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Research Article

BIOCIDAL POTENTIALS OF STEM BARK EXTRACTS OF *PSIDIUM GUAJAVA* (LINN.) ON PANEL OF BACTERIAL STRAINS ASSOCIATED WITH DIARRHEA AND DYSENTERY

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ABSTRACT

Objective: Investigations were carried out on cidal effects of crude extract and fractions obtained from stem bark of *Psidium guajava* against a panel of 74 strains of *Escherichia coli* and *Shigella dysenteriae* implicated in diarrhea and dysentery infections.

Methods: Powdered sample of the stem bark was extracted in methanol/distilled water (3:2) and then partitioned into different organic solvents. The fractions obtained were subjected to antibacterial tests against a panel of bacterial strains. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined using agar dilution method while the mode of action of the active fractions was investigated through time-kill dynamics and leakages of proteins and potassium ions from the cells.

Results: All partitioned fractions except the aqueous fraction exhibited antibacterial activities against the panel of bacterial strains at a final concentration of 10 mg/mL. The MIC exhibited by the crude extract against susceptible bacterial strains ranged between 1.56 mg/mL and 12.5 mg/mL, while the MIC for the four fractions ranged between 0.31 mg/mL and 5.0 mg/mL. The MBC ranged between 3.13–12.5 mg/mL and 0.63–5.0 mg/mL for the crude extract and fractions, respectively. The time-kill assay revealed that the percentage of the cells killed increase with an increase in the concentrations of the fractions as well as contact time intervals. Proteins and potassium ions leakages from the bacterial cells followed the same trend with that of time-kill assay.

Conclusion: The stem bark extracts of *P. guajava* exhibited appreciable bactericidal effects on bacterial strains associated with diarrhea and dysentery in humans.

Keywords: *Psidium guajava*, Antibacterial, Phytochemicals, Killing rate, Protein leakage, Potassium ions leakage.

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INTRODUCTION

Medicinal plants serve as a source of folklore remedies for many ailments, especially among the rural dwellers in Africa and many parts of the countries worldwide. Herbal medicines are gradually becoming an integral part of primary health-care delivery. Diarrhea is a very common disease condition in most tropical communities, and the use of herbal decoction is a common practice in its management [1]. *Escherichia coli* and *Shigella dysenteriae* are the causative agents of diarrhea and dysentery, respectively. Diarrhea and dysentery are known to be killer diseases especially among the children [2,3]. These vectors are now gradually developing resistant against the available antibiotics used as therapy against infections caused by these pathogens. There is an urgent need to source for other potent antimicrobials especially of natural origin to combat infections caused by these pathogens and other microorganisms as well. This will ensure a reduction in the rate of untimely death among the children. This study thus investigated the potency of *Psidium guajava* stem bark extract against *E. coli* and *S. dysenteriae*.

P. guajava is among the widely used medicinal plant in local folklore remedy. Its fruits and leaves have been studied for different medicinal values [4]. *P. guajava* belongs to the family Myricaceae and comprises 150 species. It is called apple of tropics in English and known as "Guofa" in Yoruba language. *P. guajava* is a large dicotyledonous shrub and is generally 3–10 m high with many branches. This tree is characterized by very thin skin and the fruit is ovoid or pear-shaped berry of about 4–12 cm long [5]. *P. guajava* is valued as a potential source of pectin, ascorbic acid, sugars, and minerals [6]. The main phytochemical constituents of *P. guajava* are tannins, phenolic compounds, flavonoids, sesquiterpene alcohols, and triterpenoid acids [7]. All parts of *P. guajava* including fruits, leaves, stem, bark, and roots have been used for treating stomach ache and diarrhea in many countries [8] and has been found to possess anticancer properties [9]. The seeds of *P. guajava* are used for the treatment of gastrointestinal infections and are known to possess anti-carcinogenic activity [10,11]. The *in vitro* studies of *P. guajava* leaves and stem bark revealed that they exhibited significant antimicrobial activities against *Staphylococcus aureus*, *S. dysenteriae*, *Bacillus subtilis*, *E. coli*, *Pseudomonas aeruginosa,* and *Candida albicans* [12]. The stem bark extract of *P. guajava* is an astringent and can be used to treat diarrhea in children, while the flowers have been used to treat bronchitis and eyesores [13]. Han *et al*. [14] studied the effects of extract from *P. guajava* on atopic dermatitis and found it to inhibit the chemokine expression in keratinocytes [15]. The extracts from these plants have also been found to be useful in wound healing due to the presence of tannins and flavonoids in *P. guajava*.

METHODS

Preparation of microorganisms used for this study

Various stool samples isolates used for this study were *E. coli* and *S. dysenteriae,* which were graciously donated by Department of Medical Microbiology and Parasitology, Obafemi Awolowo University, Ile-Ife, Nigeria. The typed cultures of the National Collection of Industrial Bacteria (NCIB) and American Type Culture Collection (ATCC) were collected from the culture collection of Prof D.A. Akinpelu, Department

Table 1: Susceptibility patterns exhibited by the crude stem bark extract of *Psidium guajava* and the standard antibiotics against susceptible bacterial isolates

Zones of inhibition (mm)*			
Bacterial isolates	***Psidium guajava* (25 mg/ml)**	**Streptomycin (1 mg/ml)**	**Ampicillin (1 mg/ml)**
EC1	24±2.00	21±5.03	10±1.15
EC2	24±1.13	22±4.00	0
EC3	25±1.15	17±4.16	11±1.14
EC4	23±1.14	19±5.77	0
EC5	23±1.12	20±4.00	0
EC6	27±1.11	19±7.02	0
EC7	23±2.31	18±3.46	0
EC8	25±1.14	21±6.42	0
EC9	25±1.16	23±4.61	10±1.13
EC10	25±1.15	29±1.15	12±2.00
EC11	22±2.00	28±2.00	13±1.12
EC12	24±2.00	25±5.03	13±1.15
EC13	27±1.14	20±7.21	0
EC14	25±1.13	26±4.00	12±2.00
EC15	27±1.12	24±6.92	11±1.14
EC16	27±1.15	20±8.00	11±1.16
EC17	26±2.00	21±4.16	0
EC18	26±2.00	26±5.29	11±2.31
EC19	25±1.15	27±4.16	13±1.13
EC20	26±2.00	21±5.77	12±2.00
EC21	25±1.14	19±7.02	11±1.15
EC22	22±2.00	22±9.16	13±1.16
EC23	25±1.12	25±5.03	12±2.00
EC24	25±1.13	22±7.21	11±1.13
EC25	23±1.15	22±6.00	0
EC26	17±3.05	18±2.00	0
EC27	25±2.31	21±7.02	0
EC28	23±1.15	18±5.29	0
EC29	22±2.00	19±9.23	11±1.16
EC30	23±3.05	13±1.15	0
EC31	21±1.14	13±1.16	0
EC32	23±1.16	16±4.00	0
EC33	24±2.00	16±2.00	0
EC34	23±3.05	23±5.77	0
EC35	23±1.15	23±7.57	0
EC36	20±2.00	21±9.01	0
EC37	22±2.00	25±4.16	0
EC38	19±1.13	16±5.29	0
EC39	23±2.31	20±3.46	0
EC40	23±1.15	17±4.61	10±1.15
EC41	22±2.00	16±3.46	0
EC42	22±2.00	15±3.05	0
EC43	22±2.00	13±2.31	0
EC44	24±2.00	0	0
EC45	21±1.15	0	0
EC46	22±1.14	18±6.00	0
EC47	21±2.00	13±1.15	0
EC48	22±2.00	0	0
EC49	21±1.15	12±1.16	0
EC50	25±1.16	21±4.16	11±1.15
EC51	22±1.14	25±3.05	0
EC52	24±1.16	23±1.16	0
SH1	21±1.15	18±2.00	0
SH2	23±1.16	20±2.00	0
SH3	22±2.00	0	0
SH4	23±1.15	0	0
SH5	21±1.14	22±2.00	11±1.15
SH6	21±1.17	21±1.15	0
SH7	22±2.00	0	0
SH8	24±2.00	23±1.16	0
SH9	20±2.00	0	0
SH10	24±2.00	27±3.05	0
SH11	21±1.13	0	0
SH12	19±1.15	19±1.13	10±1.14
SH13	22±2.00	21±1.15	0
SH14	21±1.17	24±2.00	0
SH15	22±2.00	23±1.17	0
SH16	24±2.00	21±3.05	0
SH17	23±1.15	21±1.15	0
SH18	22±2.00	20±2.00	0
SH19	21±1.12	18±2.00	0
SH20	19±1.14	21±1.14	11±1.16
SH21	23±1.14	19±1.14	0
SH22	21±1.13	25±2.00	0
SH9	20±2.00	0	0

EC1–EC52: Strains of *Escherichia coli*, SH1–SH22: Strains of *Shigella dysenteriae*, EC51: Typed culture (ATCC 25922), EC52: Typed culture (NCIB 86), SH21: Typed culture (ATCC 11311), SH 22: Typed culture (NCIB 197), 0: Not sensitive, *: Mean of three replicates. ATCC: American Type Culture Collection, NCIB: National Collection of Industrial Bacteria

of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. These organisms included *E. coli* (NCIB 86), *E. coli* (ATCC 25922), *S. dysenteriae* (NCIB 197), and *S. dysenteriae* (ATCC 11311). The purity of these organisms was done before been used. The preparation of the bacterial strains was done using the colony suspension method as described by European Committee on Antimicrobial Susceptibility Testing.

