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Original article

A sulfated polyphenols-rich extract from *Sabal yapa* exhibits antitumor activities in Ehrlich ascites carcinoma

Abdelgawad A. Fahmi [a], Mohamed A. El Raey [b,*], Abeer Y. Ibrahim [c], Mohamed A.O. Abdelfattah [d], Abeer M Abdelmageed [a], Mansour Sobeh [e,*]

[a] *Chemistry Department, Faculty of Science, Cairo University, Egypt*
[b] *Department of Phytochemistry and Plant Systematics, Pharmaceutical Division, National Research Centre, Dokki, Cairo 12622, Egypt*
[c] *Medicinal and Aromatic Plants Research Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki, Giza 12622, Egypt*
[d] *College of Engineering and Technology, American University of the Middle East, Kuwait*
[e] *AgroBioSciences Program, University Mohammed VI Polytechnic, Lot 660, Hay Moulay Rachid, 43150 Benguerir, Morocco*

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ABSTRACT

Cancer is the second leading cause of mortality accounting for one in every six deaths globally. Plant secondary metabolites, among them polyphenols, represent an effective and much safer alternative approach to the currently available medications. In this work, utilizing LC-MS/MS, we characterized the constituents of *S. yapa* leaves extract and evaluated its antioxidant and anticancer properties. In total, 34 secondary metabolites, mainly flavonoids (Tricin, luteolin, and apigenin and their glucosides as well as sulfated derivatives) were identified. The extract manifested substantial antioxidant activity in DPPH assay, and high total phenolic content determined by Folin Ciocalteu method. The extract was safe up to 4800 mg/kg b.wt. when administered orally in mice and neither affected the hematological parameters nor the liver enzyme levels at the studied dose (LD_{50}, 480 mg, kg b.wt.). In the treated animals, the extract surpassed the reference drug (5-flouro uracil) and significantly reduced the tumor volume and weight by 71.50 and 85.46%, respectively, increased the median survival time to 53.2 days and the lifespan by 116%. The extract improved all the hematological parameters, where it increased the hemoglobin (Hb) concentration, red blood cell (RBC) count, packed cell volume (PVC) and platelets by 58.21, 8.98, 9.89 and 120%, respectively, compared to the untreated EAC bearing animals. Additionally, the extract significantly declined the elevated levels of ALT and AST enzymes by 29.18% and 59.88%, respectively. In molecular docking, the annotated flavonoids displayed appreciable binding affinities to the active sites of VEGFR1 and VEGFR2. In conclusion, *Saba yapa* is a promising plant that can be introduced to further advanced clinical studies for the development of novel anticancer drugs with lower side effects.

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1. Introduction

Following cardiovascular diseases, cancer comes as the second leading cause of mortality accounting for one in every six deaths globally. According to recent statistics, cancer resulted in over 9 million death cases during 2018, 70% of which were recorded in low- and middle-income countries (Bray et al., 2018). The disease is characterized by rapid uncontrolled formation of abnormal cell masses that grow beyond the usual boundaries. These malicious cells can then invade other adjoining parts of the body and spread to distant organs in a process known as metastases (Hanahan and Weinberg, 2000).

Cancer etiology involves a series of mutations occurring successively in the proto-oncogenes that regulate and control the cell cycle phases including cell growth, division, and proliferation. Carcinogens such as different hazardous chemicals, pollutants, pathogens, and radiations stand as the main culprit of the cancer-initiating gene mutations (Seto et al., 2010).

Apart from the primary surgical procedure and radiotherapy, which are limited to certain tumor types and patient's conditions, the systematic drug approach comprising either standard or

* Corresponding authors.
E-mail addresses: elraiy@gmail.com (M.A. El Raey), mansour.sobeh@um6p.ma (M. Sobeh).

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metronomic chemotherapy is the most common and widespread cancer treatment strategy. However, this approach is associated with several adverse effects that, in many cases, limit the continuation of the therapy (Baskar et al., 2012). In this regard, some isolated plant secondary metabolites could represent an effective and much safer alternative.

Flavonoids represent one of the most renowned classes of polyphenols and found in high concentrations in the edible plant parts of many vegetables and fruits (Symonowicz and Kolanek, 2012). Based on their chemical structure, flavonoids are classified into the major classes flavanols, flavonols, flavanones, flavones, isoflavones, and anthocyanins in addition to many subclasses. A wide array of pharmacological activities has been reported for this class of compounds including anti-inflammatory, antioxidant, anti-aging, antimicrobial, as well as anti-cancer and cytotoxic properties (Brodowska, 2017). The flavonoids apigenin, genistein and 3-hydroxyflavone were reported to act as chemopreventive agents as they in vitro inhibited angiogenesis and consequently suppress the proliferation of tumor and endothelial cells (Kim, 2003).

The genus *Sabal*, family Arecaceae, comprises 17 species and is endemic to the new world; however, it is currently cultivated worldwide. *Sabal* species demonstrated different biological activities. For instance, *S. serrulatum* is used for managing and preventing prostate hyperplasia and nonbacterial prostatitis (Olennikov et al., 2013). In a study by (Ibrahim et al., 2017), *S. palmetto* extract and its fractions demonstrated substantial anti-inflammatory and anti-cancer activities.

Sabal yapa C. Wright ex Becc. is an evergreen palm that is cultivated in Egypt. Successive solvent extracts from the fruits exhibited promising antiandrogenic activities in castrated rats in which the non-polar extract reduced the prostate weight and plasma testosterone level and did not change the plasma levels of creatinine, aspartate transaminase and alanine transaminase. These activities were attributed the presence of several fatty acids, among them lauric, oleic and linolenic acids (Ammar et al., 2013). Another study explored anti-cancer activities of three sabal species from Egypt against glioblastoma and prostate cancer cell lines and profiled their chemical composition utilizing LC-MS (El-Hawary et al., 2020a).

