

RESEARCH ARTICLE

Chromatographic and antiproliferative assessment of the aerial root of *Ficus thonningii* Blume (Moraceae)

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ABSTRACT

Ficus thonningii (Blume) has long history of use for variety of ailments. The hot aqueous extract of *Ficus thonningii* aerial root (FT) was obtained by infusion. The antiproliferative activity of FT was evaluated using *Sorghum bicolor* seed radicle over a period of 24 h to 96 h. The mean radicle length (mm), percentage inhibition and percentage growth were calculated. Chemical characterization of FT was done using chromatographic techniques. Thin layer chromatography revealed the presence of β -sitosterol. High performance liquid chromatography showed ten peaks with gallic acid, tannins, caffeic acid, rutin, ferulic acid and morin eluting at 3.530, 3.928, 4.668, 6.706, 7.669 and 18.844 minutes respectively. Compared with negative control, FT at 1 mg/ml to 32 mg/ml significantly ($p < 0.0001$) inhibited *S. bicolor* seed radicle growth over 24 h-96 h. At 96h, FT dose-dependently inhibited *S. bicolor* seed growth, giving a percentage inhibition of 20.31%, 24.30%, 31.71%, 53.23%, 78.74%, 95.37% at 1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml, 16 mg/ml, 32 mg/ml, respectively. Methotrexate 50 μ g/ml used as the positive control gave inhibition of 70.62% at 96h. The result revealed the potential of FT to inhibit rapid proliferating cells of *S. bicolor* seed radicle and by extension cancer cells.

Keywords: *Ficus thonningii*; Antiproliferative; β -sitosterol; Caffeic acid; Ferulic acid.

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INTRODUCTION

Cancer is a generic name describing a collection of related diseases which can affect any part of the body [1]. Cancer may be malignant or neoplastic, involving the rapid growth of abnormal cells beyond their normal location or boundaries, a process called metastases which constitutes the major cause of mortality from cancer. Globally, cancer is being ranked the second leading cause of mortality, being responsible for 8.8 million deaths

in year 2015. Nearly 1 in 6 deaths globally is due to cancer and approximately 70% of mortality from cancer occurs in countries with low or middle income capacity [2].

The use of phytomedicines in disease management dates to as old as the beginning of man's existence, as various plant materials have served as medicinal recipes even to the modern man. About 75-90% of the world's population use remedies from plants as their primary health care sources [3]. Plant and their metabolites have proved to be useful in treating and managing of diseases proven by their extensive application in herbal medicine practice [4].

F. thonningii (Blume) belonging to the family Moraceae, also called the common wild fig [4] is an evergreen, multi-stemmed, deciduous tree with a crown that is round or spreading, distributed mainly in the upland forests of subtropical and tropical Africa, having altitudes varying between 1,000-2,500 m [5]. It is also found in open grasslands, rocky and riverine areas and sometimes found in Savannah. It is draught resistant occurring naturally in Tanzania, DR Congo and South Africa [6]. The local names include Chediya in Hausa, India-laurel fig in French, Odan in Yoruba; Strangler fig, common wild fig, bark-cloth fig in English [6]. *Ficus thonningii* Blume is about 6-21 m high, the leaves are alternate or whorled mid dark green and sub-glossy [7]. The leaves have smooth margins, may be leathery and papery, elongated sometimes, glabrous with the apex obtuse or sometimes rounded with stipules about 12 mm long [6]. The fruits are borne singly or in pairs and are about 10 mm in diameter usually hairy and turn yellowish and rarely pink on ripening [7]. It has dense, spreading or rounded crown. Younger branches have hairy bark with caps that are stipular covering the growth tip while older stems and branches are smooth. It is lenticellate with aerial roots often hanging down the branches. The plant exudes abundant milky latex that turns pink. Figs fruit are borne in leaf axil or below the leave sometimes. It is native to Africa, possessing diverse economic and environmental uses across many communities in Africa [8].

Research has shown that *Ficus thonningii* extracts possess biological properties such as antioxidant [9, 10], antimicrobial [11], antihelmintic [12], antiprotozoal [13], antifungal [14], anti-psychotic [15] and anti-inflammatory [16].

