid for 10 minutes on a water bath. A gray hue signifies the existence of terpenoids.

Protein Test: Five milliliter of distilled water were added individually to each extract samples, powdered lead and tuber samples and mixed well. This was allowed to stand for three hours, and filtered. To 2 ml of each sample filtrates added 0.1 ml of Million's reagent, agitated, and set aside for observation. The emergence of yellow precipitate indicates the presence of proteins.

Carbohydrate Test: Each six extract samples, powdered leaf and tuber samples was added with 5 ml of distilled water, mixed and allowed to stand for three hours. Subsequently these were filtered. Incorporated 2 drops of α-naphthol solution into samples (2 ml) within each test tubes. Added 1 ml of concentrated H2SO4 cautiously to the same each tube by pouring along the sides to create two distinct layers. A brown hue at the interface of the two layers in each sample tubes signified the presence of carbohydrate.

Antioxidant Assays

1,1-diphenyl-2-picryl hydrazyl (DPPH) Radical Scavenging Assay:

The free radical scavenging activity of samples was measured by the quenching of the stable free DPPH radical. The reduction in absorbance of DPPH in the presence of antioxidant samples, relative to the blank or untreated DPPH solution, is quantified spectrophotometrically[17]. The assay combination comprised 1.5 ml of 0.066 mM DPPH methanolic solution and methanolic solutions of varying concentrations of the test materials (AqML, ML, AqL, AqT, MT, and AqT), in dimethyl sulfoxide (DMSO). DMSO at 0.5% which did not interfere with the testing system, utilized as the solvent for samples which was not easily soluble in methanol. In addition to the extract samples, standard vitamin C (Vit. C) was also evaluated. The entire assay volume was 3 ml. Blanks and controls are also captured. The mixture is incubated at 37°c for 30 minutes, and absorbance is measured spectrophotometrically at 516 nm. The free radical scavenging activity was quantified as SC_{50} values, indicating the concentration of the sample necessary to scavenge 50% of the free radicals. The scavenging activity was determined using the formula: % DPPH Scavenging Activity = [(B-C)–(T-TC) / (B-C)] x 100, where B represents the absorbance of DPPH, C denotes the absorbance of the solvent control solution without DPPH, T indicates the absorbance of DPPH with the test sample, and TC signifies the absorbance of the test sample without DPPH.

Oxygen Radical Absorption Capacity (ORAC) Assay: The ORAC assay relies on the free radical-induced damage to the fluorescent probe fluorescein, as indicated by alterations in its fluorescence intensity. The alteration in fluorescence intensity serves as an indicator of the extent of free radical damage. The presence of antioxidants inhibits free radical damage, as seen by the preservation of probe fluorescence, serving as an indicator of their antioxidant potential against free radicals[18]. 25 µl of different sample concentrations (dissolved in 0.5% DMSO) was dispensed into each well. Subsequently adding 150 µl of a 10X 10^{-2} M AAPH reagent (2,2'-Azobis(2-amidinopropane) dihydrochloride) prepared in a 75 mM potassium phosphate buffer at pH 7.4. Subsequently, 150 µl of disodium fluorescein dye (final concentration) 4.8×10^{-7} M was introduced and mixed prior to the initial measurement (f_0) being recorded. Fluorescence measurements were conducted using the Fluostar Optima Microplate Reader at 485/520 nm per minute for a duration of 35 minutes (f1……..f35).The ORAC value is quantified as weight/weight Trolox equivalents per liter or per gram.

The Area under curve AUC = $(1 + f_1/f_0 + f_2/f_0 + \ldots. + f_{35}/f_0)$ -------- eq 1

Where f_0 is the initial fluorescence reading at 0 min and f_1 is the fluorescence reading after 1min. The data was analyzed by applying eq 1 and the AUC was calculated. The net AUC was obtained by subtracting the AUC of the blank from that of the sample.