In the present work, the chemical composition of a hydroalcoholic extract from *S. yapa* leaves was explored using HPLC-MS/MS. In addition, the antioxidant properties and the anticancer activities were investigated *in vivo* using Ehrlich ascites carcinoma (EAC). Several parameters including tumor volume and weight, viable and nonviable tumor cell count, median survival time and percentage increase in lifespan, liver enzymes activities and hematological parameters were investigated. Furthermore, an *in-silico* docking study was used to evaluate the potential of the mainly identified flavonoids in the extract to interfere with angiogenesis via blocking the vascular endothelial growth factor B receptors; VEGFR1 and VEGFR2.

2. Materials and methods

2.1. Plant material and extraction

Sabal yapa fresh leaves were collected during April 2016 from El-Orman botanical garden, Cairo, Egypt. The leaves (200 g) were air-dried at room temperature, coarsely powdered and extracted using 70% ethanol (3×1 L). The combined extracts were concentrated under reduced vacuum and lyophilized yielding a dry fine powder (16 g) with an extraction yield of 8%. The lyophilized powder was kept at -20 °C for biological and phytochemical evaluations.

2.2. LC-MS/Ms

Phytochemical analysis of *S. yapa* extract was performed using a ThermoFinnigan LC system (ThermoElectron Corporation, Austin, TX, USA) that was controlled by Xcalibur software (Xcalibur™ 2.0.7, Thermo Scientific, Waltham, MA, USA). MS operating parameters in the negative mode were set up as described in El-Hawary et al. (2020b).

2.3. Total phenolic content (TPC) and radical scavenging potential

TPC was amounted using Folin Ciocalteu method as described in Ghareeb et al. (2018) and the radical α, α-diphenyl-β-picrylhydrazyl (DPPH) scavenging potential was evaluated as detailed in Ghareeb et al. (2018).

2.4. In vivo anti-tumor activity

2.4.1. Ethicals statement

Animal experiments were carried out with strict adherence to the ethical policies and procedures approved by the Medical Research Ethics Committee for animal care and use, National Research Centre, Egypt, registration No. 6/014.

2.4.2. Acute toxicity study

Female albino mice (n = 8) were treated orally with different doses of *S. yapa* extract. The dosing pattern started with 500 mg/kg body weight (b.wt.) and increased, at a rate of 500 mg/ kg, up to a maximum dose of 6000 mg/kg b.wt. The control group received only normal saline (Bruce, 1985). The LD_{50} value was calculated using BioStat program (BioStat 2009 Build 5.8.4.3) and was recorded to be 4800 mg/kg b.wt. Therefore, the selected dose to study the *in-vivo* antitumor activity of the extract was 480 mg/kg b.wt./day as the $1/10^{th}$ of the LD_{50} according to (Ghosh, 1984).

2.4.3. Anti-tumor activity of the extract in Ehrlich ascites carcinoma model

Ehrlich ascites carcinoma (EAC) cells were used at a concentration of 2×10^6 cell/ mouse and were obtained from National Cancer Institute, Cairo, Egypt. Female albino mice (20 to 25 g) were obtained from the animal house of National Research Centre, Cairo, Egypt. Animals were kept in standard polypropylene cages, fed on standard diet and water *ad libitum*, maintained under normal room conditions (temperature of 25–30 °C and 60–65% relative humidity) for one week before experimental period for adaptation. Mice were classified into five groups, 14 animals each.

Group I: mice received the vehicle (saline solution) orally for 10 consecutive days and served as the negative control group. Group II: mice treated orally with the extract at a dose of 480 mg/kg b.wt. for 10 consecutive days and served as the extract control group. Group III: mice injected with EAC (2×10^6 cell/ mouse), incubated for 24 h, then received saline orally for 10 consecutive days and served as the tumor-bearing group. Group IV: mice injected with EAC (2×10^6 cell/ mouse), incubated for 24 h, then received orally the reference drug, 5-fluorouracil in the recommended dose (20 mg/kg b.wt./day) for 10 consecutive days. Group V: mice injected with EAC (2×10^6 cell/ mouse), incubated for 24 h, then received orally *S. yapa* extract at a dose of 480 mg/kg b.wt. for 10 consecutive days and served as the extract treated group. At the end of the experiment and after fasting overnight, mice were anesthetized by an injection of ketamine (87 mg/kg b.wt.) and xylazine (13 mg/kg b.wt.). The two drugs were dissolved in normal saline, and each mouse received 0.2 mL/100 g body weight (Van Pelt, 1977). Animals were sacrificed after anesthesia, blood samples were collected from eye blood vein from six mice/ group only for estimation of hematological parameters. Eight mice were kept

alive to check the increase in lifespan of the tumor-bearing hosts (Sivakumar et al., 2005).

2.4.4. Determination of tumor volume and weight

Ascitic fluids from the animals' peritoneal cavity were collected in a graduated centrifuge tube and the fluids' volume and weight were measured immediately (Karmakar et al., 2013).

2.4.5. Estimation of viable and nonviable tumor cell count

The ascitic fluid was placed in white blood cell pipette and diluted 100 times. A drop of the diluted suspension was placed in the Neubauer counting chamber to stain the cells with trypan blue (0.4% in normal saline) dye for distinguishing the viable cells (that were not stained) from the nonviable ones. Both cell counts were estimated by the following equation (Karmakar et al., 2013):

$$\text{Cell Count} = \text{Number of cell} \times \text{dilutionArea} \times \text{film thickness}$$

2.4.6. Determination of percentage increase in lifespan and median survival time

The percentage increase in the lifespan and median survival time of the treated animals were recorded for monitoring of mortality, by the following formula:

Increase in lifespan% = $(T - C/C) \times 100$; Where T is the number of days survived by the treated animals, and C is the number of days control mice survived (Karmakar et al., 2013).

