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Bridelia speciosa Müll.Arg. Stem bark Extracts as a Potential Biomedicine: From Tropical Western Africa to the Pharmacy Shelf

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Introduction

- Considered as a substantial source of food and medicine, plants have played a pivotal role in the progress of mankind. Traditionally used, for their curative properties among different populations of the world, medicinal plants are still considered to provide outstanding curative effects and remain the most accessible therapeutic approach to a number of ailments. In traditional medicine, herbal remedies are prepared according to “standardized formula” transmitted from elders or shamans. Some of the preparation methods include decoction, infusion, maceration, tinctures, among others which can be administered by different routes, including optical, dermal, oral, nasal, and anal. Conscious of the wealth of traditional knowledge related to medicinal use represents, the WHO has publicized the need for documentation of ethnomedicinal data on plants. Ethnomedicinal records make scientific validation easier and also provide rational regarding the use of plants/herbal preparations for the management of specific ailments.
- Recently, several endeavors have been made to probe for new sources of bioactive compounds from natural raw materials. Among them, bark of plants is one of the most important source of bioactive compounds, including phenolics, flavonoids and terpenes. In addition, extracts prepared from barks have been reported to possess broad biological activities such as antioxidant, antimicrobial or anti-cancer. Based on these data, new studies on uninvestigated bark samples, particularly from Africa, might lead to the discovery of novel bioactive compounds for potential uses in the nutraceutical and pharmaceutical industries.
- The *Bridelia* genus consists of approximately 60-70 species distributed in tropical and subtropical regions of the globe, particularly in Asia and Africa. Several species of this genus have been used in traditional medicinal systems for the management of multiple diseases including diabetes, urinary stones, lumbago, rheumatism, venereal diseases, bronchitis, gastrointestinal problems, cardiac pain, infertility, epilepsy, and diarrhoea, among others. Keeping this in view, the biological efficacy of several *Bridelia* species has been claimed in several research pieces. In earlier studies, the chemical profiles of the members of the *Bridelia* genus have been reported. For example, previous studies have reported the presence of phenolic acids (gallic acid and ellagic acid, etc.), tannins, and flavonoids in several Bridelia species, including *B. ferruginea*, *B. micrhanta* and *B. retusa*.
- As far as our literature search could ascertain, little scientific information was available on *B. speciosa*. In this perspective, the current work aims at characterizing the stem bark extracts of *B. speciosa* investigating phytocompounds and elucidating the antioxidant, enzyme inhibitory properties, antimicrobial, protective and anti-proliferative effects in experimental models of liver cancer and inflammation.

Total bioactive components of the tested samples

- Values expressed are means ± S.D. of three parallel measurements. GAE: Gallic acid equivalent; RE: Rutin equivalent; CE: catechin equivalent; CAE: caffeic acid equivalent; QE: Quillaja equivalent; EA: Ethyl acetate;
- MeOH: Methanol;
- nd: not detected. Different letters indicate significant differences in the extracts ($p < 0.05$).

Samples	Total phenolic Content (mg GAE/g Extract)	Total Flavonoid Content (mg RE/g Extract)	Total Phenolic Acid Content (mg CAE/g)	Total Flavanol Content (mg CE/g)	Total Tannin Content (mg CE/g)	Total Saponin Content (mg QE/g)
EA	38.42 ± 0.38 ^{c}	5.85 ± 0.12 ^{a}	nd	3.61 ± 0.02^{c}	3.28 ± 0.38 ^{c}	177.82 ± 14.15 ^{c}
MeOH	224.28 ± 1.08 ^{a}	1.51 ± 0.04 ^{b}	11.55 ± 1.31 ^{b}	246.28 ± 10.63 ^{a}	324.09 ± 10.99 ^{a}	1031.45 ± 48.83 ^{a}
Water	210.29 ± 0.71 ^{b}	1.44 ± 0.17 ^{b}	13.91 ± 0.42 ^{a}	6.15 ± 0.18 ^{b}	67.83 ± 3.64 ^{b}	772.56 ± 56.39 ^{b}

Values expressed are means ± S.D. of three parallel measurements. GAE: Gallic acid equivalent; RE: Rutin equivalent; CE: catechin equivalent; CAE: caffeic acid equivalent; QE: Quillaja equivalent; EA: Ethyl acetate; MeOH: Methanol; nd: not detected. Different letters indicate significant differences in the extracts ($p < 0.05$).