The relative ORAC value = $[(AUC_{sample} - AUC_{blank})/ (AUC_{trolox} - AUC_{blank})]$ X (Wt. of trolox/ Wt. of sample). ---------eq 2

Hydroxyl Radical Averting Capacity (HORAC) Assay: The HORAC test was evaluated utilizing fluorescein (FL) as the fluorescent probe[19]. The hydroxyl radical is generated by a Co (II)-mediated reaction. The fluorescent decay curve of FL is monitored in the presence and absence of the inhibitor and the area under curve (AUC) is integrated. Net AUC is calculated and it is an index of hydroxyl radical averting capacity. The values are expressed in Gallic acid equivalents. Briefly, 10µl of sample (of appropriate concentration in 0.5% DMSO) is pipetted into the respective sample well and 10µl of vehicle into the blank well of a 96 well plate layout. This is followed by the addition of 175µl of working stock of the dye into the sample wells. Immediately 20µl of 30% H_2O_2 is added into all wells, and initial fluorescence reading (F_0) is taken at 485/520 nm in FLUOstar Optima microplate reader. Finally, 5µl of cobalt (II) fluoride solution will be pipetted into all wells and the fluorescence readings are taken until 35mins (F_1, F_2, F_3 …….F_{35})

The Area under curve AUC=$0.5+f_1/f_0+f_2/f_0+\ldots\ldots+f_{34}/f_0+0.5(f_{35}/f_0)$ ------ eq3

Where f_0 = the initial fluorescence reading at 0 min and f_1 = the fluorescence reading soon after the final reagent addition. The data were analyzed by applying eq3 in a Microsoft excel spreadsheet to calculate the AUC. The net AUC is derived by deducting the AUC of the blank from that of the sample. The final HORAC values were calculated by using a regression equation (Y= a+bX+cX^2, quadratic) between gallic acid standard (Y) (µM) and the net area under the fluorescein decay curve (X). Linear regression was used in the range of 93.75-1500µM Gallic acid. The values calculated by the regression equation were divided by the respective weight in gm/liter and the values obtained are expressed as µM GAE/g

Nitric Oxide (NO) Radical Scavenging Activity: The nitric oxide radical scavenging activity was estimated where 500 µl of Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with 500 µl of different concentrations extracts (in 0.5% DMSO) and incubated at 25°C for 180 min[20]. The samples were then mixed with 500 µl Griess reagent. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with NED was read after 10 minutes of incubation at 25°C at 540 nm using a UV–VIS spectrophotometer. The NO radical scavenging activity was expressed as SC_{50} values, the concentration of the sample required for 50% of the free radical to be scavenged. The concentrations of sample which gave a scavenging activity of not more than 70% scavenging activity is taken to plot a graph, and from this graph SC_{50} was calculated using linear equation.

NO Scavenging activity was calculated as % NO Scavenging Activity = [AbB–AbT)/AbB X 100, where AbB is the absorbance of Blank, AbT is the absorbance of Test.

Anti-inflammatory Activity

Cyclooxygenase Inhibition Assay

Cyclooxygenase enzymes exhibit both cyclooxygenase and peroxidase activity. Cyclooxygenase facilitates the initial stage in the manufacture of prostaglandins, thromboxanes, and prostacyclins by converting arachidonic acid into prostaglandin H2 (PGH2). COX-1 participates in maintaining appropriate cellular homeostasis. COX-2 facilitates the