2.4.7. Determination of solid tumor volume

The solid tumor in mice was estimated according to Kuttan et al., (1990). The tumor mass was measured starting the 11th day following the tumor induction. The measurement was carried out every five days for 30 days. The volume of tumor mass was estimated using the following formula:

$$V = 4/3 \times \pi r^2$$

where r is the mean of r_1 and r_2, which are independent radii of the tumor mass.

2.4.8. Measurement of hematological parameters

Collected blood samples were used for determining the hematological parameters according to Dacie and Lewis (1975), including hemoglobin, total leukocyte count, red blood cell count, packed cell volume, platelet count, and differential white blood cell.

2.4.9. Estimation of liver enzymes activities

The liver enzymes AST and ALT activities were spectrophotometrically measured in sera according to Reitman and Frankel (1957).

2.4.10. Molecular docking

The major identified flavonoids in *S. yapa* extract were docked to the vascular epithelial growth factor B receptors; VEGFR1 and VEGFR2 that are associated with the activation and regulation mechanisms of the angiogenesis. The compounds' chemical scaffolds were drawn using the builder facility of molecular operating environment (MOE), 2013.08; Chemical Computing Group Inc., Montreal, QC, Canada, H3A 2R7, 2016. The compounds were set to their ionized state, and finally energy minimized by the aid of the molecular mechanic force-field mmff94x. The crystal structures of VEGFR1 (pdb ID: 3HNG) and VEGFR2 (pdb ID: 3EWH) with the corresponding bound inhibitor were downloaded from the protein data bank (www.pdb.org). The protein structures were prepared by adding the hydrogen atoms, assigning their protonation state and geometry. Running the docking rounds was done using the default triangle matcher placement method and London dG scoring function. Docking poses were refined by force-field energy minimization using London dG scoring function. The best three docking poses showing the minimal estimated binding free energy were virtually analyzed in terms of the interactions afforded between the amino acid residues in the binding site and the docked compounds to judge their ability to iterate the reported interactions of the reference inhibitors.

2.4.11. Statistical analysis

Data was recorded as mean ± SD and analyzed by the one-way ANOVA using IBM SPSS statistics program (version 23). $P < 0.05$ was considered as significant difference.

3. Results

3.1. LC-MS/MS analysis of S. Yapa leaves crude extract

Altogether, thirty-four compounds, mainly flavonoids and their sulphated derivatives as well as flavolignans, were identified in the hydroalcoholic extract of *S. yapa* leaves. The identification was based on molecular weight, MS^2 fragmentation, in-house library, available authentic compounds and literature (Table 1 and Fig. 1).

The *O*-methylated flavone, tricin, along with 9 hexosides and sulphated derivatives were characterized in the extract (Table 1). They showed $[M-H]^-$ at *m/z* 767, 737, 678, 571, 491, 409 and a molecular ion peak at 329; they were annotated as tricin 4′-*O*-(*erythro*-β-4 guaiacylglyceryl ether) 7-*O*- glucoside sulphate, tricin 4′-*O*-(*erythro*-β-4 hydroxyphenylglyceryl ether) 7-*O*-glucoside sulphate, tricin 4′-*O*-(*erythro*-β-4 guaiacylglyceryl ether) 7-*O*- glucopyranoside, tricin 7-*O*-glucoside-5-sulphate, tricin 7-*O*-glucoside and tricin *O*-sulphate. The corresponding retention times and fragmentation patterns are presented in Table 1 and some representative MS/MS spectra are shown in Fig. 2

Several luteolin and apigenin derivatives were also characterized along with *p*-hydroxybenzoic acid, chlorogenic acid, catechin and its isomer epicatechin, malic and quinic acids, their retention times and fragmentation pattern are displayed in Table 1. Moreover, two compounds showed a molecular ion peak at $[M–H]^-$ *m/z* 503 and three daughter ions at 197, 305, and 423; they were tentatively assigned as (epi) gallocatechin phenylacetyl sulphate, Table 1.

3.2. Antioxidant properties

The hydroalcoholic extract of *S. yapa* leaves demonstrated considerable antiradical potential against DPPH radicals (IC_{50} = 6.25 - μg/mL) when compared to the reference compound, vitamin C (IC_{50} = 3.12 μg/mL). This efficacy could be attributed to its high phenolic content, which was assayed by Folin Ciocalteu method; it represented 312 mg gallic acid equivalents/ g extract. The obtained results come in agreement with numerous plant extracts rich in polyphenols (Sobeh et al., 2019, 2020; El-Hawary et al., 2020a).

3.3. Anti-tumor properties of S. Yapa leaves extract

3.3.1. Tumor volume and weight, median survival time and lifespan

Following the carcinoma cell injection, the tumor volume and weight were significantly increased. Consequently, the lifespan of the tumor bearing mice was decreased to 20.00 ± 0.18 days. The extract significantly reduced the tumor volume and tumor weight by 71.50 and 85.46%, respectively, which in turn increased the median survival time to 53.2 day, which represents 116% increase

Table 1
Tentative identification of polyphenolics of *S. yapa* extract.

