

THE ANTIPROTOZOAL PROFILE OF TRICHOMONAS VAGINALIS, ISOLATED FROM SUSCEPTIBLE WOMEN, TO THE EXTRACTS OF JATROPHA CURCAS PLANT

Oludayo O. Oduwobi

Science Laboratory Technology Department, the Federal Polytechnic, Ilaro, Ogun State, Nigeria

DOI: <https://doi.org/10.5281/zenodo.20026945>

Keywords:

Plants,
parasites,
volunteers,
trichomonads

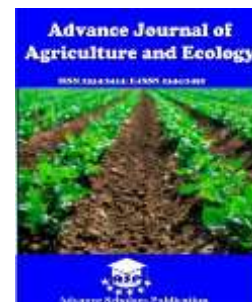
Abstract: For a long time, plants have provided a source of emerging modern medicine and drug compounds, as plant-derived medicines have made large contributions to human health. Trichomonads were identified, the phytochemical constituents of two extracts and their anti-protozoal efficacy were also determined. Both the trophozoite and amoeboid forms of *Trichomonas vaginalis* were positively identified from the vaginal swab samples collected for the investigation. From the wet-mount smears of the volunteers, the presence of the trichomonads in the midst of epithelial cells, white blood cells and red blood cells was confirmed and quantified. Only twenty-eight (28%) volunteers tested negative for the presence of *Trichomonas vaginalis* protozoan parasite. The parasitic load ranged between 5 and 13 parasites. After the plant's extracts used in this study were screened to ascertain their purity, there was visibly clear absence of microbial growth which confirmed their purity. Both extracts possessed tannins and terpenoids. While alkaloids were present in the leaf extract, they were, however, absent in the stem extract. At 100% concentration, the microscopic evaluation showed that the inhibitory property of both extracts was not disputable as the trichomonads were all sensitive to the extracts, as demonstrated by their immobilization. The control; without either extract but instead distilled water, showed no anti-protozoal activity. The minimum immobilization concentration of the leaf extract was 90mg/ml while that of the stem extract still stood at 100mg/ml. The study showed that the leaf extract was more effective than the stem extract.

INTRODUCTION

Medicinal plants have been used as a source of medicine to treat illnesses since time immemorial. For a long time, plants have provided a source of emerging modern medicine and drug compounds, as plant-

derived medicines have made large contributions to human health. All the parts of *Jatropha curcas* (seeds, leaves, barks etc.) have been used in traditional medicine and veterinary purposes for a long time (Cherpes *et al.*, 2006). The genus *Jatropha curcas* belongs

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to the family Euphorbiaceae and has a great variety of species; among them are *Jatropha multifida*, *Jatropha curcas*, *J. molissima*, *Jatropha gossypifolia*.

Jatropha curcas is an ornamental, medicinal and multipurpose shrub which is grown in home gardens in West Africa and cultivated extensively in Asia. The seeds of *Jatropha curcas* have been used medicinally as a purgative and as a remedy against syphilis. The oil has been used as a source of fuel for stimulating hair growth, making candles and soap. The viscid sap (latex) is employed for cleaning teeth to cure sores on the tongues of babies and for toothache (Devappa *et al.*, 2012a). The leaves are utilized extensively in West African ethno-medical practice in different forms to cure various ailments like fever, mouth infections, jaundice, guinea worm sores and joint rheumatism (Prasad *et al.*, 2012). The roots are used in decoction as a mouthwash for bleeding gums, toothache, eczema, ringworm, scabies and to cure dysentery and venereal diseases like gonorrhea. After colonization, the parasite causes vaginitis, urethritis and prostatitis. Moreover, the pathogen has been associated with serious consequences such as adverse pregnancy outcomes and pre-term birth, infertility, predisposition to cervical cancer and pelvic inflammatory disease (Sorvillo *et al.*, 2011). Importantly, trichomonosis acts as a co-factor in human immuno-deficiency virus (HIV) transmission and the acquisition of parasitic protozoans remain a major threat to human and animal health. There are few effective

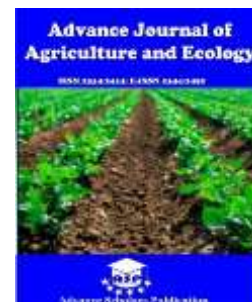
drugs for the treatment of many protozoal diseases.