manufacture of prostaglandins during acute inflammatory states. The inducible COX-2 is considered the important enzyme for the anti-inflammatory effects of nonsteroidal anti-inflammatory medications. The procedure was conducted utilizing the Cayman COX assay kit, which encompasses both ovine COX-1 and COX-2 enzymes to identify isozyme-specific inhibitors [21]. The enzymes COX-1 and COX-2 are permitted to interact with the substrate Arachidonate in both the presence and absence of the inhibitor. The quantity of prostaglandins (PGF2) generated through the decrease of COX-derived PGFH2 by stannous chloride is quantified by EIA (Cayman) utilizing a broadly specific antibody that interacts with the principal prostaglandin molecules. The reduction of PGs released in the presence of an inhibitor is directly proportional to its inhibitory activity. 950 µL of reaction buffer and 10 µL of heme solution are pipetted into each tube labeled AT (100% enzyme activity tube) and IT (inhibitor tube). Ten micro liters of enzyme are introduced to tubes AT and IT. Twenty microliters of inhibitor solution are introduced into tubes IT. All six extracts and the positive control celecoxib were evaluated at variable concentrations for its inhibitory potentials. DMSO at 0.5%, which did not influence enzyme activity, was utilized as the solvent for sample dissolution. All the tubes were incubated at 37°C for 15 minutes. Ten microliters of substrate are introduced into each tube and incubated precisely for two minutes at 37°C. After 2 minutes, the reaction is terminated by the addition of 50 µL of 0.1M HCl, followed by 100 µL of SnCl2 solution to each tube. Combine and was let to rest at ambient temperature for 5 minutes. These reactions are stable for one week when securely sealed and stored at 0-4°C. Standard prostaglandin was also maintained under identical conditions. Diluted the stock prostaglandin from 10 ng/ml to a factor of five and performed successive dilutions to obtain seven consecutive concentrations. The dilutions were utilized to conduct the reaction for constructing the standard graph. The COX reaction tubes were diluted according to the kit insert's recommendations and this was taken into account when calculating the results. Fifty microliters of suitably diluted solutions from AT and IT were aliquoted into wells covered with mouse monoclonal antibodies, respectively. Fifty micro liters of PG ache tracer and fifty micro liters of PG antiserum were poured to each well and incubated at room temperature for eighteen hours. The wells are washed 3-4 times with wash buffer, followed by the addition of 200 µL of Ellman's reagent to each well. The mixture is incubated for 1 hour at room temperature, after which the plate is read at 405 nm. Inhibition percentage was determined using the formula [A-T/B] x 100, where A represents the concentration of prostaglandins (pg/ml) in the absence of the inhibitor, and B denotes the concentration of prostaglandins (pg/ml) in the presence of the inhibitor.

Tumor Necrosis Factor-alpha (TNF-α) Inhibition Assay

TNF-α, a homodimer composed of 157 amino acid subunits, is a multifunctional cytokine that regulates inflammation, immunological response, and apoptosis. Human cells that express TNF-α include B cells, colonic columnar epithelial cells, natural killer cells, macrophages, monocytes, mast cells, neutrophils, keratinocytes, plasma cells, and adipocytes. The TNF α inhibition assay was conducted using Lipopolysaccharide (LPS), the most effective stimulus to induce TNF-α in human whole blood[22-24]. The assay utilized the quantitative sandwich enzyme immunoassay method following stimulation. Heparinized blood from healthy donors was diluted in a 1:3 ratio with RPMI 1640 culture media. Diluted blood samples were pre-incubated with the test samples and the positive control rolipram (dissolved in 0.5% DMSO, which did not influence enzyme activity) in Eppendorf tubes for one hour at 37°C in an incubator with 5% CO2. Following pre-incubation, the cells were activated by the introduction of 1 ng/mL LPS to each tube. The mixture was carefully combined and thereafter incubated for 5 hours at 37°C. The samples were centrifuged at 3000 g for 3 minutes at 4°C, and the supernatant was thereafter collected. 200 µL of supernatant from each tube was put onto a pre-coated mouse monoclonal antibody microtiter plate in the corresponding wells, followed by the addition of 50 µL of assay diluent to all wells. The plate was then covered with an adhesive strip and incubated for 2 hours at room temperature. Aspirated each well and washed, repeating the procedure three times for a cumulative total of four washes by adding 400 µL of wash buffer to each well. Inverted the plate and pressed it on a clean paper towel. Administered 200 µL of conjugate to each well. Adorned with a novel sticky strip. Incubated for two hours at ambient temperature. Reiterated the aspiration/wash as performed in the preceding phase. Administered 200 µL of substrate solution to each well. Incubated for 20 minutes at ambient temperature while shielded from light. Administered 50 µL of stop solution to each well and gently tapped the plate to ensure thorough mixing. Measured the optical density of each well within 30 minutes utilizing a microplate reader calibrated to 450 nm. Percent inhibition was estimated using the formula {[C-B] – [S-B]} / [C-B]X100, where C represents the LPS-induced TNF-α expression, S denotes the LPS-induced TNF-α expression in the presence of the test, and B signifies the baseline TNF-α expression

Quantitative examination and statistical analysis

Fifty percentage scavenging (SC_{50}) and inhibitory (IC_{50}) concentration were determined using a non- linear regression in GraphPad Prism version 5. Furthermore, data analysis was conducted using one-way analysis of variance (ANOVA), succeeded by Dunnett's test. In the study, p values less than 0.05 were deemed highly significant. All the experiments were performed in 6 replicates.