No.	R_t	$[M-H]^-$	MS^2 (*m*/*z*)	Tentatively Identified Compounds	References
1	1.47	191		Quinic acid	El-Hawary et al. (2020b)
2	2.21	133		Malic acid	Sobeh et al. (2017)
3	5.32	287	125, 241	Flavanol	
4	11.33	137	93	*p*-hydroxybenzoic acid	Ţigu et al. (2021)
5	14.16	353	191, 179	Chlorogenic acid	Ţigu et al. (2021)
6	15.18	289	245, 205, 179	Epicatechin	Sobeh et al. (2017)
7	16.69	515	357, 405	Pinoresinol derivative	
8	19.08	503	423, 305, 197	(epi)Gallocatechin phenylacetyl sulphate	
9	21.13	289	245, 205, 179	Catechin	Sobeh et al. (2017)
10	21.67	449	287, 151, 135	Eriodictyol 7-*O*-glucoside	Kachlicki et al. (2008)
11	21.83	593	473, 413, 383, 353	Apigenin 6,8-di-*C*-glucoside (Vicenin-2)	Ahmed et al. (2019)
12	23.08	503	423, 305, 197	(epi)Gallocatechin phenylacetyl sulphate	
13	24.70	643	563, 515, 443	Schaftoside sulphate*	
14	25.78	563	503, 413, 383, 353	Apigenin 6-*C*-glucosyl-8-*C*-xyloside (Schaftoside)	Sun, et al. (2013)
15	26.53	527	447, 429, 357, 327	Orientin sulphate	Ahmed et al. (2019)
16	27.79	447	357, 327, 285	Luteolin 8-*C*-glucoside (Orientin)	Ahmed et al. (2019)
17	29.58	563	443, 383, 353	Apigenin 6,8-di-*C*-glucoside (Vicenin-1)	Ahmed et al. (2019)
18	31.08	431	431, 341, 311, 269	Apigenin 6-*C*-glucoside (Isovitexin)	Ahmed et al. (2019)
19	32.84	463	343, 327, 301	Quercetin 3-*O*-glucoside	Sobeh et al. (2017)
20	33.00	447	357, 327, 285	Luteolin 7-*O*-glucoside	Ahmed et al. (2019)
21	34.43	461	371, 341	Diosmentin 8-*C*-glucoside	Ahmed et al. (2019)
22	37.15	497	417	Naringenin rhamnoside sulphate	Kachlicki et al. (2008)
23	37.93	571	491, 329, 371	Tricin 7-*O*-glucoside-5-sulphate	Ahmed et al. (2019)
24	38.18	477	315, 271, 151	Isorhamnetin 3-*O*-glucoside	El-Hawary et al. (2020b)
25	39.91	491	371, 329	Tricin 7-*O*-glucoside	Ahmed et al. (2019)
26	41.46	737	657, 567, 495, 329	Tricin 4′-*O*-(*erythro*-β-4 hydroxyphenylglyceryl ether) 7-*O*-glucoside sulphate	
27	42.86	767	687, 567, 525, 329	Tricin 4′-*O*-(*erythro*-β-4 guaiacylglyceryl ether) 7-*O*- glucoside sulphate	Ahmed et al. (2019)
29	43.29	767	687, 567, 525, 329	Tricin 4′-*O*-(*threo*-β-4 guaiacylglyceryl ether) 7-*O*- glucoside sulphate	Ahmed et al. (2019)
30	44.17	687	525, 329	Tricin 4′-*O*-(*erythro*-β-4 guaiacylglyceryl ether) 7-*O*-glucopyranoside	
31	44.66	737	657, 567, 495, 329	Tricin 4′-*O*-(*threo*-β-4-hydroxyphenylglyceryl ether) 7-*O*-glucoside sulphate	
32	46.59	687	525, 329	Tricin 4′-*O*-(*threo*-β-4 guaiacylglyceryl ether) 7-*O*-glucopyranoside	
33	50.52	409	329, 311, 295	Tricin -*O*- sulphate*	
34	58.59	329	314, 299, 285	Tricin	El-Hawary et al. (2020b)

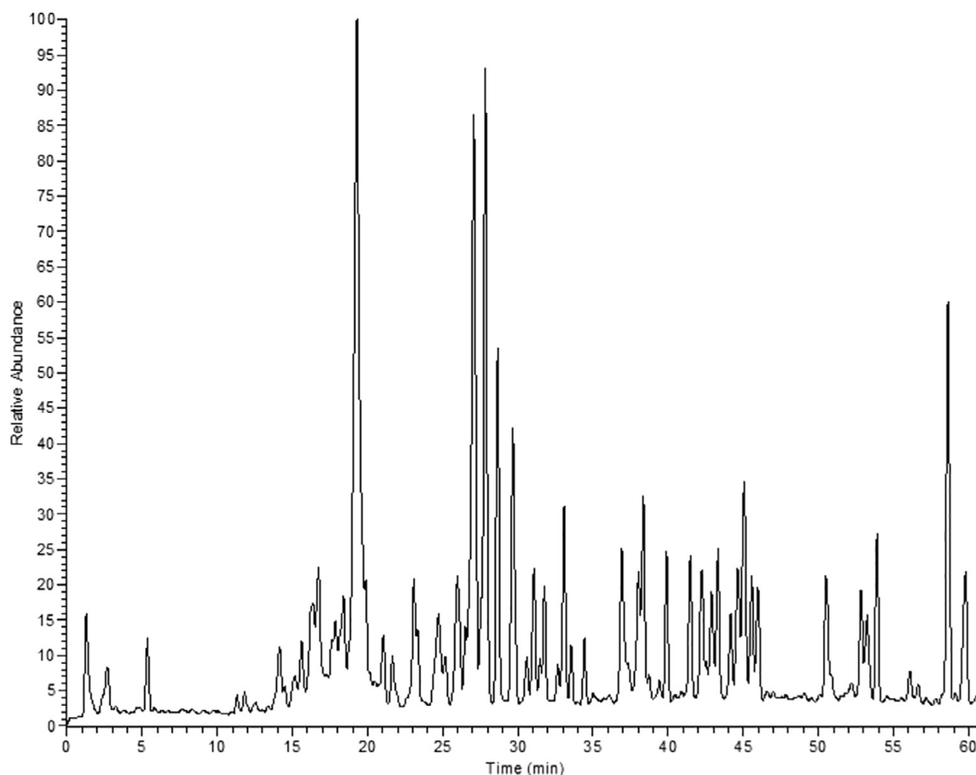

Fig. 1. LC -MS profile of the hydroalcoholic extract of *S. yapa* leaves.

