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Antibacterial Activities of Methanol Extracts of *Carica papaya, Ocimum gratissimum* and *Solanum torvum* under Normal and Osmotic Stress Conditions

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Authors' contributions

This work was carried out in collaboration between all authors. Author JDT designed the study, wrote the protocol, supervised laboratory work, and wrote the first draft of the manuscript. Authors SEE, AJN and JAM managed the literature searches and analyses of the study. Authors JDT, SEE, JAM and ICK managed the experimental. All authors read and approved the final manuscript.

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ABSTRACT

Background: Infectious diseases remain major threat to human health worldwide despite the availability of antibiotic library in drug stores, because of the emergence of new multi-drug resistant bacteria. Therefore, the discovery of new effective treatment will help in fighting such infections.
Aim: This study aimed at evaluating the antibacterial activities of extracts from three Cameroonian medicinal plants as well as the effect of osmotic stress on these activities.
Methods: Antibacterial properties of the methanol extracts of *Carica papaya*, *Ocimum gratissimum* and *Solanum torvum* were studied against five Gram positive and four Gram negative bacteria

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under normal and osmotic stress conditions. The phytochemical analysis of these plant extracts was carried out using standard methods. Broth microdilution method was used to assess the antibacterial activities of extracts as well as the effect of stress osmotic (5% NaCl) on these activities.

Results: The phytochemical analysis revealed the presence of alkaloids, polyphenols, tannins and triterpenes in all tested plant extracts. Flavonoids, anthraquinones, anthocyanins, steroids and saponins were selectively distributed in the extracts. The extract of *C. papaya* (MIC = 128 - 1024 µg/ml) was the most active followed in decreasing order by *O. gratissimum* extract (MIC = 512 - 1024 µg/ml), *S. torvum* leaf extract (MIC = 256 - 2048 µg/ml), and *S. torvum* fruit extract (MIC = 2048 µg/ml). The antibacterial activities of plant extracts and chloramphenicol increased under osmotic stress conditions while those of amoxicillin decreased under these conditions.

Conclusion: The overall results of this study provide scientific evidence of the antibacterial activity of methanol extracts of tested plants, therefore supporting their traditional use in the wound healing and infected diseases.

Keywords: Medicinal plants; organic extracts; bacteria; antibacterial activity; phytohemical analysis; osmotic stress.

1. INTRODUCTION

Infectious diseases are the world's major threat to human health and account for almost 50,000 deaths everyday [1]. Treatment of these infections is compromised worldwide by the emergence of new multi-drug resistant (MDR) bacteria. These emerging drug resistant bacterial strains, particularly vancomycin resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), and multidrug resistance (MDR) Gram-negative bacteria, are increasing worldwide and add to the gravity of the situation. Development of resistant bacteria due to the chromosomal mutations is more commonly associated with the horizontal transfer of resistance determinants borne on mobile genetic elements [2]. It is currently believed that bacterial cells sense the actions of antibiotics as just another form of environmental stress [3] and that sub-lethal concentrations of antibiotics induce stress hardening [4] and cross-protection [5]. Conversely environmental stresses (temperature, pH, osmotic pressure, depletion of nutrients) can induce resistance to antibiotics by a range of means.

The search for newer sources of antibiotics is a global challenge faced by research institutions, pharmaceutical companies, and academia, since many infectious agents are becoming resistant to synthetic drugs [6]. The local use of natural plants as primary health remedies, due to their pharmacological properties, is quite common in Africa, Latin America, and Asia [7]. Ethnomedicinal plants are important sources of natural products. They are rich in a wide variety of secondary metabolites such as steroids, anthraquinones, tannins, terpenoids, alkaloids, and flavonoids whose antimicrobial properties have been well documented [8-13]. These active principles are usually accumulated in storage organs of plant such as roots, seeds, barks, leaves. *Carica papaya L., Ocimum gratissimum L.* and *Solanum torvum L.* are medicinal plant species well known in Cameroon. Various parts of these plant species are popularly used in traditional medicine to cure wound and several infectious diseases among which those caused by bacterial species [14-17]. *C. papaya* is described as a fast growing, erect, usually unbranched tree or shrub, 7-8m tall with copious latex, trunk of about 20 cm in diameter. Papaya leaf-extracts have antimicrobial properties and phenolic compounds, such as protocatechuic acid, p-coumaric acid, 5, 7-dimethoxycoumarin, caffeic acid, kaempferol, quercetin, and chlorogenic acid [17-18]. *O. gratissimum* is widely distributed in tropical and warm temperature regions. This plant species has been reported to be active against several species of bacteria and fungi [19-21]. *S. torvum* is a bushy, erect and spiny perennial plant used horticulturally as a rootstock for eggplant. Leaves of *S. torvum* have been reported to possess antimicrobial activities and contain steroidal gluco-alkaloid, solasonine, steroidal sapogenins, neochlorogenin, neosolaspigein and solaspigenine [22].

