

http://www.herbmedpharmacol.com

doi: 10.34172/jhp.2022.47

Journal of Herbmed Pharmacology

Therapeutic potential of *Ipomoea asarifolia* on infected Swiss albino rats with *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Stephen Kayode S. Ojo^{1*®}, Gabriel Temitope Sunmonu^{1®}, Akinwunmi O. Adeoye^{2®}, Christiana Fisayo Akinwunmi^{1®}, Moses Ifeoluwa Obakunle^{1®}, Amos Olakunle Ojerinde^{3®}, Oluwakemi J. Awakan^{4®}

¹Drug Discovery and Infectious Diseases Research Group, Department of Microbiology, Federal University Oye-Ekiti, Ekiti State, Nigeria ²Department of Biochemistry, Federal University Oye-Ekiti, Ekiti State, Nigeria ³Faculty of Basic Medical Sciences, Federal University Oye-Ekiti, Ekiti State, Nigeria ⁴Department of Biochemistry, Landmark University, Omu-Aran, Kwara-State, Nigeria

ARTICLEINFO	A B S T R A C T					
<i>Article Type:</i> Original Article	Introduction: Curative misuse of medicinal plants are worrisome with the paucity of histological information. This led to the investigation of <i>Ipomoea asarifolia</i> in Swiss albino rats infected with <i>Pseudomonas aeruginosa</i> and <i>Staphylococcus aureus</i> . Methods: Extraction was done using the cold maceration method. The minimum inhibitory concentrations (MIC) of the extracts were determined using the micro-dilution method. Swiss albino rats of 6 sub-groups with 6 animals each (36 animals/organism) were administered					
Article History: Received: 16 March 2022 Accepted: 30 April 2022 <i>Keywords:</i> Medicinal plant Gram negative bacteria Gram positive bacteria Antibacterial activity Animal study Histology						
	 with 0.3 ml single oral dose of <i>P. aeruginosa</i> and <i>S. aureus</i> respectively. The animals received treatment for 5 days as follows: 0.5 ml of 5% dimethyl sulphoxide (DMSO) (negative control), 250 mg/kg of amoxicillin (positive control), 2 mg/kg of whole plant extract, 4 mg/kg of whole plant extract, 2 mg/kg of leaf extract, and 4 mg/kg of leaf extract, respectively. The packed cell volume (PCV) and white blood count (WBC) of the animals were determined before and after treatment with histology examination of vital organs. Results: MIC for <i>S. aureus</i> was 2 mg/mL; the mortality in <i>S. aureus</i> group at 2 mg/kg was 66.7%. The PCV values (50.5±0.5, 45.0±1.0, and 50.5±1.5) decreased after infection, and a corresponding increase in the PCV was observed after treatment with the extracts. Also, a significant increase in the WBC values (3.40±0.35, 4.10±0.15, and 3.30±0.40) following infection and a corresponding decrease after treatment were observed. Congestion of vessels in the kidney was also observed. Conclusion: <i>I. asarifolia</i> has a dose-dependent antibacterial and curative activity, and could 					

Implication for health policy/practice/research/medical education:

The traditional uses of *Ipomoea asarifolia* was confirmed as a potential therapeutic agent against commonly isolated hospital acquired infections.

Please cite this paper as: Ojo SKS, Sunmonu GT, Adeoye AO, Akinwunmi CF, Obakunle MI, Ojerinde AO, et al. Therapeutic potential of *Ipomoea asarifolia* on infected Swiss albino rats with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. J Herbmed Pharmacol. 2022;11(3):409-418. doi: 10.34172/jhp.2022.47.

Introduction

Medicinal plants have been an important source of medicine throughout human history and have been used in the treatment and management of several health problems. Although there have been great advances lately in modern medicine, traditional medicine practice is gaining more ground in developing countries, especially in Africa and Asia (1). This situation requires a crucial elucidation of the scientific basis for most of these underutilized medicinal plants in order to confirm the various claims asserted by local users (1). A lot of medicinal plants across different continents have been reported to exert therapeutic efficacy through their antioxidant activities (2-5).

The traditional uses, chemistry, and biological activities of *Ipomoea* species have been evaluated as a potential source

Ojo et al

of therapeutic agents by several authors. These species are used in different parts of the world for the treatment of several diseases, such as diabetes, hypertension, dysentery, constipation, fatigue, arthritis, rheumatism, hydrocephaly, meningitis, kidney ailments, and inflammations. Some of these species showed antimicrobial, antioxidant, analgesic, spasmolytic, spasmogenic, hypoglycemic, hypotensive, anticoagulant, anti-inflammatory, psychotomimetic and anticancer activities (5-7).

Ipomoea asarifolia (Desr.) Roemer & J.A. belongs to the family Convolvulaceae. It is a hairless, succulent perennial creeping or trailing plant growing on sandy soil or waste lands and usually several meters long. It possesses heart-shaped leaves and funnel-shaped purple flowers. It is a common weed in hydromorphic soils, low-lying and inland streams and river banks (1,8). It is popularly known as 'salsa' or 'salsa-brava' in South and Central America (10,11), 'Duman-kaada', 'Duman-kaafi' and' Dumang-kadu' in Northern Nigeria (5,8), and 'Gboroayaba', 'Ododooko' and 'Ododoamu' in Western Nigeria (Yoruba land) (8). It is native to tropical America but is now pan-tropical and is found throughout West Africa and Tropical Asia (8).