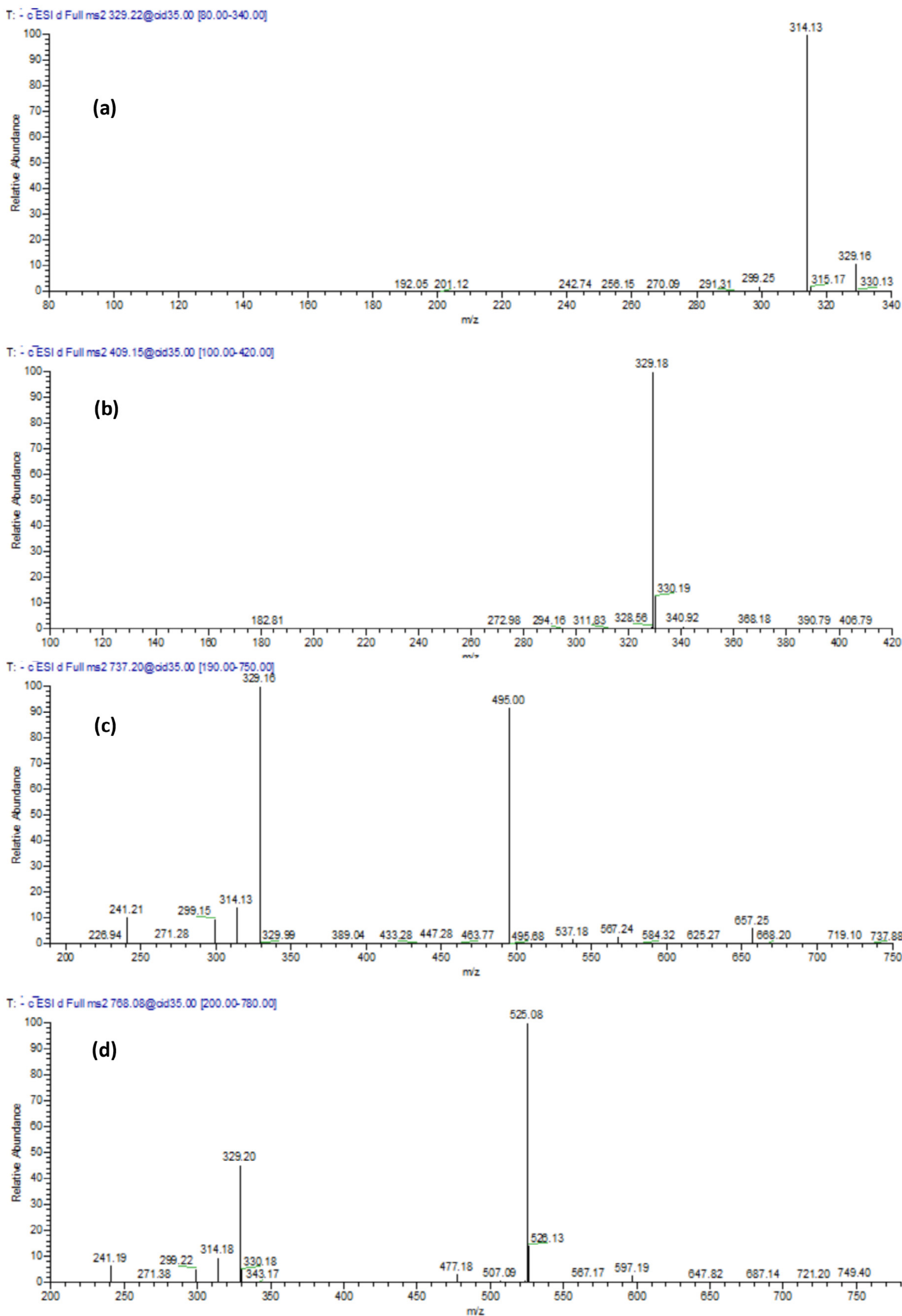

Fig. 2. MS/MS spectra of some identified compounds in the extract; (a) Tricin, (b) Tricin *O*-sulphate, (c) Tricin 4′-*O*-(*erythro*-β-4 hydroxyphenylglyceryl ether) 7-*O*-glucoside sulphate, (d) Tricin 4′-*O*-(*erythro*-β-4 guaiacylglyceryl ether) 7-*O*- glucoside sulphate.

Table 2
Effects of *S. yapa* leaves extract on survival parameters of bearing mice.

Parameters	Groups		
	Tumor bearing	FU-treated (20 mg/kg/ day)	Extract treated (480 mg/kg/day)
Tumor volume (cm^3)	3.00 ± 0.28	1.52 ± 0.16*	0.855 ± 0.028*
% change		−49.33%	−71.50%
Tumor weight	3.59 ± 0.66	0.73 ± 0.15*	0.522 ± 0.066*
% change		−79.67%	−85.46%
MST (day)	20 ± 0.18	41 ± 0.84*	53.2 ± 0.142*
Increase in lifespan %	–	105 ± 0.45	116 ± 0.817

Data are presented as mean of 5 animals ± SD followed with increasing or decreasing percentage as compared to tumor bearing mice. MST: median survival time. One-way ANOVA was used for data analysis followed by post Hoc test for multiple comparisons (n = 5, $p < 0.05$). Treated groups were compared to bearing group (*).

in the lifespan. This is considered a significant increment compared to the reference drug, 5-flouro uracil**,** Table 2.

3.3.2. Viable and nonviable tumor cells count

After 10 days, the extract significantly reduced the carcinoma cells count in the tumor-bearing group by 71.50% with more pronounced efficiency than that of the reference drug (51.40%). These observed effects were reflected by the great suppression of the viable cells' total number by 91.83% and the rise of the nonviable cells' count by 154.87%, Table 3.

