



ANTIMICROBIAL PROPERTY OF AQUEOUS AND PETROLEUM ETHER LEAF EXTRACTS OF *JATROPHACURCAS*

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ABSTRACT

The experiment was carried out to investigate the antimicrobial property of aqueous and Petroleum ether leaf extracts of *Jatropha curcas* against some gram positive micro-organisms: Staphylococcus aureus, Bacillus subtilis and some gram negative micro-organisms: Escherichia coli, Salmonella typhi using antibiotics; Gentamycin as control. The phytochemical screening of aqueous and petroleum ether extracts showed the presences of cardiac glycosides, steroids and terpenes, tannins, phlobatannins, anthraquinones and saponins. The disc diffusion techniques was used to test the sensitivity of the micro-organism to the extracts of *Jatropha curcas* the results obtained show mean zones of inhibition between (19 + 0.6mm) to (30 + 0.3mm) for aqueous extract and (24 + 0.5mm) to (35 + 0.8mm) for petroleum ether extract. Micro-organisms showed sensitivity in the following order: E.coli;(17 + 0.3mm) and (25 + 0.8mm), S.aureus; (26 + 0.2mm) and (28 + 0.6mm), B.subtilis; (16 + 0.1mm) and (20 + 0.7mm), and S.typhi (25 + 0.2mm) and (27 + 0.6mm) for aqueous and petroleum ether extracts respectively. The minimum inhibition concentration (MIC) for both extracts show that the extracts inhibited the growth of the entire test organism at concentration 0.6mg/ml. This result thus suggests the potency of *Jatropha curcas* as an antimicrobial agent especially at the concentration employed.

Keywords: Antimicrobial Property, Leaf Extracts, *Jatropha curcas*, Phytochemicals, Gentamycin.

INTRODUCTION

Plants form the main ingredient of medicine in traditional system of healing and have been the source of inspiration for several major pharmaceutical drugs. Roughly about 50,000 species of higher plants have been used medicinally. This represents by far the biggest use of the natural world in terms of number of species. In fact, they were used for treating infections ^[1], malaria ^[2], burns, edema, and allergies and prevent several diseases ^[1]. Since the last decades, researchers have begun to explain these virtues by the ability of plants to limit infections ^{[3][4]}, prevent lipid peroxidations^[5], prevent some cancers ^[6], cure allergies ^[7] and many other associated diseases ^{[8][9]}. Among all these virtues, the anti-infectious activity was considered as one of the most important activities ^[7]. *Jatropha* is a shrub or tree with spreading branches and stubby twigs, with yellowish rufescent exudates, leaves deciduous, alternate but apically crowded, 3 to 5 lobed in outline, 6-35 broad the petioles 2.5 - 7.5cm long. Male flowers have as much as 10 stamens, 5 united at the base and 5 united into a column while the female flowers borne singly ^[10]. Medicinal uses according to ^[11], the young leaves may be safely eaten, steamed or stewed. The oil has been used for illumination, soap, candles, adulteration of olive oil and making Turkey red oil. Duke and wain (1981) list it for homicide, pesticides and raticides as well. The latex was strongly inhibitory to water melon mosaic virus in south sudan the seed as well as the fruits are used as a contraceptive. Ashes of the burned root are used as a salt substitute ^[10]. The leaves are used for the treatment of inflammations, fever and itching. The seeds are bitter, useful in blood diseases and mouth sores ^[12]. They are an efficacious remedy in diarrhea ^[13]. Based on ethno botanical practice, the plant has been investigated for anti-inflammatory ^[14], antipyretic, anti-diabetic, antibacterial and diuretic properties ^{[15][7]}, and with the increased interest shown by researcher in folk medicine for new leads to develop better drugs against microbial infection ^[16], there is a need therefore, for a study on the determination of possible antimicrobial properties of extract of *Jatropha curcas*. Although the antimicrobial activity of some medicinal plants is documented, their antimicrobial activities vary widely, depending on the type of spice or herb, test medium and micro-organism. The aim of this study was to investigate the antimicrobial property of aqueous and petroleum ether leaf extracts of *Jatropha curcas* on some selected gram positive and gram negative microorganisms such as *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*.