Current treatment for cancer includes surgery, radiation therapy, chemotherapy, hormone therapy, among others, which are expensive and have many side effects, hence the growing need for new anticancer drugs that are more effective and less toxic. Cancer patients burdened with drug - induced toxicity are getting help from complementary and alternative medicines that are plant based [17].

Medicinal plants and natural products have significant roles in the prophylaxis and treatment of cancer through multiple therapeutic effects which include inhibition of cancer activating enzymes and hormones stimulation of DNA repair mechanism enhancing production of protective enzymes, antioxidant and immune boosting activities [18]. The bark of *Ficus thonningii* is useful in ethnomedicine for the treatment of dysentery, sore throat, cold, constipation, nose bleeding, wounds and the latex is useful for fever. The fibre and root are infused and taken orally to prevent abortion while the latex may also be instilled in the eye to treat cataract [6].

Ficus thonningii has been reported to contain alkaloids, flavonoids, terpenoids, tannins and saponins. It is also useful in the treatment of diabetes, arthritis, gastric discomfort, headache, asthmatic conditions, fever fungal infections and mental illness [11]. All parts of *F. thonningii* are medicinally useful, people prefer to use the leaves and bark which exudes latex, because latex has traditionally been associated with potency [19].

The aim of this study is to evaluate the phytochemical, chromatographic profile and antiproliferative effect of the aerial root of *F. thonningii* collected from Northern Nigeria. Antiproliferative effect was evaluated using rapidly growing *Sorghum bicolor* seed radicles.

MATERIALS AND METHODS

Chemicals and reagents

Unless otherwise stated all chemicals and reagents used were of analytical grade and purchased from Sigma Aldrich (Germany).

Experimental plants

Ficus thonningii leaves and aerial roots were collected from Kwandere, Nasarawa state, Nigeria. The plants were identified and authenticated by a taxonomist at the herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, where the voucher specimen was deposited.

Sorghum bicolor (Guinea corn) seeds were purchased from Karmo market, Abuja and identified and authenticated by a taxonomist of NIPRD Abuja. The seeds viability test was determined by placing them inside a beaker containing water, the seeds that floated were discarded while the totally submerged seeds were cleansed with methylated spirit and dried for usage [20, 21].

Plant preparation and extraction

The aerial roots of *F. thonningii* were air-dried at ambient temperature (28-30°C) for two weeks. The dried plant material was pulverized. Then 50 g of the powdered sample was weighed and extracted by hot water (1000 ml) infusion in air tight container for 24 h. The resultant mixture was filtered with filter paper (Whatman No. 1) under gravity [22]. The filtrate was dried at 80°C on a water bath to yield *F. thonningii* aerial root aqueous extract (FT) as brown residue.

Thin layer chromatography (TLC)

Thin layer chromatography was carried out on both the hexane and ethylacetate extracts using silica gel pre-coated glass plate. 2 g of the *Ficus thonningii* aerial root was extracted successfully with hexane and ethyl acetate at ambient temperature (28-30°C) for 24 h. Micro syringe was used to uniformly apply 10 µl of the extracts on the TLC plate and allowed to dry, β -sitosterol reference standard (Sigma) was spotted alongside as control. The plates were developed in a chromatographic tank using mobile phase comprising hexane and ethyl acetate (5:1) for the hexane extract and hexane and ethyl acetate (3:1) for the ethyl acetate extract. The developed plates were air-dried and visualized under ultraviolet light at 366 nm and iodine vapour tank. The retardation factor (R_f) for each component was calculated using the following formula:

$R_f = \text{Distance moved by the solute} / \text{Distance moved by the solvent front}$.

High performance liquid chromatography analysis

The bioactive constituents of FT were analyzed by high performance liquid chromatography (HPLC) with UV diode array detector (UV-DAD). The HPLC consisted of Ultra-Fast LC-20AB equipped with SIL-20AC auto-sampler; DGU-20A3 degasser; SPD-M20A UV-diode array detector; column oven CTO-20AC, system controller CBM-20Alite and Windows LC solution software (Shimadzu Corporation, Kyoto Japan); column, 5 µm VP-ODS C₁₈ and dimensions (4.6 x 150 mm). The chromatographic conditions included mobile phase: 0.2% v/v formic acid and acetonitrile (20:80); mode: isocratic; flow rate 0.6 ml/min; injection volume 10 µl of 100 mg/ml solution of extract in water; detection UV 254 nm. The HPLC operating conditions were programmed to give solvent B: 20%. Column oven temperature was 40°C. The total run time was 30 minutes. Flavonoids and phenolic acid standards such as apigenin, rutin, quercetin, caffeic acid, morin and ferulic acid were employed for the identification of the phytoconstituents of FT by comparing the retention time under similar experimental conditions [23].