The need of ecological safe compounds as therapeutic agents against pathogenic microorganisms has driven many studies towards the screening of aromatic plants. Hence, several plants have been evaluated not only for their inherent antimicrobial activity [23-25], but also for their action as a resistant modifying agent [26]. So, the present study was designed keeping in view the resistance of bacteria to commonly used antibiotics. It was aimed to evaluate the antibacterial activity of *C. papaya, O. gratissimum* and *S. torvum* under normal and osmotic stress condition against the most common disease causing bacteria's such as *Escherichia coli, Shigella flexneri, Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

2. MATERIALS AND METHODS

2.1 Plant Materials

The medicinal plants used in this study were *C. papaya* L. (leaves)*, O. gratissimum* L. (leaves) and *S. torvum* L. (leaves and fruits) collected from local area of Dschang (West Region of Cameroon) in February 2017, based on their traditional uses (Table 1). These plants were identified and authenticated at the Cameroon National Herbarium, where the voucher specimens were kept under their corresponding reference numbers (Table 1). The leaves/fruits of each plant was washed thoroughly under running water and dried under room temperature. They were grounded to powder using mixer grinder and stored in air tight bottles.

2.2 Preparation of Crude Extracts

The air-dried and powdered sample from each plant was macerated separately in methanol (Table 1) for 48 h at room temperature with occasional shaking. After 48 h, the extract was filtered through a Whatman no. 1 filter paper. The filtrate was then evaporated to dryness at 65°C for methanol using a rotary evaporator to give a residue which constituted the crude extract. The extraction yield was calculated (Table 1) and the crude extract was kept at +4°C until further use.

2.3 Phytochemical Screening of Extracts

The phytochemical screening of crude extracts was carried out according to the methods described by Trease and Evans [35]. The plant extract was screened for the presence of different classes of compounds including alkaloids, flavonoids, steroids, triterpenes, anthraquinones, tannins, anthocyanins, saponins and polyphenols.

2.4 Microorganisms and *in vitro* Antimicrobial Assays

2.4.1 Bacteria and growth conditions

In this study, we investigated the antibacterial properties of plant extracts against Gram-positive (*Bacillus subtilis, Staphylococcus aureus* ATCC25923, methicillin sensitive *S. aureus* MSSA01, methicillin resistant *S. aureus* MRSA03, methicillin resistant *S. aureus* MRSA04) and Gram-negative (*Escherichia coli* S2 (1), *Pseudomonas aeruginosa* PA01, *Shigella flexneri* SDINT, *Pseudomonas aeruginosa*) bacteria. These microorganisms were collected from the Department of Biochemistry, University of Calcutta in India and from the Institute of Medical Mycology, Teikyo University in Japan. The bacteria were stored and activated on nutrient agar.

2.4.2 Antibacterial assay under normal condition

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts were performed using the broth microdilution method recommended by the Clinical and Laboratory Standards Institute [36,37] with slight modifications. Each test sample was dissolved in dimethylsulfoxide (DMSO) and the solution was then added to Mueller Hinton Broth (MHB) to give a final concentration of 8192 µg/ml. This was serially diluted twofold to obtain a concentration range of 8 - 4096 µg/ml. Then, 100 µl of each concentration were added in each well (96-well microplate) containing 95 µl of MHB and 5 µl of inoculums. The final concentrations of the plant extract ranged from 4 to 2048 µg/ml. The inoculum was standardized at 10^6 CFU/ml by adjusting the optical density to 0.1 at 600 nm using a JENWAY 6105 UV/Vis spectrophotometer. The final concentration of DMSO in each well was less than 1% [preliminary analyses with 1% (v/v) DMSO did not inhibit the growth of test organisms]. The negative control well consisted of 195 µl of MHB and 5 µl of standard inoculums whereas dilutions of amoxicillin (Sigma-Aldrich, Steinheim, Germany) and ciprofloxacillin (Sigma-Aldrich, Steinheim, Germany) served as positive controls. The plates were covered with sterile lids, then agitated to mix the contents of wells using a plate shaker and incubated at 37°C for 24 h. The assay was repeated three times. The MIC values of samples were determined by adding 50 µl of a

0.2 mg/ml *p*-iodonitrotetrazolium (INT) violet solution followed by incubation at 37°C for 30 min. Viable bacteria reduced the yellow dye to a pink color. MIC values were defined as the lowest sample concentrations that prevented this change in color indicating a complete inhibition of microbial growth.