3.3.3. Hematological parameters

The ameliorative effects of *S. yapa* extract on tumor cell count was also confirmed by the measured hematological parameters, where the extract significantly reduced the deleterious effects of carcinoma cells on all these parameters. The extract significantly increased hemoglobin concentration (Hb), red blood cell count (RBC's count), packed cell volume (PVC) and platelets by 58.21, 8.98, 9.89 and 120%, respectively, compared to the tumor-bearing mice group, Table 4**.**

The extract's recovery effects were also extended to improve the deferential white blood cells status, especially neutrophils and lymphocytes. The extract reduced the elevated neutrophils by 28.75% and increased the lymphocytes by 70.34%, Table 4. The same assessments were also conducted for the positive control group that received the extract only to determine any regression effect on animals as a part of safety profile. All recorded parameters proved a good safety margin for the extract (Table 4).

Table 3
Effects *S. yapa* leaves extract on viable and non-viable tumor cell count.

Parameter	Groups		
	Tumor bearing	FU-treated	Extract treated
Total cell count (x 10^7 cell/ml)	10 ± 1.13	4.86 ± 0.87*	2.85 ± 0.50*
Change (%)		−51.40%	−71.50%
Viable cell count (x 10^7 cell/ ml)	9.18 ± 1.10	1.41 ± 0.69*	0.75 ± 0.39*
Change (%)		−84.64%	−91.83%
Non-viable cell count (x 10^7 cell/ml)	0.82 ± 0.45	3.45 ± 0.61*	2.10 ± 0.53*
Change (%)		+ 320.73%	154.87%
Viable cell %	97.23 ± 1.64	18.00 ± 0.71*	26.32 ± 0.56*
Non-viable cell %	2.77 ± 0.95	82.00 ± 0.68*	73.68 ± 0.78*

Data are presented as mean ± SD followed with decreasing or increasing percentage. One-way ANOVA was used for data analysis followed by post Hoc test for multiple comparisons (n = 5, $p < 0.05$). *Significantly different as compared to bearing mice.

3.3.4. Liver function parameters

Because of the toxic effect of the carcinoma cells injection, the liver enzymes (AST and ALT) were significantly elevated in the tumor-bearing mice. Administration of the extract significantly reduced the elevated enzyme levels by 29.18% and 59.88% for ALT and AST, respectively. Similar effects were obtained from the standard drug 5-fluorouracil. Noteworthy, *S. yapa* extract did not affect liver enzymes in the extract positive control group with respect to the negative control group (Table 5).

3.3.5. Molecular docking

Generation of blood vessels through pathological angiogenesis is a critical event for sustaining chronic inflammation and tumor progression. Induction of angiogenesis is mediated through the vascular endothelial growth factor (VEGF) family of cytokines, which exert their biological roles through activation of three tyrosine kinase receptors; namely VEGFR1, VEGFR2, and VEGFR3 (Otrock et al. 2007). Some flavonoids such as genistein, apigenin, and jaceidin were reported to block the angiogenesis cascade via interfering with VEGF signaling (Kim et al. 2003, Elhady et al., 2020). In the current work, the flavonoids identified *S. yapa* extract were docked to VEGFR1 and VEGFR2 proteins, whose structures were co-crystallized with bound chemical inhibitors, to evaluate their inhibitory potential on VEGF receptors. Docking poses of these flavonoids revealed their ability to bind to the active sites of VEGFR1 and VEGFR2 with appreciable estimated free binding energy reflected by lower docking score values relative to that of the co-crystalized inhibitor (Table 6).

In general, flavonoids of *S. yapa* showed better binding to VEGFR2, which is reflected by lower binding energy values (Table 6). *O*- and *C*-glucoside derivatives showed to be more efficient towards both receptors relative to their free aglycone congeners, most probably due to higher number of hydroxyl groups engaged in more H-bonding interactions with amino acid residues of the active sites. Vicenin 2 showed the best binding to VEGFR1 with an estimated binding energy of −19.37 kcal/mol. Orientin, on the other hand, showed the least binding energy (−24.12 kcal/mol) upon binding to VEGFR2. The sulfated derivatives, however, surpassed all the other docked compounds towards both receptors, where the sulphate group was involved in extra interactions with Lys861 in the active site of VEGFR1 and Lys868 in that of VEGFR2. The most efficient member was schaftoside sulfate that scored a free binding energy of −20.99 kcal/mol and −28.85 on VEGFR1 and VEGFR2, respectively. It iterated the major amino acid interactions reported for the co-crystallized inhibitors of both target proteins; namely the interactions with Asp1040, Glu878, and Leu882 in VEGFR1 and Asp1046 and Glu885 in VEGFR2 active sites. (Fig. 3).

4. Discussion

The polyphenolic constituents of *S. yapa* were comprehensively characterized for the first time yielding thirty-four compounds. They included flavonoids, flavonoid C-glycosides, flavonoid *O*-glycosides, flavolignans and flavonoid sulphate derivatives. The studied *S. yapa* extract exhibited antioxidant activities in vitro and anti-tumor properties in Ehrlich ascites carcinoma (EAC) model. Ehrlich ascites carcinoma is a spontaneous murine mammary adenocarcinoma. It lacks H-2 histocompatibility antigens; therefore, it proliferates rapidly in most mouse hosts and stands as good model to evaluate anti-tumor activity of newly developed drug candidates.

The extract prolonged the lifespan, ameliorated the WBC count, and showed a noticeable reduction of the viable EAC cells in the treated tumor bearing mice, which all points out its role in reducing the nutritional fluid volume and the source of tumor growth. In

Table 4
Potential effect of *S. yapa* leaves extract on hematological parameters in Ehrlich ascites carcinoma bearing mice.