Ipomoea asarifolia have been reported to be toxic in animals (cattle, sheep, and goats); however, it was also reported to be relatively safe in the rat at a sub-chronic toxicity dose of 1000 mg/kg and equally demonstrated antiulcerogenic effects on the gastric lesion (ulcer) induced in Wistar rats (12,13).

Some bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* have developed mechanisms for resisting the effects of antibiotics since the advent of antibiotics. The rates of infections by antibiotic-resistant strains have been on the increase for a few decades as a result of both the indiscriminate use of antibiotics by the population, especially in the developing world, and the microbial characteristics for resistance. Therefore, therapeutic options are increasingly limited due to the continued emergence and spread of antimicrobial-resistant strains; as a result, *P. aeruginosa* and *S. aureus* infections demonstrate high morbidity and mortality (14,15).

Besides causing different types of intoxication, especially food poisoning, *S. aureus* represents the most common etiologic agent of pyogenic infections (16,17). Foodborne diseases are of major concern worldwide and *S. aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food (18). Likewise, *P. aeruginosa* is one of the leading nosocomial pathogens worldwide. Nosocomial infections caused by this organism are often hard to treat because of both the intrinsic resistance of the species and its ability to acquire further resistance mechanisms to multiple groups of antimicrobial agents, including β -lactams, aminoglycosides, fluoroquinolones, and the polymyxins (19-20). This study aims to investigate the therapeutic effects of *I. asarifolia* in Swiss albino rats infected with *P. aeruginosa* and *S. aureus*.

Materials and Methods

Collection and identification of plant material

The whole fresh plant of *I. asarifolia* was harvested along the Faculty of Science, Federal University Oye-Ekiti (Oye Campus), Ekiti State, Nigeria (Latitude: 7.78°N and Longitude: 5.32°E). The plant was authenticated at the herbarium section of Ekiti State University, Ado-Ekiti, Ekiti State. A voucher specimen was also deposited at the herbarium with the voucher number: UHAE/2017/051.

Processing of plant

Harvested fresh whole plant of *I. asarifolia* was properly washed in running tap water and distilled water and left to air-dry for 3 weeks. The dried plant parts of *I. asarifolia* were pulverized using an electric blender (Kenwood Blender: BL440). The pulverized plant parts were stored in plastic air-tight containers and kept in a cool, dry place until required.

Sample preparation and extraction

The cold maceration extraction method was adopted as earlier described (8) with slight modifications. The extraction of the active ingredients of the plant material was done by soaking 85 g of the pulverized plant in 500 mL of absolute methanol and agitated daily for 7 days. The mixtures were filtered into conical flasks. The extracts were concentrated using a rotary evaporator at 40 rpm (SENCO Technology, China Model No: W2-1005). Extraction yield of the plant is shown in Table 1. The concentrated extracts were stored in sterile McCartney bottles and refrigerated at 40°C until further use. The extracts were prepared to achieve the desired concentration by re-dissolving in 0.5% dimethyl sulphoxide (DMSO).

Phytochemical screening of Ipomoea asarifolia

Qualitative phytochemical screening of the leaf and whole plant extract of *I. asarifolia* was carried out using established procedures (21,23).

Microbe-free proof of the extracts

The extracts were sterilized after re-dissolving in 0.5%

Table 1. Extraction yield of Ipomoea asarifolia

Plant Part	Extraction solvent	Percentage yield (%)			
	Methanol	5.80			
Leaves	Ethanol	6.75			
	Aqueous	4.59			
Whole plant	Methanol	5.99			
	Ethanol	6.83			
	Aqueous	4.7			

DMSO by filtration through Millipore membrane filter of 0.45 μ m pore size. The extract was tested for sterility after Millipore filtration by introducing 2 mL of this supposed sterile extract into 10 mL of sterile Mueller-Hinton broth. Incubation was done at 37°C for 24 hours. Absence of turbidity or clearness of broth after the incubation period indicates sterile extract (9).

Source of clinical strains

Antibiotic resistant *P. aeruginosa* and *S. aureus* were obtained from the Drug Discovery & Infectious Diseases Research Group, Department of Microbiology, Federal University, Oye-Ekiti, Ekiti State, Nigeria. The test strains were confirmed by culturing on nutrient agar, giving a green colouration (pyocyanin production) for *P. aeruginosa* while *S. aureus* gave a golden colouration on Mannitol Salt Agar (MSA). Catalase, coagulase, oxidase, and other biochemical tests were carried out as confirmatory tests on the test strains.

Standardisation of microbial cell suspension

Each strain was introduced into sterile test tubes containing sterile Mueller-Hinton broth and incubated for 18 hours. The turbidity produced by these was adjusted using distilled water and used to match 0.5 McFarland turbidity (opacity) standard equivalent to 1×10^8 cfu/mL.

