JOURNAL OF SCIENTIFIC INNOVATIONS AND CREATIVE RESEARCH
eISSN: xxxxxxxxxxxxxxxx
DOI: xxxxxxxxxxxxxxxxxx
Journal Homepage: https://jsicr.org

(RESEARCH ARTICLE)

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Quantitative Phytochemical and In vitro Antisalmonella activity of fractions of *Harungana madagascariensis* fruit Lam. Ex Poiret (Hypericaceae).

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Journal of Scientific Innovations and Creative Research, 2025, 1(1), 015–018

Publication history: Received on 5th June 2025; revised on 25th June 2025; accepted on 18th July 2025

Article DOI: xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx

Abstract

Typhoid fever caused by Salmonella species is an infectious disease of serious public health concern due to drug resistant microbial strain. This has rendered treatment with antibiotics ineffective as well as costly. This study investigated the antisalmonella activity and phytoconstituents of fractions of *Harungana madagascariensis* fruit Lam. Ex Poiret (Hypericaceae) as a source of lead agents for the discovery and development of new and affordable drugs. The fruit of *H. madagascariensis* was extracted with 70% aqueous ethanol by cold maceration. The crude 70% aqueous ethanol extract was partitioned with n-hexane to yield n-hexane soluble portions (NHF) and Aqueous portion (AQP). The quantitative phytochemical screening was done by standard methods while the antimicrobial activity (MIC) of the crude extract and fractions (NHF and AQP) against ten (10) clinical isolates of Salmonella strains was done using agar dilution method with ciprofloxacin and oxytetracycline used as reference standard drugs for comparison. The quantitative analysis of the sample revealed high content of phenolics (22.24±0.07). Other constituents include flavonoids (1.89±0.03), tannins (0.42±0.05), and carbohydrates (1.37±0.06). The trend in antibacterial activity against the ten clinical isolate of salmonella was exhibited by NHF (MIC = <0.31 – 5.00 mg/ml) > crude extract (1.57 – 6.25 mg/ml) >AQP (>10.00 mg/ml).This result may provide a rational support for the traditional use of *H. madagascariensis* in the treatment of typhoid fever.

Keywords: *Harugana madagaascariensis*, salmonella, bioactive products, typhoid fever**.**

1. Introduction

Enteric fever also called typhoid fever or typhoid is a serious bacterial bloodstream infection caused by bacterial pathogen Salmonella spps. Salmonella is one of the genera of the Enterobacteriaceae family. Among the Salmonellae species, *Salmonella typhi*, *Salmonella paratyphi* A, *Salmonella paratyphi* B, are of medical importance as causative organisms of typhoid fever. Typhoid fever is an infectious disease of serious public health concern. It is estimated that 14.3 million episodes and more than 135,000 deaths due to enteric fever occurs worldwide each year [1]. Signs and symptoms of the infection includes mostly abdominal pain, anorexia, constipation, diarrhoea, fever, frontal throbbing headache, gastrointestinal bleeding, hepatosplenomegaly, and nausea [2]. One significant complication is intestinal perforation which occurs in 1–3 % of cases when left untreated. Presently, World Health Organization (WHO) recommends the use of third-generation cephalosporines and azithromycin for its management [3]. Unfortunately, there is a widespread resistance to antimicrobial agents making them less effective in ridding off the body of these organisms [4]. This underscores the need for natural products with therapeutic potentials and mild adverse effects.

Harungana madagascariensis, a tropical shrub found in Africa known as Aranje" by the Yoruba tribe and "Uturu" by Igbo tribe has been used ethnomedicinally in the treatment of human diseases like bacterial infection [5,6] gastrointestinal diseases [7], dysentery, typhoid diarrhea, anemia [8]. This work was aimed at evaluating the in vitro antisalmonella effect of fractions of *H. madagascariensis*.

2. Material and methods

2.1 Materials

2.1.1 Culture Media and Reagents

Reagents and solvents used in this study were of analytical grade and are products of JHD. Culture media used was mueller-hinton agar for the antimicrobial (MIC) evaluation.