Parameter	Hb conc	RBC's Count	PCV	Platelets count	WBC x 1000 mm^3	Deferential white blood cells				
	mg/dl	X million/ mm^3	mm	X 1000/mm^3		Neutrophil	Lymphocytes	Monocytes	Eosinophil	Basophiles
Normal groups										
Normal control	11.5 ± 1.10	11.12 ± 1.00	41.12 ± 1.25	300 ± 1.41	7 ± 0.98	25.63 ± 0.69	65 ± 1.02	3.41 ± 1.24	4 ± 0.78	1.96 ± 0.30
S. yapa control group	13 ± 1.11	10.65 ± 1.16	46 ± 0.97	296.67 ± 0.79	6.8 ± 0.28	28 ± 1.17	65 ± 1.75	3 ± 0.21	2 ± 0.10	2 ± 0.14
Ehrlich ascites carcinoma bearing mice										
Tumor bearing mice	6.53 ± 0.96**	7.24 ± 1.11**	31.32 ± 1.52**	100 ± 1.61**	11.41 ± 1.00**	60 ± 0.84**	29 ± 0.67**	5.50 ± 0.13**	3 ± 0.09**a	2.5 ± 0.14**
5-Flourouracil	7.11 ± 1.06*	7.53 ± 0.97[a]	38.54 ± 2.16*	185 ± 1.86*	9.24 ± 1.08*	36.33 ± 2.11*	56.24 ± 1.46*	4.16 ± 0.74*	2.27 ± 0.85*[a]	1.0 ± 0.04
% change	+8.88%	+4.01%	+23.05%	+85%	−19.02%	−39.45%	+93.93%	−24.36%	−24.33%	−60%
S. yapa treated mice	10.07 ± 1.01*[a]	7.89 ± 0.90[a]	34.42 ± 1.28*	220.87 ± 1.39*	6.65 ± 0.92*	42.75 ± 0.65*	49.40 ± 1.75*	2.90 ± 0.25*	2.95 ± 0.05*	2.0 ± 0.03*
% change	+54.21%	+8.98%	+9.89%	+120%	−41.71%	−28.75%	+70.34%)	−47.27%	−1.6%	−20%

Data are presented as mean ± SD followed with increasing or decreasing percentage as compared to tumor bearing mice. ANOVA one-way was used for data analysis followed by post Hoc test for multiple comparisons (n = 5, $p < 0.05$). Bearing mice were compared to –ve control group (**) while treated groups were compared to bearing group (*). Hb; hemoglobin, RBC's; red blood cell, PCV; packed cell volume, WBC; white blood cell. Percentages of increasing or decreasing was calculated as compared to bearing mice.

Table 5
Efficacy of *S. yapa* extract on the liver function of tumor bearing mice in Ehrlich model.

Groups	ALT	AST	AST/ALT ratio
	U/L		
Normal groups			
Negative control	29.33 ± 1.02	25.83 ± 1.11	0.88 ± 0.21
Extract control group	29 ± 1.71	26.31 ± 1.31	0.91 ± 0.13
Ehrlich Ascites carcinoma bearing mice			
Tumor bearing mice	110 ± 0.05**	450 ± 2.14**	4.09 ± 1.32**
FU treated group	90 ± 1.51*	192 ± 1.05*	1.92 ± 0.31*
% change	−18.18%	−57.33%	−53.06%
Extract treated group	77.9 ± 0.199*	180.5 ± 0.437*	2.194 ± 0.95*
% change	−29.18%	−59.88%	−46.45%

Data are presented as mean of five animals ± SD. One-way ANOVA was used for data analysis followed by post Hoc test for multiple comparisons (n = 5, $p < 0.05$). Bearing mice and + ve controls were compared to –ve control group (**) while treated groups were compared to bearing group (*). FU; flouro uracil treated group, ALT; Glutamic- pyruvic transaminase, AST; Glutamic- oxaloacetic transaminase.

Table 6
Docking scores (kcal/mol) of the major compounds identified in *S. yapa* extract upon docking into VEGFR1 and VEGFR2 active sites.

Compound name	Docking score (kcal/mol)	
	VEGFR1	VEGFR2
Tricin	−13.87	−15.38
Tricin 7-*O*-glucoside	−17.28	−21.07
Tricin 7-*O*-glucoside-5-sulphate	−18.77	−26.13
Apigenin	−13.87	−14.30
Apigenin 6-*C*-glucoside (Isovitexin)	−16.34	−20.27
Apigenin 6,8-di-*C*-glucoside (Vicenin 2)	−19.37	−23.28
Apigenin 6-*C*-glucosyl-8-*C*-xyloside (Schaftoside)	−17.93	−22.09
Apigenin 6-*C*-glucosyl-8-*C*-xyloside-7-sulphate	−20.99	−28.85
Eriodictyol	−14.10	−15.69
Eriodictyol 7-*O*-glucoside	−17.64	−20.08
Quercetin	−14.83	−15.46
Quercetin 3-*O*-glucoside	−15.16	−20.52
Diosmetin	−13.97	−15.37
Diosmentin 8-*C*-glucoside	−17.20	−20.92
Luteolin	−15.18	−15.36
Luteolin 7-*O*-glucoside	−17.33	−20.09
Luteolin 8-*C*-glucoside (Orientin)	−17.20	−24.12
Luteolin 8-*C*-glucoside sulphate	−17.74	−26.93
Co-crystallized inhibitor	−13.18	−16.29

addition, the extract demonstrated protective effects on the hematopoietic system by resorting the hemoglobin content, RBC cells count, and other hematological parameters to their normal values.