Determination of growth inhibitory effects

The modified method of Okhale et al., 2017 [23] was used for this study. *Ficus thonningii* aerial root hot water extract FT (3200 mg) was dissolved in 100 ml of distilled water to obtain 32 mg/ml stock solution.

Various concentrations of FT were prepared (1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml, 16 mg/ml and 32 mg/ml). Methotrexate, an anticancer drug, was made to a concentration of 50 µg/ml as positive control. Petri dishes were layered with cotton wool and filter paper. Twenty seeds (20) of *Sorghum bicolor* were placed in each of the Petri dishes. The control was treated with 15 ml of distilled water (negative control) and methotrexate (50 µg/ml) respectively. They were made in duplicates (for each concentration, control and methotrexate two (2) Petri dishes were used) for the samples. The test seeds were treated with different preparations of FT as the seed in each specific Petri dish received 15 ml of a particular concentration (the seed in one set of Petri dishes were treated with 1 mg/ml concentration, seeds in another set of Petri dishes received 2 mg/ml, another received 4 mg/ml, another received 8 mg/ml, the next received 16 mg/ml, followed by 32 mg/ml and 64 mg/ml, 1mg/ml concentration was prepared in the first set of Petri dishes seeds, another set Petri dishes received 2 mg/ml, another received 4 mg/ml, another received 8mg/ml, the next received 16 mg/ml, followed by 32 mg/ml respectively). The seeds were incubated in a dark cupboard and observed for further growth after 24, 48, 72 and 96 h. The mean radicle lengths (mm) of the seeds were measured after 24, 48, 72 and 96h. The percentage inhibition was calculated as:

$$\frac{(\text{Mean radicle length of Control} - \text{Mean radicle length treated}) \times 100}{\text{Mean radicle length Control}}$$

Percentage growth was calculated as 100 - percentage inhibition. Percentage inhibition and percentage growth at 24, 48, 72 and 96 h for seed treated with 1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml, 16 mg/ml, 32 mg/ml of FT and the positive control methotrexate at 50 µg/ml are shown in Table 1.

Statistical analysis

The data obtained were expressed as mean ± standard error of mean and analyzed using GraphPad Prism (version 7.03).

RESULTS

Extraction of 50 g of *Ficus thonningii* aerial root powdered sample with hot water yielded 3.42g (6.84% w/w) of the dried extract (FT).

Thin layer chromatography (TLC)

The chromatogram of the hexane extract showed a total of 8 spots of which 5 were detected under ultraviolet light at 366 nm with R_f of 0.03 (pink), 0.07 (pink), 0.16 (pink), 0.25 (pink), 0.46 (white) and 3 of the spots were detected in iodine vapour with R_f of 0.29 (β-sitosterol), 0.51 and 0.77. The ethyl acetate extract showed 6 spots on TLC of which 5 were detected under ultraviolet light at 366 nm with R_f of 0.25 (pink), 0.38 (pink), 0.56 (pink), 0.68 (pink), 0.71 (white) and 1 of the spots was detected in iodine vapour with R_f of 0.45 (β-sitosterol).

High performance liquid chromatography analysis

From the HPLC chromatogram of FT ten peaks were detected as the bioactive constituents with retention time in minutes of 3.530, 3.928, 4.668, 6.706, 7.669, 8.727, 10.517, 12.475, 14.904 and 18.844. Compounds with retention time in minutes of 3.530, 3.928, 4.668, 6.706, 7.669 and 18.844 corresponded to gallic acid (33.95%), tannin (28.74%), caffeic acid (23%), rutin (5.25%), ferulic acid (3.18%) and morin respectively (Table 1 and Fig. 1).