Culture media were prepared according to the instructions of the manufacturers. Standard control drugs used include Ciprofloxacin and oxytetracycline.

2.1.2 Equipment

Microscope (Olympus, UK), Autoclave [Equitron Partially Automatic Autoclave (Medica Instrument Manufacturing CO., India)], Incubator [Incubator (Genlab, UK)], Hot air oven [(Hot Air Oven (Genlab, UK)].

2.1.3 Test microorganisms

The microorganisms used in this study were ten different isolates of Salmonella spp. These are clinical isolates previously purified and standardized to Mac Farland**.**

2.2 Methods

2.2.1 Sample Collection and Extraction

The sample, fruit of *H. madagascariensis* was collected from the Medicinal plant Garden of Pharmacognosy and Phytotherapy Department, UNIPORT and identified by a Taxonomist. It was air dried and pulverized. A 600g of the pulverized sample was extracted with 70% aqueous ethanol by cold maceration for 72hours with filtration and change of solvent done every 24hours. After the extraction, it was concentrated with rotary evaporator at 400C to at least one-tenth of its volume. The crude extract was further concentrated by putting it in glass desiccator until all solvents evaporated. The crude aqueous ethanol extract was defatted with n-hexane to give n-hexane portion (NHP) and aqueous portion (AQP).

2.2.2 Isolation of Test Samples

Pure cultures of ten Salmonella species for the in-vitro antimicrobial/minimum inhibitory (mic) determination assay was obtained from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical sciences, Nnamdi Azikiwe University Awka-Nigeria after sub-culturing on salmonella shigella agar. The cultures were maintained on nutrient agar slants for a period of 48 hours in a refrigerator before it was sub-cultured into freshly prepared salmonella shigella agar for well diffusion method.

2.2.3 Determination of Minimum Inhibitory Concentration (MIC) by agar dilution assay

The Minimum Inhibitory Concentration (MIC) of the extracts was determined for each of the test organisms using agar dilution method. Briefly in the method, the media i.e. Mueller Hinton Agar MHA (Hi-Media, USA) was prepared and treated according to the manufacturer's specification, were 38 g of the media was mixed with 1L of sterile distilled water and sterilized at 1210C for 15 mins. The media was allowed to cool to 500C and later specified volumes was used to dilute the extracts appropriately.

Stock solutions of 1000 mg/ml of the various extracts were prepared. Then, two-fold serial dilutions were made to get 500, 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, 1.9 and 0.9 mg/mL, thereafter 10-fold dilutions of each of the concentration was made using 9 mL sterile molten agar (50 OC) this was allowed to solidify. The microbial inoculums which has been standardized to 0.5 McFarland turbidity is streaked on the agar appropriately. The plates were incubated at 370C for 24 hrs.

After incubation, the plates were examined for microbial growth by checking for growths using a plus sign (+) indicating growth while a negative sign (-) indicates no growth. * indicates no MIC carried out because there was no antibacterial activity.

The antimicrobial potential for each extract were determined by visually observing the plates for the presence and/or absence of growth. Each extract was tested against all the bacterial isolates

3. Results

The phytoconstituents analysis of the methanol crude extract of *H. madagascariensis* is as shown in Fig. 1. below. While the percentage amounts of alkaloids and glycosides in the extract were zero, phenolics were mostly abundant at 22.24%. Flavonoids, tannins and carbohydrates are present at the percentages of 1.89, 0.42 and 1.37 respectively.

Antimicrobial activity of crude extract of *H. madagascariensis* against isolated salmonella species is as shown in Table 1 below.