Antioxidant activities of the tested samples.

- Values expressed are means ± S.D. of three parallel measurements. TE: Trolox equivalent; EDTAE: EDTA equivalent; EA: Ethyl acetate; MeOH: Methanol.
- Different letters indicate significant differences in the extracts ($p < 0.05$).

Samples	Phosphomolybdenum (mmol TE/g)	DPPH (mg TE/g Extract)	ABTS (mg TE/g Extract)	CUPRAC (mg TE/g Extract)	FRAP (mg TE/g Extract)	Metal Chelating Ability (mg EDTAE/g)
EA	2.24 ± 0.07 ^{c}	18.62 ± 0.39 ^{c}	14.82 ± 0.45 ^{c}	94.34 ± 0.82 ^{c}	46.13 ± 0.58 ^{c}	32.08 ± 1.60 ^{a}
MeOH	5.89 ± 0.37 ^{a}	495.45 ± 0.53 ^{a}	902.33 ± 2.41 ^{a}	1325.89 ± 30.05 ^{a}	952.68 ± 23.61 ^{a}	12.98 ± 0.10 ^{b}
Water	5.17 ± 0.14 ^{b}	463.86 ± 14.04 ^{b}	581.14 ± 33.94 ^{b}	1082.42 ± 3.72 ^{b}	850.05 ± 5.35 ^{b}	14.28 ± 2.15 ^{b}

Values expressed are means ± S.D. of three parallel measurements. TE: Trolox equivalent; EDTAE: EDTA equivalent; EA: Ethyl acetate; MeOH: Methanol. Different letters indicate significant differences in the extracts ($p < 0.05$).

- HPLC-MS/MS was employed to evaluate the phytocomposition of *B. speciosa* stem bark extracts more accurately. With regards to the different extracts, 36 compounds were identified from the ethyl acetate extract , 44 from the methanol, and 38 from the water extract of *B. speciosa* stem bark. Ellagic, quinic, shikimic, gallic, and ferulic acids were characterized in all extracts.

