


RESEARCH ARTICLE

Open Access



Isolation, characterization and in vitro anti-salmonellal activity of compounds from stem bark extract of *Canarium schweinfurthii*

Jean Baptiste SOKOUDJOU^{1,2}, Olubunmi ATOLANI^{2,3}, Guy Sedar Singor NJATENG¹, Afsar KHAN², Cyrille Ngoufack TAGOUSOP⁴, André Nehemie BITOMBO^{2,5}, Norbert KODJIO¹ and Donatien GATSING^{1*} 

Abstract

Background: Bacteria belonging to the *Salmonella* genus are major concern for health, as they are widely reported in many cases of food poisoning. The use of antibiotics remains a main stream control strategy for avian salmonellosis as well as typhoid and paratyphoid fevers in humans. Due to the growing awareness about drug resistance and toxicities, the use of antibiotics is being discouraged in many countries whilst advocating potent benign alternatives such as phyto-based medicine. The objective of this work was to isolate, characterise the bioactive compounds of *Canarium schweinfurthii*; and evaluate their anti-salmonellal activity.

Methods: The hydro-ethanolic extract of *Canarium schweinfurthii* was fractionated and tested for their anti-salmonellal activity. The most active fractions (i.e. chloroform and ethyl acetate partition fractions) were then explored for their phytochemical constituents. Fractionation on normal phase silica gel column chromatography and size exclusion chromatography on Sephadex LH-20 led to the isolation of four compounds (maniladiol, scopoletin, ethyl gallate and gallic acid) reported for the first time in *Canarium schweinfurthii*.

Results: Result indicated that scopoletin and gallic acid had greater activity than the crude extracts and partition fractions. Among the isolated compounds, scopoletin showed the highest inhibitory activity with a MIC of 16 µg/ml against *Salmonella* Typhimurium and *Salmonella* Enteritidis.

Conclusions: The overall results of this study indicates that the hydro-ethanolic extract as well as some of isolated compounds have interesting anti-salmonellal activities that could be further explored for the development of potent therapy for salmonellosis. Furthermore, the study adds credence to the folkloric applications of the plant.

Keywords: Ethnomedicine, Salmonellosis, *Canarium schweinfurthii*, Natural substances

* Correspondence: gatsingd@yahoo.com

¹Research Unit of Microbiology and Antimicrobial substances, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon
Full list of author information is available at the end of the article



© The Author(s). 2020 **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Background

Salmonella is a major source of food-borne illness in humans and a major cause of morbidity, mortality and economic loss both in the poultry and human health sectors. The disease caused by bacteria belonging to *Salmonella* genus is often called salmonellosis. This pathology remains one of the limiting factors in the development of poultry farming especially in developing countries of Asia and Africa [1] because it causes huge direct and indirect losses [2]. The genus *Salmonella* is very diverse and today it is composed of more than 2500 serotypes, many of which cause enteric diseases in humans and animals. Many serotypes of *Salmonella* can infect chickens and some serotypes are well adapted although, *Salmonella* Gallinarum and *Salmonella* Pullorum cannot be transmitted to human. However, some serotypes can infect both poultry and human and among these serotypes *Salmonella* Enteritidis and *Salmonella* Typhimurium are more prevalent in chickens and notable in human disease outbreaks. These serotypes are most commonly implicated in the human *Salmonella* infections [3, 4]. The poultry is considered one of the main sources of *Salmonella* human infection usually through poorly cooked foods [5–9] and foodstuffs of avian origin [10]. *Salmonella* infection represents a considerable burden in both developing and developed countries. Ubiquitous non-typhoidal *Salmonella* (NTS) which includes *Salmonella* Enteritidis and *Salmonella* Typhimurium annually cause more than 93.8 million illnesses and 155,000 deaths each year [11]. *Salmonella* Enteritidis and *Salmonella* Typhimurium, both NTS are the most frequently occurring serotypes from poultry causing infection in human [3]. Similarly, each year worldwide, typhoidal serotypes among which *Salmonella* Typhi and *Salmonella* Paratyphi, cause approximately 22 million cases of typhoid and 216,500 deaths [12].

Resistance of *Salmonella* to commonly used antimicrobial agents is increasing both in the veterinary and public health sectors and has emerged as a global health challenge. Several *Salmonella* serotypes are multidrug resistant, and there is evidence of the spread of these strains from animals to humans. Antimicrobial resistance in NTS is considered one of the major public health threats related with food-animal production, as well as the poultry production chain and poultry meat, which is an additional concern in the management of salmonellosis [13]. Many authors [14–17] have reported that several strains of *Salmonella* isolated from chicken have shown resistance to many antibiotics commonly used in human medicine and some of these strains have been found in humans [14]. Moreover, antibiotic residues in poultry products intended for consumption may lead to hypersensitivity or poisoning in consumers. Due to the growing awareness of resistance issues, the use of

antibiotics is strongly discouraged in many countries whilst encouraging the use of plants as a better alternative due to their diverse nature of bioactive principles [18–20]. The large majority of salmonellosis in humans is carried by foodstuffs; mainly those of avian origin [10, 20, 21], therefore controlling avian salmonellosis by using plant could significantly reduce the prevalence of human gastroenteritis [20]. Several studies have focused on medicinal plants as new control strategies for human salmonellosis [22, 23] or avian salmonellosis [24–28]. But, to our knowledge, no phytomedicine has yet been formulated to control avian salmonellosis. *Canarium schweinfurthii* Engl. (Burseraceae), is a tree with a cylindrical bole, native to tropical West Africa and grows to about 50 m high [29]. This plant is mainly found in equatorial forest regions from Cameroon, Central African Republic, Gabon to Congo [30] and is used in folk medicine for the treatment of various diseases including malaria, diarrhea and Typhoid fever [31, 32]. Previous studies of Sokoudjou et al. [20, 28] showed that the hydroethanolic extracts of *Canarium schweinfurthii* were active both in vitro and in vivo against several serotypes of *Salmonella*. The objective of this work was to isolate, characterise the bioactive compounds of *Canarium schweinfurthii*; and evaluate their anti-salmonellal activity.

Methods

General experiment

Reagents which include ammonium cerium sulphate, were of analytical grade. Solvents were distilled before being used (St Louis, MO, USA). Thin Layer Chromatography (TLC) was performed on pre-coated silica gel with thickness 0.20 mm 60 F₂₅₄ plates (MerckKGaA, Germany) and viewed under the UV light (254 and 365 nm). NMR analyses which included ¹H NMR, ¹³C NMR, DEPT 90, DEPT 135, 2D NMR (COSY, HSQC), NOESY and ROESY were performed using deuterated solvents (Acétone-*d*₆, CD₃OD and/or CDCl₃) on 400 MHz NMR (Ascend™ 400, Bruker) with TMS as internal reference. ESI-MS spectra of the compounds were recorded on a Bruker-Ion Trap MS (MicroTOF-Q mass spectrometer, Bruker) using the positive mode.