Some factors could be responsible or induce a high prevalence and transmission of trichomoniasis in potentially endemic areas and highly sexually-active individuals, particularly females. Those factors may include; poverty, harsh economic conditions, poor sanitation and personal hygiene but in the case of the target individuals for this current investigation, it could simply just be due to youthful exuberance and indiscriminate sexual activities. Schirm *et al.* (2007) posit that the damage caused by *Trichomonas vaginalis* to vaginal epithelium increases a woman's susceptibility to HIV infection. Currently, antibiotic resistance has become a global concern as it is being threatened by emergence of multidrug-resistant pathogens. Due to possible cases of drug resistance by resistant trichomonads, cost, toxicity and perhaps allergies from the use of synthetic commercial antiprotozoal agents and asymptomatic cases, phytoremediation is now being considered as an effective and more health-friendly alternative.

MATERIALS AND METHODS

Sample Collection

This investigation was carried out at the Medical Centre and Microbiology Laboratory of The Federal Polytechnic, Ilaro, Ogun State, Nigeria. Ethical clearance was sought at the Research and Development Centre of the institution prior to the collection of vaginal fluid from the volunteer females at the institution's Medical Centre. The assistance of a female medical staff was sought in obtaining



the vaginal fluid from the participants within two weeks, under a strict condition of confidentiality and the use of pseudonyms. The plant used was obtained from a local farm after its identification with the help of a local farmer. It was then authenticated by a certified horticulturist from Leisure and Tourism Department of the institution. Appropriate precautionary measures were adopted during the course of the investigation which includes the use of a pair of hand gloves and a nose mask. The collected plant sample was kept in a moist-free area in the Microbiology Laboratory until when required.

Identification of Trichomonads

Motile trichomonads from the vaginal fluid of the volunteer female participants were isolated and identified using sterile disposable swab sticks (Tine *et al.*, 2019). While wearing a pair of hand gloves, a swab stick apiece was carefully and gently inserted into the vagina of each participant and then rotated clockwise and anti-clockwise intermittently for about 10 seconds, ensuring that the stick touches the walls of the vagina to extract adequate fluid for analysis. The swab sticks were carefully placed into their labeled sachet containers and gently sealed for onward transit to the Microbiology Laboratory for microscopy.

Wet-mount smears to detect possible trichomonads from the specimens were prepared by placing each inoculated swab stick in vials containing 1ml of normal saline. A drop from each vial was centrally placed on each microscope slide for the wet mount and then viewed under a microscope with x10 low-power magnification and x40 high-power

magnification to detect *T. vaginalis*. The presence of the trichomonads was confirmed by the detection of visibly-large motile parasites with flagella and undulating membranes on a side of the organisms.

Preparation of Extracts

The *Jatropha curcas* plant's leaves and stems were cut out and washed with sterile distilled water. They were then air-dried for twenty-eight days before they were considered brittle enough. A warring industrial blender was used to crush the leaves and stems and then kept in separate airtight containers before use. Cold-extraction method was employed according to Joseph and Raj (2010).

Evaluation of Extracts' Purity

The plant's extracts used in this study were screened to ascertain their purity; this was done by streaking the plant's extracts separately onto sterile plates of growth media (nutrient and potato dextrose agar).

Phytochemical Screening

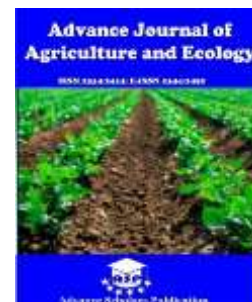
The phytochemical screening was carried out in order to determine the active ingredients in the plant's extracts which could be responsible for their anti-protozoal effect. The method of Ogboke and Akamo (1998) was employed.

Tannin test

1g of the sample was extracted with 10ml of boiling water for five minutes, filtered and cooled. 1ml of 1% ferric chloride was added to the filtrate portion and then observed.

Saponin test

1g of the sample was added to 10ml of hot distilled water in different test tubes. The sample was filtered and the following were performed;



i. Frothing: 2ml of the filtrate was diluted with 10ml distilled water and then shaken vigorously for about 2minutes.

ii. Emulsification: 2 drops of oil were added to 2ml of the filtrate and then shaken vigorously.

Phlobatannin test

1g of the sample was added to 10ml of distilled water in different test tubes and then boiled. The sample was filtered hot, cooled and 1ml of aqueous hydrochloric acid was added to the filtrate. They were shaken vigorously, re-boiled and then allowed to cool.

Salkowski test

0.5g of the sample was added to 10ml of chloroform, after which it was shaken vigorously and then filtered. 1ml of 10% sulphuric acid was added to the filtrate.