Table 1. Chemical constituents of the hot aqueous extract of *Ficus thonningii* aerial root (FT) from HPLC analysis.

Peak number	Name	Ret. time (minute)	Peak area	Percentage composition*
1	Gallic acid	3.530	15543282	33.949887
2	Tannin	3.928	13158311	28.740595
3	Caffeic acid	4.668	10535024	23.007693
4	Rutin	6.706	2401990	5.2464653
5	Ferulic acid	7.669	1455720	3.1796071
6	ND	8.727	1572372	3.4344003
7	ND	10.517	822115	1.7956768
8	ND	12.457	235737	0.5149006
9	ND	14.904	38192	0.0834196
10	Morin	18.844	20272	0.0442784
Total			45783015	99.9969231

ND = Not Detected; * Percentage composition of each peak = Peak Area / Total Peak Area x 100

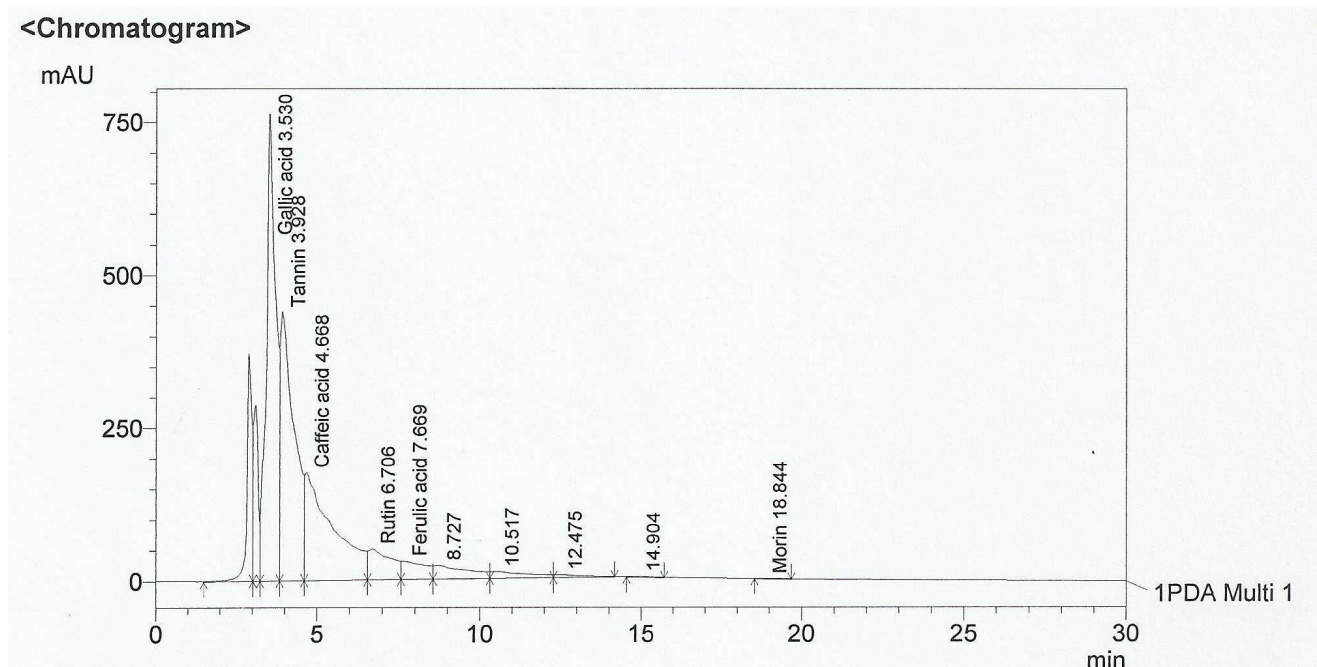


Figure 1. HPLC spectrum of hot aqueous extract of *Ficus thonningii* (FT) aerial root. Compounds with retention time in minutes of 3.530, 3.928, 4.668, 6.706, 7.669 and 18.844 corresponded to gallic acid (33.95%), tannin (28.74%), caffeic acid (23%), rutin (5.25%), ferulic acid (3.18%) and morin, respectively.