Plant collection, identification and extraction

Canarium schweinfurthii stem bark was harvested in West region of Cameroon and identified at the National Herbarium at Yaoundé-Cameroon, where a voucher specimen was deposited under the reference Number 16929/SRF/Cam. The air-dried plant material (3 Kg) was powdered and macerated at room temperature with 12 L of ethanol-water system (50/50, v/v). After 48 h, the mixture was filtrated using Whatman №1 filter paper. The filtrate was evaporated using a Rotary evaporator (Büchi

R200) at reduced pressure to afford the crude extract (265 g, 8.8%).

We needed no permission to collect the sample since *Canarium schweinfurthii* is not a protected species in Cameroon.

Fractionation and isolation of bioactive compounds of *Canarium schweinfurthii*

The profiling of the hydro-ethanolic extract of *Canarium schweinfurthii* on TLC plates with several solvent systems showed no promising separation. In order to facilitate isolation, 260 g of extract was dissolved in distilled water (700 mL) and successively extracted with hexane (500 mL \times 2), chloroform (500 mL \times 2), ethyl acetate (500 mL \times 2) and *n*-butanol (500 mL \times 2) yielding respectively 5.56 g, 25.97 g, 25.92 g and 90.89 g of fractions after evaporation to dryness. These partition fractions were explored for their antibacterial activity and only the most active fractions were selected for the isolation of bioactive principles. Figure 1 below shows the protocol for isolating the bioactive principles of *Canarium schweinfurthii*.

Part of Chloroform fraction (23 g) was subjected to silica gel column chromatography using *n*-hexane-EtOAc (85:15 \rightarrow 00:100) and MeOH, gradient elution. 40 sub-fractions of 100 mL each were collected and combined on the basis of their TLC profiles to give 5 fractions: A (1–3), B (4–12), C (13–22), D (23–25) and E (25–40). Sub-fraction A (4.5 g) was purified on silica gel column chromatography eluted with *n*-hexane-EtOAc (95:5 \rightarrow 80:20) to give compound 1 (42 mg). The purification of

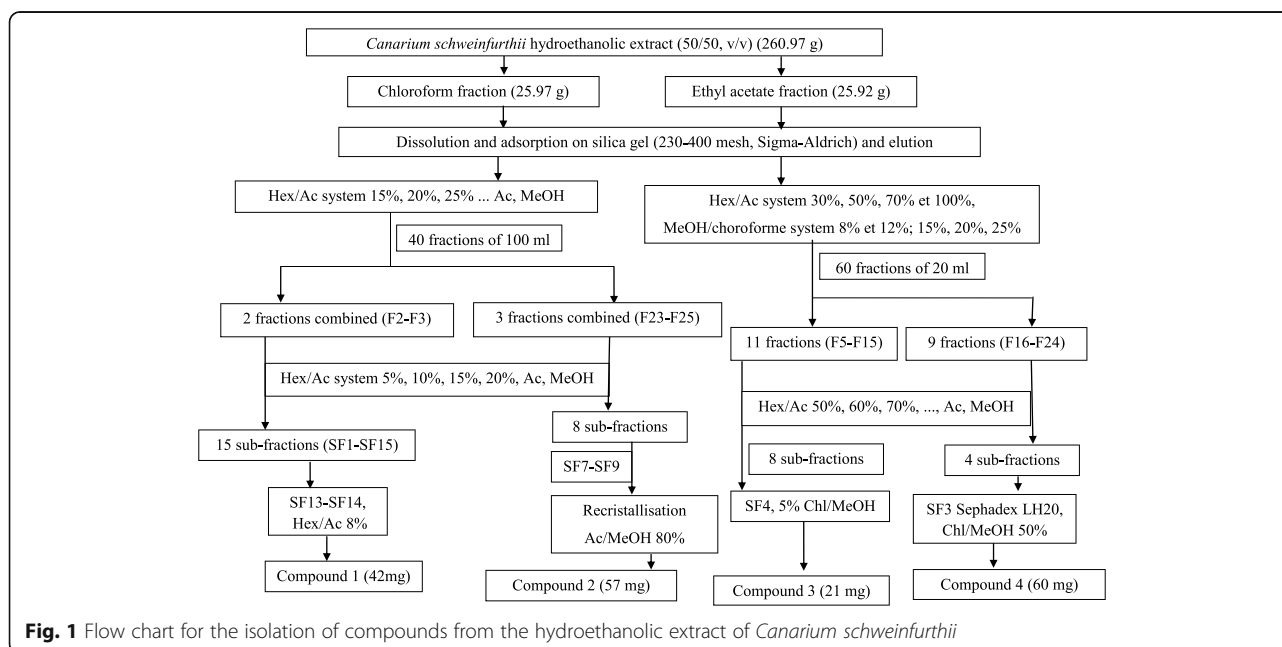
sub-fraction D (4 g) on silica gel column chromatography using *n*-hexane-EtOAc (70:30 \rightarrow 20:80) afforded compound 2 (57 mg) which was recrystallized in EtOAc-MeOH (20:80).

Part of EtOAc fraction (23 g) was also subjected to silica gel column chromatography eluted with a gradient of *n*-hexane-EtOAc (70:30 \rightarrow 00:100) and chloroform-MeOH (92:5 \rightarrow 75:25) to afford 60 sub-fractions of 20 mL which were combined to four sub-fractions: F (1–4), G (5–15) H (16–24), I (25–60) on the basis of their TLC profile. Sub-fraction G (3.5 g) was purified on silica gel column chromatography using *n*-hexane-EtOAc (50:50 \rightarrow 00:100) to yield compound 3 (21 mg) while purification of sub-fraction H (2.6 g) on sephadex LH-20 column eluted with chloroform-methanol (50:50) afforded compound 4 (60 mg). The structures of the isolated compounds were elucidated by combining various techniques comprising 1D Nuclear Magnetic Resonance (NMR): ^1H NMR, ^{13}C -NMR, DEPT 90, DEPT 135 and 2D NMR (COSY, HSQC), NOESY and ROESY as well as Mass Spectrometry analysis (TOF-ESI-MS). The data of the established structures were compared with those existing in literature.