The anti-tumor activities of the extract might be attributed to the presence of flavonoids, flavonoid C-glycosides and their sulphated derivatives, hence, $C_2 = C_3$ double bond significantly contributes to the molecular planarity and the conjugation between rings C and A/B, which is essential for potent tumor inhibition (Chidambara Murthy et al., 2012). Many sulphated phenolic compounds were identified in *S. yapa* leaves extract in the present work, they include schaftoside sulphate, orientin sulphate, tricin 7-glucoside sulphate, tricin 4′-*O*-(*erythro*-β-4 hydroxyphenylglyceryl ether) 7-*O*-glucoside sulphate isomers, tricin 4′-*O*-(*erythro*-β-4 guaiacylglyceryl ether) 7-*O*-glucoside sulphate isomers and tricin -*O*- sulphate. This sulphation renders polyphenols more hydrophilic, increases their bioavailability, and affects their interactions with other biomolecules (Manach et al., 2004).

Jaceidin, a flavonoid from *Chiliadenus montanus*, attenuated EAC tumor progression in mice and interfered with angiogenesis by lowering VEGF levels (Elhady et al., 2020). Our molecular docking study revealed the strong potential of *S. yapa* flavonoids to bind to the inhibitor's site of VEGFR1 and VEGFR2, thus hindering the receptors activation by VEGF cytokines and consequently could interfere with angiogenesis. Among the identified compounds, orientin; it exhibited solid cytotoxic properties against several cancer cell lines, and its apoptotic effects were explained by their interference with caspases 3/7 and caspase-9 pathways (Law et al., 2014).

The identified phenolic *p*- hydroxybenzoic acid was reported to possess antimicrobial, estrogenic, cytotoxic effects as well as antioxidant properties (Pugazhendhi et al., 2005). Chlorogenic, ferulic and caffeic acids were also proved as potent anticancer and antimutagenic substances (Cai et al., 2004). Quercetin identified in this extract as well, demonstrated substantial antiproliferative properties, activated apoptosis in many human cancer cell lines (HT-29, LNCaP, HCT and MCF7) and induced cell cycle arrest (Rahman et al., 2011; Plaumann et al., 1996). Another promising anticancer flavonoid, tricin, characterized in the *S. yapa* leaves extract is used to treat colorectal cancer. Moreover, it competitively inhibits the cytosolic sulfotransferases, thus increases the flavonoids' bioavailability in tissues and blood (Wen et al., 2017). Vitexin identified in our extract, was reported for its antineoplastic effect in PC12 and U937 cells due to induction of apoptosis via

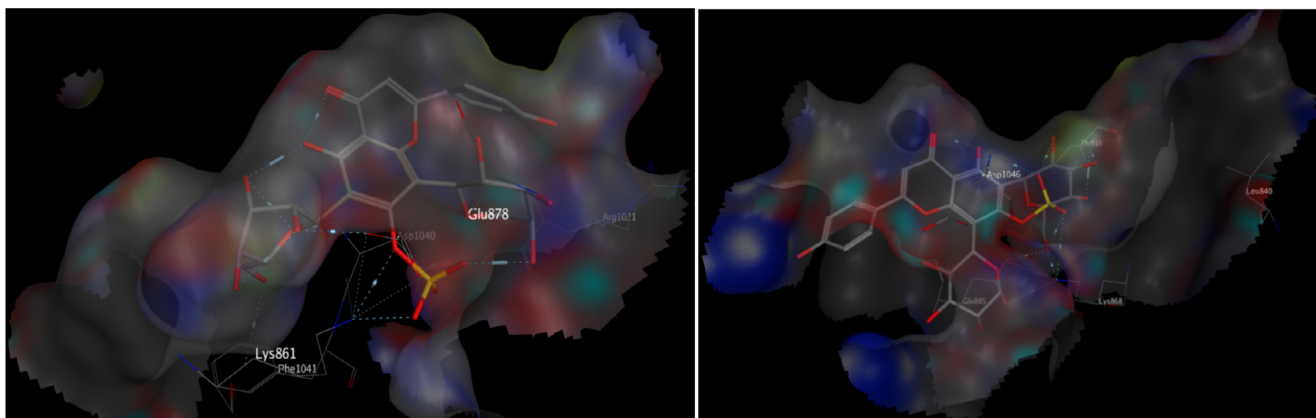

Fig. 3. 3D poses of schaftoside sulphate docked to VEGFR1 (left) and VEGFR2 (right).

interference with Bcl-2, caspase-3 and caspase-9 pathways (Choi et al., 2006).

The identified flavonoids, luteolin, luteolin- 7-*O*-glucoside, and luteolin-4-*O*-glucoside that were previously isolated from *Olea Europaea* showed antiproliferative effect on cancer and endothelial cell lines with IC_{50} value between 3 and 50 µM for luteolin (Seelinger et al., 2008, Goulas et al., 2009). Similar cytotoxic activities were reported from several extracts rich in flavonoids and polyphenols. For instance, a study by Islam et al. (2012) reported antineoplastic activity against Ehrlich ascites carcinoma by *Eucalyptus* extracts. Another study by Rahman et al. (2017) documented solid antioxidant and anticancer properties of *Aponogeton undulates* extracts.

5. Conclusions

The phytochemical analysis of a hydroalcoholic extract from *Sabal yapa* leaves revealed thirty-four compounds among which different flavonoids along with their sulfated derivatives and *C*- and *O*-glucoside derivatives prevailed the extract. In the biological assays, the extract turned to be safe to experimental animals up to a dose as high as 4800 mg/kg b.wt. It revealed high phenolic content and showed substantial antioxidant potential. The extract showed a solid anticancer potential against EAC through reducing tumor weight and volume, increasing the animals lifespan, improving various hematologic parameters, and restoring a normal liver enzymes level. Molecular docking showed that the identified flavonoids were able to block VEGFR1 and VEGFR2 and thus might be able to interfere with angiogenesis. In conclusion, *S. yapa* leaves extract could be considered as a safe anticancer regimen that is worth to be a subject for further studies to prove its therapeutic potential.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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