Growth inhibitory effect of FT on *Sorghum bicolor* seeds

There was appreciable and observable reduction in the radicle length of *Sorghum bicolor* seeds treated with various concentrations of the extract. The seed radical length increased over the incubation period of 24h-96h. There was an observable, progressive and rapid growth of the seed radical length in the negative control (distilled water). At 96h, the mean radicle length (mm) of the control seed was 96.10 ± 3.49 while the mean radicle length of the seeds treated with 1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml, 16 mg/ml and 32 mg/ml were 76.58 ± 1.37 , 72.75 ± 2.20 , 65.63 ± 3.52 , 44.95 ± 4.09 , 20.43 ± 1.49 , 4.450 ± 0.66 , respectively (Fig. 2) corresponding to percentage inhibition of 20.32%, 24.30%, 31.71%, 53.23%, 78.74% and 95.37% showing that the growth inhibitory effect of FT was concentration-dependent. Radicle lengths were measured at 24h, 48h, 72h and 96h. The negative control used was distilled water while the positive control was methotrexate (50 μ g/ml). Mean

radicle length, percentage inhibition and percentage growth at 24h, 48h, 72h and 96h for seeds treated with 1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml, 16 mg/ml and 32 mg/ml of FT as well as negative control (H₂O) and positive control (methotrexate) 50 µg/ml as shown in Table 1.

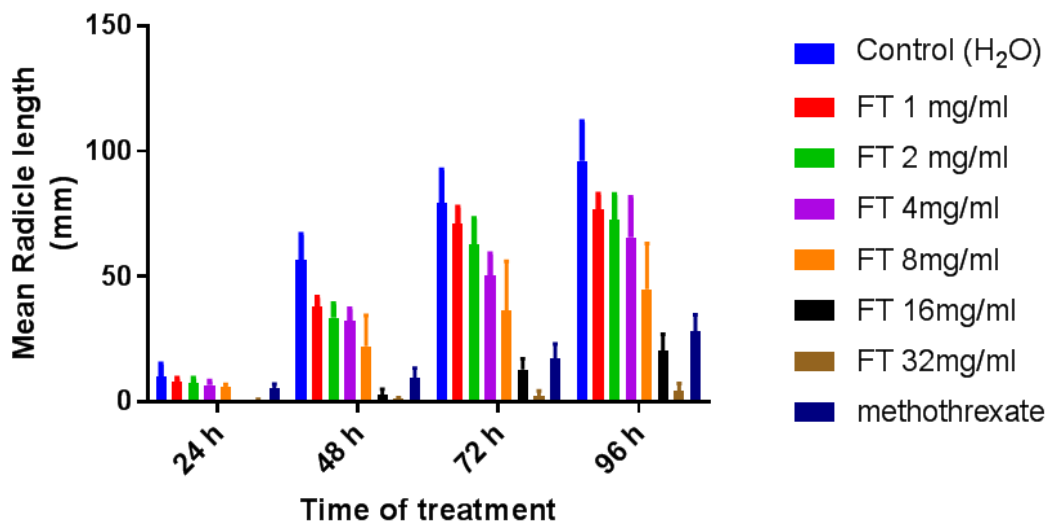


Figure 2. Growth inhibitory effects of *Ficus thonningii* aerial root aqueous extract (FT) on *Sorghum bicolor* seed radical.

Table 1. Mean radicle length, percentage inhibition and percentage growth for *Sorghum bicolor* seeds treated with FT.

