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Antimicrobial and toxic potential of aqueous extracts of *Allium sativum*, *Hibiscus sabdariffa* and *Zingiber officinale* in Wistar rats

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Abstract

Allium sativum, Hibiscus sabdariffa and *Zingiber officinale* are medicinal plants with wide use in traditional medicine; however, the increasing use of crude extracts for traditional medicine applications raises safety concerns. We made a preliminary determination of the phytochemical constituents and antimicrobial and safety profiles of aqueous extracts of *A. sativum, H. sabdariffa* and *Z. officinale*. The extracts were administered orally to Wistar rats for 30 days: a control group received distilled water, three groups received the three extract, and a fifth group received a combination of the three extracts. All three extracts, either individually or in combination, had antimicrobial activity, and all extracts influenced the activities of marker enzymes. The evidence lends credence to use of these plants in traditional medicine but also suggests the probable toxic potential of crude plant extracts.

Keywords: Medicinal plants; Anti-infective agent; Toxicity; Traditional medicine

1. Introduction

Plant-derived compounds are being used more and more widely for their potential chemotherapeutic and nutritional value [1,2]. Administration of medicinal plants by traditional practitioners remains the mainstay of some health care systems, especially in the rural areas of developing countries [3,4]. The World Health Organization reported [5] that 74% of 119 plant-derived pharmaceutical remedies are used in modern medicine in ways that correlate directly with their traditional uses as medicinal plants, and major pharmaceutical companies are undertaking extensive research to determine the medicinal value of plant materials [5]. Medicinal herbs, vegetables [6,7] and spices contain chemical constituents such as tannins, alkaloids and terpenes, and their secondary metabolites are a potential source of novel compounds with various health-promoting effects [3,8,9].

Plant extracts are commonly used to treat infections in traditional African medicine, and numerous plants and herbs are used in Nigeria [10]. Traditional medicine practices vary from one country to another [11], and herbal formulations may include extracts from the root, bark or leaves of various plants [12]. The various applications of these herbs in traditional medicine system [10] underscore the need for scientific investigations to

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affirm or disprove the claims of traditional healers. The increasing consumption of crude extracts of medicinal plants is becoming a major concern because of safety issues. Many people believe that, as plants are natural, they can be consumed safely; unfortunately, this has proven otherwise [4], and growing concern about the safety of consuming crude extracts of medicinal plants was a motivation for this study [3]. We studied aqueous extracts of *Allium sativum*, *Hibiscus sabdariffa* and *Zingiber officinale* for antimicrobial activity *in vitro* and for biochemical indices in rats *in vivo*.

A. sativum, commonly known as garlic, belongs to the Alliaceae family, which also includes leek, onion and shallot. Garlic is used widely in food and medicine [3,13–16], like other herbs and spices [17]. Use of *A. sativum* in alternative medicine has increased over the years [18–20]. Garlic can be prepared in various forms, namely oil, powder, raw juice and extracts [21–23]. The therapeutic effect of garlic has been attributed to its organosulfur constituents, which also are responsible for its typical flavour and odour [1,17,24]. Other studies have implicated thiosulfinates in the antibiotic activity of garlic [22,25–28].

H. sabdariffa, hibiscus, is a variety of true roselle, belonging to the Malvaceae family. The plant is an erect, sparsely branched annual 4.8 m in height, which is cultivated for its jute-like fibre in India, the East Indies, Nigeria and to some extent in other parts of tropical Africa. The stems of this variety are green or red, and the leaves are green, sometimes with red veins. Its flowers are yellow, and the red or green, non-fleshy, spiny calyxes can be used for food [29,30]. Hibiscus tea is consumed both hot and cold by people around the world [31] as an infusion made from the crimson or deep magenta-coloured calyxes of the *H. sabdariffa* flower [32]. It has a tart, cranberry-like flavour, and sugar is often added to sweeten the beverage. The tea has been shown to contain vitamin C and minerals and is traditionally used as a mild medicine. In west Sudan, white hibiscus flowers are favoured for their bitter taste and are not sold but used by the owner's family and guests [31,33].

Z. officinale is a rhizome commonly known as ginger or ginger root. It can be consumed as a delicacy, medicine or spice. It lends its name to its genus and family (Zingiberaceae). Other notable members of this plant family are turmeric, cardamom and galangal [34–36]. Ginger cultivation began in South Asia and then spread to East Africa and the Caribbean. Historically, the traditional medical form of ginger was called Jamaica ginger, which was classified as a stimulant and carminative [37]. Ginger has also frequently been used to disguise the taste of medicines [3]. Studies indicate that ginger may provide short-term relief of pregnancy-related nausea and vomiting [34–36].