Determination of minimum inhibitory concentration (MIC) against test strains

The *in vitro* antibacterial activity/MICs of the extracts (whole plant and leaves) were determined in four concentrations; 2, 4, 6, and 8 mg/mL of each extract using the micro-dilution method. Mueller-Hinton broth of 100 μ L was dispensed into each well of a microtitre plate, and 50 μ L volume of the final concentrations (2, 4, 6, and 8 mg/mL) of each extract was added to each well and properly labelled. A 50 μ L of the broth culture of the clinical strains, adjusted to 0.5 McFarland turbidity standard, was dispensed into each well containing different extract concentrations. Incubation was done at 37°C for 16 hours and the minimum inhibitory concentration was taken as the lowest concentration showing no turbidity (15).

Experimental animals

Healthy Swiss albino male rats weighing 60-150 g were obtained from the Animal House of Federal University of Technology Akure, Ondo State. The animals were maintained under controlled conditions of temperature ($26 \pm 2^{\circ}$ C), relative humidity (55-60%), and a 12-hour light-dark cycle in an animal house. They were housed in sanitized standard polypropylene cages, with each cage containing 6 animals. These animals were allowed to acclimatise to the cage for 5 days and were fed with growers' mash feed and water *ad libitum*. The experimental protocol of the study was approved by the Faculty of Science Ethics

& Research Committee, Federal University Oye-Ekiti (with approval number: FUOYE/FS/2021/07/003) before the commencement of the experimental study and the "guidelines for ethical conduct in the care and use of nonhuman animals in research" was also employed (24).

In vivo assay on Swiss albino rats

A total of 72 Swiss albino rats were used and grouped into 12 groups of 6 animals, each according to their sex and weight range (6 groups each for P. aeruginosa and S. aureus). The animals were fasted overnight prior to the experiment. Swiss albino rats in every six groups were given a single oral administration of 0.3 mL of P. aeruginosa and S. aureus, respectively, and observed for behavioural/physiological/pathological any changes within 7 days before commencement of treatment. Blood samples were collected from the animals through the tail in EDTA bottles and cultured on Tryptone Soy Yeast Extract Agar to ensure the proliferation of the organisms in the animals. Packed cell volume (PCV) was determined by micro haematocrit technique using capillary tubes, and white blood cells (WBCs) were determined as previously described (25,26).

The animals in each group (A-F) received treatment as follows: group A (Pseudomonas curative/Staphylococcus curative) (APC/ASC) received 0.5 ml of 0.5% DMSO (negative control), group B (Pseudomonas curative/ Staphylococcus curative) (BPC/BSC) received 250 mg/kg of amoxicillin (positive control), group C (Pseudomonas curative/Staphylococcus curative) (CPC/CSC) received 2 mg/kg of whole plant extract, group D (Pseudomonas curative/Staphylococcus curative) (DPC/DSC) received 4 mg/kg of whole plant extract, group E (Pseudomonas curative/Staphylococcus curative) (EPC/ESC) received 2 mg/kg of leaf extract and group F (Pseudomonas curative/ Staphylococcus curative) (FPC/FSC) received 4 mg/kg of leaf extract for both P. aeruginosa (P) and S. aureus (S) categories. They received the treatment for 5 days. PCV and WBC count of the animals were determined before, during, and after treatment (25). Blood samples were collected in duplicate.

After the treatments, the animals were anaesthetized with chloroform and sacrificed. The abdomen was opened with a midline incision. The integrity of the internal organs such as stomach, liver, kidneys, and heart were observed, rinsed in normal saline to remove adhered blood, and preserved in 10% formalin for histopathological studies (27,28).

Statistical analysis

Results were expressed as mean \pm standard error of the mean (SEM). The significance of differences between the means of control and treated groups was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Results were regarded as significant with P < 0.05.

Ojo et al

Results

Phytochemicals in *I. asarifolia* leaf and whole plant extract Phytochemicals present in *I. asarifolia* leaf and whole plant extracts included alkaloids, saponins, phenolics, tannins, and anthraquinones, while flavonoids were only present in the whole plant extract.

MIC of *Ipomoea asarifolia* against *Pseudomonas* aeruginosa and *Staphylococcus* aureus

The antibacterial activities of the extracts against the tested organisms are presented in Table 2. All concentrations of the methanol extract, except 2 mg/mL, showed antibacterial activity on the two *P. aeruginosa* strains tested. Also, all concentrations of the methanol extract showed antibacterial activity on 8 tested strains of *S. aureus*. The MIC of *I. asarifolia* methanol extract against *P. aeruginosa* was 4 mg/mL, while in *S. aureus*, it is 2 mg/mL. Similarly, all concentrations of the ethanol extract, except 2 mg/mL, showed antibacterial activity on the two *P. aeruginosa* test strains, while all the concentrations of the ethanol extract showed antibacterial activity on the 8 tested strains of *S. aureus*, except *S. aureus* B strain.