No.	Name	Class [3]	Formula	Rt ± 0.03 min	$[M+H]^+$	$[M-H]^-$	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Detected in Extract [2]
1	Quinic acid	a	C7H12O6	1.23		191.05557	173.0447	127.0388	111.0438	93.0331	85.0280	A,B,C
2	Shikimic acid	a	C7H10O5	1.31		173.04500	155.0338	137.0234	111.0439	93.0331	73.0280	A,B,C
3	Citric acid	a	C6H8O7	1.57		191.01918	173.0082	129.0181	111.0074	87.0073	85.0280	B,C
4	Prodelphinidin B	b	C30H26O14	1.73		609.12444	441.083	423.073	305.0672	177.0185	125.0231	B,C
5 [1]	Gallic acid (3,4,5-Trihydroxybenzoic acid)	c	C7H6O5	2.29		169.0137	125.0231	97.0282	81.0332	79.0175	69.0329	A,B,C
6	Gallocatechin (Casuarin, Gallocatechol)	d	C15H14O7	4.52		305.06613	261.0767	219.0651	167.0341	137.0234	125.0232	A,B,C
7 [1]	Tryptamine	e	C10H12N2	8.44	161.107875		144.0810	143.0732	117.0703	115.0546	103.0547	B,C
8	Syringic acid-4-O-glucoside	f	C15H20O10	10.57		359.09783	197.0451	182.0214	153.0546	138.031	123.0073	C
9 [1]	Catechin (Catechol, Catechuic acid)	d	C15H14O6	13.28		289.07121	245.082	203.0711	151.0389	125.0233	109.028	B,C
10 [1]	Epigallocatechin (Epigallocatechol)	d	C15H14O7	13.57		305.06613	261.0767	219.0658	167.0339	137.0234	125.0232	A,B,C
11 [1]	Vanillin (4-Hydroxy-3-methoxybenzaldehyde)	g	C8H8O3	15.47	153.05517		125.0601	111.0445	110.0367	93.0341	65.0393	A,B,C
12 [1]	Epigallocatechin-3-O-gallate (Teatannin II)	d	C22H18O11	16.39		457.07709	305.0661	169.0131	161.0238	125.0231		A,B,C
13 [1]	Gallocatechin-3-O-gallate	d	C22H18O11	16.40		457.07709	305.067	169.0133	161.0233	125.0231		C
14	Dihydrokaempferol-O-hexoside	d	C21H22O11	17.02		449.10839	287.0568	269.0447	259.0607	125.023		A,B,C
15 [1]	Epicatechin	d	C15H14O6	17.04		289.07121	245.0818	203.0706	151.0388	125.0231	109.028	B,C
16	3,5-Dimethoxy-4-hydroxybenzaldehyde (Syringaldehyde)	g	C9H10O4	17.24	183.06574		155.0705	140.0469	123.0444	105.0341	95.0498	A,B
17	Corilagin	h	C27H22O18	17.49		633.07279	463.0526	419.0627	300.9995	275.0205	169.0134	A,B,C
18	Mangiferin (Aphloiol, Chinonin)	i	C19H18O11	18.41		421.07709	343.0459	331.0464	301.0358	272.033	259.0249	A,B,C
19	Unidentified tannin 1	h	C34H26O22	18.96		785.08375	633.0741	300.9992	275.0205	125.0229		B
20	Ferulic acid	c	C10H10O4	19.25		193.05009	178.0259	149.0594	137.023	134.0364	121.028	A,B,C
21	Mallotinic acid or isomer	h	C34H26O23	19.28		801.07867	757.0872	633.0753	613.047	463.0517	300.9995	B,C
22 [1]	Epicatechin-3-O-gallate	d	C22H18O10	19.37		441.08218	289.0725	271.0614	245.0808	169.0132	125.023	B,C
23	Loliolide	j	C11H16O3	19.47	197.11777		179.1071	161.0963	135.1172	133.1016	107.0861	A,B,C
24	Unidentified tannin 2	h	C41H30O27	19.63		953.08963	300.9994	275.02	249.0387			B,C