2. Materials and methods

All reagents were of analytical grade and prepared with distilled water, unless otherwise stated.

2.1. Plant materials

A. sativum, *H. sabdariffa* and *Z. officinale* were purchased on a commercial market, Oja-Oba, in Ilorin, Kwara State, Nigeria. The plant materials were identified and authenticated at the herbarium of the Botany Department, University of Ilorin, Ilorin, Nigeria, where specimen vouchers have been deposited.

2.2. Test organisms

Test organisms were obtained from the culture collection centre of the Microbiology and Parasitology laboratory of the University of Ilorin Teaching Hospital, Ilorin, Nigeria. They were isolated from sputum samples submitted by patients with suspected respiratory tract infections. The isolates were further subjected to Gram staining and other biochemical tests according to standard procedures and were identified as *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureaus* and *Pseudomonas aeruginosa.*

2.3. Experimental animals

Wistar rats of an average weight of 180 g were obtained from the small animal unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were housed in a well-ventilated, hygienic environment and allowed free access to feed (rat pellet purchased from Bendel Feeds, Ewu, Nigeria) and clean water. The animals were acclimatized for 2 weeks before the start of the experiment. Handling was consistent with the ethical guidelines approved by the University of Ilorin Ethics Committee for scientific and medical research.

2.4. Preparation of plant extracts

Fresh samples were cut into pieces with a sterile knife and dried at room temperature until a constant weight was obtained. The dried sample was ground to powder, and 85 g of each sample were percolated in 850 ml distilled water and allowed to stand for 72 h. The extracts were then filtered through Whatman no. 1 filter paper, and the filtrate was vacuum-dried at the Nigerian Stored

Product Research Institute, Ilorin, Nigeria. The extracts were stored at 4 °C until further use. The yields of the extracts were 30% for *H. sabdariffa*, 20% for *A. sativum* and 15% for *Z. officinale*.

2.5. Phytochemical screening

Phytochemical tests were carried out on the extracts to identify the constituents by standard procedures [38–42].

2.6. Antibacterial susceptibility

Antibacterial activity was tested by the agar well diffusion method. Sterile Mueller–Hinton agar was poured into sterile Petri dishes at 45 °C and allowed to solidify; then, 1 ml of each organism adjusted to 0.5 MacFarland standard were swabbed onto the agar with a sterile cotton swab. Four wells (5 mm in diameter) were bored into the agar with a sterile cork borer, and 0.25 ml of reconstituted extract was pipetted into the well; streptomycin and distilled water served as positive and negative controls. The plates were held for 2 h at room temperature for diffusion of extracts into the agar and then incubated at 37 °C for 18 h. The diameters of the zones of inhibition were then measured. Each experiment was conducted in duplicate, and the mean values were taken.

2.7. Animal treatment

The rats were randomized into five groups of three. One group served as the control and received distilled water; three groups received 10 mg/kg of extract of *A. sativum*, *H. sabdariffa* or *Z. officinale*, and a final group received 10 mg/kg of all three extracts. All extracts were administered orally for 30 days. Rats were weighed weekly.

2.8. Necroscopy

After cessation of treatment, the rats were sacrificed under slight diethylether anaesthesia, and blood was collected into a clean centrifuge tube and spun (Heraeus Labofuge 300, Thermo Scientific, Hampshire, United Kingdom) at 4000 × *g* for 10 min to obtain serum. Tissues of interest (liver, kidneys and heart) were harvested, weighed and homogenized with a Teflon homogenizer (Sigma–Aldrich Chemie GmbH, Munich, Germany) in a cold 0.25 mol/L sucrose solution (1:5, w/v). The tissue homogenates were then centrifuged at 5000 × *g* for 10 min to remove unbroken particulate.

Table 1
Phytochemical analysis of extracts of *Allium sativum*, *Hibiscus sabdariffa* and *Zingiber officinale*.

Phytochemical	*A. sativum*	*H. sabdariffa*	*Z. officinale*
Saponins	+	+	+
Phlobatanins	–	+	–
Terpenoids	–	+	–
Anthraquinones	–	+	–
Flavonoids	+	–	+
Tannins	–	+	–
Glycosides	+	–	–
Steroids	–	+	–
Alkaloids	+	–	–
Phenolics	–	+	–

2.9. Biochemical assays

Alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) activities were assayed with Randox assay kits [43–45] in a spectrophotometer (Spectrumlab model 752s).