In contrast, no antibacterial activity was shown by the aqueous extract on the two *P. aeruginosa* test strains at all concentrations for the leaves and whole plant extracts, while all concentrations of the aqueous extract showed no antibacterial activity on most of the tested strains of *S. aureus* at 2, 4, and 6 mg/mL concentrations of both aqueous leaves and aqueous whole plant extracts, except strain *S. aureus* A, which were susceptible at 6 mg/mL concentration of both extracts. Also, strains "*S. aureus* AC Orange 20b" and "*S. aureus* B" were not susceptible at all concentrations of both aqueous leaves and aqueous leaves and aqueous leaves and aqueous between the strain st

Haematological parameters

The mean packed cell volume (%PCV) and WBC counts of Swiss albino rats are shown in Figures 1 to 4. The PCV

level of all the Swiss albino rats after infection with the organisms was evaluated before initiation of treatment and was found to be significantly lower than their respective normal values before challenging them with organisms i.e. statistical decrease was observed in the PCV values obtained from 50.0-51.5 (day 0) to 37.5-45.0 (day 5) and a corresponding increase to 50.0-52.0 after treatment (day 10) except the negative control group whose PCV value decreased further from 43.5 to 40.0 (Figures 1 and 2).

The WBC (10⁹/cm³) showed a significant increase in value for all the Swiss albino rats after 5 days of organism challenge ranging from 3.40-5.00 (day 0) to 4.10-6.90 (day 5) and a corresponding decrease to 3.30-5.20 (day 10) after 5 days of treatment with the plant extracts except the negative control group whose WBC value increased from 4.40 ± 0.40 to 4.90 ± 1.00 and further to 5.15 ± 0.45 (WBC ×10⁹/cm³) (Figures 3 and 4).

Mortality rate in Swiss albino rats

The mortality rate in Swiss albino rats before and after administration of *I. asarifolia* extracts is presented in Table 3. The percentage mortality recorded in Swiss albino rats was found to be dose dependent for both *P. aeruginosa* and *S. aureus* categories. Groups administered with low doses of extract experienced a higher mortality rate than the groups administered with higher doses of extract. It was also observed that Swiss albino rats treated with the methanol leaves extract had a lower percentage mortality compared to those treated with the methanol whole plant extract of the same concentration. However, it was observed that *P. aeruginosa* was more susceptible to both extracts than *S. aureus* in vivo, since the mortality rate in Swiss albino rats, administered with *P. aeruginosa*, was less than those administered with *S. aureus*.

Histopathological pattern of the test plant

The kidneys examined showed congestion and, in some cases, haemorrhagic lesion; in fact, some glomeruli

Table 2	. Minimum inhibitor	y concentration	(mg/mL) of .	Ipomoea	asarifolia against	Pseudomonas aeru	<i>iginosa</i> and	Staphylococcus aureus
								•	

0	Metha	anol extract	Ethan	ol extract	Aqueous extract		
Strains	Leaves	Whole plant	Leaves Whole plant		Leaves	Whole plant	
P. aeruginosa							
Pseudo A	4	4	4	4	Ν	Ν	
Pseudo B	4	4	4 4		Ν	Ν	
S. aureus							
AC Orange 12b	2	2	2	2	8	8	
AC Orange 20b	2	2	2	2	Ν	Ν	
W273	2	2	2	2	8	8	
W392	2	2	2	2	8	8	
AC Orange 4b	2	2	2	2	8	8	
AC Orange 10c	2	2	2	2	8	8	
AC Orange 4ai	2	2	2	2	8	8	
S. aureus B	Ν	Ν	Ν	Ν	Ν	Ν	
S. aureus A	2	2	2	2	6	6	

Key: N - No MIC value (i.e., there was growth in all the concentrations tested).















Figure 4. Effect of *Ipomoea asarifolia* whole plant extract (WPE) and leaf extract (LE) on the white blood cell (WBC) count of Swiss albino rats infected with *Staphylococcus aureus*. Data are expressed as mean of replicates ± SEM. The ones with different alphabets are significantly different (*P* < 0.05).

showed congestion as well, but this may not be suggestive of any deleterious effect (Figure 5). It can be observed that the hearts were essentially not affected by the administration of crude extracts (Figure 6). The liver sample had disseminated infiltration of inflammatory cells and focal lymphoid aggregate with the hepatocytes showing vesicular nuclei tending towards necrosis and particularly showing hyperplasia (Figure 7).

Discussion

The traditional use of medicinal plants for curative measures by the local populace is an accepted practice since ancient times and continues until the present day. In many countries, especially developing nations, access to medicines is still limited, and therefore, many people resort to the use of medicinal plants, seeking relief or cure from pain caused by physiological or pathological means. Our study on the secondary metabolites in *I. asarifolia* revealed the presence of alkaloids, saponins, phenolics, tannins, and anthraquinones for the leaf and whole plant extract, while flavonoids were only present in the whole plant extract. This was partly supported by an earlier report on the hydroethanolic leaf extract of *I. asarifolia*, which revealed flavonoids, reducing sugars, glycosides, terpenoids, steroids, and phlobatannins (not present in our findings), aside saponins, phenols, and