Anti-salmonellal assay

Chemicals for anti-salmonellal assay

Ciprofloxacin (BDH Chemicals, England) and oxytetracyclin (BDH Chemicals, England) were used as reference antibiotics. P-iodonitrotetrazolium chloride (Sigma-Aldrich, Germany) was used as microbial growth indicator.



Test bacteria and culture media

Three clinical isolates (*Salmonella* Typhi, *Salmonella* Enteritidis and *Salmonella* Typhimurium from Pasteur Center, Yaoundé-Cameroon) and one bacterial strain (*Salmonella* Typhi ATCC6539 from American Type Culture Collection) were used for antimicrobial evaluation. The culture media used were Salmonella-Shigella Agar (SSA from HiMedia Laboratories, India) and Mueller Hinton Broth (MHB from HiMedia Laboratories, India).

Determination of minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs)

The MIC values of the fractions obtained from partition and compounds from *Canarium schweinfurthii* were determined in 96-wells microplates using rapid INT colorimetric assay [33, 34]. Briefly, each sample was dissolved in 5% Dimethyl-sulfoxide (DMSO)/MHB. The obtained solution was then added to 100 μ L of MHB, and followed by two-fold serial dilution. Then 100 μ L of inoculum (1.5×10^6 CFU/mL) prepared in MHB were added to each well except the negative control wells. The plates were covered with a sterile plate sealer and incubated at 37 °C for 18 h. The wells containing either MHB or MHB and 100 μ L of inoculum served as control. After the incubation, 40 μ L of INT (0.2 mg/mL) was added to each well and plates were re-incubated at 37 °C for 30 min, and the MIC of each sample was recorded. MIC was defined as the lowest concentration of the sample that prevented change in colour and exhibited complete inhibition of microbial growth. The MBC was determined by adding 50 μ L aliquots of the preparations, which did not show any growth after incubation during MIC assays, to 150 μ L of MHB. These preparations were then incubated at 37 °C for 48 h. The MBC was recorded as the lowest concentration of test sample which did not produce a colour change after addition of INT as previously described. The tests were performed in triplicates.

Results

The yield and physical appearance of each partition fraction of *Canarium schweinfurthii* extract are as shown below (Table 1).

Characterization of isolated compounds

The four compounds isolated and characterized from the stem bark extract of *Canarium schweinfurthii* are as depicted in Fig. 2.

Compound 1: Maniladiol (42 mg) white solid, soluble in methanol, with molecular weight 442 calculated for $C_{30}H_{50}O_2$ (ESI-MS: m/z 465.1 [M + Na]).

Compound 2: Scopoletin (57 mg) yellowish crystals, soluble in acetone, with molecular weight 192 calculated for $C_{10}H_8O_4$ (ESI-MS: m/z 214.9 [M + Na]).

Compound 3: Ethyl gallate (21 mg) white solid, soluble in methanol, with molecular weight 198 calculated for $C_9H_{10}O_5$ (ESI-MS: m/z 221.0 [M + Na]).

Compound 4: Gallic acid (60 mg) white solid, soluble in methanol, with molecular weight 170 calculated for $C_7H_6O_5$ (ESI-MS: m/z 193.1 [M + Na]).

The 1H -NMR and ^{13}C -NMR data of isolated compounds are presented in the Tables 2, 3, 4 and 5.

Anti-salmonellal activity of partition fractions and isolated compounds from stem barks extract of *Canarium schweinfurthii*

Table 6 shows the inhibition parameters (MIC, MBC, MBC/MIC ratio) of the crude extract, partition fractions and isolated compounds of *Canarium schweinfurthii* against pathogenic *Salmonella*. The isolated compounds have variable activity ($16 \leq MIC \leq 1024$ μ g/mL) on the tested *Salmonella* serotypes. It appears that the activity of isolated compounds is greater than those of the crude extract and partitions. Among the partition fractions, chloroform and ethyl acetate fractions showed the best anti-salmonellal activity while among the isolated compounds, scopoletin showed the highest inhibitory activity with a MIC of 16 μ g/mL against *Salmonella* Typhimurium and *Salmonella* Enteritidis. MIC values of other compounds and extract ranged between 128 and 1024 μ g/mL, while hexane and residual fractions are the less active substances with MICs of 512 or 1024 μ g/mL.

Discussion

The antimicrobial effects of some plants and their extracts are well known today [39, 40]; the diversity of plant species is a valuable source for the search for new classes of antibiotics. These plants may proffer valuable alternative to address certain human and veterinary health challenges. It is in this perspective that the hydro-ethanolic extract of *Canarium schweinfurthii* has been explored for its anti-salmonellal activity and its bioactive compounds. Several plants are traditionally used against human salmonellosis [41–46] and avian salmonellosis [24–26, 47]. Plants with

Table 1 Yield and physical appearance of each partition fraction of *Canarium schweinfurthii* stem barks extracts

Partitioned fractions	Yields (%)	Physical characteristics	
		Color	Physical appearance
Hexane fraction	2	Green	Oily
Chloroform fraction	10	Dark brown	Oily
Ethylacetate fraction	10	Brown	Solid
n-butanol fraction	34	Blackish	Cristalline powder
Residual fraction	38	Blackish	Sticky semi-solid (Syrup)

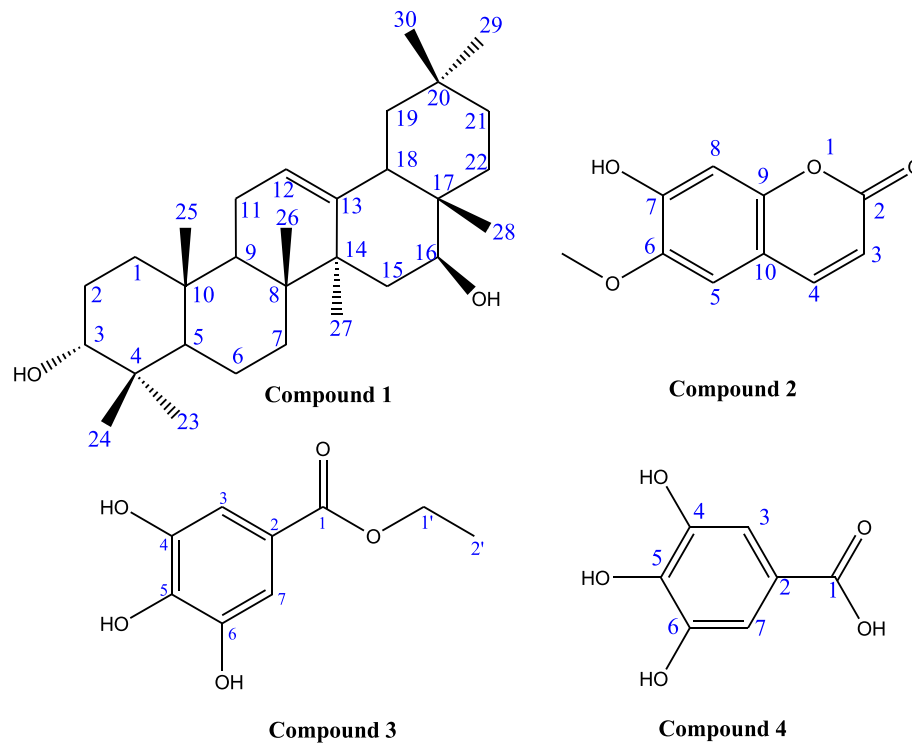


Fig. 2 Chemical structures of isolated compounds from *Canarium schweinfurthii* stem barks extract