2.10. Data analysis

Data are presented as group means ± SEM ($n = 3$). Significant differences among group mean values were evaluated by one-way analysis of variance. Post-test analyses were conducted with the Dunnett multiple comparison test at 99.5% significance level ($\alpha = 0.05$).

3. Results

The phytoconstituents of the three extracts are presented in Table 1. Saponins were present in all the extracts. Phlobatanins, terpenoids, anthraquinones, tannins, steroids and phenolics were detected in extracts of *H. sabdariffa*, and flavonoids were found in extracts of both *A. sativum* and *Z. officinale.* Glycosides and alkaloids were found only in *A. sativum* extracts.

The aqueous extract of *Z. officinale* was active against *P. aerugenosa* and *S. aureus*, with highest activity against the former at a concentration of 200 μg (Table 2). The *A. sativum* extracts showed no inhibitory activity, while *H. sabdariffa* was active against all test organisms, with the highest activity against *P. aeruginosa* at a concentration of 200 μg. The combination of the three extracts had antimicrobial activity against all test organisms, with the highest activity against *P. aerugenosa* at a maximum concentration of 200 μg. The control drug, streptomycin, showed dose-dependent antimicrobial activity against all test organisms except *P. aeruginosa.* At lower streptomycin concentrations

Table 2
Antibacterial activity of *Hibiscus sabdariffa* and *Zingiber officinale extract* combinations and streptomycin.

Extract	Test organism	Concentration of extract (mg/ml)			
		25	50	100	200
		(Diameter of zone of inhibition (mm))			
Zingiber officinale	*S. aureus*	6.5	7.2	13.0	13.5
	K. pneumonia	–	–	–	–
	P. aeruginosa	13.0	13.5	14.0	15.5
	E. coli	–	–	–	–
Hibiscus sabdariffa	*S. aureus*	8.0	12.0	13.0	13.5
	K. pneumonia	10.5	12.5	14.0	14.0
	P. aeruginosa	9.0	11.0	12.5	17.0
	E. coli	8.5	9.0	12.0	13.5
Combination	*S. aureus*	5.6	7.5	8.3	9.2
	K. pneumonia	4.5	5.1	6.6	7.3
	P. aeruginosa	7.5	9.0	10.8	12.8
	E. coli	4.8	5.0	6.0	6.5
Streptomycin	*S. aureus*	9.8	11.5	16.0	19.5
	K. pneumonia	–	–	16.0	18.5
	P. aeruginosa	–	–	–	–
	E. coli	10.5	10.5	11.5	17.0

–, no noticeable inhibition.

(25 and 50 mg/ml), no inhibitory activity was observed against *K. pneumonia*.

Table 3 shows significant increases in the body weight of rats given extracts of *A. sativum* or *Z. officinale* and in controls, whereas rats given *H. sabdariffa* extract and those given the combination of extracts had lower weights than controls. The organ-to-body weight ratios of the treated rats are presented in Table 4. No significant difference was found among the treatment groups relative to the control, except that the heart, liver and kidney to body ratios were higher in rats that received *Z. officinale*.

Administration of aqueous extracts of all three plants significantly increased ($p < 0.05$) the activity of AST in serum when compared with controls (Table 5), especially by *Z. officinale* (23.66 U/I) and by the combination (29.00 U/I) of extracts. The highest AST activities in liver, kidney and heart were seen with the combination (135 U/I), *A. sativum* (118.33 U/I) and *Z. officinale* (136.67 U/I) extracts. *A. sativum* extracts also increased the serum ALT relative to the control and the other treatment groups (Table 6). Liver ALT activity was increased after administration of *A. sativum* and *Z. officinale* extracts. In contrast, the activity of ALT in rat heart and kidney was lowered in all test groups after extract administration. The *A. sativum* extracts caused a significant reduction in ALP activity in serum (151.57 U/I), heart (34.96 U/I), kidney (38.64 U/I) and liver (55.20 U/I) (Table 7). Serum ALP was significantly reduced with *H. sabdariffa* (92.69 U/I) and *Z. officinale* (92.00 U/I).

4. Discussion

Phytochemical analysis of the aqueous extracts of *A. sativum*, *H. sabdariffa* and *Z. officinale* revealed the presence of flavonoids, terpenoids, alkaloids, tannins, saponins, anthraquinones and phenolics. Other investigators have reported that extracts of *H. sabdariffa* contained a number of bioflavonoids, including phlobatanins, terpenoids, anthraquinones, tannins, steroids and phenolics [46,47]. Our phytochemical findings are also consistent with previous reports [8,9,48].