Group	Mean Weight (g)	Administered dose (mg/kg)	х	Y	Z	% Mortality
P. aeruginosa A						
APC	110 ± 0.5	0.5 mL of 5% DMSO	6	2	4	66.7
BPC	85 ± 1.4	250 mg/kg of AMX	6	6	0	0
CPC	85 ± 1.0	2 mg/kg of WPE	6	3	3	50
DPC	85 ± 1.5	4 mg/kg of WPE	6	5	1	16.7
EPC	135 ± 2.0	2 mg/kg of LE	6	5	1	16.7
FPC	85 ± 0.5	4 mg/kg of LE	6	6	0	0
S. aureus W273						
ASC	95 ± 1.0	0.5 ml of 5% DMSO	6	0	0	100.0
BSC	75 ± 0.5	250 mg/kg of AMX	6	5	1	16.7
CSC	65 ± 1.5	2 mg/kg of WPE	6	1	5	83.3
DSC	75 ± 0.5	4 mg/kg of WPE	6	4	2	33.3
ESC	65 ± 0.5	2 mg/kg of LE	6	2	4	66.7
FSC	65± 1.0	4 mg/kg of LE	6	4	2	33.3

Table 3. Mortality rate in Swiss albino rats

APC, Group A *Pseudomonas* curative; BPC, Group B *Pseudomonas* curative; CPC, Group C *Pseudomonas* curative; DPC, Group D *Pseudomonas* curative; EPC, Group E *Pseudomonas* curative; FPC, Group F *Pseudomonas* curative; ASC, Group A *Staphylococcus* curative; BSC, Group B *Staphylococcus* curative; CSC, Group C *Staphylococcus* curative; DSC, Group D *Staphylococcus* curative; ESC, Group E *Staphylococcus* curative; FSC, Group F *Staphylococcus* curative; CMSO, dimethylsulfoxide; AMX, amoxicillin; WPE, whole plant extract; LE, leaves extract; X, number of animals at the start of the experiment; Y, number of animals at the end of the experiment; Z, number of deaths (X-Y).

%Mortality = number of dead animal(s) (Z)/total number of animal (X) x 100



Figure 5. (A) CK: Curative effect of *I. asarifolia* crude extract on Kidney. Plates show congestion of vessels (blue arrows). (CK- Curative kidney) (B) CK-Control: Effect of administered 0.5% DMSO on Kidney. Plates show congestion (blue arrows).

tannins (present in our findings) (29). This variation in the secondary metabolites of *I. asarifolia* may be due to the season of harvest or soil environment, which calls for a research focus.

At all concentrations tested, except 2 mg/mL, the methanol and ethanol extract exhibited antibacterial activities on P. aeruginosa with a MIC value of 4 mg/mL, while the MIC value of methanol and ethanol extract against S. aureus was 2 mg/mL. Only S. aureus was susceptible to the aqueous extract at the concentrations tested. This was in partial consonance with Aliyu et al (6), who reported zone sizes of 18.0 and 20.0 mm for S. aureus to be susceptible to the 100 mg/mL and 200 mg/mL aqueous, respectively, and methanol extract of I. asarifolia (14.0 mm) at 200 mg/mL, while P. aeruginosa was resistant to the aqueous and methanol extracts of the plant at a zone size of 6 mm for all the concentrations (12.5-200 mg/ mL). The results of the inhibition of bacterial growth have shown that the extracts are active at high concentrations and inactive at very low concentrations. Thus, the study suggests that the inhibition of bacterial growth activity of the extracts is dose dependent.

The activity of the extracts against both gram-negative and gram-positive bacteria tested may indicate a broad spectrum of activity. This observation is important because of the possibility of developing therapeutic substances that will be active against multidrug-resistant organisms. Aqueous extracts have shown little or no antimicrobial activity in contrast to organic solvent extracts (30,31). The success in traditional medicines may



Figure 6. (A) CH: Curative effect of *Ipomoea asarifolia* crude extract on Heart. Plates shows no significant lesion. (CH – Curative Heart). (B) CH-Control: Effect of administered DMSO on Heart. Plates show no significant lesion.



Figure 7. CL: Curative effect of *Ipomoea asarifolia* crude extract on liver. Plates show focal area of hemorrhagic lesion (dotted arrow) and the architecture of the hepatocytes appears distorted with hyperplasia (black arrows). (CL- Curative liver).

be due to administration of the extracts in large quantities and over a long period of time (32).

The haematological profile determined on the *in vivo* antibacterial activity of the methanol leaf and whole plant extract was observed in Swiss albino rats using a curative therapeutic approach. The steady increase in the haematological values among the two major groups demonstrates a progression in disease development, which was corroborated with previous studies (33,34). The mean PCV values ($40.5 \pm 1.5 - 45.0 \pm 2.0$ and $40.5 \pm 1.5 - 45.0 \pm 1.0$ for *P. aeruginosa* and *S. aureus* treatment groups, respectively) of the animals after organism challenge were found to be lower than the respective normal values ($50.0 \pm 1.0 - 51.5 \pm 2.5$ and $50.0 \pm 1.0 - 51.5 \pm 1.5$ for *P.*

aeruginosa and *S. aureus* groups, respectively) before the organism challenge and a gradual increase during treatment with PCV values between 40.0 ± 1.0 and 52.5 ± 1.5 for *P. aeruginosa* treatment group and 50.0 ± 1.0 – 52.5 ± 1.5 for *S. aureus* treatment group.