high anti-salmonellal potential that show promise for the control of avian salmonellosis include *Aloe secundiflora* [47], *Thymus vulgaris* [48], *Curcuma longa* and *Scutellaria baicalensis* [25] and *Erica mannii* [27]. Plant extracts as well as traditionally improved drugs are one of the promising ways to combat human salmonellosis [23, 47, 49]. Several authors [22, 23, 28, 50–53] have shown that plant extracts depending on their concentrations are active both in vitro and in vivo against several *Salmonella* serotypes. Most of these extracts treat salmonellosis in the same range of time as conventional medicines. These findings corroborate our results which showed that the hydroethanolic extract of *Canarium schweinfurthii* is active against *Salmonella* serotypes with MIC range from 64 to 128 $\mu\text{g/ml}$, moreover this extract have previously demonstrated an in vivo anti-salmonellal activity [20], curing avian salmonellosis on day 9 and with the doses 19 and 75 mg/kg bw of the extract. In addition to the therapeutic efficacy of the hydroethanolic extract of *Canarium schweinfurthii*, the antibacterial activity of its partitions was evaluated. Among the partitions, chloroform and ethyl acetate fractions showed the best anti-salmonellal activity. It also appears that the activity of isolated compounds is greater than those of the crude extract and partitions. This could be due to the low concentration of these compounds in the plant extract or to the antagonism effect of other compounds present in the same extract. The anti-salmonellal activity of plants is linked to the diversity and complexity

of their secondary metabolites. The in vitro anti-salmonellal effect of hydroethanolic extract of *Canarium schweinfurthii* found in this study and its therapeutic efficacy [20] can be linked to a combined action of its secondary metabolites. Indeed, at the molecular level, compounds such as gallic acid and scopoletin found in plants belonging to *Canarium* genus [54] could act synergistically and could be partly responsible for the anti-infectious activity of *Canarium schweinfurthii*. In order to verify this possibility and to have a clear idea on the active principles of this plant, the fractionation of its stem bark extract was performed.

Gallic acid, ethyl gallate, scopoletin and maniladiol were isolated from the *Canarium schweinfurthii* stem bark extract, these compounds were reported for the first time in this medicinal plant species and belong to the classes of polyphenols, triperpenes and coumarins. From the previous reports [54], only gallic acid and scopoletin have been isolated from other plants belonging to the same genus as *Canarium schweinfurthii* and these compounds were reported to have antibacterial and antioxidant properties. The isolated compounds have variable activities ($16 \leq \text{MIC} \leq 1024 \mu\text{g/mL}$) against the tested *Salmonella* serotypes. Among the pure isolated compounds, scopoletin showed the highest inhibitory activity with a MIC of 16 $\mu\text{g/mL}$ against *Salmonella* Typhimurium and *Salmonella* Enteritidis. The activity of most of the isolated compounds was less than those of

Table 2 ^1H -NMR and ^{13}C -NMR of compound 1

Compound 1			Maniladiol, Quijano et al. [35]	
Positions	δ_c ($\text{CD}_3\text{OD} + \text{CDCl}_3$, 100 MHz)	δ_H (mult; J) ($\text{CD}_3\text{OD} + \text{CDCl}_3$, 400 MHz)	δ_c (CD_3Cl , 125 MHz)	δ_H (mult; J) (CD_3Cl , 500 MHz)
1	32.9	1.40 (1H; m) 1.12 (1H; m)	38.5	1.64 (1H; m) 0.98 (1H; m)
2	24.5	1.99 (1H; m) 1.52 (1H; m)	27.1	1.62 (1H; m) 1.58 (1H; m)
3	75.4	3.35 (1H; dd; 11.9; 4.8)	78.9	3.22 (1H; dd; 11.5; 4.5)
4	37.1	–	38.7	–
5	48.8	1.30 (1H; m)	55.1	0.74 (1H; dd; 11.5; 1.5)
6	18.0	1.45 (1H; m) 1.44 (1H; m)	18.3	1.58 (1H; t; 3.6) 1.41 (1H; dd; 15.5; 12.0)
7	32.4	1.62 (1H; m) 1.38 (1H; m)	32.6	1.54 (1H; t; 3.5) 1.33 (1H; t; 3.6)
8	39.9	–	39.8	–
9	46.5	1.06 (1H; m)	46.8	1.51 (1H; dd; 11.0; 6.5)
10	36.6	–	37.3	–
11	23.4	1.91 (2H; m)	23.5	1.92 (1H; ddd; 18.5; 11.0; 3.5) 1.86 (1H; ddd; 18.5; 7.0; 4.0)
12	122.3	5.26 (1H; t; 3.4)	122.3	5.25 (1H; t; 3.5)
13	143.7	–	143.5	–
14	43.5	–	43.7	–
15	34.9	1.71 (1H; m) 1.26 (1H; m)	35.5	1.67 (1H; d; 13.0) 1.31 (1H; dd; 13.0; 5.0)
16	65.0	4.16 (1H; dd; 11.5; 4.9)	66.0	4.20 (1H; dd; 11.5; 5.0)
17	37.0	–	36.8	–
18	49.2	2.16 (1H; dd; 11.5; 4.9)	49.0	2.15 (1H; dd; 14.0; 4.5)
19	46.5	1.71 (1H; m) 1.06 (1H; m)	46.5	1.68 (1H; t; 14.0) 1.06 (1H; ddd; 13.5; 4.5; 2.5)
20	30.4	–	30.9	–
21	34.0	1.41 (1H; m) 1.13 (1H; m)	34.1	1.36 (1H; t; 3.7) 1.15 (1H; t; 3.6)
22	30.5	1.91 (1H; m) 1.88 (1H; m)	30.5	1.83 (1H; t; 3.4) 1.20 (1H; t; 3.5)
23	27.8	0.95 (3H; s)	28.0	1.00 (3H; s)
24	21.7	0.86 (3H; s)	15.6	0.79 (3H; s)
25	14.8	0.99 (3H; s)	15.5	0.94 (3H; s)
26	16.24	1.03 (3H; s)	16.8	0.99 (3H; s)
27	26.4	1.27 (3H; s)	27.1	1.22 (3H; s)
28	21.4	0.80 (3H; s)	21.4	0.80 (3H; s)
29	32.6	0.90 (3H; s)	33.2	0.89 (3H; s)
30	23.2	0.92 (3H; s)	23.9	0.90 (3H; s)

