



Assessment of Phytochemical, Antimicrobial and Haematological Activity of Synergistic Ethanolic Extracts of *Azadirachta indica* and *Psidium guajava* against Selected Enteric Pathogens

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ABSTRACT: *Azadirachta indica* (neem) and *Psidium guajava* (guava) are well known therapeutic plants used to manage various diseases. Hence, this paper is an assessment of phytochemical, antimicrobial and haematological activity of synergistic ethanolic extracts of *Azadirachta indica* and *Psidium guajava* against selected enteric pathogens using standard procedures. Results of the phytochemical analysis showed the presence of tannins, saponins, flavonoid and phenols in the plants. The ethanolic extract of the leaves were active against the organisms, with *Salmonella* having the highest zone of inhibition and *Staphylococcus aureus* having the least zones of inhibition at all concentrations tested. There was no significant weight change ($p > 0.05$), toxicity nor mortality recorded for the period. The results of the haematological parameters for the experimental rats showed increases in red blood cells and haemoglobin; and decrease in white blood cells, lymphocytes, granulocytes and platelets, but not statistically significant ($p > 0.05$). The study established that the synergistic effect of ethanol extract of the plant leaves could be a possible source of novel broad spectrum medicine for treating diseases.

DOI: <https://dx.doi.org/10.4314/jasem.v28i7.7>

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Cite this Article as: ISICHEI-UKAH, O. B; UWAH, E. S (2024). Assessment of Phytochemical, Antimicrobial and Haematological Activity of Synergistic Ethanolic Extracts of *Azadirachta indica* and *Psidium guajava* against Selected Enteric Pathogens. *J. Appl. Sci. Environ. Manage.* 28 (7) 1983-1988

Dates: Received: 21 May 2024; Revised: 17 June 2024; Accepted: 23 June 2024 Published: 02 July 2024

Keywords: *Azadirachta indica*; *Psidium guajava*; ethanol extract; antimicrobial; toxicological effect.

The increasing rate of antibiotic resistance of bacterial pathogens has led to the need for alternative therapeutics. These circumstances have inspired scientists to explore new antimicrobial elements from many resources for instance medicinal plants (Cordell, 2000). Numerous studies have described different type of plants such as herbs, shrubs and trees with the aim of knowing their phytochemical constituents and using them for the management of diseases as possible substitutes to the synthetic drugs (Bouamama *et al.*, 2006). The selection of plants for therapeutic purposes requires a struggle to discover novel, reliable, and more active drugs with the ability to combat pathogens (Abubakar, 2010). Herbal plants are generally

believed to be harmless and reliable in contrast with expensive synthetic drugs which have undesirable side effects along with beneficial effects (Alviano and Alviano, 2009). These plants have been in use in traditional medicine worldwide for a long time but are still understudied (Karou *et al.*, 2005). In past few decades, the curiosity to assess plants having antimicrobial, antifungal, anti-inflammatory action for several diseases have improved, and a large number of bioactive compounds have been characterized (Kupeli *et al.*, 2007). Several studies have established that many plants contain bioactive substances like flavonoids, essential oils, peptides, alkaloids, tannins, and phenols amongst others, which have antimicrobial

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properties (Okigbo and Omodamiro, 2006). *Azadirachta indica* (neem) is an evergreen popular tree commonly found in India, Africa and America. Neem tree contains a large number of organically active compounds that chemically are diverse as well as complex structurally. Numerous bioactive compounds which include ketones, phenolic compounds, carotenoids, alkaloids, flavonoids, triterpenoids, steroids, azadirachtin and nimbin have been obtained from numerous parts of neem plant (Subapriya and Nagini, 2005). Almost every part of the tree is used as traditional drug for treating different diseases. The tree and its extracts have also been described to possess insecticidal, antifungal, antiviral, and antibacterial properties (Hoque *et al.*, 2007). Extracts of neem seed have been observed to inhibit bacterial pathogens causing eyes and ear infections (El-Mahmood *et al.*, 2010). *Psidium guajava* belongs to the family Myrtaceae. It is a tropical plant found mainly in Africa, India, Indonesia, Pakistan, Bangladesh and South America. It is popularly called guava. The guava is a phytotherapeutic plant, which contains components that are active and also effective in treating diseases such as dysentery, ulcer, wounds, sore throat, toothache, inflamed gums, malaria, diarrhea, vomiting, gastroenteritis and many other conditions (Abdelrahim *et al.*, 2002). The components present in guava include lectins, phenols, tannins, flavanoids, essential oils, fatty acids, vitamins, etc. The leaves have been shown to exhibit antibacterial properties by inhibiting the growth of some enteric pathogens (Biswas *et al.*, 2013; Ratnakaran *et al.*, 2020). Studies conducted in the past had been reported that these plants individually have been used to treat infections and other ailments. Some researchers have combined each of the plants with commercial antibiotics, and have been shown to have antibacterial effects against the tested organisms (Bhinge *et al.*, 2022; Mitra *et al.*, 2024). Therefore, this paper assesses the phytochemical, antimicrobial and haematological activity of the synergistic ethanolic extracts of *Azadirachta indica* and *Psidium guajava* against some enteric pathogens.