For the determination of MBC values, each well that showed no growth of microorganism was mixed with the pipette tips, then 10 µl was loaded and spread on Mueller Hinton Agar (MHA) followed by incubation at 37°C for 24 h. The lowest concentrations that lead to failure in bacterial growth after this subculture process were considered as the MBC values.

2.4.3 Antibacterial assay under osmotic stress (5% NaCl) condition

Osmotic stress condition was induced by adding 5% NaCl (w/v) to MHB. The MHB supplemented with 5% NaCl was then sterilized and used for the determination of new MIC and MBC values of the samples as described above. The incubation time was increased from 24 hours to 48 hours at 37°C.

3. RESULTS

3.1 Phytochemical Analysis

The phytochemical tests revealed the presence of alkaloids, polyphenols, tannins and triterpenes in the plant extracts (Table 2). Flavonoids, anthraquinones, anthocyanins, steroids and saponins are selectively distributed in the extracts. Steroids, anthocyanines and saponins were mainly found in *C. papaya* (leaves), *S. torvum* (fruits) and *O. gratissimum* (leaves). Flavonoids were revealed only in *S. torvum* (fruits).

3.2 Antibacterial Activity of Extract under Normal Condition

The antibacterial activity of plant extracts was evaluated through the determination of minimum inhibitory concentrations (MIC) and minimum bactericidal (MBC) concentrations against multi-drug-resistant pathogenic bacteria. Analysis of the results (Table 3) shows a variability of the antibacterial activity according to the extracts and bacterial species (MIC = 128 -2048 µg/ml).

Table 1. Botanical identification, parts used, extraction solvent/yield and traditional therapeutic indications of studied medicinal plants

Scientific name (Family)	Voucher specimen	Part used; extraction solvent and yield	Traditional therapeutic indications
Carica papaya L. (Caricaceae),	18647/SRF/Cam	Leaves; methanol; 7.32%	Typhoid, urinogenital disorder, Intestinal parasites, chronic skin ulcer, warts, cancer, tumors, nervous pains, hypotension, wound, malaria [17,27], gastrointestinal tract disease, oxidization of cholesterol, nausea and morning sickness, weight loss, looting of body immunity, recovery of kidney, dengue fever and menstrual irregularities in women, pest control [28].
Ocimum gratissimum L. (Lamiaceae)	5817/SRF/Cam	Leaves; methanol; 4.57%	Upper respiratory tract infections, diarrhea, headache, ophthalmic, skin diseases, pneumonia, cough, fever, and conjunctivitis [14,15], abdominal pains, ear infections, barrenness, convulsions, and tooth gargle, regulation of menstruation and prolapse of the rectum [29-32].
Solanum torvum L. (Solanaceae)	10742/SRF/Cam	Leaves and fruits; methanol; 10.70% and 5.98%	Pains, coughs, colds, toothache, cracks in feet, liver and spleen enlargement [16,33-34]. Also used as tonic and haematopoetic agent [29], haemostatic, sedative, diuretic and digestive [16].

The extract of *C. papaya* (MIC = 128 - 1024 µg/ml) was the most active followed in decreasing order by *O. gratissimum* (MIC = 512 - 1024 µg/ml), *S. torvum* leaf (MIC = 256 - 2048 µg/ml), and *S. torvum* fruit (MIC = 2048 µg/ml). The lowest MIC value (128 µg/ml) corresponding to the highest activity was obtained with *C. papaya* extract against *S. flexneri* and *B. subtilis*.

On the basis of MBC values (Table 3), the antibacterial activities of studied plants also varied according to the extracts and microorganisms (MBC = 1024 - 2048 µg/ml). Apart from the methanol extract of *C. papaya* which showed bactericidal activity against *S. aureus* (MBC = 2048 µg/ml), the other extracts from the tested plants displayed bacteriostatic activities (MBC > 2048 µg/ml) against the tested microorganisms.