RESULTS

The sample were extracted with different solvents based on polarity. The AqT extract had the highest yield, followed by AqML, AqMT, ML, AqL, and MT extracts in that order. The percentage yields are given in Table 1.

Phytochemical analysis revealed a rich diversity of phytochemical composition:

Qualitative analysis of *D. transversa* leaf, tuber and its extracts samples revealed the presence of significant phytochemicals, as summarized in Table 2. The leaf sample exhibited a significant concentration of phenolic compounds, tannins, flavonoids, and terpenoids, but the tuber samples and its extracts was abundant in saponins, proteins, and steroids. All examined phytochemicals were found in the leaf, tuber and the extracts, except for protein, which was lacking in the leaf and its extracts but prominently present in the tuber and its extract.

Dioscorea transversa extracts are rich in antioxidants.

Different in-vitro experiments carried out using the *D. transversa* showed highantioxidant activities. Vitamin C was used as a positive control. All six extracts exhibited dose-dependent DPPH radical scavenging activity, as presented in Figure 2. Among the extracts AqML extract exhibited markedly enhanced DPPH radical scavenging activity ($P< 0.05$) in comparison to the other four extractsand ML ($P < 0.5$). The concentration (µg/ml) at which each tested material exhibited 50% scavenging activity (SC_{50}) was determined using non-linear regression curve fittingusing GraphPad Prism 5. The SC_{50} values are presented in Table 3 and Figure 2. The SC_{50} values of the investigated samples were arranged in ascending order as follows: AqML ≤Vit.C < ML < AqL < AqMT < MT < AqT. A lowerSC_{50} indicates superior scavenging activity. The comparative DPPH radical scavenging activity (SC_{50}) of all examined samples are illustrated in Figure 4A.

A dose-dependent NO radical scavenging activity is summarized in Figure 3. AqML, ML AqL extracts demonstrated better NO radical

Table 1. Yield of *D. Transversa* leaf and tuber extracts

Part	Solvent		Code of extract	% Yield
	Aqueous methanol	(1:1: water: methanol)	AqML	86.3
Leaf	Methanol		ML	51.6
	Aqueous (water)		AqT	43.8
	Aqueous methanol	(1:1: water: methanol)	AqMT	67.4
Tuber	Methanol		MT	35.6
	Aqueous (water)		AqT	89.2

Table 2. Phytochemicals in *D.transversa* leaf and tuber extracts

	Leaf	AqML	ML	AqL	Tuber	AqMT	MT	AqT
Phenolics	++++	+++++	++++	++++	+	+	+	+
Tannins	++++	++	+	++	+	+	-	+
Saponins	+	++	+	++	++++	++++	+	++++
Resins	+	-	++	-	+	-	+	-
Alkaloids	++	++	+	++	+	+		+
Glycosides	+	+	-	+	++	++	+	++
Flavonoids	++++	+++	++	++	+	+	+	+
Steroids	+	-	+	-	+++	+++	+	+++
Terpenoids	++++	++	++++	++	+	-	+	-
Proteins	-	-	-	-	++++	++	+	++++
Carbohydrates	+	+	-	+	++	++	-	++

Table 3. Comparative antioxidant activity of *D.transversa* leaf and tuber extracts

	Vit. C	AqML	ML	AqL	AqMT	MT	AqT
DPPH Radical Scavenging Activity as SC_{50}(µg/ml)	2.86	2.26	11.02*	81.48**	92.61***	335.0***	1201***
NO Radical Scavenging Activity as SC_{50}(µg/ml)	29.58	11.37	12.84	17.59	31.12	31.56	180.3
ORAC value µmol TE/g	137.2	11782***	9590***	1818**	1191**	724*	62
HORAC value µmol GAE/g	1516	17026***	5988**	1507	2340*	1816	717

*P<0.5; **P< 0.05; ***P< 0.001

Table 4. Comparative anti-inflammatory activity of *D.transversa* leaf and tuber extracts