No.	Name	Class [3]	Formula	Rt ± 0.03 min	$[M+H]^+$	$[M-H]^-$	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Detected in Extract [2]
25	Ellagic acid-4-O-glucoside	k	C20H16O13	19.90		463.05127	300.9995	299.9915				A,B,C
26	4-Hydroxy-3-methoxycinnamaldehyde (Coniferyl aldehyde)	g	C10H10O3	19.97	179.07082		161.0599	147.0443	133.0654	119.0496	55.0187	A,B,C
27	Unidentified tannin 3	h	C34H26O22	20.08		785.08375	633.0734	300.9994	275.0207			B
28	Isoferulic acid	c	C10H10O4	20.30		193.05009	178.0264	149.06	137.0232	134.0362	121.0283	A
29	Unidentified tannin 4	h	C34H26O22	21.25		785.08375	300.9996	275.0205	249.0402	125.0228		B,C
30	Myricitrin (Myricetin-3-O-rhamnoside)	d	C21H20O12	21.96		463.08765	317.0292	316.023	287.0213	271.0255	178.9978	B,C
31	Di-O-methylellagic acid-O-hexoside	k	C22H20O13	22.16		491.08257	476.0599	328.023	312.9996	297.9761		A,B,C
32	Ellagic acid-O-pentoside	k	C19H14O12	22.76		433.04071	300.9994	299.9916	283.9974	257.0082		A,B,C
33	Eschweilenol C (Ellagic acid-4-O-rhamnoside)	k	C20H16O12	23.09		447.05636	300.9994	299.9916				A,B,C
34	Pentahydroxyflavone-C-hexoside	d	C21H20O12	23.11	465.10331		447.0935	429.0806	369.0611	327.0503	303.0504	A
35	Ellagic acid	k	C14H6O8	23.38		300.99845	283.9967	257.0094	229.0138	201.0187	185.0237	A,B,C
36	Dimethoxy-trihydroxyflavone-O-hexoside	d	C23H24O12	24.29		491.11895	328.0586	313.0352	299.0195	285.0397	271.0252	B,C
37	Di-O-methylflavellagic acid O-hexoside	k	C21H18O13	24.70		507.07749	344.0187	328.994	313.97			A
38	Ducheside A (3-O-Methylellagic acid-4′-O-xyloside)	k	C20H16O12	24.74		447.05636	315.0151	314.0074	299.9917	298.983	270.9886	A,B,C
39	3,3′-Di-O-methylellagic acid-O-pentoside	k	C21H18O12	25.32		461.07201	446.0498	328.0228	312.9995	297.9757		A,B,C
40	3,3′,4-Tri-O-methylflavellagic acid-4-O-glucoside	k	C23H22O14	25.55		521.09314	506.0705	491.0473	358.0327	343.0098	327.9864	A,B,C
41	Eschweilenol A or isomer	k	C20H10O11	25.90		425.01449	300.9993	299.9917	298.9837			B
42	Dihydroactinidiolide	j	C11H16O2	26.58	181.12286		163.112	145.1015	135.1172	121.1015	107.0861	A,B,C
43	Di-O-methylellagic acid acetylhexoside	k	C24H22O14	27.49		533.09313	328.0231	312.9999	297.9756	269.9827		A
44	3,3′-Di-O-methylellagic acid	k	C16H10O8	27.84		329.02975	314.0073	298.9837	270.9887			A,B,C
45	Sebacic acid	a	C10H18O4	27.96		201.11268	183.102	157.1229	139.1117	111.0801		A
46	3,3′,4-Tri-O-methylellagic acid	k	C17H12O8	30.18		343.0454	328.0231	312.9995	297.9758	285.0038		A
47	Undecanedioic acid	a	C11H20O4	30.85		215.12834	153.1273	125.0956				A
48	3,3′,4-Tri-O-methylflavellagic acid	k	C17H12O9	31.21		359.04031	344.0171	328.9948	313.9717	300.9995		A,B,C
49	3,3′,4,4′-Tetra-O-methylellagic acid	k	C18H14O8	32.00	359.0767		344.0533	343.0448	329.0295	313.0347		A,B,C
50	Dihydroxy-trimethoxyflavone	d	C18H16O7	33.10		343.08178	328.0585	313.0359	298.0118			B
51	Bruguierol A	l	C12H14O2	36.06	191.10721		173.0965	161.0966	147.0801	135.0807	107.0496	A,B
52 [1]	Linoleic acid	a	C18H32O2	45.69		279.23241	261.2231	59.0124				A,B
53	Pheophytin A	m	C55H74N4O5	62.94	871.57375		593.277	533.2559	460.2264			A,B

[1] Confirmed by standard. [2] A: Ethyl acetate extract; B: Methanol extract; C: water extract. [3] a: carboxylic acid; b: polyflavonoid; c: phenolic acid; d: flavonoid; e: alkaloid; f: phenolic acid glucoside; g: phenolic aldehyde; h: tannin; i: xanthon; j: benzofuran; k: benzopyrane; l: phenolic heterocycle; m: porphyrin.

Enzyme inhibitory properties of the tested extracts

- Values expressed are means ± S.D. of three parallel measurements. AChE: acetylcholinesterase; BChE: butyrylcholinesterase; GALAE: Galantamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; na: not active;
- EA: Ethyl acetate; MeOH: Methanol. Different letters indicate significant differences in the extracts ($p < 0.05$).

Samples	AChE (mg GALAE/g Extract)	BChE (mg GALAE/g Extract)	Tyrosinase (mg KAE/g Extract)	α-Amylase (mmol ACAE/g Extract)	α-Glucosidase (mmol ACAE/g Extract)
EA	4.56 ± 0.20 [b]	3.59 ± 0.05 [b]	119.80 ± 1.30 [c]	0.86 ± 0.03 [b]	3.56 ± 0.03
MeOH	4.98 ± 0.04 [a]	5.14 ± 0.08 [a]	157.25 ± 0.48 [a]	1.20 ± 0.01 [a]	na
Water	3.60 ± 0.15 [c]	2.61 ± 0.31 [c]	137.49 ± 0.35 [b]	0.59 ± 0.04 [c]	na

Values expressed are means ± S.D. of three parallel measurements. AChE: acetylcholinesterase; BChE: butyrylcholinesterase; GALAE: Galantamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; na: not active; EA: Ethyl acetate; MeOH: Methanol. Different letters indicate significant differences in the extracts ($p < 0.05$).