Extracts of the three plants and the combination of extracts had potent antimicrobial activity against the test organisms, thus corroborating previous investigations [36,49]. *Z. officinale* extracts have been reported to have antifungal and antibacterial activity *in vitro* [19,25] and to be effective in combating post-operative nausea and vomiting [34]. Our results agree with previous reports [2,49–54], in which the antimicrobial activity of *Z. officinale* was attributed to the presence of specific chemical constituents. Extracts of *H. sabdariffa* showed remarkable activity against all the test organisms, with the highest activity against *P. aerugenosa* (17.0 mm zone) at 200 mg/ml, thereby supporting the findings of Tseng et al. [55] and Walker et al. [56]. In previous studies, the least activity was found with *S. aureus* (8.0 mm zone),

Table 3
Average weight (g) of rats treated with extracts of *Hibiscus sabdariffa*, *Zingiber officinale* and *Allium sativum*.

Week	Control	*H. sabdariffa*	*Z. officinale*	*A. sativum*	Combination
0	160.0 ± 24.1	196.7 ± 16.0[*,£]	106.7 ± 11.0[*,£]	166.7 ± 27.0[*,£]	139.3 ± 13.9[*,£]
1	163.0 ± 24.2	189.0 ± 20.0[*,£]	116.7 ± 15.0[*,£]	179.3 ± 30.0[*,£]	134.7 ± 13.8[*,£]
2	166.7 ± 23.7	185.3 ± 19.0[*,£]	114.0 ± 11.0[*,£]	172.7 ± 24.0[*,£]	130.3 ± 13.4[*,£]
3	168.7 ± 24.4	179.3 ± 19.0[*,£]	117.7 ± 11.0[*,£]	175.3 ± 26.0[*,£]	128.3 ± 13.4[*,£]
4	167.7 ± 21.4	177.0 ± 18.0[*,£]	119.3 ± 10.0[*,£]	175.0 ± 24.0[*,£]	126.0 ± 13.1[*,£]
5	167.3 ± 20.6	180.7 ± 13.0[*,£]	120.0 ± 10.0[*,£]	172.7 ± 23.0[*,£]	123.7 ± 13.2[*,£]

Values are mean ± SEM ($n = 3$).
[*] Significantly different at $p < 0.01$ relative to control.
[£] Significantly different relative to the combination treatment at $p < 0.001$.

Table 4
Organ-to-body weight ratio of rats treated with extracts of *Hibiscus sabdariffa*, *Zingiber officinale* and *Allium sativum*.

Organ	Control	*H. sabdariffa*	*Z. officinale*	*A. sativum*	Combination
Heart	0.31 ± 0.01	0.32 ± 0.02	0.42 ± 0.04	0.31 ± 0.04	0.38 ± 0.03
Liver	0.61 ± 0.07	0.56 ± 0.04	0.84 ± 0.08	0.60 ± 0.08	0.83 ± 0.08
Kidney	0.59 ± 0.06	0.56 ± 0.04	0.77 ± 0.12	0.56 ± 0.59	0.74 ± 0.05

Values are means ± SEM ($n = 3$).

Table 5
Aspartate transaminase activity in rat serum and tissues after administration of extracts of *Hibiscus sabdariffa*, *Zingiber officinale* and *Allium sativum*.

Source	Control	*H. sabdariffa*	*Z. officinale*	*A. sativum*	Combination
Serum	16.33 ± 5.89[a]	18.00 ± 2.08[a,b]	23.66 ± 2.90[c]	14.66 ± 4.40[b]	29.00 ± 3.05[d]
Heart	40.66 ± 24.5[a]	103.33 ± 50.3[b]	136.67 ± 29.2[d]	129.67 ± 34.1[c]	132.00 ± 50.83[d]
Kidney	91.33 ± 11.9[c]	86.00 ± 11.93[b]	148.67 ± 24.6[c]	118.33 ± 68.5[d]	64.67 ± 52.54[a]
Liver	69.67 ± 11.5[a]	132.67 ± 17.1[c]	167.00 ± 27.1[d]	90.33 ± 23.32[b]	135.00 ± 45.0[c]

Values are means ± SEM ($n = 3$). Values with different superscripts across the same row are significantly different at $p < 0.05$.

Table 6
Alanine transaminase activity in rat serum and tissues after administration of extracts of *Hibiscus sabdariffa*, *Zingiber officinale* and *Allium sativum*.

