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Screening phytochimicals, biological activity and antioxidant activity of the methanol fractions of the leaves of *Jatropha chevalieri* beille

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Abstract
Jatropha chevalieri beille's foliage possesses haemostatic and vulnerary properties, capable of stopping bleeding by being applied directly onto wounds. Recently, the identification of novel antimicrobial phytoconstituents has become increasingly imperative. The pursuit of new sources of natural antioxidants, antimicrobials, and antifungal agents is of utmost importance. In this study, we explore the antioxidant, antimicrobial, and antifungal activity of methanol fractions derived from *Jatropha chevalieri* beille.
We conducted a test for antimicrobial activity on a range of clinical isolates. We tested Gram-positive strains such as *Listeria monocytogenes* ATCC9144, *Staphylococcus aureus* and *Salmonella typhimurium* ATCC14028, as well as Gram-negative strains including *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa*. Additionally, we tested for antifungal activity against Fusarium oxysporum and assessed antioxidant activity using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method.
A significant discovery was made in the methanol extract of *Jatropha chevalieri* beille as it contained various secondary metabolites including flavonoids, polyphenols, saponins, and tannins. Furthermore, this extract demonstrated strong potential in terms of antioxidant, antimicrobial, and antifungal properties.
Our results indicate that the methanol fraction derived from *Jatropha chevalieri* Beille is highly effective in combatting *Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC14028, and *Staphylococcus aureus*, and shows potential as an excellent agent for combating fusarium fungi. Moreover, this fraction displayed the greatest antioxidant activity, likely resulting from the presence of secondary metabolites. Additional research should be conducted to better identify and characterize these compounds and their biological activity.

Keywords: *Jatropha chevalieri* beille, Antimicrobial activity, antifungal, antioxidant activity

Introduction
Jatropha chevalieri beille is a type of turmeric plant that was first described by Lucien Beille. It belongs to the lawn plant family and the Jatropha genus, which is a group of flowering plants in the Euphorbiaceae family. The plant's name is derived from the Greek words "iatros," meaning "doctor," and "trophe," meaning "nutrition," and it is locally known as Gindfer.
The leaves of *Jatropha chevalieri* beille have haemostatic and vulnerary properties, which means that they are able to stop bleeding when applied directly to wounds. Some phytochemicals found in the plant, such as phenols, tannins, flavonoids, saponins, and alkaloids, are known to exhibit medicinal and physiological activities.
Through our study, we found that the crude methanol extract from *Jatropha chevalieri* beille has both antioxidant and biological activity.

Materials and Methods
Preparation of plant extract
The *Jatropha chevalieri* beille leaves were air-dried in the laboratory at 25-30 °C and shielded from light for a few days. Subsequently, the leaves were ground into a fine powder and used for extractions through maceration. Specifically, 19.424 g of the powder was blended with 200 mL of methanol and allowed to infuse for 24 hours. The resulting extract was then filtered using Whatman filter paper and concentrated under reduced pressure using a rotavapor. The raw extracts were preserved at 4 °C until they were analyzed.

Microbial isolates

Microorganisms with multidrug resistance are often identified, and specific species are commonly isolated. Among these isolates were three Gram-positive strains: *Listeria monocytogenes* ATCC9144, *Staphylococcus aureus*, and *Salmonella typhimurium* ATCC14028. Two Gram-negative strains were also identified: *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* (ATCC 27853). In addition, a fungal isolate known as Fusarium oxysporum was also identified.

Phytoconstituents screening

Screening the qualitative phytoconstituents for secondary plant metabolites, including flavonoids, polyphenols, alkaloids, saponins, and tannins, was performed by applying the standard phytogenic compounds' qualitative analysis approach, as detailed in the works of Trease and Evans [1] and Harborne [2].

Antioxidant DPPH method

The method used to determine the scavenging activity of DPPH radical in the extract solution was based on Torey *et al*. approach [3], with some modifications. In this method, a mixture of 0.1 mM DPPH in methanol, along with 0.003-1 mg/ml of the plant extracts and standard BHT, was prepared. The mixture was thoroughly vortexed and kept in the dark at room temperature for 30 minutes, after which the absorbance was measured spectrophotometrically at 517 nm. The scavenging ability of the plant on DPPH was then calculated using a formula.

$$(\%)DPPH = \frac{(A_{DPPH} - A_{SIMPLE})}{A_{DPPH}}$$

A_{DPPH}: Absorbance of DPPH

A_{SIMPLE}: Absorbance of DPPH radical + sample extract or standard

Antibacterial activity

To determine the antibacterial properties of the crude methanol extract, an agar well diffusion method was utilized [4]. The bacterial strains were initially cultivated on Mueller-Hinton Agar MHA overnight at 37 °C, with a bacterial suspension concentration of 106 UFC. Using a Graham tube with a uniform diameter of 6 mm, wells were punched onto the Mueller-Hinton Agar. Sterile cotton buds were then employed to spread 100 µL of bacterial suspensions onto the agar. The DMSO-diluted crude Methanol extract was prepared at 20 mg/ml, 10 mg/ml, and 5 mg/ml. 50 µl of the extract was dispensed into the wells and allowed to dry overnight, while incubated at 37 °C. The test was replicated three times.