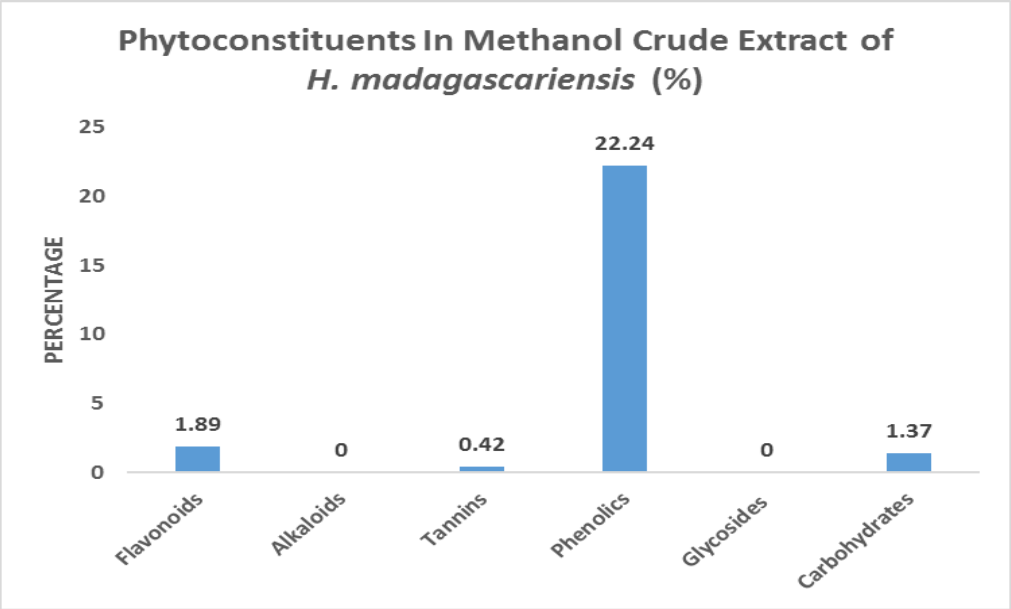

Fig 1. Phytoconstituents of *H. madagascariensis* Methanol crude extract

Table 1: Antimicrobial activity of crude extract/ fractions of *H. madagascariensis* against isolated salmonella species

Extract	Minimum inhibitory concentration (mg/mL)									
	S1	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*	*S8*	*S9*	*S10*
Crude	3.13	3.13	3.13	1.57	6.25	3.13	6.25	6.25	6.25	6.25
NHF	<0.31	2.50	2.50	<0.31	<0.31	<0.31	<0.31	<0.31	5	<0.31
AQP	10	>10	10	10	10	10	>10	>10	10	>10

Key: NHF = n-hexane fraction; AQP = aqueous fraction; S1-10 = clinically isolated strains of salmonella

4. Discussion

In the present investigation secondary metabolites from the fruits of H. madagascariensis were quantitatively established thus; flavonoids (1.89±0.03), tannins (0.42±0.05), phenolics (22.24±0.07) and carbohydrates (1.37±0.06) in the crude extract. Qualitatively the presence of flavonoids, glycosides, anthraquinones, tannins, carbohydrates,

terpenoids and steroids were confirmed while alkaloids and saponnin were absent. The presence of these phytochemicals in the fruits of H. madagascariensis makes it beneficial as they have potent medicinal values, including analgesic, antiplasmodial, bactericidal, wound healing, hypoglycemic, anti-inflammatory, and antioxidant properties, among others [9]. The trend in antibacterial activity (MIC) against the ten clinical isolates of salmonella was exhibited by NHF (<0.31 – 5.00 mg/ml) > crude extract (1.57 – 6.25 mg/ml) >AQP (>10.00 mg/ml). The result showed that n-hexane fraction has a better activity than aqueous portion thus the major constituent with antisalmonella activity of H. madagascariensis resides in the non-polar solvent.

5. Conclusion

The quantitative analysis of the sample revealed high content of phenolics while the assay result showed that NHF has a better activity than aqueous fraction. This result may provide a rational support for the traditional use of *H. madagascariensis* in the treatment of typhoid fever.

Compliance with ethical standards

Disclosure of conflict of interest

All authors declare that there is no conflict of interest.

Contributions of the Authors

OBB, OEO and AKA are all involved in the design and execution the project.

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