MATERIALS AND METHODS

Sample Collection and Extract Preparation: Fresh leaves of *Azadirachta indica* and *Psidium guajava* were obtained from the University of Benin, Benin City, Nigeria. They were destalked, air dried and grounded (pulverized) into powdered form using a dry electric blender (SAISHO, Model-S-748). The powdered leaves were kept in airtight containers and stored at room temperature (28 ± 2 °C) until when they were needed for use. The powdered form of the leaf samples (*Azadirachta indica* and *Psidium guajava*) were weighed separately. Then, 50g of each plant leaf

powder were mixed together and dissolved in 500ml of ethanol to make 1:5 dilution ratio. The leaves were extracted by the Soxhlet extraction method using ethanol (Hatbamu *et al.*, 2010).

Qualitative analysis of the phytochemicals constituents: Phytochemical tests to detect the presence of tannins, saponins, flavonoids, phenols and terpenoids were done using the standard protocol, as earlier described by Biswas *et al.* (2013).

Test Organisms: Pure cultures of the test organisms used for this study were obtained from the Department of Medical Microbiology, University of Benin Teaching Hospital, Benin City. The organisms used were *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* and *Candida albicans*. All the test organisms were cultured and maintained on agar slants.

Antimicrobial Assay: The ethanol extracts of the leaves of *A. indica* and *P. guajava* were screened for antimicrobial activity by agar well diffusion method. The medium used was Mueller Hinton agar (MHA). The agar surface was cut with a sterile cork borer having a diameter of 6.0 mm size. The bacterial and fungal strains were grown in nutrient broth and Sabouraud dextrose broth respectively for 4-6 hours.

The turbidity of the broth culture was adjusted to 0.5 McFarland units. An aliquot (0.02 ml) of microbial culture was added to molten MHA at 45°C and poured onto the petri plates. After solidification of the agar on the plates, wells were made on agar surface using sterile cork borer (3 wells per 90 mm diameter plate). Bacterial cultures were incubated at 37 °C for 24 h and fungal cultures at 25 °C for 48 h. Antimicrobial activity was determined by measuring the zones of inhibition (in mm) around the well. Ciprofloxacin (5µg/disc) and Amphotericin B (100µg/disc) were used as positive controls for bacteria and fungi respectively. This process was repeated for the different extract concentrations which were 100mg/ml, 200mg/ml and 300mg/ml. Each concentration was done in replicates and the results were average of three independent experiments.

Experimental Animals and Treatment: Sixteen (16) male Wistar rats (*Rattus norvegicus*) weighing 200-250 g were obtained from the Anatomy Department of the University of Benin, Benin City. The rats were individually housed in cages and maintained under controlled environmental conditions such as temperature (20 ± 2 °C), relative humidity (45-55%) and 12 h dark/light cycle. All the rats were fed with rodent meal and water *ad libitum* under strict hygienic

conditions. After one week of acclimatization according to set criteria the rats were randomly divided into four different groups (Groups A-D), with each having four rats. Group A rats were used as the control group and given distilled water only. Group B were given the extract at a dosage of 50mg/kg, while group C and D were given the extract at dosages of 100 and 200 mg/kg of body weight respectively. The administration was done three (3) times a week for twenty-eight (28) days. During this period the animals were weighed to determine if the extract has any effect on their weight. All animals were observed daily for signs of toxicity and mortality (Daradka, 2016).

Hematological Studies: After the administration of the extracts on the twenty-eighth (28th) day, the blood samples of the animals were obtained for haematological analysis. The blood samples were collected from the heart (cardiac puncture) using sterile needles and syringes and placed in anticoagulant bottles containing ethylene diamine tetraacetic acid (EDTA). The bottles containing the blood samples were transported to the Department of Haematology, University of Benin Teaching Hospital, Benin City for analysis. The parameters analyzed were white blood cells, lymphocytes, monocytes, granulocytes, red blood cells, haemoglobin, hematocrits and platelets. This haematological analysis was carried out using an automated haematology analyzer (AHA) (Tefferi *et al.*, 2005).