Culture media used

The culture media used for this study were nutrient broth (LAB M) and nutrient agar (LAB M) for sub-culturing the bacterial strains. Mueller-Hinton agar (LAB M) was used for sensitivity testing, eosin methylene blue agar (LAB M) was used to re-confirm *E. coli* and *S. dysenteriae,* respectively. All media except *Salmonella-Shigella* agar were sterilized in an autoclave at 121°C for 15 min.

Collection and preparation of plant sample

The fresh stem bark of *P. guajava* used for this study was collected at Abeokuta, Ogun State, Nigeria, in the month of May 2015. The plant was identified in the Herbarium of Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The voucher sample of the plant was prepared and deposited in the herbarium for future reference purposes with voucher number IFE-14753. The plant sample was oven-dried at 40°C until a constant weight of the sample was obtained. The dried sample was later ground into a fine powder and stored in an air-tight container for further use.

Extraction of biological active components of the plant sample

One thousand and five hundred grams of the powdered sample of the plant were soaked in a mixture of methanol and sterile distilled water in ratio 3:2 (v/v) and left on the laboratory bench for 4 days with regular agitation of the aliquot. The solution collected was filtered into a clean sterile flask and the supernatant collected was concentrated *in vacuo* using a rotatory evaporator to eliminate the methanol leaving aqueous portion. The aqueous part was then lyophilized to collect the crude extract of the plant sample.

Assessment of photochemical components in the extract from *P. guajava* stem bark

The assessment of the phytochemicals in the plant extract was done using Trease and Evans [16] and Harborne [17] methods. A small portion of the dry extract was screened for the presence of alkaloids, tannins, steroids, cardiac glycosides, reducing sugars, and saponins.

Fractionation of the crude extract

The crude extract was fractionated using a different organic solvent in order of their polarity, starting with low polar solvent and graduated to the highest polar solvent that is, starting with n-hexane and finished

Table 2: Sensitivity patterns exhibited by fractions obtained from stem bark extract of *Psidium guajava* against susceptible bacterial isolates

Zones of inhibition (mm)*					
Bacterial isolates	**N-HEX (10 mg/ml)**	**CHLORO (10 mg/ml)**	**ETHYL (10 mg/ml)**	**BUT (10 mg/ml)**	**AQU (10/mg/ml)**
EC1	17±3.05	19±4.16	15±2.31	17±3.05	0
EC2	14±2.00	15±3.05	15±1.15	15±1.15	0
EC3	17±3.06	17±5.03	15±2.31	17±1.17	0
EC4	18±3.46	21±1.15	19±1.13	23±1.13	0
EC5	15±3.05	14±2.00	14±2.00	21±1.14	0
EC6	17±3.03	16±2.00	19±1.14	23±2.31	0
EC7	17±3.02	14±2.00	16±2.00	19±1.15	0
EC8	17±3.04	19±1.16	21±1.15	21±2.31	0
EC9	17±3.07	18±2.00	16±2.00	21±1.13	0
EC10	17±4.16	19±1.15	19±1.17	22±2.00	0
EC11	17±5.03	17±2.31	15±3.05	20±2.00	0
EC12	15±4.16	15±2.33	13±1.14	15±1.15	0
EC13	16±7.21	19±3.05	20±3.46	21±1.16	0
EC14	16±2.00	19±4.16	21±1.16	21±2.31	0
EC15	19±1.15	17±4.61	21±5.03	23±3.05	0
EC16	18±3.46	19±3.05	16±2.31	21±1.15	0
EC17	17±5.03	18±3.46	18±2.00	17±3.05	0
EC18	14±2.00	17±4.16	13±1.15	17±2.31	0
EC19	13±3.05	16±2.00	15±1.17	17±3.03	0
EC20	13±2.31	15±2.31	17±1.14	13±1.15	0
EC21	14±2.00	16±2.00	16±4.00	17±3.05	0
EC22	12±2.00	15±3.05	13±1.15	19±3.06	0
EC23	15±1.15	13±3.07	17±3.05	20±2.00	0
EC24	15±4.16	17±3.03	15±3.03	21±4.16	0
EC25	17±6.11	17±1.15	17±5.03	18±2.00	0
EC26	17±3.05	17±3.05	19±1.15	19±1.17	0
EC27	15±3.06	13±2.31	18±2.00	17±1.16	0
EC28	17±4.16	16±2.00	17±3.05	22±2.00	0
EC29	15±1.15	13±1.15	17±3.07	17±3.05	0
EC30	17±4.16	19±1.14	16±4.00	19±1.15	0
EC31	17±3.05	17±2.31	19±2.31	18±2.00	0
EC32	17±3.07	17±2.30	20±2.00	22±2.00	0
EC33	13±3.05	18±4.00	16±2.00	19±1.15	0
EC34	13±3.03	18±2.00	16±3.46	19±1.16	0
EC35	15±1.15	17±3.05	19±3.05	21±1.13	0
EC36	16±2.00	22±2.00	19±1.15	19±1.17	0
EC37	13±3.05	18±2.00	15±2.31	19±1.14	0
EC38	15±3.07	20±2.00	20±2.00	17±1.13	0
EC39	13±3.05	20±2.00	21±1.15	22±2.00	0
EC40	15±3.07	22±2.00	19±2.31	21±1.15	0
EC41	13±3.03	20±2.00	18±2.00	22±2.00	0
EC42	16±5.29	21±1.15	19±3.05	21±1.16	0
EC43	15±5.03	19±3.05	19±1.15	19±1.17	0
EC44	16±2.00	17±3.07	19±2.31	21±1.15	0
EC45	16±3.46	17±1.15	17±3.05	19±1.14	0
EC46	13±3.05	13±4.16	17±3.06	18±2.00	0
EC47	15±3.06	19±4.17	19±3.07	20±2.00	0
EC48	17±2.31	18±2.00	21±2.31	19±1.15	0
EC49	16±2.00	16±2.00	22±2.00	17±1.16	0
EC50	17±3.05	24±2.00	25±3.05	20±2.00	0
EC51	17±3.05	17±2.31	19±2.31	18±2.00	0
EC52	15±3.07	20±2.00	20±2.00	17±1.13	0
SH1	19±1.15	20±2.00	19±1.15	24±2.00	0
SH2	19±3.05	19±1.15	17±3.05	21±1.15	0
SH3	21±1.16	18±2.00	17±1.13	24±2.00	0
SH4	17±1.17	18±2.00	19±1.16	23±2.31	0
SH5	17±3.05	14±2.00	14±2.00	19±2.30	0
SH6	18±2.00	19±1.14	16±2.00	20±2.00	0
SH7	19±1.14	19±1.13	17±2.31	23±1.14	0
SH8	20±2.00	21±1.17	16±2.00	21±1.16	0
SH9	19±1.15	20±0.00	17±1.15	23±1.17	0
SH10	19±1.16	18±2.00	15±1.16	23±2.31	0
SH11	19±1.17	19±1.13	18±3.46	20±2.00	0
SH12	21±1.12	17±1.16	16±2.00	19±1.15	0
SH13	21±1.13	19±1.15	15±1.16	21±1.17	0
SH14	17±1.15	21±1.17	15±1.17	23±1.13	0
SH15	19±1.17	20±2.00	14±2.00	21±1.12	0

(contd...)

Table 2: *(Continued)*

Zones of inhibition (mm)*					
Bacterial isolates	**N-HEX (10 mg/ml)**	**CHLORO (10 mg/ml)**	**ETHYL (10 mg/ml)**	**BUT (10 mg/ml)**	**AQU (10/mg/ml)**
SH16	17±3.05	21±1.17	17±1.13	21±1.16	0
SH17	18±2.00	20±2.00	19±1.17	23±1.14	0
SH18	20±2.00	19±1.16	19±1.14	24±2.00	0
SH19	19±1.15	18±2.00	17±1.15	23±1.15	0
SH20	21±1.14	19±1.15	19±1.16	23±1.17	0
SH21	21±1.16	18±2.00	17±1.13	24±2.00	0
SH22	20±2.00	21±1.17	16±2.00	21±1.16	0

EC1–EC52: Strains of *Escherichia coli*, SH1–SH22: Strains of *Shigella dysenteriae*, EC51: Typed culture (ATCC 25922), EC52: Typed culture (NCIB 86), S21: Typed culture (ATCC 11311), SH22: Typed culture (NCIB 197), N-HEX: n-Hexane fraction, CHLORO: Chloroform fraction, ETHYL: Ethyl acetate fraction, BUT: Butanol fraction, AQU: Aqueous fraction, 0: Not sensitive, *: Mean of three replicates. ATCC: American Type Culture Collection, NCIB: National Collection of Industrial Bacteria

Table 3: The minimum inhibitory concentrations exhibited by crude stem bark extract and active fractions of *Psidium guajava* against susceptible bacterial isolates