Antifungal Activity

In order to determine the antifungal properties of the extracts, the radial growth method was employed [5]. The extracts, at a concentration of 80 mg/ml (w/v), were diluted to three different concentrations (10, 1.25, and 0.15 mg/ml) using a 1:8 ratio with PDA. The resulting mixtures were then poured into separate petri dishes and allowed to solidify before inoculating them with a 5mm diameter of fungal culture. A control experiment was also conducted without the extracts. The plates were then incubated at 25 °C for 72 hours, and the antifungal activities were measured by recording the diameter of growth.

$$\text{Antifungal activity } (\%) = \frac{D_T - D_S}{D_T}$$

Where D_T is the diameter of growth in control plate and D_S is the diameter of growth in the plate containing tested antifungal agent.

Results and Discussions

Methanol is a versatile solvent that has the ability to dissolve compounds with different polarities, including polar, semi-polar, and non-polar substances [6]. To determine the extraction efficiency, the yield was measured using the following formula:

$$\frac{\text{DWe}}{\text{DWs}} \times 100$$

Where, DWe is the dry weight of extract after evaporation and DWs, the weight of the dry powdered leaves.

The yield of the methanol extract was highest. Table1 showed that.

Table 1: Fractionation yield of J.Chevalieri methanol.

Fraction	Dry powdered leaves (g)	Dry weight of extract (g)	Yields, %
Methanol	19.424	1.041	5.35

Preliminary phytochemical screening showed that *J. Chevalieri* contain higher amount of Tannins, Saponin, Poly phenols and Flavonoids. (Table 2). These phytochemicals are known to be behind the antimicrobial activities, antifungal. M. O. Oyama [7] was working in the phytochemical screening of *Jatropha Curcas* leaf and observed the presence of Tannins, Saponin and Flavonoids.

Table 2: Phytochemical screening tests for the methanol of J Chevalieri

Phytochemical Compounds	Alkaloids	Flavonoids	poly phenols	saponins	tannins
Methanol fraction	-	++	+++	+++	+++

Blois' method can be used to classify the antioxidant activity of a test substance. According to this method, if the IC50 value is less than 50 µg/mL, it indicates a highly active intensity. Similarly, if the value ranges from 50-100 µg/mL, it indicates active intensity. A value between 101-250 µg/mL denotes a moderate intensity, while 250-500 µg/mL reflects weak intensity. Finally, if the IC50 value is greater than 500 µg/mL, it suggests an inactive intensity [8].