A similar study on anti-diarrhoeal effect of *Guiera* senegalensis root extracts on male mice showed similar trend, with our results with PCV values before treatment ranging between 50.10 ± 2.00 and 52.00 ± 1.87 and during treatment ranging from 48.00 ± 2.15 to 54.50 ± 0.55 (35). A similar result was also reported (26) using oral administration of methanolic extract of wild *Ganoderma* sp. having PCV values before treatment as $46.6 \pm 43 - 50.8 \pm 1.6$ and $44.4 \pm 4.0 - 53.3 \pm 3.1$ after treatment. Odeghe et al (36) reported the PCV of the control or untreated group on the investigation of *Anthocleista grandiflora* methanol extract as 44.67 ± 4.37 , which was still within the range of our study.

From our study, it could be revealed that there was a relationship between PCV value and dosages of the extracts administered but not with the plant parts used, which shows no significant difference. This is also reflected in the positive and negative control. It was also observed from the PCV results that during the period of treatment with plant extracts, there was a gradual increase of the blood level to the normal indicating that the plant under study could have a blood-boosting agent.

The WBC counts showed a significant increase in value after 5 days of infection with the organisms compared to the animal's normal WBC value. Prior to being challenged with *P. aeruginosa*, the normal values ranged between 3.40 ± 0.35 and 5.00 ± 0.35 , while that of *S. aureus* group had normal values of 3.90 ± 0.20 and 5.00 ± 0.50 . The results of our study were not in tandem with the report of a previous study (26) reporting WBC values of $8.4 \pm 1.2 - 9.0 \pm 1.2 \times 10^3$ /mm³ and another study (36) reporting a WBC value of $7.23 \pm 0.92 \times 10^9$ /L. In another related research, animals treated with different doses of the aqueous extract before peritonitis induction presented significant decreased leukocyte migration (70.4%, 78.48%, and 83.54%) into the peritoneal cavity at the doses of 10, 20 and 30 mg/kg, respectively (11).

It was clearly observed that there was a gradual rise in the value of WBC from normal values after the organism challenge test for *P. aeruginosa* test group $(4.10\pm0.15$ – 5.70 ± 1.10) and a gradual decrease after plant extract administration $(3.30\pm0.40 - 4.50\pm0.30)$. This trend was also observed in the *S. aureus* test group with a gradual increase after organism challenge $(4.60\pm0.50-6.90\pm1.60)$ and a gradual decrease after plant extract administration $(3.70\pm0.30 - 4.50\pm0.90)$. This was not in consonance with a previous report (26) reporting gradual rise with WBC values ranging from 9.6 ± 1.3 to 14.8 ± 0.2 , without any organism challenge on the Swiss albino rats. However, Odeghe et al (36), reported a gradual rise ranging between 4.02 ± 4.95 and 9.13 ± 0.45 with *Plasmodium berghei* as organism challenge. These contrasting results could, however, depend on the severity or virulent nature of the organism being used, especially in this study. In another study, the treatment with aqueous extract of *I. asarifolia* was reported to significantly reduce the inflammatory infiltrate of leucocytes into the air pouch at all tested doses (20, 30, and 40 mg/kg) at 58%, 67%, and 53%, respectively, compared with the group that received only zymosan and sterile saline (11), which was in favour with the results from our study. Also, it was observed that the extracts effect were dose-dependent in their in vivo curative activity against the tested organisms.

Lower mortality rate was recorded among animals in groups treated with higher doses of the extract than those treated with lower doses in comparison with the positive control group treated with 250 mg/kg of amoxicillin, while the negative control treated with 0.5 mL of 0.5% DMSO had a high mortality rate. In addition, the leaf and whole plant extracts of I. asarifolia were found to have more curative ability in the animal group infected with P. aeruginosa than S. aureus-infected group judging from their mortality rate. The leaf extract was found to have more curative potential as compared with the whole plant extract, which is in relation to their mortality rate. In another study on the subchronic toxicity of I. asarifolia in rats by Akindele et al (12), it was reported that the hydroethanolic extract administered through intraperitoneal was relatively safe up to a dose of 1000 mg/ kg. In another similar study, the authors reported that *I*. asarifolia extract administered orally did not produce any mortality at the dose of 5000 mg/kg. However, there was mortality at 4000 mg/kg intraperitoneally (i.p.), while the i.p. LD_{50} value was obtained to be 1000 mg/kg (29).

The histological indices were in consonance with the above reports. However, the liver was worst affected with a variety of lesions; but not severe. The extracts administration for curative activities had no effect on the heart. The kidney also showed haemorrhagic lesions but was not suggestive of anything serious. Aiwonegbe et al (13) noted that there was no observable haemorrhage on the stomach lining at a dose of 400 mg/kg *I. asarifolia* extract on ethanol-induced gastric lesion and was not dose dependent, thus having significant antiulcerogenic activity, which was similar to the report of this study.

Findings from an anti-inflammatory and histological study on the aqueous extracts of *I. asarifolia* on DNBS-induced colitis in rats revealed that there was an improvement in the preservation of intestinal tissue at the doses of 50 and 100 mg/kg, and the mucosa architecture appeared to be preserved with the presence of goblet cells with their preserved mucin content. It equally showed a reduction in inflammation and infiltration of leucocytes. This shows similar activity of the plant under study from our report (37).