Bacterial isolates	CE (mg/ml)	N-HEX (mg/ml)	CHLORO (mg/ml)	ETHYL (mg/ml)	BUT (mg/ml)
EC1	1.56	0.31	0.31	1.25	0.31
EC2	1.56	0.31	0.63	0.31	0.63
EC3	3.13	2.50	2.50	2.50	2.50
EC4	3.13	2.50	1.25	2.50	0.63
EC5	12.5	5.00	2.50	1.25	2.50
EC6	1.56	1.25	2.50	1.25	1.25
EC7	6.25	2.50	5.00	2.50	0.63
EC8	1.56	0.63	2.50	1.25	0.31
EC9	1.56	0.63	2.50	1.25	2.50
EC10	6.25	5.00	1.25	2.50	0.63
EC11	6.25	2.50	2.50	1.25	1.25
EC12	3.13	1.25	2.50	2.50	1.25
EC13	1.56	0.63	1.25	1.25	0.31
EC14	3.13	1.25	2.50	2.50	0.63
EC15	1.56	0.63	1.25	1.25	0.31
EC16	3.13	2.50	1.25	2.50	1.25
EC17	6.25	2.50	0.63	1.25	2.50
EC18	1.56	0.31	1.25	1.25	0.63
EC19	3.13	1.25	2.50	1.25	1.25
EC20	1.56	0.31	0.63	0.63	0.31
EC21	3.13	1.25	2.50	2.50	1.25
EC22	12.5	5.00	2.50	5.00	2.50
EC23	6.25	2.50	5.00	2.50	0.63
EC24	3.13	2.50	1.25	2.50	1.25
EC25	6.25	2.50	2.50	1.25	1.25
EC26	12.5	5.00	2.50	5.00	2.50
EC27	3.13	1.25	1.25	2.50	0.63
EC28	6.25	2.50	2.50	1.25	1.25
EC29	6.25	1.25	2.50	2.50	1.25
EC30	3.13	1.25	1.25	1.25	1.25
EC31	12.5	5.00	2.50	2.50	1.25
EC32	3.13	1.25	2.50	1.25	0.63
EC33	1.56	0.31	1.25	0.63	0.31
EC34	6.25	2.50	2.50	2.50	1.25
EC35	3.13	1.25	1.25	1.25	0.63
EC36	12.5	2.50	5.00	2.50	2.50
EC37	3.13	1.25	1.25	0.63	1.25
EC38	12.5	5.00	2.50	5.00	1.25
EC39	3.13	1.25	2.50	2.50	1.25
EC40	6.25	2.50	0.31	0.63	0.63
EC41	3.13	1.25	0.63	2.50	1.25
EC42	6.25	2.50	2.50	1.25	0.31
EC43	3.13	1.25	1.25	0.63	1.25
EC44	3.13	2.50	2.50	1.25	1.25
EC45	12.5	5.00	2.50	5.00	2.50
EC46	6.25	1.25	5.00	2.50	1.25
EC47	12.5	5.00	2.50	5.00	2.50
EC48	12.5	2.50	5.00	2.50	2.50
EC49	6.25	1.25	0.63	0.63	1.25
EC50	3.13	0.63	2.50	1.25	2.50
EC51	6.25	1.25	2.50	2.50	1.25
EC52	3.13	0.63	0.31	2.50	0.63
SH1	6.25	1.25	1.25	2.50	1.25
SH2	3.13	2.50	2.50	1.25	2.50
SH3	6.25	0.63	1.25	0.63	1.25
SH4	1.56	0.31	0.63	1.25	0.31
SH5	3.13	0.63	1.25	2.50	1.25
SH6	6.25	1.25	0.63	5.00	0.63
SH7	3.13	0.63	2.50	1.25	1.25
SH8	1.56	0.31	1.25	0.63	0.31
SH9	12.5	5.00	2.50	2.50	1.25
SH10	1.56	0.31	1.25	1.25	0.63
SH11	3.13	0.63	0.31	1.25	0.63
SH12	12.5	2.50	5.00	5.00	2.50
SH13	3.13	0.63	0.63	2.50	1.25
SH14	6.25	1.25	2.50	1.25	0.63
SH15	6.25	2.50	2.50	1.25	1.25
SH16	3.13	0.31	0.63	0.63	0.31
SH17	3.13	0.63	1.25	0.63	0.63
SH18	12.5	5.00	2.50	5.00	2.50
SH19	12.5	2.50	2.50	5.00	2.50
SH20	3.13	0.63	1.25	0.63	0.63
SH21	3.13	0.31	2.50	1.25	1.25
SH22	6.25	1.25	2.50	1.25	0.63

(contd...)

Table 3: *(Continued)*

EC1–EC52: Strains of *Escherichia coli*, SH1–SH22: Strains of *Shigella dysenteriae*, EC51: Typed culture (ATCC 25922), EC52: Typed culture (NCIB 86), SH21: Typed culture (ATCC 11311), SH22: Typed culture (NCIB 197), N-HEX: n-Hexane fraction, CHLORO: Chloroform fraction, ETHYL: Ethyl acetate fraction, BUT: Butanol fraction. ATCC: American Type Culture Collection, NCIB: National Collection of Industrial Bacteria

up with n-butanol. The fractions obtained were kept in an air-tight container in a refrigerator at 5°C for further use.

Determination of the antibacterial potency of the crude extract and fractions obtained from it

The antibacterial tests were done using agar-well diffusion methods [18]. The test organisms were re-activated in nutrient broth for 18 h before use. Exactly 0.1 ml of standardized test bacterial strains (10^6 cfu/mL of 0.5 McFarland standard) was transferred into Mueller-Hinton agar medium at 40°C. This was thoroughly mixed together and later poured into pre-sterilized Petri dishes. The plates were allowed to set and wells were bored into the medium using 6 mm sterile cork borer. These wells were then filled up with the prepared solutions of the crude extract and the fractions. Care was taken not to allow the solution to spill on the surface of the medium. The final concentrations of the crude extract and the fractions used were 25 mg/mL and 10 mg/mL, respectively. On the other hand, ampicillin and streptomycin were used

Table 4: The minimum bactericidal concentrations exhibited by crude stem bark extract and active fractions of *Psidium guajava* against susceptible bacterial isolates

Bacterial isolates	CE (mg/ml)	N-HEX (mg/ml)	CHLORO (mg/ml)	ETHYL (mg/ml)	BUT (mg/ml)
EC1	3.13	0.63	0.63	2.50	0.63
EC2	3.13	0.63	1.25	0.63	1.25
EC3	6.25	5.00	5.00	5.00	5.00
EC4	6.25	5.00	2.50	5.00	1.25
EC5	ND	ND	5.00	2.50	5.00
EC6	3.13	2.50	5.00	2.50	2.50
EC7	12.5	5.00	ND	5.00	1.25
EC8	3.13	1.25	5.00	2.50	0.63
EC9	3.13	1.25	5.00	2.50	5.00
EC10	12.5	ND	2.50	5.00	1.25
EC11	12.5	5.00	5.00	2.50	2.50
EC12	6.25	2.50	5.00	5.00	2.50
EC13	3.13	1.25	2.50	2.50	0.63
EC14	6.25	2.50	5.00	5.00	1.25
EC15	3.13	1.25	2.50	2.50	0.63
EC16	6.25	5.00	2.50	5.00	2.50
EC17	12.5	5.00	1.25	2.50	5.00
EC18	3.13	0.63	2.50	2.50	1.25
EC19	6.25	2.50	5.00	2.50	2.50
EC20	3.13	0.31	0.63	0.63	0.31
EC21	6.25	2.50	5.00	5.00	2.50
EC22	ND	ND	5.00	ND	5.00
EC23	12.5	5.00	ND	5.00	1.25
EC24	6.25	5.00	2.50	5.00	2.50
EC25	12.5	5.00	5.00	2.50	2.50
EC26	ND	ND	5.00	ND	5.00
EC27	6.25	2.50	2.50	5.00	1.25
EC28	12.5	5.00	5.00	2.50	2.50
EC29	12.5	2.50	5.00	5.00	2.50
EC30	6.25	2.50	2.50	2.50	2.50
EC31	ND	ND	5.00	5.00	2.50
EC32	6.25	2.50	5.00	2.50	1.25
EC33	6.25	0.63	2.50	1.25	0.63
EC34	12.5	5.00	5.00	5.00	2.50
EC35	6.25	2.50	2.50	2.50	1.25
EC36	ND	5.00	ND	5.00	5.00
EC37	6.25	2.50	2.50	1.25	2.50
EC38	ND	ND	5.00	ND	2.50
EC39	6.25	2.50	5.00	5.00	2.50
EC40	12.5	5.00	0.63	1.25	1.25
EC41	6.25	2.50	1.25	5.00	2.50
EC42	12.5	5.00	5.00	2.50	0.63
EC43	6.25	2.50	2.50	1.25	2.50
EC44	6.25	5.00	5.00	2.50	2.50
EC45	25.0	ND	5.00	ND	5.00
EC46	12.5	2.50	ND	5.00	2.50
EC47	ND	ND	5.00	ND	5.00
EC48	ND	5.00	ND	5.00	5.00
EC49	12.5	2.50	1.25	1.25	2.50
EC50	6.25	1.25	5.00	2.50	5.00
EC51	12.5	2.50	5.00	5.00	2.50
EC52	6.25	1.25	0.63	5.00	1.25
SH1	12.5	2.50	2.50	5.00	2.50
SH2	6.25	5.00	5.00	2.50	5.00
SH3	12.5	1.25	2.50	1.25	2.50
SH4	3.13	0.63	1.25	2.50	0.63
SH5	6.25	1.25	2.50	5.00	2.50
SH6	12.5	2.50	1.25	ND	1.25
SH7	6.25	1.25	5.00	2.50	2.50
SH8	3.13	0.63	2.50	1.25	0.63
SH9	ND	ND	5.00	5.00	2.50
SH10	3.13	0.63	2.50	2.50	1.25
SH11	6.25	1.25	0.63	2.50	1.25
SH12	ND	5.00	ND	ND	5.00
SH13	6.25	1.25	1.25	5.00	2.50
SH14	12.5	2.50	5.00	2.50	1.25
SH15	12.5	5.00	5.00	2.50	2.50
SH16	6.25	0.63	1.25	1.25	0.63
SH17	6.25	1.25	2.50	1.25	1.25
SH18	ND	ND	5.00	ND	5.00
SH19	ND	5.00	5.00	ND	5.00
SH20	6.25	1.25	2.50	1.25	1.25
SH21	6.25	0.63	5.00	2.50	2.50
SH22	12.5	2.50	5.00	2.50	1.25

(contd...)