MATERIALS AND METHODS

Source and Collection of Plant Parts:

The leaves of *Jatropha curcas* plant were collected within Jos environment, identified and authenticated at the Forest Research Institute of Nigeria in Jos, Plateau State.

Preparation of Plant Extracts:

To a volume of 1500ml of distilled water and petroleum ether was added 300g of the powdered plant extract. The suspension was allowed to stand in position for 3 days in the laboratory. The mixture was agitated at intervals on each day. On the third day, the extract was filtered out into a clean sterile flask with the aid of millipore filter and later concentrated to dryness in a rotatory evaporator in vacuum.

Standardization of Isolates:

Test organisms were sub-cultured onto fresh plates of MacConkey agar and incubated aerobically at 37°C for 24 hours. Colonies from these plates were suspended in Mueller- Hinton broth to a turbidity matching 0.5 McFarland standard (108cfu/ml). Mueller-Hinton agar was then used for antimicrobial assay. All the broth cultures were incubated at 37°C.

Phytochemicals screening:

The phytochemical screening of the *jatropha curcas* leaf extract was carried out using Standard qualitative procedures.

Antimicrobial Assay:

Suspensions of the bacteria obtained contained approximately 1×10^8 cfu/ml. Each labelled plate was uniformly seeded with a test organism by means of sterile swab stick rolled in the culture medium. Aliquots were dropped in each well to fullness. Each plate was kept in the refrigerator for 1 hour to allow the extracts to diffuse into the culture medium while the immediate growth of the organism was stopped from taking place. These plates were then incubated at 37°C for 24hours. The zones of inhibition around the wells were measured in millimeter (mm). Control antibiotics and petroleum ether solvent were placed in a well on each plate along with the test extracts as control.

Determination of Minimum Inhibitory Concentration:

The Minimum Inhibitory Concentration (MIC) of the extracts was determined for each of the test organisms in triplicate in test tubes. To 0.5 ml of varying concentrations of the extracts (0.5, 0.4, 0.3, 0.2 and 0.1mg/ml) in test tubes, Nutrient broth (2ml) was added and then a loopful of the test organism, previously diluted to 0.5 McFarland turbidity standard, was introduced. The procedure was repeated on the test organisms using the standard antibiotics (Erythromycin). A tube containing Nutrient broth only was seeded with the test organisms, as described above, to serve as controls. The culture tubes were then incubated at 37°C for 24 hours. After incubation the tubes were then examined for microbial growth by observing for

turbidity.

RESULTS

Phytochemicals	Aqueous Extract	Petroleum Ether Extract
Saponins	+	+
Phlobatannins	+	+
Anthraquinone	-	+
Steroids & terpenes	+	+
Tannins	+	+
Cardiac glycosides	+	+

Table I: Results of phytochemicals screening of *Jatropha curcas* extracts

+ = Present, **-** = Absent

Group	Test Isolates	Gentamycin	Aqueous Extract	Petroleum Ether Extract
1	E. coli	20 ± 0.4	17 ± 0.3	25 ± 0.8
2	S. aureus	30 ± 0.2	26 ± 0.2	28 ± 0.6
3	S. typhii	22 ± 0.2	25 ± 0.2	27 ± 0.6
4	B. subtilis	19 ± 0.5	16 ± 0.1	20 ± 0.7

Table II: Mean zones of inhibition (mm) of extract and Gentamycin on test isolates

Group	Micro organism	Extract	0.6mg/ml	0.5mg/ml	0.4mg/ml	0.3mg/ml	0.2mg/ml
1	E. coli	AE	-	+	+	+	++
		PE	-	-	-	-	+
2	S. aureus	AE	-	+	+	++	++
		PE	-	-	-	+	+
3	S. typhii	AE	+	++	++	++	++
		PE	-	-	-	+	+
4	B. subtilis	AE	-	-	+	++	++
		PE	-	-	-	+	+

Table III: Minimum inhibitory concentration of leaf extracts obtained from aqueous and Petroleum ether extracts on micro-organisms.