oxyphylline B (10 $\mu\text{g}/\text{mL}$) isolated from *Zizyphus oxyphylla* Edgew against *Salmonella* Typhi [55] and lespeidin (12.25 $\mu\text{g}/\text{mL}$) isolated from *Brillanta isialamium* against *Salmonella* Typhi [56]. However the anti-salmonellal activity of gallic acid and scopoletin against *Salmonella* Typhi (32 $\mu\text{g}/\text{mL}$) was better than those of Bafoudiosbulbins A and Bafoudiosbulbins B isolated

from *Dioscorea bulbifera* L. var. sativa [57]. These results corroborate the finding of Lunga et al. [44] who showed that the anti-salmonellal activity of isolated compounds from *Paullinia pinnata* Linn ranged from 0.781 to 100 $\mu\text{g}/\text{mL}$. According to the Kuete's classification scale [39], the antibacterial activity of a compound is significant when the MIC < 10 $\mu\text{g}/\text{mL}$; moderate when 10 <

Table 3 ^1H -NMR and ^{13}C -NMR of compound 2

Compound 2			Scopoletin, Mogana et al. [36]	
Positions	δ_{C} (acétone- d_6 , 100 MHz)	δ_{H} (mult; J) (acétone- d_6 , 400 MHz)	δ_{C} (CD_3Cl , 100 MHz)	δ_{H} (mult; J) (CD_3Cl , 400 MHz)
1	–	–	–	–
2	160.4	–	161.6	–
3	112.5	6.20 (1H; d; 9.5)	111.6	6.30 (1H; d; 9.5)
4	143.6	7.86 (1H; d; 9.5)	143.3	7.63 (1H; d; 9.5)
5	102.8	6.81 (1H; s)	103.2	6.87 (1H; s)
6	144.9	–	144.6	–
7	150.8	–	150.2	–
8	108.9	7.20 (1H; s)	107.4	6.95 (1H; s)
9	150.0	–	149.7	–
10	112.1	–	113.5	–
6-OCH ₃	55.9	3.92 (3H; s)	56.4	3.98 (3H; s)
7-OH	–	8.78 (1H; s)	–	–

MIC \leq 100 $\mu\text{g}/\text{mL}$ and low when MIC $>$ 100 $\mu\text{g}/\text{mL}$. With regard to this scale, the anti-salmonellal activities of the isolated compound from *Canarium schweinfurthii* are moderate (10 < MIC \leq 100 $\mu\text{g}/\text{mL}$). Scopoletin and gallic acid are significantly active against *Salmonella* Typhi, *Salmonella* Typhi ATCC6539 and *Salmonella* Typhimurium. These results corroborate those of Okoli et al. [58] who showed that 3 β -hydroxyolean-12,18-diene isolated from *Canarium schweinfurthii* was active on *Salmonella* Typhi with a MIC of 12.5 $\mu\text{g}/\text{mL}$ against *Salmonella* Typhi. It has been shown that in addition to its immunomodulatory effect [59], scopoletin reduces the intracellular survival of *Salmonella* Typhi within U937 human macrophage cell line [60]. Gallic acid has in addition to its in vitro and in vivo antibacterial effect against *Salmonella* Typhimurium [61, 62], an antioxidant activity. These compounds related properties corroborate the findings of Sokoudjou et al. [20] who reported that the ability of the extract of *Canarium schweinfurthii* to cure salmonellosis in broilers could be

explained by its ability to directly kill *Salmonella* and/or boost the immune system of the host. The dosage of the compounds isolated from this plant can be used to normalize the extract during the phytomedicine evaluation and preparation.

Conclusion

Gallic acid, ethyl gallate, scopoletin and maniladiol were isolated from the *Canarium schweinfurthii* stem bark extract. These compounds were reported for the first time in this plant species. The four isolated compounds showed in vitro anti-salmonellal activity against *Salmonella* serotypes and particularly scopoletin was the most active and highly selective against both non-typhoidal *Salmonella* and typhoidal *Salmonella* with MIC of 16 or 32 $\mu\text{g}/\text{mL}$. The anti-salmonellal activity of the compounds isolated from *Canarium schweinfurthii* justifies the use of this plant in traditional medicine and confirms the anti-salmonellal effect of the hydroethanolic extract thus adding credence to its use in the treatment

Table 4 ^1H -NMR and ^{13}C -NMR of compound 3

Compound 3			Ethyl gallate, Ooshiro et al. [37]	
Positions	δ_{C} (CD_3OD , 100 MHz)	δ_{H} (mult; J) (CD_3OD , 400 MHz)	δ_{C} (CD_3OD , 150 MHz)	δ_{H} (mult; J) (CD_3OD , 600 MHz)
1	168.8	–	168.5	–
2	121.7	–	121.7	–
3/7	110.0	7.07 (2H; s)	110.0	7.04 (2H; s)
4/6	146.2	–	146.4	–
5	139.6	–	139.7	–
1'	61.6	4.28 (2H; q; 7.1)	61.6	4.28 (2H; q; 7.3)
2'	14.7	1.35 (3H; t; 7.1)	14.6	1.33 (3H; t; 7.3)

Table 5 $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ of compound 4

Compound 4			Gallic acid, Chanwitheesuk et al. [38]	
Positions	δ_{C} (CD_3OD , 100 MHz)	δ_{H} (mult; J) (CD_3OD , 400 MHz)	δ_{C} (acétone- d_6 , 100 MHz)	δ_{H} (mult; J) (acétone- d_6 , 400 MHz)
1	168.8	–	167.3	–
2	120.7	–	120.8	–
3/7	108.0	7.08 (2H; s)	109.1	7.15 (2H; s)
4/6	145.0	–	144.9	–
5	138.1	–	137.7	–

Table 6 Inhibition parameters (MIC, MBC) of partition fractions and isolated compounds from *Canarium schweinfurthii* against different test microorganisms