Table 4: *(Continued)*

EC1–EC52: Strains of *Escherichia coli*, SH1–SH22: Strains of *Shigella dysenteriae*, EC51: Typed culture (ATCC 25922), EC52: Typed culture (NCIB 86), SH21: Typed culture (ATCC 11311) SH22: Typed culture (NCIB 197); N-HEX: n-Hexane fraction, CHLORO: Chloroform fraction, ETHYL: Ethyl acetate fraction, BUT: Butanol fraction, ND: Not done. ATCC: American Type Culture Collection, NCIB: National Collection of Industrial Bacteria

Table 5: Phytochemical compounds exhibited by the extract

Phytochemical test	Results
Alkaloids	Positive
Saponins	Positive
Tannins	Positive
Steroids	Positive
Flavonoids	Positive
Reducing sugars	Positive
Cardiac glycosides	Positive

as positive controls at a concentration of 1 mg/mL. The plates were left on the laboratory bench for 1 h to allow proper in-flow of the solution into the medium before incubating them at 37°C for 24 h. The plates were not stock-piled to allow even distribution of temperature round the plates to avoid false results. The plates were later observed for zones of inhibition which is an indication of susceptibility of the test organisms to the extracts.

Assessment of minimum inhibitory concentrations (MIC) of the crude extract and fractions against susceptible organisms

Two-fold dilution of the crude extract and fractions was adopted to determine their MIC [19]. Two milliliters of different concentrations of the solution were added to 18 mL of pre-sterilized molten nutrient agar to give the final concentrations ranging between 0.31 mg/mL–5.00 mg/mL for the fractions and 1.56 mg/mL–12.5 mg/mL for crude extract. The mixture was then poured into well-labeled sterile Petri dishes and allowed to solidify. The plates were left on the laboratory bench overnight to ascertain their purity. The surfaces of the plates were allowed to dry before streaking with the standardized inoculums of the organisms and incubated aerobically at 37°C for 48 h. The plates were later examined for the presence or absence of bacterial growth. The MIC was taken as the lowest concentration of the fraction that inhibited the growth of the organisms.

Assessment of minimum bactericidal concentration (MBC) of the crude extract and fractions against susceptible organisms

The MBC of the fractions and the crude extract was assessed by taken sample from streaked line of the MIC test and cultured on fresh sterile nutrient agar medium. The plates were incubated at 37°C for 72 h. The MBC was taken as the concentrations of the fraction that did not support any bactericidal growth of the medium.

Determination of the killing rate of bacterial strains by the fractions

The test was carried out using test bacterial strains, that is, *E. coli* and *S. dysenteriae* on their viability against different concentrations of fractions relative to their MIC [18]. Viable counts of the test organisms were initially determined to standardize the inoculums. Exactly 0.5 mL volume of unknown cell density (by viable counts 10^6 cfu/mL) was added to 4.5 mL of different concentrations of the fraction relative

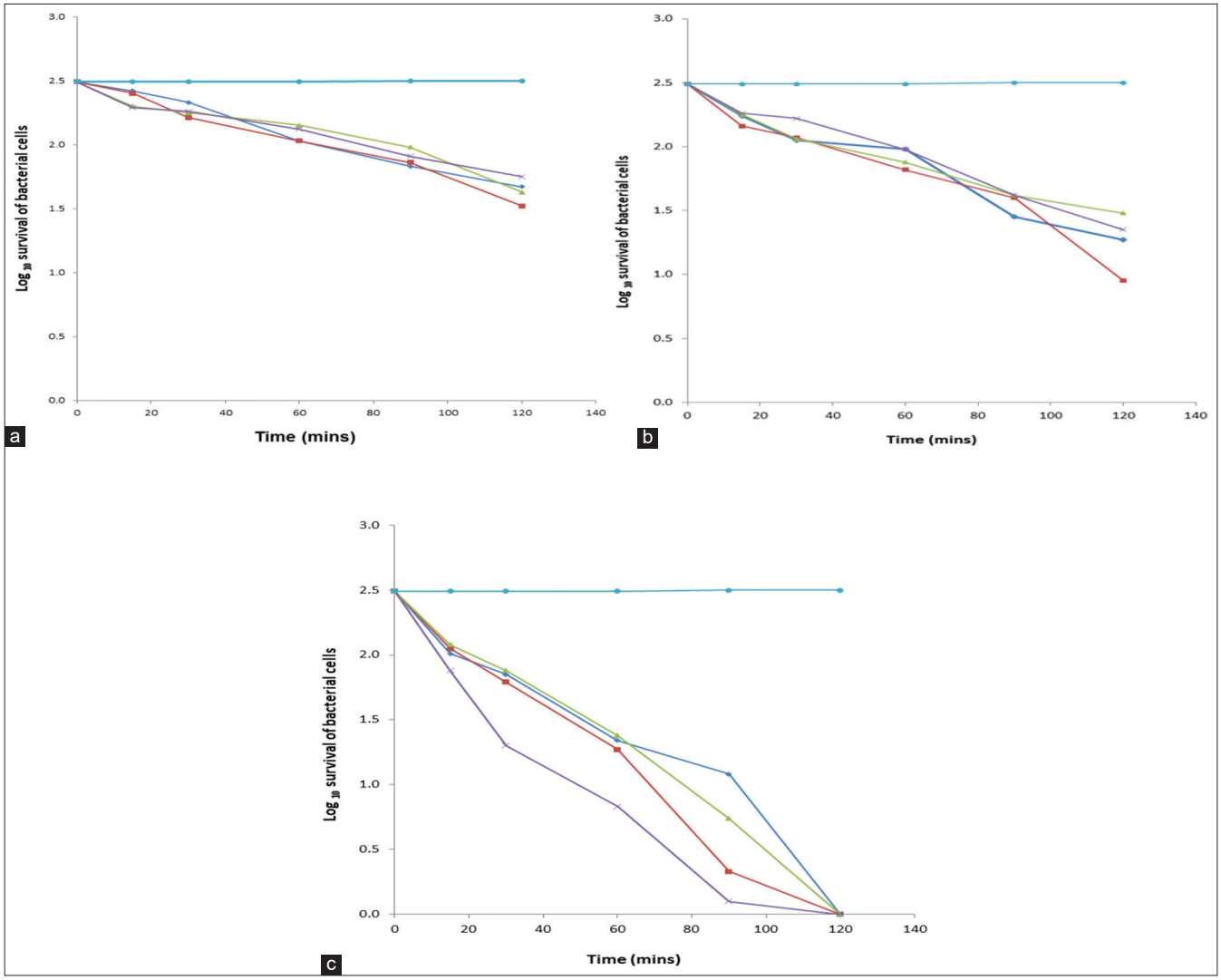

Fig. 1: (a) The extent and the rate of killing of *Escherichia coli* by n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) at ×1 minimum inhibitory concentrations (MIC). Each point represents the $\log_{10}$ of mean survival of bacterial cells at a particular time interval in the presence of the fractions. (b) The extent and the rate of killing of *E. coli* by n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) at ×2 MIC. Each point represents the $\log_{10}$ of mean survival of bacterial cells at a particular time interval in the presence of the fractions. (c) The extent and the rate of killing of *E. coli* by n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) at ×3 MIC. Each point represents the $\log_{10}$ of mean survival of bacterial cells at a particular time interval in the presence of the fractions

to MIC. The suspension was thoroughly mixed and held at room temperature (28–30°C) for 2 h to determine the killing rate of the test organisms. Exactly 0.5 mL of each suspension was withdrawn at the appropriate time intervals and transferred to 4.5 mL nutrient broth recovery medium containing 3% "Tween 80" to neutralize the effects of the fractions carry-over from the bacterial cells. The suspension was shaken properly and then serially diluted up to 10^{-5} in sterile physiological saline and plated out for viable counts. The plates were incubated at 37°C for 48 h before determining the survival cells. Control experiment was set up without the inclusion of the fractions. Viable counts were made in triplicates for each sample and compared with the counts of the control. Depression in viable counts indicated killing by the fractions.