Source	Control	*H. sabdariffa*	*Z. officinale*	*A. sativum*	Combination
Serum	12.00 ± 0.58[b]	9.67 ± 0.67[a]	10.33 ± 3.92[a,b]	14.33 ± 2.40[c]	11.67 ± 0.91[b]
Heart	91.67 ± 1.45[d]	84.00 ± 6.08[c]	76.33 ± 11.79[b]	68.67 ± 10.17[a]	81.33 ± 10.27[c]
Kidney	79.33 ± 2.40[b]	72.00 ± 14.11[a]	73.33 ± 10.39[a]	78.00 ± 8.39[b]	79.00 ± 5.29[b]
Liver	82.00 ± 5.56[a]	83.00 ± 5.56[a]	85.67 ± 4.91[b]	93.67 ± 0.33[c]	81.67 ± 10.39[a]

Values are means ± SEM ($n = 3$). Values with different superscripts across the same row are significantly different at $p < 0.05$.

Table 7
Alkaline phosphatase activity in rat serum and tissues after administration of extracts of *Hibiscus sabdariffa*, *Zingiber officinale* and *Allium sativum*.

Source	Control	*H. sabdariffa*	*Z. officinale*	*A. sativum*	Combination
Serum	497.72 ± 43.27[b]	92.69 ± 4.48[a]	92.00 ± 23.30[a]	151.57 ± 2.97[a]	526.67 ± 32.24[c]
Heart	782.00 ± 53.96[a]	816.96 ± 89.63[a,b]	720.36 ± 89.48[a,b]	34.96 ± 0.92[b]	554.76 ± 24.11[b]
Kidney	709.32 ± 53.97[a]	689.08 ± 45.36[b]	785.68 ± 46.29[b]	38.64 ± 6.95[b]	633.88 ± 39.81[b]
Liver	753.48 ± 18.24[a]	732.32 ± 40.81[b]	648.60 ± 08.24[b]	55.20 ± 6.94[b]	687.24 ± 26.08[b]

Values are means ± SEM ($n = 3$). Values with different superscripts across the same row are significantly different at $p < 0.05$.

the highest concentration of which (200 mg/ml) yielded a 13.5-mm zone of inhibition [57]. The *Z. officinale* extract was active against *P. aerugenosa* and *S. aureus* [57,58], with the highest activity against *P. aeruginosa* (15.5 mm zone) at 200 mg/ml [56,59]. The combination of extracts showed the highest inhibition against *P. aerugenosa* (12.8 mm zone) and the least inhibition against *K. pneumonia* (4.5 mm zone) [60,61]. The antibacterial activities of *Z. officinale* and *H. sabdariffa* against *P. aeruginosa* were better than that of the control drug, streptomycin.

The marker enzymes AST, ALT and ALP were selected because of their relevance in assessing the integrity of tissues in cases of disease or toxicity consequent to drug or compound assault [62,63]. Administration of the aqueous extracts either individually or in combination altered the relative weights of the heart, liver and kidney of the animals when compared with controls, which may be an indication of a toxic effect [64]. Administration of the extracts individually or in combination significantly altered serum and tissue AST relative to the control, as reported previously [65]. Inconsistent alterations in serum and tissue ALT and ALP activity were found. The elevated ALP activity seen after administration of the combination of extracts might predict liver cholestasis. The alterations in AST and ALT activity might reflect a response or adaptation of tissues to the stress imposed by administration of the extracts. Previous studies have shown that drugs or extracts can promote a stressful environment in tissues or cells and thereby lower or raise enzyme activity as part of an adaptation mechanism [62,66]. Several studies have shown that *H. sabdariffa* can increase serum AST and ALP activity [67–73]. The reductions in the activities of AST, ALT and ALP consequent to exposure to individual or combined extracts may also be due to inactivation of the enzyme proteins by compounds present in the extracts or their metabolites. Drugs or compounds can react with enzymes, causing activation or inactivation [63].

5. Conclusion

We present further evidence of the antimicrobial properties of crude extracts of *H. sabdariffa* and *Z. officinale*; however, *A. sativum* had little or no antimicrobial activity. *H. sabdariffa and Z. officinale* were more effective against *P. aeruginosa* than streptomycin, which reinforces the scientific basis for their inclusion in traditional medicine systems. Extracts of *H. sabdariffa, Z. officinale* and *A. sativum*, however, appear to have toxic potential, with a demonstrated capability to alter biochemical indices in vital tissues. Thus, caution is required in using unrefined extracts of these herbs in traditional settings.

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