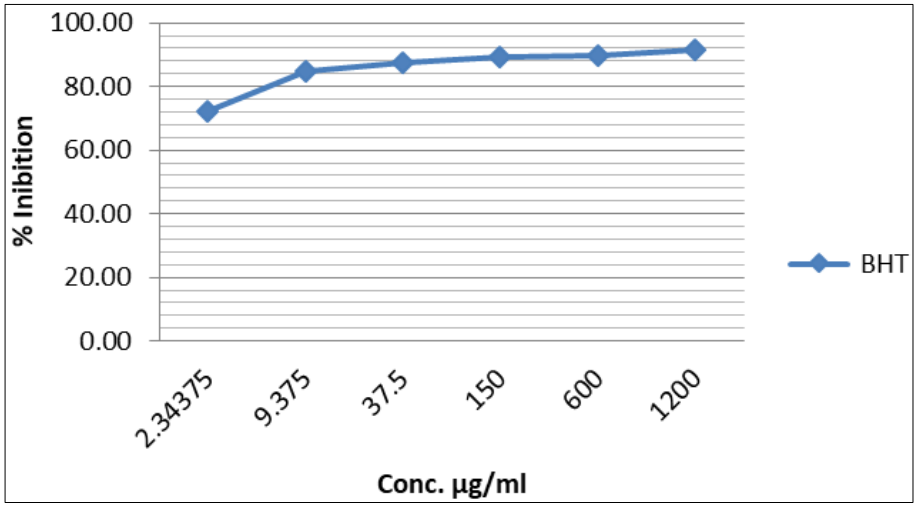

Fig 1: Inhibition activity of BHT reference

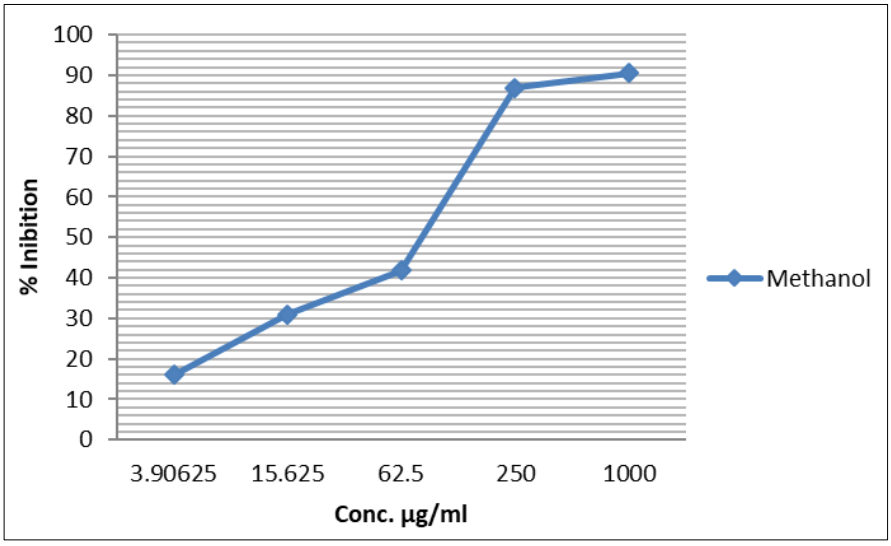

Fig 2: Inhibition activity of methanol fractions.

The methanol crude extracts of J Chevalieri were found to exhibit strong antibacterial activity against Gram-positive strains, including *Salmonella typhimurium* ATCC14028 and *Staphylococcus aureus*, as well as Gram-negative *Escherichia coli* ATCC25922, which are responsible for common hospital infections. In a study by Leonard G. O. Adamu [9], the ethanol extract of *J. curcas* leaves was used and demonstrated significant antimicrobial activity against *E. coli* and *S. aureus*. Similarly, in a study by Kumar Arun *et al.* [10], the methanolic leaf extract of *Jatropha Curcas* L. showed a zone of inhibition of 4.0 mm against *E. coli* at a concentration of 20 mg/10 ml, and a zone of inhibition of 3.0 mm against *Staphylococcus aureus* at the same concentration. Our own results showed that at a concentration of 20 mg/ml, the activity against *E. coli* was 9.33±1.53 and against *S. aureus* was 14±4.36.

Table 3: Phytochemical screening tests for the methanol of J Chevalieri

Conc. of extracts (mg/ml)	Microorganisms/zone of inhibition (mm)				
	EC	LM	PA	ST	SA
20	9,33±1,53	0	0	8,66±0,58	14±4,36
10	9±1	0	0	8±1,7	9,66±0,58
5	8,83 ±0,58	0	0	0	9,33±0,58
DMSO	0	0	0	0	0

EC: *Escherichia coli* ATCC25922; LM: *Listeria monocytogenes* ATCC9144; PA: *Pseudomonas aeruginosa* ATCC27853; ST: *Salmonella typhimurium* ATCC14028; SA: *Staphylococcus aureus*

Fungal infections have a significant impact on public health [11], resulting in high levels of illness and death worldwide and increasing healthcare expenditure. Our study has indicated that the methanol-based extract has a potent inhibitory effect at a concentration of 10 mg/ml. It is likely that the presence of saponins and phenolic compounds contribute to its antifungal activity.

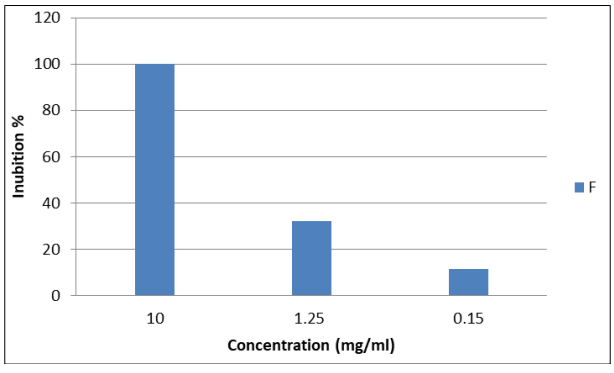

Fig 3: Antifungal activity of *J. Chevalieri* plant against Fusarium oxysporum

Conclusion

This study showed the *J. Chevalieri* had activity as an antioxidant and the methanol extracted showed good inhibition gains one gram-negative and tow gram-positive and the responsible of this activity is the metabolite which is present in the extracted.

In the future we will separate the metabolite and repeated this test so determined the realer responsible of this activity.

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