Key:

+ = small growth

++ = major growth

- = No growth

PE = petroleum ether extract, AE = aqueous extract.

DISCUSSION

The Table I show result obtained from the preliminary phytochemical screening of aqueous and petroleum ether extracts, studies which showed the presences of cardiac glycosides, phlobatannins, tannins, saponins, anthraquinone, terpenes and steroids. it is suspected in this study, that the mechanism of its antimicrobial potency may not be unconnected to the presence of tannins, phlobatannins, saponins, steroids, Cardiac glycosides and reducing sugars associated with the phytochemical screening/analysis of most Nigerian medicinal and traditional herbs ^{[17][18]}. The use of plants extract; water (aqueous) and other alcoholic concentrations has become a common practice among traditional medical practitioners ^[19], Although, the active potential phytochemicals present in both aqueous and alcohol extracts of leaves and herbs are the same, there is always a variation in their inhibition potency especially at different concentrations ^[20]. Therefore, the comparison of these aqueous and alcoholic extracts at their different concentrations serves to propose a model for the pharmacological studies of plants/herbs ^[20]. Table II shows the Mean zones of inhibition (mm) of extracts and Gentamycin (control) on test isolates, the result shows the levels of inhibition observed ranged between 19mm – 30mm Gentamycin, 16mm – 26mm for aqueous extract and 20 – 28mm for petroleum ether extracts of *Jatropha curcas*. For E.coli, the petroleum ether extract shows greater inhibition (27mm) than aqueous (25mm) and antibiotics (22mm) (Gentamycin). For S.aureus, the antibiotics (30mm) (Gentamycin) shows greater inhibition that aqueous (26mm) and petroleum ether (28mm) extracts. Also, For S. typhii, the petroleum ether extract shows greater inhibition (25mm) than aqueous (17mm) and antibiotics (20mm) (Gentamycin), and for B. subtilis, the petroleum ether extract shows greater inhibition (20mm) than aqueous (16mm) and antibiotics (19mm) (Gentamycin). Comparison of aqueous and petroleum ether extracts of *Jatropha curcas* against microorganisms showed that petroleum ether extract have greater inhibitory potency. Table III shows result obtained for Minimum Inhibitory Concentration (MIC), the MIC of both aqueous and petroleum ether extract against E.coli, S.aureus, S.typhii, and B.subtilis, at concentration 0.6mg/ml shows that the growth of the microorganisms are inhibited expect for aqueous extract on S. typhii. On the other hand E.coli, S.aureus, and S.typhi appeared to be resistant to the aqueous plant extract at concentrations lower than 0.6mg/ml. The minimum inhibitory concentration (MIC) of the aqueous and petroleum ether extracts shows values indicating a possible adoption of *Jatropha curcas* extract as an

antibacterial agent. Also, the results obtained from the study may not be unexpected as natural plants/herbs have been shown to possess various medicinal potency as well as their inhibitory effects on various human-disease causing microorganisms. Ethanolic leaf extract of *Aloe veraburm* have been shown to inhibit the growth of *E.coli*, *K.pneumoniae*, *P.aeruginosa*, *B.subtiilis* and *S.aureus*^[21]. It was showed that *E.coli*, *B.subtiilis*, *P.aeruginosa*, and *S.aureus* are strongly inhibited by nine Nigerian spices/herbs of which this result is consistent as all microorganisms were strongly inhibited by both the aqueous and petroleum ether extracts of *Jatrophacurcas*.

CONCLUSION

In conclusion, the study shows that all the micro-organism investigated were inhibited and highly sensitive to petroleum ether leaf extract of *Jatrophacurcas*. *Jatrophacurcas* can be employed in the treatment of ailments and diseases caused by tested microbes at concentration 0.6 mg/ml. Further studies on animal toxicity are recommended.

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