Tested samples	Studied parameters ($\mu\text{g/mL}$)	Strain/isolates			
		ST	STs	STM	SE
HEE 50/50	MIC	256	128	64	128
	MBC	512	512	256	512
	MBC/MIC	2	4	4	4
Hexane partition	MIC	1024	1024	512	> 1024
	MBC	> 1024	> 1024	> 1024	> 1024
	MBC/MIC	–	–	–	–
Chloroform partition	MIC	512	1024	256	1024
	MBC	1024	> 1024	> 1024	> 1024
	MBC/MIC	2	–	–	–
Ethyle acetate partition	MIC	256	256	128	32
	MBC	> 1024	1024	> 1024	128
	MBC/MIC	–	4	–	4
n-butanol partition	MIC	> 1024	1024	512	> 1024
	MBC	> 1024	> 1024	> 1024	> 1024
	MBC/MIC	–	–	–	–
Residual partition	MIC	> 1024	> 1024	> 1024	1024
	MBC	> 1024	512	256	> 1024
	MBC/MIC	–	–	–	–
Compound 1 Maniladiol	MIC	512	512	32	64
	MBC	> 1024	> 1024	128	256
	MBC/MIC	–	–	4	4
Compound 2 Scopoletin	MIC	32	32	16	16
	MBC	64	128	32	64
	MBC/MIC	2	4	2	4
Compound 3 Ethyl gallate	MIC	128	1024	64	1024
	MBC	> 1024	> 1024	> 1024	> 1024
	MBC/MIC	–	–	–	–
Compound 4 Gallic acid	MIC	32	32	64	128
	MBC	32	32	128	256
	MBC/MIC	1	1	2	2
Oxytetracycline	MIC	8	8	4	2
	MBC	32	64	32	16
	MBC/MIC	4	8	8	8
Ciprofloxacin	MIC	0,5	1	4	4
	MBC	2	2	8	8
	MBC/MIC	4	2	2	2

ST *Salmonella* Typhi, STs *Salmonella* Typhi ATCC6539, STM *Salmonella* Typhimurium, SE *Salmonella* Enteritidis, MIC Minimum inhibitory concentration, MBC Minimum bactericidal concentration.

of avian salmonellosis. Further studies will be necessary to verify the in vivo activity of these compounds and to elucidate their mechanisms of action.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s12906-020-03100-5>.

Additional file 1.

Acknowledgements

Authors thank the researchers of the Natural Products Chemistry Laboratory of CUI, Abbottabad Campus, Pakistan for their useful suggestions.

Authors' contributions

All the authors contributed to carry out this study. JBS was the principal investigator, OA and GSSN contributed to evaluate the anti-salmonellal activities. CNT, ANB and NK contributed to the fractionation purification and structural elucidation of isolated compounds. NK revised the manuscript, AK and DG co-supervised the work. All authors read and approved the final manuscript.

Funding

This research work was supported in part by The Academy of Sciences for the Developing World (TWAS) in collaboration with COMSATS University Islamabad (CUI) under grant FR number 3240299471 (TWAS-CIIT Postgraduate fellowship). The obtained fund was used for compound isolation and characterization.

Availability of data and materials

They are available as Supporting information.

Ethics approval and consent to participate

Not applicable in this section.

Consent for publication

All authors read and approved the final manuscript.

Competing interests

Authors have declared that no competing interests exist.

Author details

¹Research Unit of Microbiology and Antimicrobial substances, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon. ²Natural Products Chemistry Laboratory, Department of Chemistry, COMSATS University Islamabad, Abbottabad Campus-22060, Islamabad, Pakistan. ³Department of Chemistry, Faculty of Physical Sciences, University of Ilorin, P.M.B, Ilorin 1515, Nigeria. ⁴Department of Basic Scientific Studies, University Institute of Technology, University of Ngaoundere, P.O.Box 455, Ngaoundere, Cameroon. ⁵Department of Organic Chemistry, Faculty of Science, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon.

Received: 20 December 2019 Accepted: 29 September 2020

Published online:

References

- Rajagopal R, Mini M. Outbreaks of salmonellosis in three different poultry farms of Kerala, India. *Asian Pac J Trop Biomed*. 2013;3(6):496–500.
- Awa DN, Achukwi MD. Livestock pathology in the central African region: some epidemiological considerations and control strategies. *Anim Health Res Rev*. 2010;11:235–44.
- Ali MZ, Sultana S. Avian salmonellosis, Newcastle disease and aspergillosis. *Tech Rep*. 2012;25. <https://doi.org/10.13140/RG.2.1.1981.0404>.
- Threlfall EJ, Wain J, Peters T, Lane C, De Pinna E, Little CL, et al. Egg-borne infections of humans with *Salmonella*: not only an *S. Enteritidis* problem. *Worlds Poult Sci J*. 2014;70:15–26.
- Carli KT, Unal CB, Caner V, Eyigor A. Detection of *Salmonellae* in chicken feces by a combination of tetrathionate broth enrichment, capillary PCR, and capillary gel electrophoresis. *J Clin Microbiol*. 2001;39:1871–6.
- Crilly J, Power EP, Cowman HJ, Cryan B, Buckley JF. Epidemiology of *Salmonella* infection in the south of Ireland. *Ir J OfAgri and Food Resea*. 2001;40:215–26.
- INFOSAN (Réseau international des autorités de sécurité sanitaire des aliments). Lutte contre les salmonelles à la source. Note d'information INFOSAN n° 03/2007– Salmonelles. 2007;4.
- Danan C, Granier S, Bohnert M, Piquet C, Lalande F, Fremy S, et al. Surveillance active de la résistance aux antibiotiques des *Salmonella* isolées de la filière « poulet de chair » à différentes étapes de la chaîne alimentaire (données 2008–2009). *Bull Épidémiol Sante Anim Aliment*. 2008–2009;44:13–7.
- Batz MB, Hoffmann S, Morris JG. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. *J Food Prot*. 2012;75:1278–91.
- Mercado M, Ávila J, Rey M, Montoya M, Gamboa A, Carrascal AK, et al. Brotespor *Salmonella* spp., *Staphylococcus aureus* y *Listeria monocytogenes* asociados al consumo de pollo. *Biomédica*. 2012;32(3):375–85.
- Majowicz SE, Musto J, Scallan E, Angulo FJ, Kira M, O'Brien SJ, et al. The global burden of non typhoidal *Salmonella* gastroenteritis. *Clin Infect Dis*. 2010;50:882–9.
- WHO (World Health Organisation). Fièvre typhoïde et utilisation des Vaccins en Asie du Sud-Est et la région de l'ouest du pacifique occidental, 2009–2013. *Relevé Epidémiol Hebd* 2014;89(40):429–440.
- Antunes P, Mourão J, Campos J, Peixe L. Salmonellosis: the role of poultrymeat. *Clin Microbiol Infect*. 2016;22:110–21.
- Cardinale E, Perrier JD, Aidara A, Tall F, Coudert C, Gueye IL, et al. Identification d'une nouvelle salmonelle multirésistante dans Une viande de poulet de chair au Sénégal. *Revue Élev Méd vét Pays trop*. 2000;53(1):5–8.
- Ngoune TL, Tanedjeu KS, Mbofung CMF. Impact de l'utilisation des antibiotiques Sur la sensibilité des bactéries pathogènes de poules dans la ville de Ngaoundéré. *Cameroon J Exp Biol*. 2009;5(2):52–61.
- Chen MH, Hwang WZ, Wang SW, Shih YC, Tsen HY. Pulsed field gel electrophoresis (PFGE) analysis for multidrug resistant *Salmonella entericaserovar Schwarzengrund* isolates collected in six years (2000–2005) from retail chicken meat in Taiwan. *Food Microbiol*. 2011;28(3):399–405.
- Lamas A, Fernandez-No IC, Miranda JM, Vazquez B, Cepeda A, Franco CM. Prevalence, molecular characterization and antimicrobial resistance of *Salmonella* serovars isolated from northwestern Spanish broiler flocks (2011–2015). *Poult Sci*. 2016;95:2097–105.
- Bada-Alamedji R, Cardinal E, Biagui C, Akakpo AJ. Recherche de résidus de substances à activité antibactérienne dans la chair de poulet consommée dans la région de Dakar (Sénégal). *Bull Acad Vet Fr*. 2004;157(2):67–70.
- Dahan M, Hanotaux P, Durand F, Liebert F. Encadrement des pratiques commerciales pouvant influencer la prescription des antibiotiques vétérinaires. *RAPPORT N°RM2013-078P/GF2013-M-006-02/CGAAER N°13014*, 119. 2013;123.
- Sokoudjou JB, Fodouop SPC, Djouedam FG, Kodjio N, Kana JR, Fowa AB, et al. Antisalmonellal and antioxidant potential of hydroethanolic extract of *Canarium schweinfurthii* Engl. (Burseraceae) in *Salmonella enterica* serovar Typhimurium-infected chicks. *Asian Pac J Trop Biomed*. 2019;9(11):474–83.
- Patrick ME, Adcock PM, Gomez TM, Altekruse SF, Holland BH, Tauxe RV, et al. *Salmonella* Enteritidis infections, United States, 1985–1999. *Emerg Infect Dis*. 2004;10:1–7.
- Tala DS, Gatsing D, Fodouop SPC, Fokunang C, Kengni F, Djimeli MN. *In vivo* anti-salmonella activity of aqueous extract of *Euphorbia prostrata* Aiton (Euphorbiaceae) and its toxicological evaluation. *Asian Pac J Trop Biomed*. 2015;5(4):310–8.
- Kodjio N, Atsafack SS, Njateng GSS, Sokoudjou JB, Kuatie J-R, Gatsing D. Antioxidant effect of aqueous extract of *Curcuma longa* rhizomes (Zingiberaceae) in the typhoid fever induced in Wistar rats model. *J Adv Med Pharm Sci*. 2016;7(3):1–13.
- Ayachi A, Alloui N, Bennoune O, Yakhlef G, DaasAmiour S, Bouzid W, et al. Antibacterial activity of some fruits, berries and medicinal herb extracts against poultry strains of *Salmonella*. *American-Eurasian J Agric Environ Sci*. 2009;6(1):12–5.
- Varmuzova K, Matulova ME, Gerzova L, Cejkova D, Gardan-Salmon D, Panhéleux M, et al. *Curcuma* and *Scutellaria* plant extracts protect chickens against inflammation and *Salmonella* Enteritidis infection. *Poultry Sci*. 2015; 94:2049–58.
- Abiala M, Olayiwola J, Babatunde O, Aiyelagbe O. Akinyemi. Evaluation of therapeutic potentials of plant extracts against poultry bacteria threatening public health. *BMC Complement Altern Med*. 2016;16(417):1–8.