Statistical Analysis of Data: Data obtained were expressed as Mean \pm SEM (standard error of mean) and analyzed using the Statistical Package of Social Sciences (SPSS) program version 17. For all parameters, comparison among groups was carried out using one way analysis of variance (ANOVA). All P-values less than 0.05 ($p < 0.05$) were considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical Screening of Plant Extracts: The phytochemical screening of chemical constituents of combination of *A. indica* and *P. guajava* ethanolic leaves extract showed the presence of bioactive compounds in the extracts (Table 1). It indicated the presence of tannins, saponins, flavonoids and phenols, but absence of terpenoids. The phytochemical analysis of the ethanol extract shown in this study indicated the presence of the phytochemicals of high therapeutic value. The phytochemical constituents present in this study correlated with the study of Ali *et al.* (2022), who observed the presence of tannins, phenols, saponins, flavonoids, alkaloids and terpenoids, in the leaves of *A. indica*. In a cohort study by Oncho *et al.* (2021), the researchers discovered the presence of

tannins, saponins, alkaloids and steroids in *P. guajava* leaves.

Table 1: Phytochemical constituents of ethanolic extract of *A. indica* and *P. guajava*.

Phytochemical constituents	Ethanolic extract
Tannin	+
Saponin	+
Flavonoid	+
Phenol	+
Terpenoid	-

KEY: + = Presence of constituents; - = Absence of constituents

Tannins have been shown to have antibacterial activity, they are polyphenolic compounds that bind to proline rich protein and interfere with protein synthesis. Saponins are glycosides have been found to have inhibitory effects on *Staphylococcus aureus*. Phenols and flavonoids are hydroxylated polyphenolic compounds produced by plants in response to microbial infections. Their antibacterial activity has been attributed to their ability to form complexes with extracellular and soluble proteins and bacterial cell walls (Cowan, 1999). It has therefore been observed from the phytochemical analysis that the ethanolic extract of the mixture of *A. indica* and *P. guajava* possess chemical compounds which have been seen to possess antibacterial actions. Phytochemicals have been attributed as the reason why herbal plants have their protective effects (Chauchan, 2014).

Antimicrobial Activity of Plant Extracts: The ethanolic extract of the combination of *A. indica* and *P. guajava* was tested for their antimicrobial activity at different concentrations ranging from 100mg/ml to 300mg/ml and the results are shown in Figure 1.

The antimicrobial action of ethanolic extract indicated that at all concentrations, it was effective on the test microbes with varying inhibition zones except *Candida albicans* which showed no activity against any of the concentrations of the extract. At the least concentration of 100mg/ml, the highest inhibition zone was recorded against *Salmonella* with diameter of 6.00 ± 1.00 mm and the least in *Staphylococcus aureus* with a diameter of 4.00 ± 0.00 mm.

At 200mg/ml, the highest inhibition zone was recorded against *Salmonella* having a diameter of 10.00 ± 1.73 mm while *Staphylococcus aureus* recorded inhibition zones of 7.33 ± 0.58 mm. At concentration of 300mg/ml, the highest clearance zone was obtained from the extract against *Salmonella* with a diameter of 12.33 ± 2.31 mm while the least zone of inhibition at this concentration was obtained from the extract against *Staphylococcus aureus* with a diameter of 10.00 ± 0.00 mm.

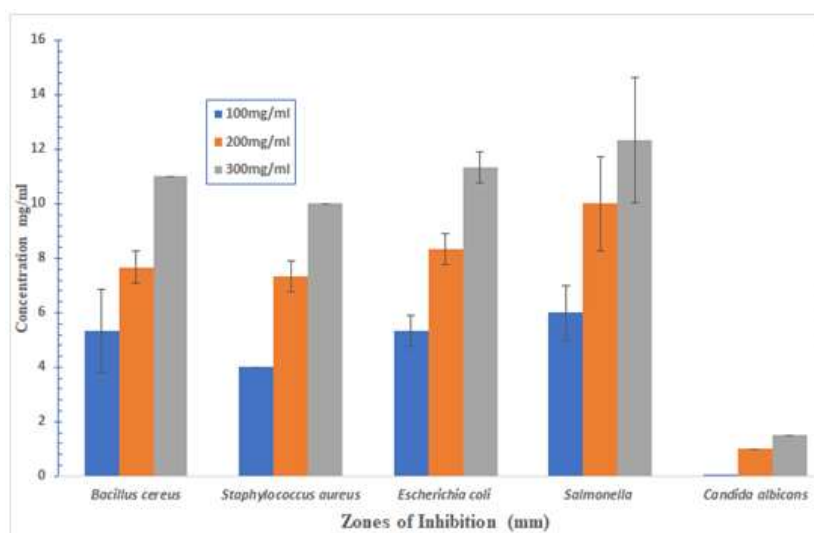


Table 2: Antimicrobial activity of ethanolic extract of *A. indica* and *P. guajava* leaves ($P < 0.05$)