Determination of protein leakage from the bacterial strains by the fractions

The test bacterial strains cells were (*E. coli* and *S. dysenteriae*) separately washed in 0.9% (w/v) normal saline to remove any presence of slime layer from the cells. These washed cells (inoculum size approximately 10^6 cells 0.5 McFarland standards) were treated with various concentrations of the fraction relative to the MIC at various time intervals for 2 h. Each suspension was later centrifuge at 7000 rpm to collect the supernatant for protein assaying [20]. The standard curve for protein analysis was constructed to quantify the quality of protein leaked from the bacterial cells. Exactly 0.4 mL Bradford reagent was added to 1.6 mL sample (0.2 mL supernatant added to 1.4 mL sterilized distilled water) to make up 2 mL total volume. Optical density (OD) of the resulting solution was thereafter taking at 595 nm after 5 min of preparation but not later than 1 h. The OD of each of the samples was calculated from the equation of the best-fit linear regression line obtained from the graph of the bovine serum albumin (BSA) standard curve.

Preparation of the BSA standard curve for the quantification of protein leaked out of the bacterial cells

Exactly 100 ug/mL concentration of BSA stock solution was prepared and varying concentrations were made from the stock solution. A 0.4 mL of Bradford reagent was added to the various BSA concentrations. This

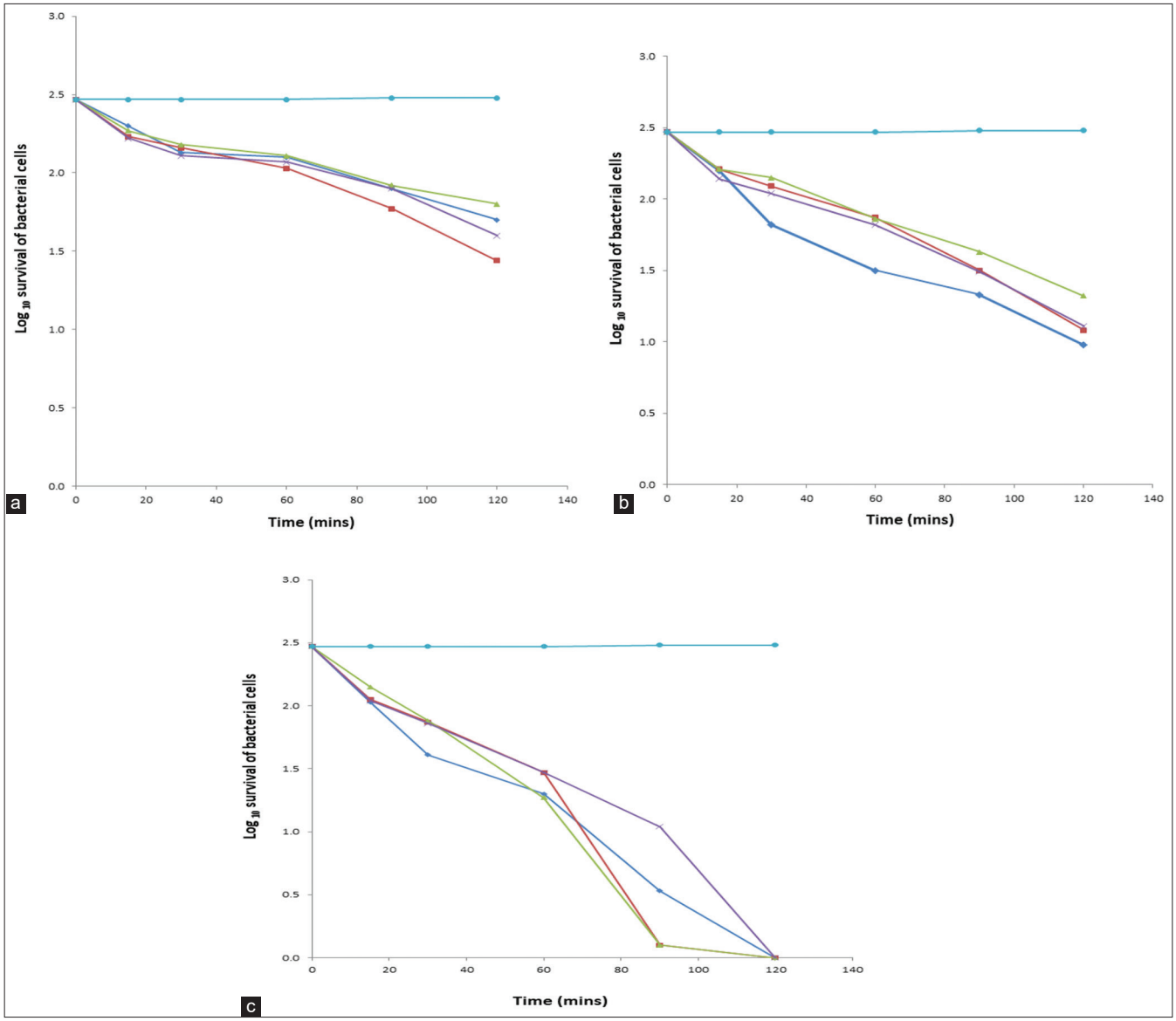

Fig. 2: (a) The extent and the rate of killing of *Shigella dysenteriae* by n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) at ×1 minimum inhibitory concentrations (MIC). Each point represents the $\log_{10}$ of mean survival of bacterial cells at a particular time interval in the presence of the fractions. (b) The extent and the rate of killing of *S. dysenteriae* by n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) at ×2 MIC. Each point represents the $\log_{10}$ of mean survival of bacterial cells at a particular time interval in the presence of the fractions. (c) The extent and the rate of killing of *S. dysenteriae* by n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) at ×3 MIC. Each point represents the $\log_{10}$ of mean survival of bacterial cells at a particular time interval in the presence of the fractions

was allowed to stand for 5 min after which the OD was measured at 600 nm. The various OD values obtained were then plotted against BSA concentrations to form a standard albumin curve. The concentrations of proteins in the samples were then calculated from the equation of the best-fit linear regression line obtained from the graph of the BSA standard curve.

Determination of potassium ions leakage from the bacterial cells by the fractions

Eighteen-hour old cells of *E. coli* and *S. dysenteriae* were washed 3 times in physiological saline to remove slime layers from the cells. Exactly 50 mL of washed cells (OD 470 nm=1.5) was dispensed into a clean beaker which was magnetically stirred. Fifty milliliters of ionic strength adjustment buffer (18.37 g of tetraethylammonium chloride in deionized water and made up to 100 mL) were added to the beaker to make the background ionic strength of all solution kept constant. The potassium ion sensing electrode (Qualiprobe QSE 314, EDT instruments Waldershare Park, Dover, UK) and its reference electrode (Qualiprobe double junction reference electrode E 8092 EDT instruments) were placed into the cell suspension. The potential difference (mV) derived by the electrodes was measured using a Whatman Maidstone, UK. *E. coli* and *S. dysenteriae* cells were treated with various concentrations of the fractions relative to the MIC. The potassium ions leaked out from the cells of the organisms were measured at time interval over a period of 2 h as a potential difference in mV. These values were later converted to concentrations of potassium ions by reference to a conversion graph which had been constructed using potassium chloride standard solutions. The concentrations of potassium ions released were plotted against time.