	PS	AqML	ML	AqL	AqMT	MT	AqT
COX-1 Inhibition as IC_{50}(µg/ml)	66.64	473.2**	1.99**	26.73*	NA	NA	NA
COX-2 Inhibition as IC_{50}(µg/ml)	5.52	2.94	18.9*	101**	NA	NA	NA
TNF-α inhibition as IC_{50}(µg/ml)	4.61	5.04	32.19*	1473***	1357***	NA	NA

*P<0.5; **P< 0.05; ***P< 0.001

Figure 1. Image of *Dioscorea transversa* leaf and tuber

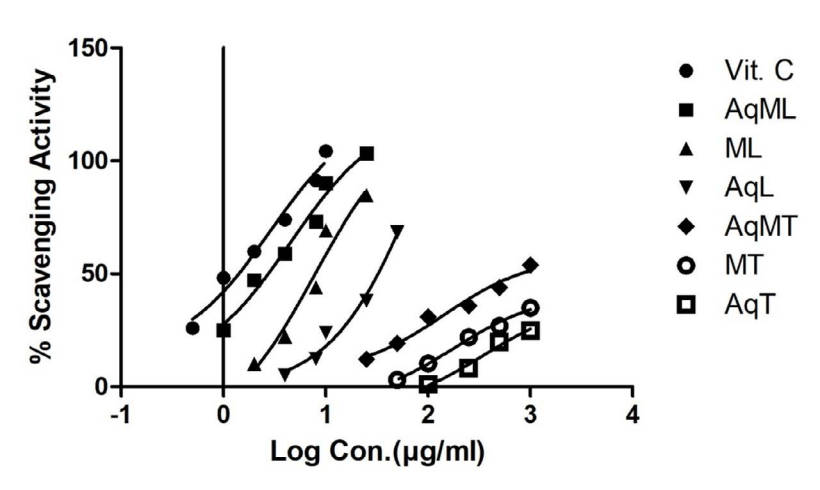

Figure 2. Graph showing the percentage DPPH radical scavenging activity of D. transversa extracts as compared to the positive control Vitamin C.

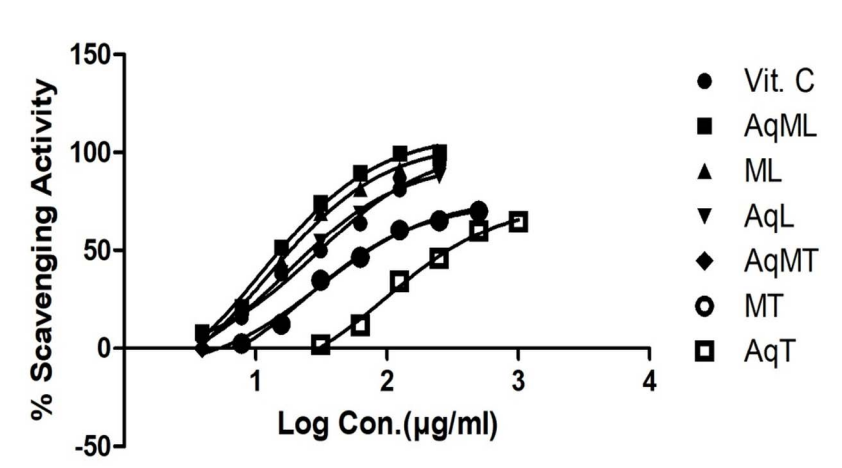

Figure 3. Graph showing the percentage NO radical scavenging activity of *D. transversa* extracts as compared to the positive control Vitamin C.