All the tested Gram-positive and Gram-negative bacteria exhibited varying zones of inhibition against the extracts, indicating the antimicrobial effects of the ethanol extracts of the combination of the two plants. It was observed that increase in the concentration of the extracts resulted in significant increase in the zones of inhibition ($p < 0.05$). The fungi, *Candida albicans* did not show any inhibition. Results from this study correlated and conflicted with some reported researches from literature. Raut *et al.* (2014) conducted a study in Loni, India, on the leaves and bark extracts of *A. indica*, they discovered that the extracts were inhibitory against *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae* and *Bacillus subtilis*. In the study by Khan *et al.* (2021), the ethanol extracts of *A. indica* inhibited the test organisms (*Staphylococcus aureus*, *Escherichia coli*, *Enterococcus* and *Pseudomonas aeruginosa*). In a study by Biswas *et al.* (2013), the antimicrobial potential of guava (*Psidium guajava*) leaf extracts against two gram-negative bacteria (*Escherichia coli* and *Salmonella enteritidis*) and two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) was evaluated. Only two of the crude solvent extracts made from *Psidium guajava* leaves, methanol and

ethanol, had inhibitory efficacy against bacteria, according to the study's findings. The two extracts only demonstrated inhibition against the two gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus*, while the gram-negative bacteria did not show any inhibition at all. In another study by Mitra *et al.* (2024), the extracts of *P. guajava* combined with antibiotics inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus*, *Citrobacter*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter* and *Candida albicans*. But in this study, the ethanol extract of the combined plants did not inhibit the growth of *Candida albicans*. These results contradicted the outcomes of the present study.

Toxicity and Haematological Tests: The toxicity test of the ethanol extracts of combination of *A. indica* and *P. guajava* on the Wistar rats are shown in Table 2. The changes in the body weight of the treated groups (Group 2 to 4) were similar to that of the control group. There was no significant difference ($p > 0.05$) in the changes in body weight. The rats did not show any physical sign of toxicity and no death was recorded during the period of study.

Table 2: Toxicity test of ethanol extracts of *A. indica* and *P. guajava* on the Wistar rats

Treatment Groups	Dosage (mg/kg)	Body Weight	Body Weight (g)	Mortality
Group 1 (Control)	Distilled Water		225.50+15.20 ^a	-
Group 2	50mg/kg extract		228.10+21.56 ^a	-
Group 3	100mg/kg extract		224.53+18.21 ^a	-
Group 4	150mg/kg extract		233.81+15.62 ^a	-

$p > 0.05$ – Not statistically significant; - Indicates no death recorded

The results of the haematological analysis showed that there was increase in the values of the monocytes, red blood cells, haemoglobin and hematocrit readings. Then, there was decrease in the white blood cells,

lymphocytes, granulocytes and platelets. The increase and decrease in the parameters were not statistically significant ($p > 0.05$) compared to the control (Table 3).

Toxicity studies in animal model are commonly used to evaluate potential health risk in humans caused by intrinsic opposing effects of chemical composites/plant extracts (Daradka, 2016). There was no significant changes in the body weight of the animals ($p>0.05$) and no recorded death during the period of experiment. Assessment of hematological

parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal. In this study, the changes observed in the haematological parameters were not statistically significant ($p>0.05$).

Table 3: Haematological parameters of ethanolic extract of *A. indica* and *P. guajava* leaves on Wistar rats.

PARAMETERS	EXTRACT (mg/kg body weight)				P-value
	CONTROL	50	100	200	
White Blood Cell	12.38±0.69	10.08±1.87	9.68±0.27	5.20±1.25	$p>0.05$
Lymphocyte	7.24±0.43	4.99±1.07	4.70±0.36	4.33±0.98	$p>0.05$
Monocyte	2.35±0.25	2.05±0.38	2.60±0.07	2.90±0.12	$p>0.05$
Granulocyte	2.59±0.12	5.99±0.68	4.70±0.14	3.26±0.39	$p>0.05$
Red Blood Cell	6.22±0.13	6.63±0.40	6.87±0.97	6.98±1.02	$p>0.05$
Haemoglobin	12.17±0.29	12.23±0.99	13.00±1.81	13.66±1.22	$p>0.05$
Hematocrit	33.61±0.40	36.65±2.82	37.56±1.48	38.50±4.35	$p>0.05$
Platelets	268.28±7.42	326.99±47.71	295.46±46.09	282.90±34.97	$p>0.05$

Mean ± S.E.M. values; $p>0.05$ – Not statistically significant

Conclusion: The ethanolic extracts of *A. indica* and *P. guajava* leaves exhibited clear zones of inhibition against the tested bacteria, which indicates that the combination of both herbal plants have great potentials as antimicrobial agents, hence as an antibiotic to treat bacterial infections. The synergistic influence from the association of various plant extracts on pathogenic microbes will lead to new adoptions of plants with medicinal properties for the management of infectious diseases.

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