Fungal Strains	MIC (μg mL^{-1}) *			
	Methanol Extract	Water Extract	Fluconazole	Griseofulvin
Candida albicans (YEPGA 6183)	396.85 (250–500)	198.42 (125–250)	2	>8
Candida albicans (YEPGA 6379)	49.6 (31.25–62.5)	78.74 (62.5–125)	1	>8
Candida tropicalis (YEPGA 6184)	629.96 (500–1000)	396.85 (250–500)	4	>8
Candida parapsilosis (YEPGA 6551)	78.74 (62.5–125)	99.21 (62.5–125)	2	>8
Arthroderma crocatum (IHEM 5251)	157.49 (125–250)	78.74 (62.5–125)	8	>8
Arthroderma crocatum (CCF 5207)	99.21 (62.5–125)	78.74 (62.5–125)	>16	>8
Arthroderma insingulare (CCF 5417)	157.49 (125–250)	39.37 (31.25–62.5)	>16	>8
Arthroderma quadrifidum (CCF 5792)	198.42 (125–250)	78.74 (62.5–125)	>16	>8
Trichophyton erinacei (CCF 5930)	314.98 (250–500)	157.49 (125–250)	>16	0.25
Trichophyton interdigitale (CCF 4823)	99.21 (62.5–125)	49.61 (31.25–62.5)	>16	1
Trichophyton rubrum (CCF 4879)	78.74 (62.5–125)	78.74 (62.5–125)	8	2
Trichophyton tonsurans (CCF 4834)	157.49 (125–250)	39.58 (31.25–62.5)	2	0.125

* MIC values are reported as geometric means of three independent replicates (n = 3); MIC range concentrations are reported within brachets. CCF, Culture Collection of Fungi, Department of Botany, Charles University, Prague, Czech Republic; IHEM, Belgian Coordinated Collections of Micro-organisms (BCCM/IHEM), Brussels, Belgium; YEPGA, yeast extract-peptone-glucose agar.

Bacterial Strains	MIC (μg mL^{-1}) *		
	Methanol Extract	Water Extract	Ciprofloxacin
Escherichia coli (ATCC 10536)	396.85 (250–500)	629.96 (500–1000)	<0.12
Pseudomonas aeruginosa (ATCC 15442)	629.96 (500–1000)	314.98 (250–500)	1.23 (1.95–0.98)
Salmonella typhimurium (clinical isolate)	793.70 (500–1000)	793.70 (500–1000)	0.40 (0.25–0.5)
Bacillus cereus (ATCC 12826)	198.42 (125–250)	157.49 (125–250)	<0.12
Bacillus subtilis (environmental isolate)	314.98 (250–500)	793.70 (500–1000)	0.01 (0.125–0.062)
Staphylococcus aureus (ATCC 6538)	198.42 (125–250)	396.85 (250–500)	0.62 (0.98–0.49)

* MIC values are reported as geometric means of three independent replicates (n = 3); MIC range concentrations are reported within brachets.