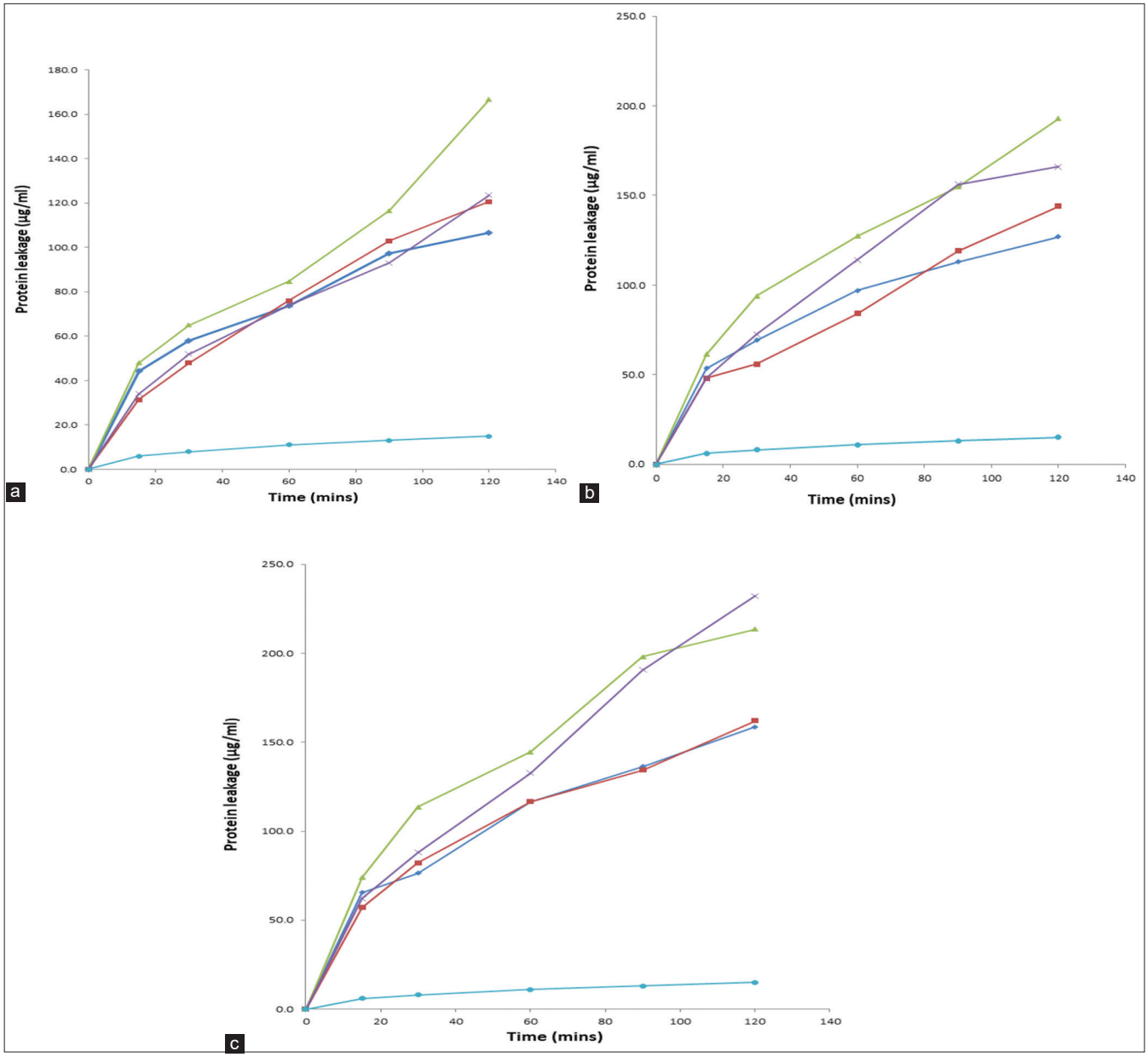

Fig. 3: (a) The effect of n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) on protein leakage from *Escherichia coli* at ×1 minimum inhibitory concentrations (MIC). Each point represents the quantity of protein leaked (µg/ml) from the bacterial cells at a particular time interval in the presence of the fractions. (b) The effect of n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) on protein leakage from *E. coli* at ×2 MIC. Each point represents the quantity of protein leaked (µg/ml) from the bacterial cells at a particular time interval in the presence of the fractions. (c) The effect of n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) on protein leakage from *E. coli* at ×3 MIC. Each point represents the quantity of protein leaked (µg/ml) from the bacterial cells at a particular time interval in the presence of the fractions

Statistics and data processing

The experiments were carried out in triplicates. Data were analyzed by a 4×4 Latin square designated with the statistical program using the GLM model (Statistical Analysis Systems Institute, Cary NC, USA, 2001). Results were contrasted with negative and a positive control. The mean of the values was compared using independent t-test of significance ($p<0.05$).

RESULTS

Exactly 130.5 g of crude extract was obtained from 1500 g of powdered stem bark of *P. guajava*. This gave 8.7% yield of the total weight of powdered sample used and the extract was dark brown in color. Four fractions were obtained from the crude extract and these include n-hexane, chloroform, ethyl-acetate, and butanolic fractions. The crude extract along with the four fractions exhibited appreciable antimicrobial activities against all the 72 bacterial strains used for this study. The crude extract exhibited antimicrobial activity at a final concentration of 25 mg/mL while all the four fractions exhibited antimicrobial activities at a final concentration of 10 mg/mL. On the other hand, streptomycin at a concentration of 1 mg/mL inhibited the growth of 64 test organisms while ampicillin at the same concentration with streptomycin inhibited the growth of 22 organisms only (Tables 1 and 2). Overall, the crude extract along with the four fractions compared favorably with the two

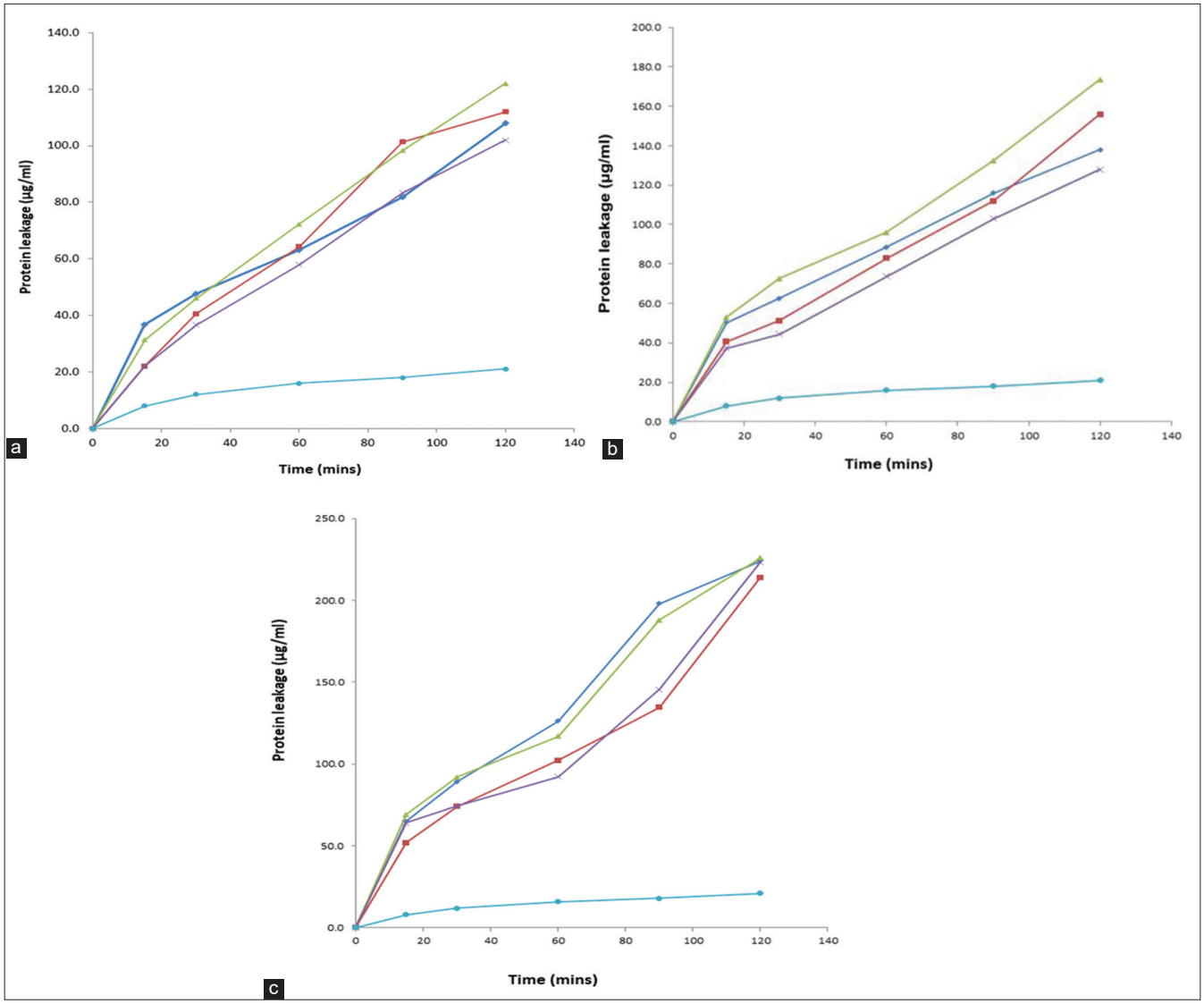

Fig. 4: (a) The effect of n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) on protein leakage from *Shigella dysenteriae* at ×1 minimum inhibitory concentrations (MIC). Each point represents the quantity of protein leaked (µg/ml) from the bacterial cells at a particular time interval in the presence of the fractions. (b) The effect of n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) on protein leakage from *S. dysenteriae* at ×2 MIC. Each point represents the quantity of protein leaked (µg/ml) from the bacterial cells at a particular time interval in the presence of the fractions. (c) The effect of n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) on protein leakage from *S. dysenteriae* at ×3 MIC. Each point represents the quantity of protein leaked (µg/ml) from the bacterial cells at a particular time interval in the presence of the fractions

standard antibiotics, namely, streptomycin and ampicillin used as a positive control.

The MIC and MBC of the crude extract along with those of the four fractions were also assessed. The crude extract and the fractions exhibited varying degrees of MIC and MBC against test organisms used for this study, as indicated in Tables 3 and 4. The MIC exhibited by the crude extract against both *E. coli* and *S. dysenteriae* ranged between 1.56 mg/mL and 12.5 mg/mL. On the other hand, the range exhibited by the fractions against susceptible organisms ranged between 0.31 mg/mL and 5.0 mg/mL.

The MBC exhibited by the crude extract against the bacterial strains followed the same trend with those observed for the MIC. The MBC ranged between 3.13 mg/mL and 12.5 mg/mL. On the other hand, the MBC observed for the fractions against *E. coli* and *S. dysenteriae* ranged between 0.63 mg/mL and 5.0 mg/mL.