Conclusion

Ipomoea asarifolia was proved to have a dose-dependent antibacterial and curative activity, and could enhance innate immunity. The evaluation of the curative effectiveness of crude extract of *I. asarifolia* on *P. aeruginosa* and *S. aureus* in Swiss albino rats revealed imminent information on the medicinal importance of the extract over infections caused by the test organisms under study at low concentrations. The traditional uses of *I. asarifolia* was confirmed as a potential therapeutic agent against commonly isolated hospital acquired infections.

Acknowledgement

The authors wish to acknowledge the head of department, Animal and Environmental Biology for the release of their animal house for this research.

Authors' contribution

SKSO conceived, designed and performed the experiments; GTS, CFA, AOA, and MIO performed the experiments; OJA analyzed the data; SKSO and GTS wrote the manuscript, while OJA proofread and edited the paper. All authors read and confirmed publication of the paper.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

Ethical issues including plagiarism, misconduct, data fabrication, falsification, double publication or submission have been carefully checked by authors. All experimental procedures conform to the guiding principles for research as recommended by "Guidelines for Ethical Conduct in the Care and Use of Nonhuman Animals in Research" (American Psychological Association, 2012). The experimental protocol of the study was approved by the Faculty of Science Ethics & Research Committee, Federal University Oye-Ekiti (with approval number: FUOYE/ FS/2021/07/003).

Funding/Support

None.

References

- Lawal U, Ibrahim H, Agunu A, Abdulahi Y. Antiinflammatory and analgesic activity of water extract from *Ipomoea asarifolia* Desr (Convolvulaceae). Afr J Biotechnol. 2010;9(51):8877-80. doi: 10.5897/ajb10.649.
- Park EJ, Pezzuto JM. Botanicals in cancer chemoprevention. Cancer Metastasis Rev. 2002;21(3-4):231-55. doi: 10.1023/a:1021254725842.
- Aruoma OI. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutat Res. 2003;523-524:9-20. doi: 10.1016/s0027-5107(02)00317-2.

- Zhu YZ, Huang SH, Tan BK, Sun J, Whiteman M, Zhu YC. Antioxidants in Chinese herbal medicines: a biochemical perspective. Nat Prod Rep. 2004;21(4):478-89. doi: 10.1039/ b304821g.
- 5. Atawodi SE. Antioxidant potential of African medicinal plants. Afr J Biotechnol. 2005;4(2):128-33.
- Aliyu MS, Lawal U, Tijjani MB, Doko MH, Garba I, Kokya HA, et al. Phytochemical and antibacterial properties of leaf extracts of *Ipomoea asarifolia*. Nig J Basic Appl Sci. 2011;19(2):236-40.
- Meira M, da Silva EP, David JM, David JP. Review of the genus *Ipomoea*: traditional uses, chemistry and biological activities. Rev Bras Farmacogn. 2012;22(3):682-713. doi: 10.1590/s0102-695x2012005000025.
- Jegede IA, Nwinyi FC, Ibrahim J, Ugbabe G, Dzarma S, Kunle OF. Investigation of phytochemical, antiinflammatory and anti nociceptive properties of *Ipomoea asarifolia* leaves. J Med Plants Res. 2009;3(3):160-5. doi: 10.5897/jmpr.9000827.
- Sule IO, Agbabiaka TO. Antibacterial effect of some plant extracts on selected *Enterobacteriaceae*. Ethnobot leafl. 2008;2008(1):137.
- Lopes JR, Riet-Correa F, Cook D, Pfister JA, Medeiros RM. Elimination of the tremorgenic toxin of *Ipomoea asarifolia* by milk. Pesqui Vet Bras. 2014;34(11):1085-8. doi: 10.1590/ s0100-736x2014001100009.
- Furtado AA, Torres-Rêgo M, Lima M, Bitencourt MAO, Estrela AB, Souza da Silva N, et al. Aqueous extract from *Ipomoea asarifolia* (Convolvulaceae) leaves and its phenolic compounds have anti-inflammatory activity in murine models of edema, peritonitis and air-pouch inflammation. J Ethnopharmacol. 2016;192:225-35. doi: 10.1016/j. jep.2016.07.048.
- Akindele AJ, Unachukwu EG, Osiagwu DD. 90 Days toxicological assessment of hydroethanolic leaf extract of *Ipomoea asarifolia* (Desr.) Roem. and Schult. (Convolvulaceae) in rats. J Ethnopharmacol. 2015;174:582-94. doi: 10.1016/j.jep.2015.03.044.
- Aiwonegbe AE, Omenai IF, Ogbeide KO, Benjamin GO, Eikpe IR. Antiulcerogenic activity of methanol extract from *Ipomoea asarifolia* leaves in Wistar rats. Bayero J Pure Appl Sci. 2020;12(2):89-95. doi: 10.4314/bajopas.v12i2.12.
- Gellatly SL, Hancock RE. *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. Pathog Dis. 2013;67(3):159-73. doi: 10.1111/2049-632x.12033.
- 15. Ojo SKS, Nwachuckwu PO, Igumbor EO, Okon CM. Preliminary Study on the Antimicrobial Potency of *Acalypha godseffiana* Leaf (Ornamental Plant) within Ukwuani L.G.A of Delta State on Selected Clinical Isolates. Niger J Appl Sci. 2013;31:109-15.
- Shojaei Moghadam M, Maleki S, Darabpour E, Motamedi H, Seyyed Nejad SM. Antibacterial activity of eight Iranian plant extracts against methicillin and cefixime restistant *Staphylococcous aureus* strains. Asian Pac J Trop Med. 2010;3(4):262-5. doi: 10.1016/s1995-7645(10)60063-6.
- Abd El-Kalek HH, Mohamed EA. Synergistic effect of certain medicinal plants and amoxicillin against some clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA). Int J Pharm Appl. 2012;3(3):387-98.
- 18. Le Loir Y, Baron F, Gautier M. *Staphylococcus aureus* and food poisoning. Genet Mol Res. 2003;2(1):63-76.