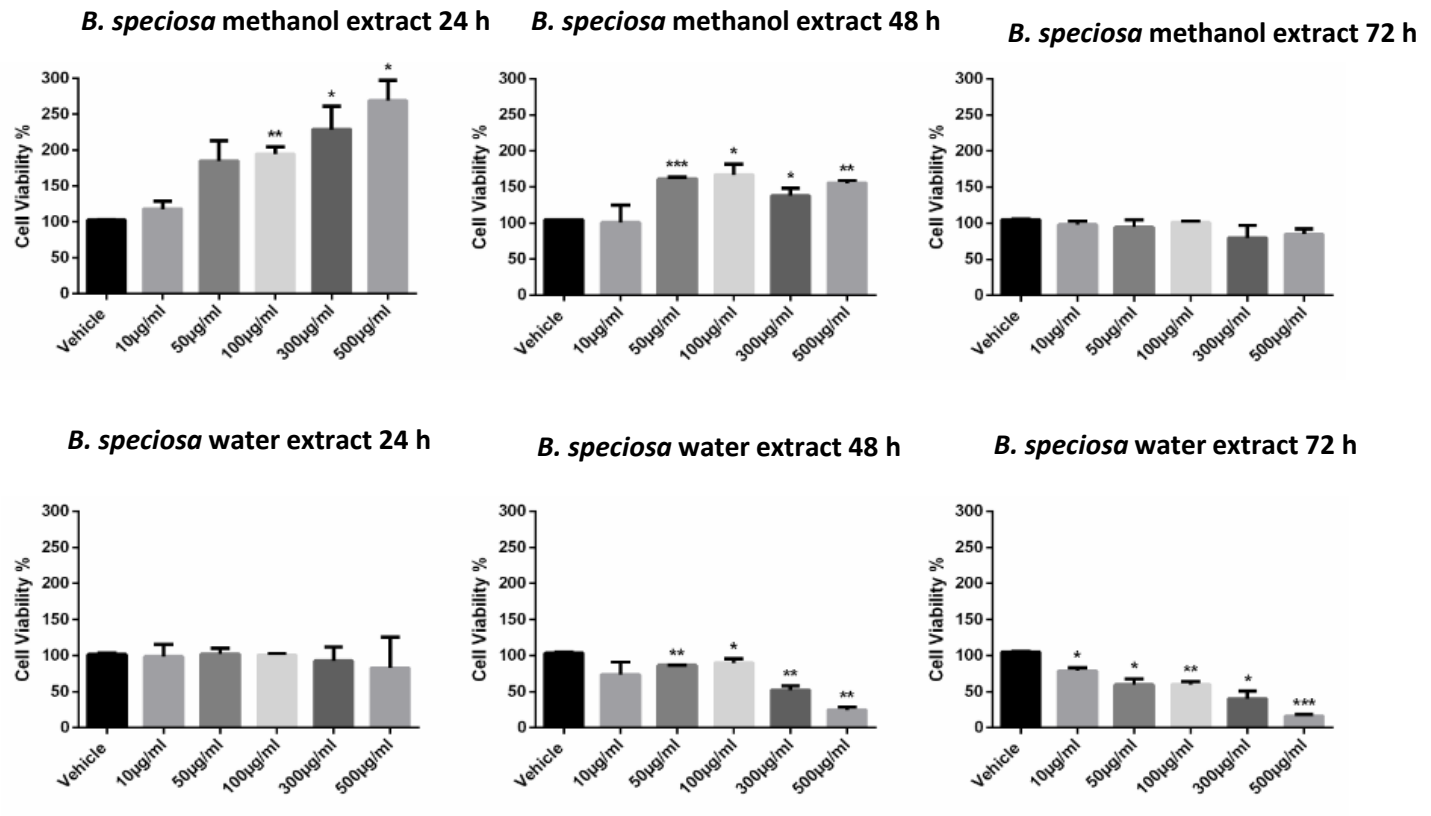

Figure 1. B. speciosa methanol and water extracts affect cell viability in human hepatocellular carcinoma HepG2. Cell viability was assessed by MTT assay after incubation for 24, 48 or 72 hours, with the extracts at various concentrations as indicated, or with vehicle (control). Data shown are the means + SD of two independent experiments with quadruplicate determinations. Statistical analyses were performed using GraphPad Prism version 5.01 software (San Diego, CA). Comparisons of mean values between control and each drug concentration were performed by an unpaired Student's t-test. A p-value ≤ 0.05 was considered statistically significant (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001).