3.3 Antibacterial Activity of Plant Extracts under Osmotic Stress Condition

The effect of osmotic stress on minimum inhibitory concentrations of plant extracts was evaluated (Table 4). The results clearly indicated that the MIC values of plant extracts obtained under osmotic stress (in the presence of 5% NaCl) conditions are generally smaller than those obtained under normal conditions (0% NaCl). This result suggests an increase in the activities of extracts under osmotic stress. In the presence of 5% NaCl, the *O. gratissimum* extract was the most active, followed in decreasing order by *C. papaya*, *S. torvum* leaves and *S. torvum* fruits. The MIC values of chloramphenicol determined under osmotic stress condition are smaller than those determined under normal conditions. However, most of MIC values of amoxicillin determined under osmotic stress are higher than those determined under normal conditions in particular against *P. aeruginosa* PA01, *S. aureus* MSSA01, *S. aureus* MRSA04, *S .aureus, S. flexneri.* Under osmotic stress, the antibacterial activity of *O. gratissimum* extract against *S. aureus* ATCC25923 (MIC = 16 µg/ml) and *B. subtilis* (MIC = 16 µg/ml) was higher than that of amoxicillin (MIC = 32 and 256 µg/ml). Similarly, at 5% NaCl, the activity of *C. papaya* extract against *S. aureus* ATCC25923 (MIC = 16 µg/ml) was greater than that of amoxicillin (MIC = 32 µg/ml).

4. DISCUSSION

The phytochemical screening revealed the presence of alkaloids, polyphenols, anthocyanins, saponins, steroids, triterpenes and tannins in all the extracts and the absence of anthraquinones and flavonoids in the methanol extract of *O. gratissimum.* These results partially disagree with the findings of Mbata and Saikia [38], who revealed the presence of alkaloids, resins, tannins, flavonoids, glycosides, saponins, cardiac glycosides, and terpenes as well as the absence of anthraquinones in the aqueous and alcoholic leaf extracts of *O. gratissimum.* The phytochemical analysis of *C. papaya* extract showed the presence of alkaloids, polyphenols, triterpenes, steroids, saponins and anthocyanins. These results partially corroborate those found by Kongo-Nzuzi [39], who revealed the presence of alkaloids, polyphenols, flavonoids, quinones and saponins in the methanol extract of the leaves of this plant. The results on the chemical composition of the methanol extract of *S. torvum* fruits showed the presence of alkaloids, anthraquinones, polyphenols, flavonoids, triterpenes, steroids, tannins and saponins. As for the extract of leaves of this plant, we have demonstrated the presence of alkaloids, polyphenols, anthraquinones, anthocyanins, tannins and triterpenes. The presence of these groups of compounds in plant organs was also

Table 2. Main classes of secondary metabolites identified in the plant extracts

Groups of compounds	*C. papaya* (leaves)	*S. torvum* (leaves)	*S. torvum* (fruits)	*O. gratissimum* (leaves)
Alkaloids	+	+	+	+
Polyphenols	+	+	+	+
Flavonoids	-	-	+	-
Anthraquinones	-	+	+	-
Anthocyanines	+	+	-	+
Tannins	+	+	+	+
Triterpenes	+	+	+	+
Steroids	+	-	+	+
Saponins	+	-	+	+

+: present; -: absent

Table 3. Antibacterial activities (MIC and MBC in µg/ml) of extracts and reference antibacterial drugs under normal conditions

Bacterial strain	Inhibition parameters	*C. papaya*	*S. torvum* (leaves)	*S. torvum* (fruit)	*O. gratissimum*	Amoxicillin	Ciprofloxacin
B. subtilis	MIC	128	256	>2048	>2048	32	1
	MBC	>2048	>2048	>2048	>2048	32	1
	MBC/MIC	/	/	/	/	1	1
E. coli	MIC	1024	2048	>2048	1024	64	1
	MBC	>2048	>2048	>2048	>2048	64	1
	MBC/MIC	/	/	/	/	1	1
P. aeruginosa	MIC	512	2048	>2048	>2048	128	2
	MBC	>2048	>2048	>2048	>2048	256	2
	MBC/MIC	/	/	/	/	2	1
S. flexneri	MIC	128	2048	2048	512	1	16
	MBC	>2048	>2048	>2048	>2048	4	16
	MBC/MIC	/	/	/	/	4	1
S. aureus ATCC 25923	MIC	512	>2048	>2048	>2048	1	4
	MBC	2048	>2048	>2048	>2048	1	4
	MBC/MIC	4	/	/	/	1	1
P. aeruginosa PA01	MIC	256	>2048	>2048	512	2	1
	MBC	>2048	>2048	>2048	>2048	2	1
	MBC/MIC	/	/	/	/	1	1
S. aureus MSSA 01	MIC	256	2048	>2048	>2048	4	1
	MBC	>2048	>2048	>2048	>2048	4	1
	MBC/MIC	/	/	/	/	1	1
S. aureus MRSA03	MIC	512	>2048	>2048	512	16	1
	MBC	>2048	>2048	>2048	>2048	16	1
	MBC/MIC	/	/	/	/	1	1
S. aureus MRSA 04	MIC	1024	>2048	>2048	512	16	2
	MBC	>2048	>2048	>2048	>2048	16	2
	MBC/MIC	/	/	/	/	1	1