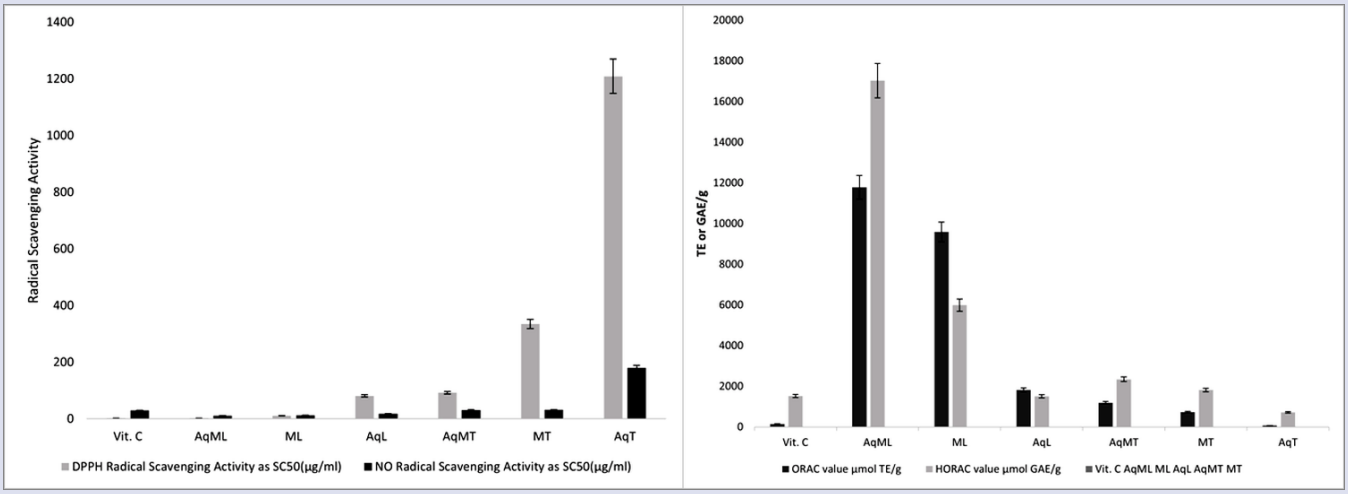

Figure 4. Figure showing (A) SC_{50} values obtained for *D. transversa* extracts for antioxidant assays (B) ORAC and HORAC values for *D. transversa* extracts as compared to positive control Vit C.

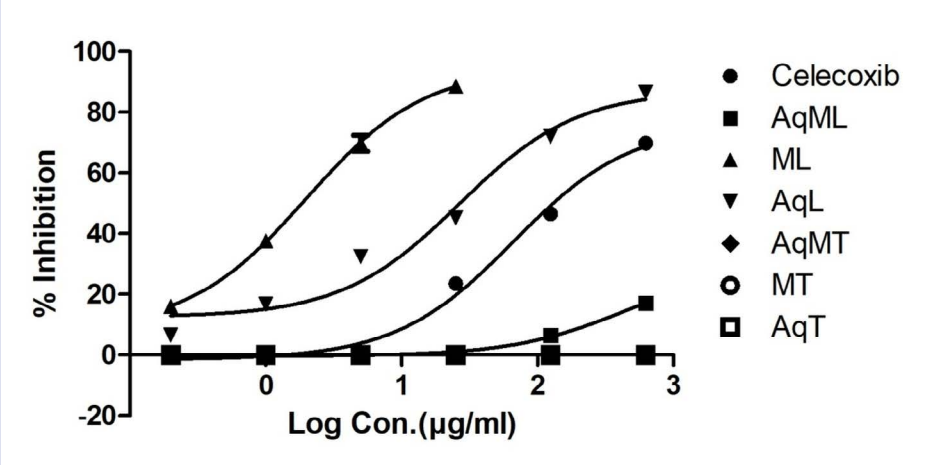

Figure 5. Log graph representing percentage COX-1 inhibition by *D. transversa* extracts compared to positive control celecoxib