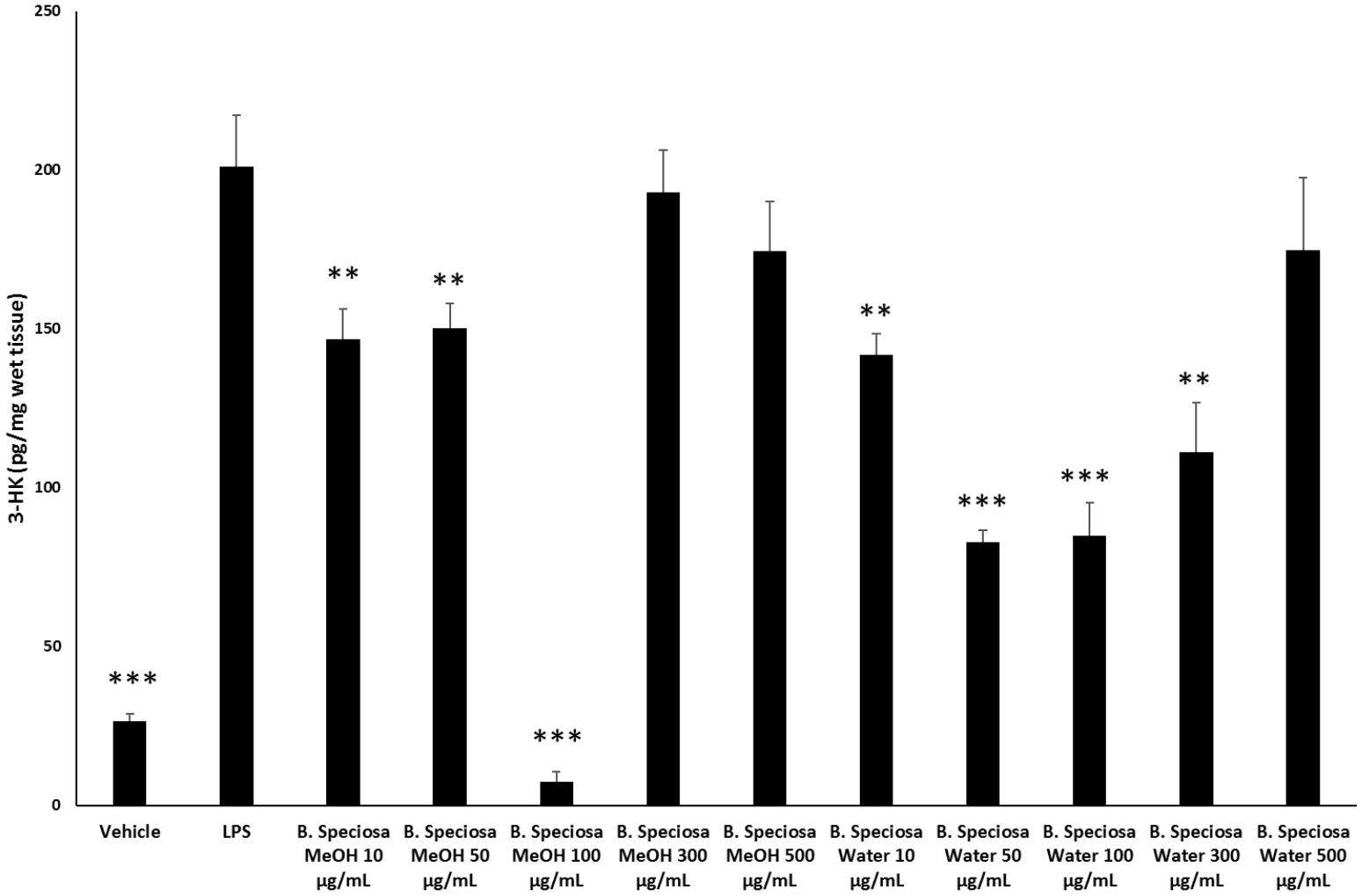

Figure 2. Effects of B. speciosa methanol and water extracts on LPS-induced 3-HK level in isolated rat liver specimens. ANOVA, $P<0.0001$; *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. LPS control group.

Conclusions

- This is the first report about the biological and phytochemical profiles of *B. speciosa* stem bark extracts. In this respect, our findings can be considered as a first attempt to provide new scientific information on the *Bridelia* genus. Among the three extracts studied, the methanol extract showed antioxidant and inhibitory properties against enzymes related to Alzheimer's disease and epidermal hyperpigmentation conditions. The antioxidant effects displayed by the methanol extract are also consistent with the observed protective effects in the liver and the anti-mycotic effect against the *C. albicans* (YEPGA 6379) strain. On the other hand, the water extract reduced the HepG2 cell viability, thus suggesting potential anti-proliferative effects. Further studies are strongly recommended to explore more biological properties through in vivo animal studies.