/: not determined; **- : >** *2048 µg/ml. MIC: Minimum Inhibitory Concentration; MBC Minimum Bactericidal Concentration.*

Table 4. Effect of osmotic stress on the antibacterial activities (MIC in µg/ml) of the studied plant extracts

Bacteria	***C. papaya***		***S. torvum* (leaves)**		***S. torvum* (fruit)**		***O. gratissimum***		**Chloramphenicol**		**Amoxicillin**	
	0% NaCl	**5% NaCl**	**0% NaCl**	**5% NaCl**	**0% NaCl**	**5% NaCl**	**0% NaCl**	**5% NaCl**	**0% NaCl**	**5% NaCl**	**0% NaCl**	**5% NaCl**
B. subtilis	128	16	256	16	-	16	-	16	16	8	32	2
E. coli S2(1)	1024	2048	2048	-	-	-	1024	512	4	4	64	16
P. aeruginosa	512	512	2048	2048	-	-	-	2048	64	64	128	64
S. flexneri SDINT	128	512	2048	512	2048	1024	512	512	64	1	1	256
S. aureus ATCC25923	512	16	-	-	-	2048	-	16	32	1	1	32
P. aeruginosa PA01	256	1024	-	512	-	2048	512	512	8	2	2	256
S. aureus MSSA 01	256	2048	2048	2048	-	-	-	256	32	8	4	256
S. aureus MRSA 03	512	2048	-	2048	-	-	512	2048	64	16	16	4
S. aureus MRSA 04	1024	512	-	512	-	-	512	512	64	16	16	128

- : *> 2048 µg/ml*

reported by the previous works which showed that this plant contained flavonoids, alkaloids, saponins, glycosides and tannins [40]. The differences in chemical composition of plant extracts between the observations of this study and those reported in previous findings can be attributed to the extraction solvents, the part of the plant analyzed, the place and period of harvesting of the plant or the variety of plant species tested.

Differences in antibacterial activity were noted between the studied extracts. These differences may be due to the different groups of secondary metabolites found in these extracts. Indeed, the antimicrobial activity of medicinal plants is correlated with the presence in their extracts of one or more classes of bioactive secondary metabolites [41]. Nevertheless, previous report carried out showed that the antimicrobial activity of plant extracts does not necessarily depend on the number of active compounds present in these extracts [42]. This observation would also justify the antibacterial activity of the methanol extract of *C. papaya* which contains the same number of groups of compounds as the extracts of *O. gratissimum* and *S. torvum* but is more active than the latter. All these observations suggest that the antibacterial activity of a plant extract does not depend solely on the presence of the secondary metabolites, but also on the types present, their quantity and possible interactions with constituents. These phytochemical constituents grouped into three main classes namely alkaloids, terpenoids and phenolic compounds have been reported for their antibacterial properties [43].

According to Tamokou et al. [43], a plant extract is considered to be highly active if the MIC < 100 µg/ml; significantly active when 100 ≤ MIC ≤ 512 µg/ml; moderately active when 512 < MIC ≤ 2048 µg/ml; weakly active if 2048 < MIC ≤ 10000 µg/ml and not active when MIC > 10000 µg/ml. Based on this classification, *C. papaya* extract showed significant antibacterial activity against *B. subtilis, S. flexneri*, *P. aeruginosa*, *S. aureus*, *P. aeruginosa* PA01, *S. aureus* MSSA01 and *S. aureus* MRSA03 and moderately elevated activity against *E. coli* and *S. aureus* MRSA04. The antibacterial activities of the *C. papaya* methanol extract confirmed those of the previous works [17,44] that the aqueous and organic extracts of this plant exhibit important inhibitory activities with respect to a wide variety of bacterial strains. *O. gratissimum* extract demonstrated significantly elevated activities on *S. flexneri, P. aeruginosa* PA01, *S. aureus* MRSA03 and *S. aureus* MRSA04 and moderately elevated activities against *E. coli S2*. The antibacterial activity of seeds and fruits of *O. gratissimum* has been previously demonstrated against *N. gonorrhoeae S. typhi, P. aeruginosa, K. pneumoniae, V. cholera, E. coli* and *S. aureus* [45]. The extract of *S. torvum* leaves showed significantly elevated activities against *B. subtilis* and moderately elevated activities against *S. flexneri*, *E. coli*, *P. aeruginosae* and *S. aureus* MSSA01. The antibacterial activity of *S. torvum* concurs with that reported in the literature [46]. However, to the best of our knowledge, this is the first report on the antibacterial activities of methanol extracts of *O. gratissimum, S. torvum* and *C. papaya* against multi-drug-resistant pathogenic bacteria.