Ojo et al

- Rossolini GM, Mantengoli E. Treatment and control of severe infections caused by multiresistant *Pseudomonas aeruginosa*. Clin Microbiol Infect. 2005;11 Suppl 4:17-32. doi: 10.1111/j.1469-0691.2005.01161.x.
- Strateva T, Yordanov D. Pseudomonas aeruginosa a phenomenon of bacterial resistance. J Med Microbiol. 2009;58(Pt 9):1133-48. doi: 10.1099/jmm.0.009142-0.
- Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol. 2005;4(7):685-8. doi: 10.5897/ajb2005.000-3127.
- 22. Sofowara A. Medicinal Plants and Traditional Medicine in Africa. Ibadan, Nigeria: Spectrum Books; 1993. p. 289.
- Trease GE, Evans WC. Drugs of biological origin. In: Pharmacognosy. 12th ed. United Kingdom: Balliere Tindall; 1989. p. 309-540.
- 24. American Psychological Association (APA). Guidelines for Ethical Conduct in the Care and Use of Nonhuman Animals in Research. 2012. Retrieved January 19, 2021 from http:// www.apa.org/ethics/code/index.aspx.
- 25. Enye JC, Chineke HN, Onubeze DP, Nweke I. Evaluation of the healing effects of aqueous extracts of *Musa paradisiaca* (unripe plantain) and *Brassica oleracea* (cabbage) on peptic ulcer. IOSR J Dental Med Sci. 2013;8(6):40-6.
- 26. Shamaki BU, Ogbe AO, Abdulrahman FI, Sandabe UK. Haematological parameters in normal albino rats following oral administration of methanolic extract of wild *Ganoderma* sp. International Journal of Pharmacological Screening Methods. 2014;4(2):94-9.
- 27. Nanjappaiah HM, Hugar S. Prophylactic and curative effects of *Moringa oleifera* Lam. pods in CCl4 damaged rat liver. Indian J Nat Prod Resour. 2012;3(4):541-6.
- Bharadwaj S, Nayak S. Experimental evaluation of prophylactic and curative effect of a herbal drug *Hemidesmus indicus* R. Br. in drug induced ulcers in Wistar albino rats. Int J Res Med Sci. 2013;1(3):243-7. doi: 10.5455/2320-6012. ijrms20130816.

- Akindele AJ, Palmer EL. Effects of hydroethanolic leaf extract of *Ipomoea asarifolia* (Convolvulaceae) in doxorubicin and isoproterenol-induced toxicity in rats. Trop J Nat Prod Res. 2018;2(2):59-66. doi: 10.26538/tjnpr/ v2i2.2.
- Parekh J, Chanda SV. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol. 2007;31(1):53-8.
- Kitonde CK, Fidahusein DS, Lukhoba CW, Jumba MM. Antimicrobial activity and phytochemical screening of *Senna didymobotry* used to treat bacterial and fungal infections in Kenya. Int J Educ Res. 2014;2(1):1-12.
- 32. Yineger H, Kelbessa E, Bekele T, Lulekal E. Plants used in traditional management of human ailments at Bale Mountains National Park, Southeastern Ethiopia. J Med Plants Res. 2013;2(6):132-53. doi: 10.5897/jmpr.9001023.
- Weatherall DJ, Miller LH, Baruch DI, Marsh K, Doumbo OK, Casals-Pascual C, et al. Malaria and the red cell. Hematology Am Soc Hematol Educ Program. 2002:35-57. doi: 10.1182/asheducation-2002.1.35.
- Chang KH, Stevenson MM. Malarial anaemia: mechanisms and implications of insufficient erythropoiesis during blood-stage malaria. Int J Parasitol. 2004;34(13-14):1501-16. doi: 10.1016/j.ijpara.2004.10.008.
- Williams ET, Barminas JT, Akinniyi J, William A. Antidiarrhoeal effects of the root extracts of *Guiera* senegalensis in male mice. Afr J Pure Appl Chem. 2009;3(8):152-7. doi: 10.5897/ajpac.9000016.
- Odeghe OB, Uwakwe AA, Monago CC. Some biochemical and haematological studies on the methanolic extract of *Anthocleista grandiflora* stem bark. Int J Appl Sci Technol. 2012;2(5):58-65.
- 37. da Silva VC, de Araújo AA, de Souza Araújo DF, Souza Lima MCJ, Vasconcelos RC, de Araújo Júnior RF, et al. Intestinal anti-inflammatory activity of the aqueous extract from *Ipomoea asarifolia* in DNBS-induced colitis in rats. Int J Mol Sci. 2018;19(12):4016. doi: 10.3390/ijms19124016.