The phytochemical compounds that were responsible for the biological activities of the stem bark extract of *P. guajava* were also investigated as shown in Table 5. The extract revealed the presence of saponins, alkaloids, flavonoids, tannins, steroids, cardiac glycosides, and reducing sugars.

The biocidal potentials of the fractions obtained from the stem bark extract of *P. guajava* were also investigated. This was assessed through the killing rate effects of the test cells protein and potassium ions leakages from the test cells. Fig. 1a-c shows the extent and killing rate of *E. coli* when subjected to the effect of the four fractions, namely, n-hexane, chloroform, ethyl acetate, and butanol fractions at different concentrations relative to the MIC of these fractions. The percentage of the cells of *E. coli* killed by n-hexane and chloroform fractions in 15 min at ×1 MIC was 14.9% and 17.2%, respectively. The killing rate exhibited by ethyl acetate and butanol fractions at the same time interval of 15 min was 35.3%. The rate at which these cells were killed increases

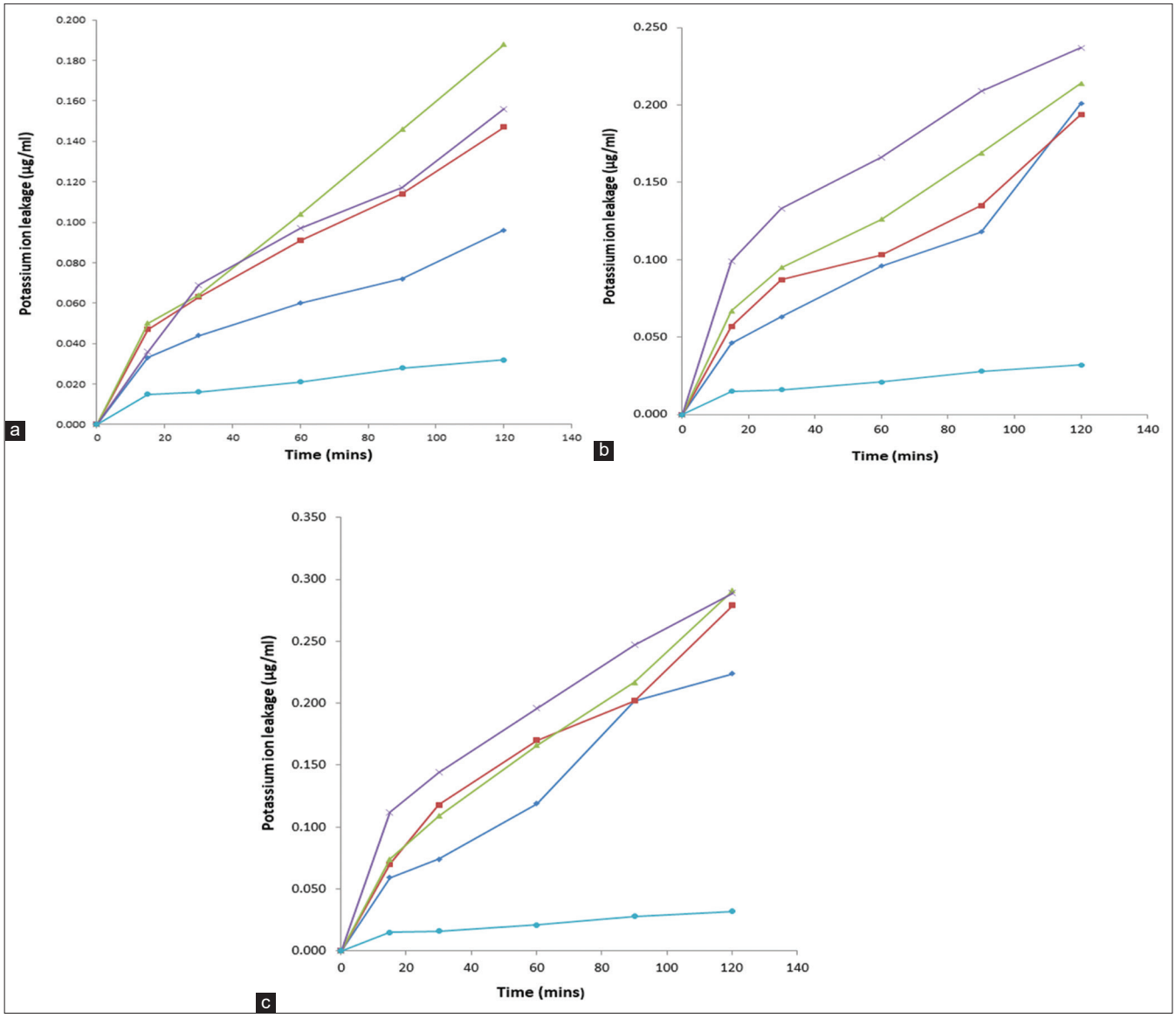

Fig. 5: (a) The effect of n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) on potassium ion leakage from *Escherichia coli* at ×1 minimum inhibitory concentrations (MIC). Each point represents the quantity of potassium leaked (µg/ml) from the bacterial cells at a particular time interval in the presence of the fractions. (b) The effect of n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) on potassium ion leakage from *E. coli* at ×2 MIC. Each point represents the quantity of potassium leaked (µg/ml) from the bacterial cells at a particular time interval in the presence of the fractions. (c) The effect of n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) on potassium ion leakage from *E. coli* at ×3 MIC. Each point represents the quantity of potassium leaked (µg/ml) from the bacterial cells at a particular time interval in the presence of the fractions

with a contact time of these cells with the fractions. Finally, when the contact time was increased to 120 min, the percentage of cells killed by butanol, n-hexane, ethyl acetate, and chloroform fractions at ×1 MIC increased to 81.8%, 84.9%, 86.4%, and 89.1%, respectively. The same trend of reactions was observed when the concentrations of the fractions increased to ×2 MIC and ×3 MIC.

As shown in Fig. 2a-c, results obtained for killing rate on *S. dysenteriae* cells when subjected to n-hexane, chloroform, ethyl-acetate, and butanol followed the same trend as exhibited in the tests carried out on *E. coil*.

The biocidal effect of the four fractions, namely, n-hexane, chloroform, ethyl-acetate, and butanol fraction was also investigated by assaying for leakage of protein from the protoplasm of the test organisms, that is, *E. coli* and *S. dysenteriae*. The protein leakage from *E. coli* cells due to the effect of n-hexane, chloroform, ethyl acetate, and butanol fractions at different concentrations relative to the MIC at ×1 MIC, ×2 MIC, and ×3 MIC is shown in Fig. 3a-c. The leakage effect on *E. coli* cells at ×1 MIC in 15 min contact time interval by n-hexane, chloroform, ethyl acetate, and butanol fractions was 44.3 µg/mL, 31.3 µg/mL, 48.0 µ/mL, and 34.0 µg/mL, respectively. The contact time of the cells with the fractions was later increased to 30 min, 60 min, 90 min, and finally 120 min. When the contact time of the cell with the fractions at 1×MIC concentration reached 120 min, the percentage of protein leaked out of the cells increased. The percentage increase for n-hexane, chloroform, ethyl acetate, and butanol fractions was 127.0 µg/mL, 144.0 µg/mL, 193.5 µg/mL, and 166.0 µg/mL, respectively. The same trend of reactions was observed when the concentrations of the fractions increased to ×2 MIC and ×3 MIC.