During the MIC and MBC determinations, we have noted that most of extracts had MBC values > 2048 µg/ml; suggesting their bacteriostatic effect on the corresponding microorganisms [47].

The antibacterial activities of MeOH extracts and chloramphenicol increased under osmotic stress (5% NaCl) whereas those of amoxicillin decreased under this condition. The current findings have key implications in disinfectants, antiseptics and wound-dressing formulations for topical treatments since *C. papaya*, *O. gratissimum* and *S. torvum* are traditionally used as wound healing in Cameroonian medicinal system. Early reports have shown that certain bacteria (*E. coli, S. aureus, P. aeruginosa*) can survive and develop under osmotic stress conditions [48]. At low water activity, lipid composition of bacterial cell membrane was changed [49]. This incident might lead to occurrence of more antibacterial binding site on cell membrane of bacteria and cause less resistance to antibacterial substance. Therefore, the presence of the salt triggered changes in the membrane lipid composition. This is possible to increase the microbial activity of plant extracts and chloramphenicol. However, the mechanisms that make bacteria more sensitive to certain antibiotics/extracts under osmotic stress conditions are still unknown. The results on amoxicillin activity are similar to those of McMahon et al. [50] who demonstrated a decrease in the activity of amikacin, ceftriaxone and trimethoprim with respect to *E. coli* and *S. aureus* under osmotic stress conditions. Plant extracts contain a multitude of compounds that can act individually or interact on several targets [51]. This could make it difficult to develop mechanisms of resistance by bacteria to the tested extracts.

5. CONCLUSION

The phytochemical analysis revealed that the plant extracts of *C. papaya*, *O. gratissimum* and *S. torvum* possess alkaloids, polyphenols, tannins and triterpenes whereas flavonoids, anthraquinones, anthocyanins, steroids and saponins are selectively distributed in these extracts. The extract of *C. papaya* (MIC = 128 - 1024 µg/ml) was the most active followed in decreasing order by *O. gratissimum* extract (MIC = 512 - 1024 µg/ml), *S. torvum* leaf extract (MIC = 256 - 2048 µg/ml), and *S. torvum* fruit (MIC = 2048 µg/ml). The antibacterial activities of plant extracts and chloramphenicol increased under osmotic stress (5% NaCl) while those of amoxicillin decreased under this condition. The overall results of this study showed that the methanol extracts of the tested plants possess antibacterial activity that can justify their traditional use in the treatment of infected diseases.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian Medicinal plants against multidrug resistant human pathogens. J Ethnopharm. 2001;74:87-91.
2. Samy RP, Manikandan J, Al Qahtani M. Evaluation of aromatic plants and compounds used to fight multidrug resistant infections. Evid Based Complement Altern Med. 2013;2013:1-17.
3. McDowell DA. Food processing stresses in the spread of antibiotic resistance, p. 243–265. *In* F. J. M. Smulders and J. D. Collins (ed.), Safety assurance during food processing: food safety assurance and veterinary pub lic health, vol. 2. Wageningen Academic Publishers, Wageningen, The Netherlands; 2004.
4. Blackburn CD, Davies AR. Development of antibiotic resistant strains for the enumeration of foodborne pathogenic bacteria in stored foods. Int J Food Microbiol. 1994;24:125-136.
5. Hastings PJ, Rosenberg SM, Slack A. Antibiotic-induced lateral transfer of antibiotic resistance. Trends Microbiol. 2004;12:401-404.
6. Doughari JH, Elmahmood AM, Mansara S. Studies on the antibacterial activity of root extracts of *Carica papaya* L. Afr J Microbiol Res. 2007;2007:33-41.
7. Bibitha B, Jisha VK, Salitha CV, Mohan S, Valsa AK. Antimicrobial activity of different plant extracts. Indian J Microbiol. 2002;42:361-363.
8. Tamokou JD, Tala FM, Wabo KH, Kuiate JR, Tane P. Antimicrobial activities of methanol extract and compounds from stem bark of *Vismia rubescens*. J Ethnopharmacol. 2009;124:571-575.
9. Chouna JR, Tamokou JD, Nkeng-Efouet-Alango P, Lenta NB, Sewald N. Antimicrobial triterpenes from the stem bark of *Crossopteryx febrifuga.* Z Naturforsch. *C.* 2015;70(7-8)c:169-173.
10. Djouossi MG, Tamokou JD, Ngnokam D, Kuiate JR, Tapondjou AL, Harakat D, et al. Antimicrobial and antioxidant flavonoids from the leaves of *Oncoba spinosa* Forssk. (Salicaceae). BMC Complement Altern Med. 2015;15:134.
11. Tatsimo NSJ, Tamokou JD, Lamshöft M, Mouafo TF, Lannang MA, Sarkar P, et al. LC-MS guided isolation of antibacterial and cytotoxic constituents from *Clausena anisata*. Med Chem Res. 2015;24(4):1468-1479.
12. Pagning NAL, Tamokou JD, Lateef M, Tapondjou AL, Kuiate JR, Ngnokam D, et al. New triterpene and new flavone glucoside from *Rhynchospora corymbosa* (Cyperaceae) with their antimicrobial, tyrosinase and butyrylcholinesterase inhibitory activities. Phytochem Lett. 2016;16(1):121-128.