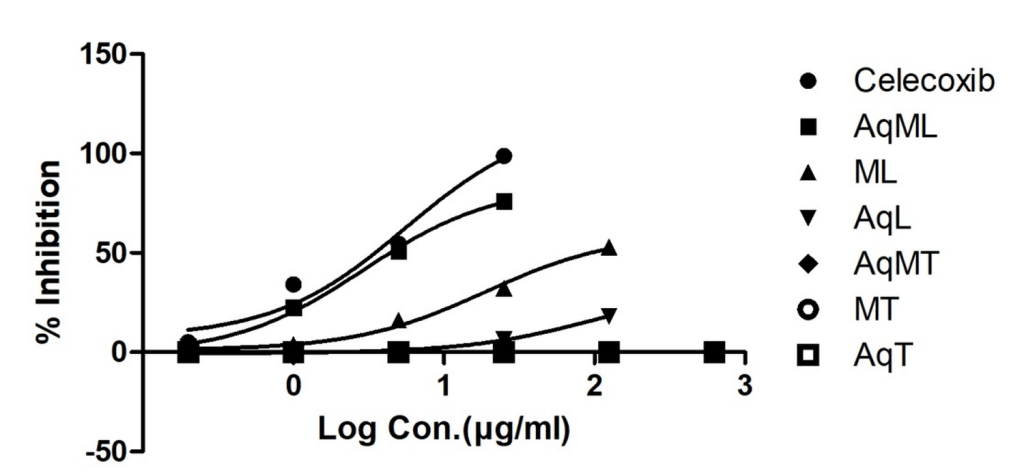

Figure 6. Log graph representing percentage COX-2 inhibition by *D. transversa* extracts compared to positive control celecoxib

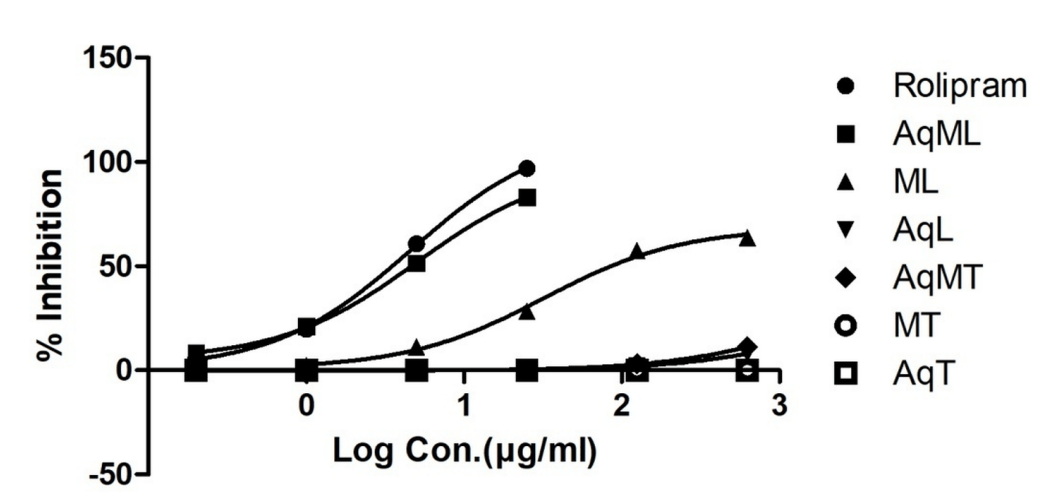

Figure 7. Log graph representing percentage TNF-α inhibition by *D. transversa* extracts compared to positive control rolipram

scavenging activity than the tuber extracts and the positive control, Vitamin C. The concentration (µg/ml) at which each tested material exhibited 50% scavenging activity (SC_{50}) was determined using non-linear regression curve fitting. The SC_{50} values are presented in Table 3 and Figure 3. The SC_{50} values of the investigated samples were arranged in increasing order AqML < ML < AqL < Vit. C < MT < AqMT < AqT. A lower SC_{50} indicates superior scavenging activity. The comparative NO radical scavenging activity of all examined samples are illustrated in Figure 4A.

The ORAC values of the examined extracts and Vitamin C are summarized in Table 3. The higher the ORAC value indicates superior antioxidant capacity. AqML extract had a markedly superior (P< 0.001) oxygen radical absorption capacity, followed by ML extract(P< 0.001), when compared to AqL and AqMT (P<0.05). The ORAC value of Vitamin C waslower to other samples tested except for AqT extract. The ORAC values of the examined samples were ranked in descending order as follows: AqML > ML > AqL > AqMT > MT > Vit. C > AqT. The comparative ORAC values of all examined samples are illustrated in Figure 4B.