27. Tchoua N. Evaluation des activités antisalmonelles et antioxydantes des extraits de *Erica mannii* chez le poulet de chair. Thèse de Mater, Université de Dshang. Faculté des sciences, école doctorale des Sciences Fondamentales et Technologiques. 2016;97.
28. Sokoudjou JB, Njateng GSS, Fodouop SPC, Kodjio N, Atsack SS, Fowa AB, et al. *In vitro* antisalmonellal and antioxidant activities of *Canarium schweinfurthii* stem bark extracts. *Acad J Med Plants*. 2018;6(10):331–41.
29. Kamdem RS, Wafo O, Yousuf S, Ali Z, Adhikari A, Rasheed S, Khan IA. Canarene: a triterpenoid with a unique carbon skeleton from *Canarium schweinfurthii*. *Org Lett*. 2011;13:5492–5.
30. Coronel RH. Pili nut *Canarium ovatum* Engl. Promoting the conservation and use of underutilized and neglected crops. *Inst. Plant. Genet. Crop Plant Res. Gaters/International Plant Genet. Resour.* Institute, Rome, Italy. 1996;
31. Koudou J, Abena AA, Ngaissona P, Bessiere JM. Chemical composition and pharmacological activity of essential oil of *Canarium schweinfurthii*. *Fitoterapia*. 2005;76:700–3.
32. Yousuf S, Kamdem RS, Wafo P, Ngadjui BT, HoongKun F. A cocrystal of 3 α -hydroxy-tirucalla-8,24-dien-21-oic acid and 3 β -fluoro-tirucalla-7,24-dien-21-oic acid. *Acta Cryst*. 2011;E67:1015–6.
33. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Med*. 1998;64:711–3.
34. Mativandele SPN, Lall N, Meyer JJM. Antibacterial, antifungal and antitubercular activity of *Pelargonium reniforme* (CURT) and *Pelargonium sidoides* (DC) (Geraniaceae) root extracts. *S Afr J Bot*. 2006;72:232–7.
35. Quijano L, Rios T, Franczek FR, Fischer NH. The molecular structure of maniladiol from *Baccharis salicina*. *Phytochemistry*. 1998;49(7):2065–8.
36. Mogana R, Teng-Jin K, Wiart C. Anti-inflammatory, anticholinesterase, and antioxidant potential of scopoletin isolated from *Canarium patentinervium* Miq. (Burseraceae Kunth). *Evidence-Based Complement. Alt. Med*. 2013;2013:1–7.
37. Ooshiro A, Hiradate S, Kawano S, Takushi T, Fujii Y, Natsume M, et al. Identification and activity of ethyl gallate as an antimicrobial compound produced by *Geranium carolinianum*. *Weed Biol Manage*. 2009;9:169–72.
38. Chanwitheesuk A, Teerawutgulrag A, Kilburn JD, Rakariyatham N. Antimicrobial gallic acid from *Caesalpinia mimosoides* Lamk. *Food Chem*. 2007;100:1044–8.
39. Kuete V. Potential of Cameroonian plants and derived products against microbial infections: a review. *Planta Med*. 2010;76:1–13.
40. Kuete V, Efferth T. Cameroonian medicinal plants: pharmacology and derived natural products. *Frontiers in Pharmacol*. 2010;1(123):1–19.
41. Pasha C, Sayeed S, Ali MS, Khan MZ. Antisalmonella activity of selected medicinal plants. *Turk J Biol*. 2009;33:59–64.
42. Kengni F, Tala DS, Djimeli MN, Fodouop SPC, Kodjio N, Magnifouet HN, et al. *In vitro* antimicrobial activity of *Harungana madagascariensis* and *Euphorbia prostrata* extracts against some pathogenic *Salmonella* sp. *Int J Biol Chem Sci*. 2013;7(3):1106–18.
43. Tsobou R, Mapongmetsem PM, Van Damme P. Medicinal plants used against typhoid fever in Bamboutos division, Western Cameroon. *Ethnobotany Res Appl*. 2013;11:163–74.
44. Lunga PK, Tamokou JDD, Fodouop SPC, Kuate JR, Tchoumboue J, Gatsing D. Antityphoid and radical scavenging properties of the methanol extracts and compounds from the aerial part of *Paullinia pinnata*. *Springer Plus*. 2014;3(302):1–9.
45. Porte SM. Overview of folk medicine used for typhoid in India. *Int J Res Ayurveda Pharm*. 2014;5(2):219–24.
46. Noghogne LR, Gatsing D, Kodjio N, Sokoudjou JB, Kuate JR. *In vitro* antisalmonellal and antioxidant properties of *Mangifera indica* L. stem bark crude extracts and fractions. *Br J Pharm Res*. 2015;5(1):29–41.
47. Waihenya RK, Mtambo MMA, Nkwengulila G, Minga UM. Efficacy of crude extract of *Aloe secundiflora* against *Salmonella gallinarum* in experimentally infected free-range chickens in Tanzania. *J Ethnopharmacol*. 2002;79(3):317–23.
48. Khan RU, Naz S, Nikousefat Z, Tufarelli V, Laudadio V. *Thymus vulgaris*: alternative to antibiotics in poultry feed. *World Poult Sci J*. 2012;68:401–8.
49. Atsack SS, Kodjio N, Njateng GSS, Sokoudjou JB, Kuate J-R, Gatsing D. anti-infectious and *in vivo* antioxidant activities of *albizia gummifera* aqueous stem bark extract against *salmonella typhi*-induced typhoid fever in rats. *Int J Pharm*. 2016;6(2):20–30.
50. Iroha IR, Ilang DC, Ayogu TE, Oji AE, Ugbo EC. Screening for anti-typhoid activity of some medicinal plants used in traditional medicine in Ebonyi state, Nigeria. *Afr J Pharm Pharm*. 2010;4(12):860–4.
51. Akinyemi OI, Dada EO. *In vivo* antityphoid activities and proximate analysis of ethanolic leaf extracts of *Parquetina nigrescens*. *J Pharm Biol Sci*. 2014;9(5):115–2.
52. Gul S, Eraj A, Ashraf Z. *Glycyrrhiza glabra* and *Azadirachta indica* against *Salmonella* Typhi: herbal treatment as an alternative therapy for typhoid fever. *iMedPub J (Arch Med)*. 2015;7(6/4):1–5.
53. Ngoudjou TD, Arfat YM, Njateng GSS, Fokunang C, Nyemb JN, Nighat F, et al. GC/MS analysis, antisalmonellal potential of methanol leaf extracts of *Tristemma mauritanum* and effects on hematological parameters on *Wistar* rats infected with *Salmonella* Typhi. *Int J Pharm*. 2017;7(2):120–31.
54. Mogana R, Wiart C. *Canarium* L.: A phytochemical and pharmacological review. *J Pharm Res*. 2011;4(8):2482–9.
55. Kaleem WA, Nisar M, Qayum M, Zia-Ul-Haq M, Adhikari A, Feo VD. New 14-membered cyclopeptide alkaloids from *Zizyphus oxyphylla* Edgew. *Int J Mol Sci*. 2012;13:11520–9.
56. Tamokou JDD, Kuate JR, Tene M, Nwemegueta TJK, Tane P. The antimicrobial activities of extract and compounds isolated from *Brillanta isialamium*. *Iran J Med Sci*. 2011;36(1):24–31.
57. Teponno RB, Tapondjou AL, Gatsing D, Djoukeng JD, Abou-Mansour E, Tabacchi R, et al. Bafoudiosbulbins a, and B, two anti-salmonellal clerodane diterpenoids from *Dioscorea bulbifera* L. Var sativa. *Phytochemistry*. 2006; 67(17):1957–63.
58. Okoli BJ, Ndukwe GI, Ayo RG, Habila JD. Inhibition of the developmental stages of *ascaris suum* and antimicrobial activity of 3 β -hydroxyolean-12,18-diene isolated from the aerial parts of *Canarium schweinfurthii* (Engl). *American Chem Sci J*. 2016;11(3):1–11.
59. Manuele MG, Ferraro G, Arcos MLB, López P, Cremaschi G, Anesini C. Comparative immunomodulatory effect of scopoletin on tumoral and normal lymphocytes. *Life Sci*. 2006;79:2043–8.
60. Acharya D, Bogati B, Risal P. Scopoletin reduces intracellular survival of *Salmonella typhi* within U937 human macrophage cell line *in vitro*. *Sky J Microbiol Res*. 2013;1(6):47–51.
61. Reyes AWB, Hong TG, Hop HT, Arayan LT, Huy TXN, Min W, et al. The *in vitro* and *in vivo* protective effects of tannin derivatives against *Salmonella enterica* serovar Typhimurium infection. *Microb Pathog*. 2017;109:86–93.
62. López-Romero JC, Valenzuela-Melendres M, Juneja VK, García-Dávila J, Camou JP, Peña-Ramos A, et al. Effects and interactions of gallic acid, eugenol and temperature on thermal inactivation of *Salmonella* spp. in ground chicken. *Food Res Int*. 2018;103:289–94.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