13. Tebou PLF, Tamokou JD, Ngnokam D, Voutquenne-Nazabadioko L, Kuiate JR, Bag PK. Flavonoids from *Maytenus buchananii* as potential cholera chemotherapeutic agents. S Afr J Bot 2017;109:58–65.
14. Corrêa MP. Dicionário das Plantas Úteis do Brasil, IBDF, Ministério da Agricultura, Rio de Janeiro. 1932;63. Portugais.
15. Onajobi FD. Smoth muscle contracting lipidicsoluble principles in chromatographic fractions of *Ocimum gratissimum.* J Ethnopharmacol. 1986;18:3-11.
16. Yuanyuan LU, Jianguang L, Xuefeng H, Lingyi K. Four steroidal glycosides from *Solanum torvum* and their cytotoxic activities. Steroids. 2009;74:95-101.
17. Peter JK, Kumar Y, Pandey P, Masih H. Antibacterial activity of seed and leaf extract of *Carica Papaya* var. *Pusa dwarf* Linn. IOSR J Pharm Biol Sci. 2014;9(2):29-37.
18. Romasi EF, Karina J, Parhusip AJN. Antibacterial activity of papaya leaf extract against pathogenic bacteria. Makara, Teknology. 2011;15(2):173–177.
19. Adebolu TT, Oladimeji SA. Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhea causing bacteria in Southwestern Nigeria. Afr J Biotech. 2005;4(7):682-684.
20. Lemos JA, Passons XS, Fernande OFL, Paula JR, Ferri PH, Souza LKH, Lemos AA, Silva RRM. Antifungal activity from *Ocimum gratissimum* L. towards *Cryptococcus neoformans.* Mem. Inst Oswaldo Cruz. 2005;100(1):55-58.
21. Matasyoh LG, Matasyoh JC, Wachira FN, Kinyua MG, Muigai Thairu AW, Mukiama TK. Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. growing in Eastern Kenya. Afr J Biotechnol. 2007;6(6):760-765.
22. Bari MA, Islam W, Khan AR, Manda A. Antibacterial and Antifungal Activity of *Solanum torvum* (Solanaceae). Int J Agri Biol. 2010;12(3):386-390.
23. Tamokou JD, Mpetga Simo DJ, Lunga PK, Tene M, Tane P, Kuiate JR. Antioxidant and antimicrobial activities of ethyl acetate extract, fractions and compounds from the stem bark of *Albizia adianthifolia* (Mimosoideae). BMC Complement Altern Med. 2012;12:99.
24. Tatsimo NSJ, Tamokou JD, Tsague TV, Lamshoft M, Sarkar P, Bag PK, et al. Antibacterial-guided isolation of constituents from *Senna alata* leaves with a particular reference against Multi-Drug-Resistant *Vibrio cholerae* and *Shigella flexneri.* Int J Biol Chem Sci. 2017;11(1): -53.
25. Joubouhi C, Tamokou JD, Ngnokam D, Voutquenne-Nazabadioko L, Kuiate JR. Iridoids from *Canthium subcordatum* iso-butanol fraction with potent biological activities. BMC Complement Altern Med. 2017;17:17.
26. Phatthalung PN, Chusri S, Voravuthikunchai PS. Thai ethnomedicinal plants as resistant modifying agents for combating *Acinetobacter baumannii* infections. BMC Complement Altern Med. 2012;12:56.
27. Afolayan AJ. Extracts from the shoots of *Aretottis arotoides* inhibit the growth of bacteria and fungi. Pharm Biol. 2003;41(1):22-25.
28. Vijayalakshmi K, Subhashini B, Koul SV. Plant in pest control (Tobacco, Papaya and Thumai), Centre for Indian Knowledge Systems, Channai. 1997;P.18.
29. Watt JM, Breyer-Brandwijk MG. Medicinal and poisonous plants of Southern and Eastern Africa. E&S. Livingstone, Ltd. London; 1962.
30. Harjula H. Mirau and his practice: A study of the ethnomedicinal repertoire of a Tanzanian herbalist pg 223. Tri-med. books London; 1980.
31. FAO. Some medicinal forest plants of Africa and Latin America. Forestry Paper 67; 1986.
32. Kokwaro JO. Medicinal plants of East Africa, East Africa Literature Bureau, Kampala, Nairobi, and Dar-es-Salaam. 1993;106-115.
33. Bhakuni DS, Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN. Screening of Indian plants for biological activity: Part II. Indian J Exp Biol. 1969;7:250-262.
34. Bhakuni DS, Srivastava SN, Sharma VN, Kaul KN. Chemical examination of the fruits of *Coccinia indica* W. and A. J Sci Ind Res. 1962;21B:237-238.
35. Trease GE, Evans WC. A text book of pharmacognosy. 13e édition, Baillière Tindall Ltd., Londres; 1989.
36. CLSI. Methods for determining bactericidal activity of antimicrobial agents. Approved guideline, M26-A. Clinical and Laboratory Standards Institute, Wayne, Pa; 1999.

37. CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standards, M7A4. Clinical and Laboratory Standards Institute, Wayne, PA; 1997.
38. Mbata T, Saikia A. Antibacterial activity and phytochemical screening of crude ethanolic extract of leaves of *Ocimum gratissimum L* on *Listeria monocytogenes.* Internet J Microbiol. 2007;4(2):1-6.
39. Kongo-Nzuzi Y. Evaluation *in vitro* des pouvoirs antifongique des extraits de feuille de papayer sur des souches de *Candida albicans*. ISTM Kinshasa - Graduée en techniques de laboratoire; 2009. French.
40. Lu Y, Luo J, Xu D, Huang X, Kong L. Characterization of spirostanol saponins in *Solanum torvum* by high-performance liquid chromatography/evaporative light scattering detector/electrospray ionization with multistage tandem mass spectrometry. Magn Reson Chem. 2008;47:808-812.
41. Reuben KD, Abdulrahman FI, Akan JC, Usman H, Sodipo OA, Egwu GO. Phytochemical screening and *in vitro* antimicrobial investigation of the methanolic extract of *Croton Zambesicus* Muell ARG. stem bark. Eur J Sci Res. 2008;23(1):134-140.
42. Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol. 2000;31:247-256.
43. Tamokou JD, Mbaveng TA, Kuete V. Antimicrobial activities of African medicinal spices and vegetables. In: Medicinal Spices and Vegetables from Africa: Therapeutic Potential against Metabolic, Inflammatory, Infectious and Systemic Diseases, 1st Edition, Elsevier, 2017, Chapter 8, Pp207-237.
44. Tewari BB, Subramanian G, Gomathinayagm R. Antimicrobial properties of *Carica papaya* (Papaya) different leaf extract against *E. coli*, *S. aureus* and *C. albicans.* Am J Pharmacol Pharmacother. 2014;1(1):025-039.
45. Abdullahi M. Phytochemical constituents and antimicrobial and grain protectant activities of clove basil (*Ocimum gratissimum L.*) grown in Nigeria. Int J Plant Res. 2012;2(1):51-58.
46. Yuan-Man H, Jing-Ru W, Tsurng-Juhn H, Chih-Ho L, Chiu-Hsiang S, Chang-Hung C. *Solanum torvum* inhibits *Helicobacter pylori* growth and mediates apoptosis in human gastric epithelial cells. Oncol Rep. 2010;23:1401-1405.
47. Mims CA, Playfair JHL, Roitt IM, Wakelin D, Williams R. Antimicrobials and chemotherapy. In: Mims, C.A., et al. (Eds.). Med Microbiol Rev. 1993;35:1-34
48. Besten HMW, Mols M, Moezelaar R, Zwietering MH, Abee T. Phenotypic and transcriptomic analyses of mildly and severely salt-streesed *Bacillus cereus* ATCC 14579 cells. Appl Environ Microbiol. 2009;75:111-119.
49. Beales N. Adaptation of microorganisms to cold temperatures, weak acid reservatives, low pH, and osmotic stress: A review. Compr Rev Food Sci Food Saf. 2004;3(1):1-20.
50. McMahon MAS, Xu J, Moore JE, Blair IS, McDowell DA. Environmental stress and antibiotic resistance in food-related pathogens. Appl Environ Microbiol. 2007;73:211-217.
51. Lopez-Malo VA, Palou E, Alzamora SM. Naturally occurring compounds-plant sources. In: Antimicrobials in Food. 3rd edition. Davidson, P.M.; Sofos, J.N.; and Branen, A.L. (eds.) CRC Press, Taylor & Francis Group, Boca Raton, FL, USA. 2005;429-451.

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