The HORAC values of the evaluated extracts and Vitamin C are given in Table 3. AqML extract had a markedly superior hydroxyl radical averting capacity (P< 0.001), followed by ML extract (P < 0.05), when compared to all other samples. The HORAC value of Vitamin C was relatively lower. A higher HORAC score indicates superior antioxidant capacity. The comparative HORAC values for all examined samples are illustrated in Figure 4 B. The ORAC values of the evaluated samples were ranked in descending order as follows: AqML > ML > AqMT > MT > Vit. C > AqL > AqT.

Dioscorea transversa showed anti-inflammatory activity by inhibiting the key inflammatory enzyme and inflammatory cytokine

Six extracts, in addition to the positive control celecoxib, were evaluated for COX-1 and COX-2 inhibition, as depicted in Figures 5 and 6. The IC_{50} values are presented in Table 4. Celecoxib, AqML, ML, and AqL extracts exhibited suppression of COX-2 and COX-1. AqML exhibited similar COX-2 inhibition to that of celecoxib positive control. COX-2 inhibition of AqML was superior to ML(P value <0.5) and AqL (P value <0.05) extracts. Nonetheless, ML and AqL exhibited a more pronounced suppression of COX-1 compared to COX-2. However, the tuber extracts i.e., AqMT, MT, and AqT shown no ability for inhibiting COX-1 nor COX-2.

Six extracts, in addition to the positive control rolipram, were evaluated for their capacity to suppress TNF-α. Rolipram and AqML exhibited comparable TNF-α inhibition, succeeded by ML, AqMT, and AqL extracts. The findings are encapsulated in Figure 7 and Table 4. MT and AqT shown no potential for TNF-α inhibition.

DISCUSSION

Our in vitro study on antioxidant and anti-inflammatory properties revealed that *D. transversa* leaf and tuber extracts exhibited both antioxidant and anti-inflammatory activity. Extracts of *D. communis* are documented to exhibit acetylcholinesterase and butyrylcholinesterase activity, in addition to antioxidant properties[25]. The anthocyanin fraction of *D. alata* demonstrated significant anti-inflammatory benefits in a mouse model of inflammatory bowel illness[26]. *D. nipponica* rhizomes containing Trillin exhibit protective benefits against hyperlipidemia and oxidative stress[27]. The anti-inflammatory and antioxidant properties of phenanthrene derivatives extracted from *D. batatas* were investigated[28]. A diverse array of *Dioscorea* species, as indicated by Aura et al. [12], had demonstrated possible antioxidant action; nonetheless, *D. transversa* is infrequently examined for its health advantages, despite its consumption by indigenous populations for health purposes. This plant thrives abundantly as a natural species in northern Kerala. Our analysis of the leaf and tuber of *D.transversa* demonstrated that each contained distinct phytochemicals. The leaf sample exhibited a significant concentration of phenolic compounds, tannins, flavonoids, and terpenoids, but the tuber was abundant in saponins, proteins, and steroids. The aqueous tuber extract yielded 89.2%, followed by the aqueous methanolic leaf extract at 86.3%. The research on antioxidant and anti-inflammatory activities indicated that the aqueous methanolic extract of *D. transversa* leaf exhibited superior antioxidant and anti-inflammatory properties compared to other leaf and tuber extracts (ML, AqL, AqMT, MT, and AqT). The SC_{50} (µg/ml) values for DPPH and NO radical scavenging activity of AqML were 2.26 and 11.37, respectively. These values were similar to the activity of Vit. C, 2.86 and 29.58. Nonetheless, the ORAC and HORAC values, namely 11782 TE/g and 17026 GAE/g, indicate that AqML exhibited greater activity than Vit. C, i.e. 113.2 TE/g and 1516 GAE/grespectively. Furthermore, it was noted that AqML extract exhibited selective COX-2 inhibition, with an IC_{50} of 2.94 (µg/ml), comparable to celecoxib at 5.52. The IC_{50} of AqML extract for TNF-α suppression was 5.04 µg/ml, comparable to rolipram's 4.61 µg/ml, indicating its enhanced anti-inflammatory efficacy. In conclusion this study highlights the pharmacological potential of *D. transversa* as a valuable source of natural bioactive compounds and supports its traditional medicinal uses. This study paves the way for future studies on *D. transversa*active photochemical constituent standardization to support for its clinical application.

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