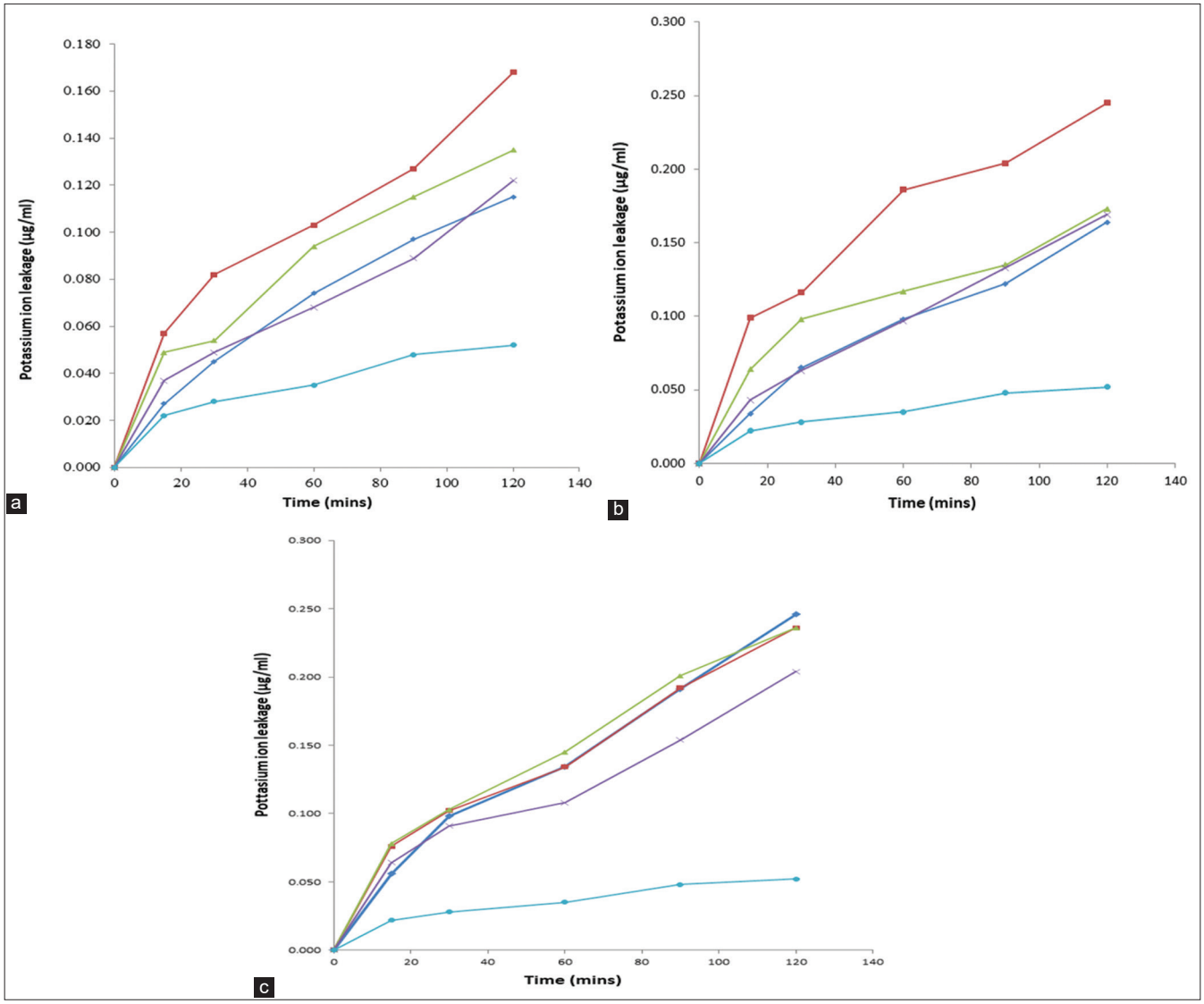

Fig. 6: (a) The effect of n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) on potassium ion leakage from *Shigella dysenteriae* at ×1 minimum inhibitory concentrations (MIC). Each point represents the quantity of potassium leaked (µg/ml) from the bacterial cells at a particular time interval in the presence of the fractions. (b) The effect of n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) on potassium ion leakage from *S. dysenteriae* at ×2 MIC. Each point represents the quantity of potassium leaked (µg/ml) from the bacterial cells at a particular time interval in the presence of the fractions. (c) The effect of n-hexane fraction ( ), chloroform fraction ( ), ethyl acetate fraction ( ), butanol fraction ( ), and control ( ) on potassium ion leakage from *S. dysenteriae* at ×3 MIC. Each point represents the quantity of potassium leaked (µg/ml) from the bacterial cells at a particular time interval in the presence of the fractions

S. dysenteriae cells were also subjected to the effect of protein leakage from their cells by n-hexane, chloroform, ethyl acetate, and butanol fraction. The concentrations of the protein leaked out of the test cells continue to rise as the concentration of the fractions increases. Fig. 4a-c represents the leakages from *S. dysenteriae* through the action of the four fractions.

Finally, the cidal effects of the four fractions on potassium ions leakage from the test cells were investigated. The effect of n-hexane, chloroform, ethyl acetate, and butanol fractions on potassium ion leakage from *E. coli* cells at ×1 MIC, ×2 MIC, and ×3 MIC is shown in Fig. 5a-c. The effect of the four fractions on potassium ions leakages from *E. coli* cells at a concentration of ×1 MIC at 15 min contact time is shown in Fig. 5a. The n-hexane, chloroform, ethyl acetate, and butanol fractions leaked out 0.033 µg/mL, respectively, after 15 min of contact time with *E. coli* cells. The leakage of potassium ions continued to increase with an increase in a contact time of the fraction with the bacterial cells. When the contact time reached 120 min at ×1 MIC concentration, the quantity of potassium ions leaked out of the test cells rose up to 0.096 µg/mL, 0.147 µg/mL, 0.188 µg/mL, and 0.156 µg/mL when treated with n-hexane, chloroform, ethyl acetate, and butanol fractions, respectively. The same trend of reactions was observed for ×2 MIC and ×3 MIC concentrations of the fractions.

S. dysenteriae cells were also subjected to the effects of the fractions for potassium ions leakage. Appreciable quantities of potassium ion were also leaked out of the cells and follow the same trend with those observed for the *E. coli* cells. Fig. 6a-c shows the effects of the four fractions on *S. dysenteriae* cells. The higher the concentrations of the extract and contact time, the more potassium ions got leaked out of this bacterial cells as observed for *E. coli.*

DISCUSSION

The biocidal effects of *P. guajava* stem bark extract were investigated against a panel of *E. coli* and *S. dysenteriae* that are causative agent of diarrhea and dysentery [2]. Four fractions were obtained from the crude extract of *P. guajava* stem bark. The crude extract along with the four fractions exhibited broad spectrum activities on all the 72 bacterial strains used for this study. In contrast to the positive control used, that is, streptomycin and ampicillin, streptomycin showed an almost similar trend of activity with both the crude extract and the fractions. On the other hand, ampicillin showed less activity in comparison to the crude extract and all the four fractions. Thus, the crude extract and the four fractions exhibited appreciable antimicrobial activities against *E. coli* and *S. dysenteriae*. Hence, the stem bark extract of *P. guajava* could serve as a pointer towards the development of drugs of natural origin for the treatment of diarrhea and dysentery. Our findings thus support the usefulness of *P. guajava* in folklore remedy for the treatment of infections caused by microorganisms. The MIC and MBC of the crude extract and those of the fractions were investigated. The crude extract revealed the lowest MIC of 1.56 mg/mL and MBC of 3.13 mg/mL. On the other hand, the lowest MIC exhibited by the fractions was 0.31 mg/mL and the lowest MBC was 0.63 mg/mL. Plant extract with very low MIC and MBC is known to possess high antimicrobial potency [21]. According to Shanmughapriya *et al*. [22], plants extract with MIC index which is equal or <2 mg/mL is considered as bactericidal while those above 2 mg/mL but <16 mg/mL are said to be bacteriostatic. This observation showed that *P. guajava* stem bark extract is bactericidal in action. As a matter of fact, such a plant could be a good source of antimicrobial drug of natural origin that can be used to combat diarrhea and dysentery that are killer diseases especially among the children in underdeveloped countries. Some phytochemical compounds which include tannins, flavonoids, alkaloids, cardiac glycosides, saponins, and reducing sugars were detected in *P. guajava* stem bark extract. Such phytochemicals are known to contribute to the biological activities of medicinal plants [16]. This was revealed in the cidal effect of the studied extract of *P. guajava* on bacterial strains used for this study. The cidal effects on cells of *E. coli* and *S. dysenteriae* which were observed through the killing rate of the test cells along with protoplasmic leakage of proteins and potassium ions might be enhanced by phytochemicals present in this plant extract. These phytochemicals usually form a complex with extracellular bacterial cell walls causing lysis of the cell wall and disruption of cell membranes of the organisms [23]. All the fractions used for the investigations of the killing rate of *E. coli* and *S. dysenteriae* cells achieved 100% killing of these cells at low concentrations within the shortest period of time. This ability of *P. guajava* stem bark extract to kill these organisms within the shortest period of time indicates a bactericidal effect. This observation supported the findings of Pankey and Sabath [24]. The killing of these test bacterial isolates could be attributed to damage caused to the cytoplasmic membrane of these organisms. Damage to the cytoplasmic membrane will lead to the leakage of protoplasmic inclusion. Hence, leakage of protein and potassium ions from the protoplasms of *E. coli* and *S. dysenteriae* used for this study led to the death of these organisms. Leakage of potassium ions from protoplasms of bacteria may lead to a serious consequence on such organisms and hence the death of such an organism. Potassium is responsible for the activation of intracellular enzymes and the maintenance of a constant internal pH and membrane potential [25]. Potassium ions transport a critical determinant of growth and survival through its role in regulating cytoplasmic pH [26,27]. Leakage of such important ions from bacterial protoplasm will have an adverse effect on such organisms and lead to their death. All these evidences contributed to the death of the test bacterial isolates, that is, *E. coli* and *S. dysenteriae* when subjected to the effects of *P. guajava* stem bark extracts.

CONCLUSION

P. guajava stem bark extracts exhibited bactericidal activities against a panel of organisms associated with diarrhea and dysentery. This showed a significant therapeutic potential of this plant for the treatment of dysentery and diarrhea in addition to combating infections caused by other microorganisms. This observation supported the usefulness of *P. guajava* in folklore remedies for the management of infections caused by pathogens. The ability of this plant extract to kill test bacteria used for this study at low concentration and minimal contact time has established the potential of *P. guajava* as a template for future drugs of natural origin. Such a drug could go a long way in health-care delivery to combat infections caused by pathogens.

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AUTHORS' CONTRIBUTIONS

DAA conceptualized the study, ORA collected, and preparation of the isolates and plant materials used. ORA, MOA, and KAA carried out the microbiological assays. The manuscript was drafted by DAA, prove read, and approved by all authors.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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