Treatment	Mean radicle length (mm)				% Inhibition*				% Growth†			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control (H ₂ O)	10.32±1.07	56.65±2.25	79.33±2.95	96.10±3.47	0	0	0	0	100	100	100	100
Methotrexate	5.600±0.35	9.775±0.84	17.25±1.33	28.23±1.48	45.74	82.74	78.26	70.62	54.26	17.26	21.74	29.38
FT (1 mg/ml)	7.925±0.34	37.93±0.84	71.15±1.43	76.58±1.37	23.21	33.05	10.31	20.31	76.79	66.95	89.69	79.69
FT (2 mg/ml)	7.500±0.43	33.70±1.19	62.90±2.27	72.75±2.20	27.33	40.51	20.71	24.30	72.67	59.49	79.29	75.70
FT (4 mg/ml)	6.750±0.31	32.43±1.04	50.38±1.90	65.63±3.52	34.59	42.75	36.49	31.71	65.41	57.25	63.51	68.29
FT (8 mg/ml)	6.025±0.25	22.35±2.72	36.28±4.44	44.95±4.09	41.62	60.55	54.27	53.23	58.38	39.45	45.73	46.77
FT (16 mg/ml)	1.000±0.00	3.050±0.48	12.86±1.00	20.43±1.49	90.31	94.62	83.79	78.74	9.690	5.380	16.21	21.26
FT (32 mg/ml)	0.950±0.05	1.350±0.11	2.425±0.44	4.450±0.66	90.79	97.62	96.94	95.37	9.210	2.380	3.060	4.630

*Percentage Inhibition = [(mean radicle length of control - mean radicle length of treated) / mean radicle length of control] x 100. †Percentage growth = 100 - percentage inhibition, n = 20. p<0.0001

DISCUSSION

One distinguishing feature between cancer cells and normal body cells is the ability of cancerous cells to proliferate without responding to cell feedback mechanism or apoptotic mechanisms that regulate cell death, which is replicated by meristematic stems of seeds and hence the choice of *Sorghum bicolor*. Under favourable environmental conditions meristematic cells of *Sorghum bicolor* seeds retain ability to proliferate similar to cancer cells [24]. The extractive value obtained for hot aqueous extract of *Ficus thonningii* aerial root (FT) was 6.84%. Thin layer chromatography of the hexane and ethyl acetate extracts of *Ficus thonningii* aerial root revealed the presence of β -sitosterol. HPLC chromatogram of FT revealed gallic acid, tannin, caffeic acid, rutin and morin. Gallic acid and its derivatives have been implicated as antimutagenic, anticarcinogenic, antiangiogenic, antimicrobial and anti-inflammatory agents [25]. Gallic acid is present in almost every part of plants such as roots, seeds, bark, wood and leaf and is also a known antioxidant [26]. Tannins are polyphenolic

compounds of high molecular weight found in roots, stems, barks and outer layers of plant tissues [27]. They possess antioxidant, antitumour and antibacterial activities [28]. Tannin had been reported to have anticancer properties as in maplexin A-1 in red maples [29], cuphiin DI [30], ellagitannins [31]. Corilagin, tannin, inhibits growth of ovarian cancer cell lines [32]; tannic acids also prevent the activation of PARP-1, thereby preventing doxorubicin-induced cell death [33]. Cancer can be linked to oxidative stress [34]. Caffeic acid (3,4-dihydrocinnamic acid) is a polyphenolic acid possessing antioxidant and anticancer properties [35]. Plant phenolic such as morin, tannins, ferulic and caffeic acids serve as potent antioxidants [36]. Rutin, a polyphenol, is implicated in cytoprotection, antioxidant, anticarcinogenic cardioprotective and neuroprotective effects [37]. The bark of aerial roots of *Ficus elastica* (Moraceae) had growth inhibitory activity against the human A549 lung cancer cell line [38]. Aerial root of *Ficus microcarpa* had been reported to contain compounds with anticancer activities [39]. *Ficus thonningii* stem bark and aerial root are used as cancer remedy in Northern Nigeria [40]. This study provided preliminary scientific support for folkloric use of *Ficus thonningii* aerial root as anticancer agent.

CONCLUSION

The hot aqueous aerial root extract of *Ficus thonningii* exhibited antiproliferative activity on the fast proliferating meristematic cells of *Sorghum bicolor* and hence can be said to be potential inhibitor of cancerous growth. This claim may be attributed to antioxidant rich secondary metabolites such as gallic acid, tannin, ferulic acid, rutin and morin which have been reported to possess anticancer potentials.

AUTHOR'S CONTRIBUTION

MOA wrote the initial draft of the manuscript; SEO designed and supervised the study; SFA assisted with the antiproliferative evaluation; UOE wrote the final draft of the manuscript and did the statistical analysis; SEO proof read, and edited the word. All authors were involved in the execution of the research plan. The final manuscript was read and approved by all authors.

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