Anthraquinone test

5ml of benzene was added to 5ml of the filtrate and then shaken gently. Benzene solution was decanted into another test tube and 5ml of dilute ammonia was added.

Alkaloids test

1g of the sample was extracted with 10ml of 12% H₂SO₄. The pH of the filtrate was adjusted to between 6 and 7 and few drops of Hoyer's and Meyer's reagent were added separately to aliquots of the filtrate in a test tube.

Anti-Protozoal Efficacy

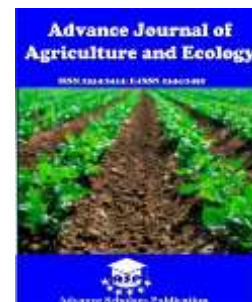
The inocula utilized were less-than-24hr-old trichomonads. A drop each of 100% concentration of the *Jatropha curcas* leaf and stem aqueous extracts was placed on wet-mount smears containing confirmed *T. vaginalis* accordingly to ascertain their anti-protozoal potency. This was determined by the

loss of motility of the trichomonads, which in turn suggests their mortality. Thereafter, 90%, 80%, 70% and 60% of the two extracts were prepared to determine their minimum immobilization concentrations (MIC) on the test trichomonads, *in-vitro*. The ability of the extracts to inhibit the movement of the trichomonads was indicated by their non-motility even in the presence of their flagella in the wet mount smears and recorded as mortality, otherwise, resistance vice-versa. Distilled water was used as control.

RESULTS AND DISCUSSION

The study was conducted using a total of 100 volunteer female participants. Vaginal fluid samples were collected from them at the institution's medical centre within those two weeks, with the help of female medical staff for microscopy. At the end of the study, both the trophozoite and amoeboid forms of *T. vaginalis* were positively identified from the vaginal swab samples, which were processed shortly after collection. While the amoeboid form was qualitatively shapeless and quantitatively scanty, the trophozoite form was fairly abundant per the samples that tested positive for the presence of the parasite. The trophozoite-form parasite appeared as a pear-shaped organism, about 15µm in length, with four whip-like flagella at the anterior end and an undulating membrane almost half of its body length and also possesses a posterior axostyle.

From their wet-mount smears, the presence of the trichomonads in the midst of epithelial cells (which usually appear as squamous, cuboidal, columnar or ciliated columnar), white blood



cells (which usually appear as nucleated and irregular in shape) and red blood cells (which usually appear as anucleated and biconcave-disc in shape) was confirmed and quantified, as the trichomonads were present in some samples while absent in the others. In the samples where they were present, they showed

varied population. The parasitic load ranged between 5 and 13 parasites. After the microscopy, 72 out of the 100 volunteers tested positive for the presence of *T. vaginalis* parasite while the remaining 28 tested negative, as shown in table 1.

Table 1: The Trichomonad Status of the Female Volunteers

Category	Statistics	Freq. of Occurrence (%)
Infected	72	72
Un-infected	28	28
Total	100	100

After the plant's extracts used in this study were screened to ascertain their purity by streaking the extracts separately onto sterile plates of the test growth media (nutrient and

potato dextrose agar) and examined for possible growth of bio-contaminants, there was visibly clear absence of microbial growth which confirmed the purity of the two extracts.

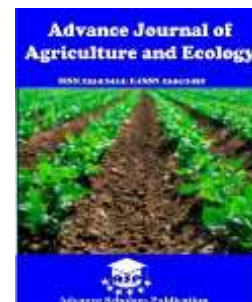
Table 2: The Phytochemical Constituents of *Jatropha curcas* Plant's extracts

Constituent	Leaf Extract	Bark Extract
Tannins	+	+
Saponins	-	-
Phlobatannins	-	-
Terpenoids (Salkowski)	+	+
Anthraquinone (Glycosides)	-	-
Alkaloids	+	-

Keys: + = Positive, - = Negative

Table 2 shows the result of the phytochemical screening carried out on the leaf and stem extracts of *Jatropha curcas* (Physic nut) plant in order to determine their active constituents with supposedly anti-parasitic efficacy. Coincidentally, both extracts possess tannins and terpenoids. The extracts both also showed a negative result for the presence of saponins, phlobatannins and anthraquinone glycosides i.e. saponins, phlobatannins and

anthraquinone glycosides were all absent. While alkaloids are present in the leaf extract, they were, however, absent in the stem extract. It was revealed that the formation of green precipitate is a positive result for the presence of tannins, stable foam for saponins, reddish brown precipitate for terpenoids and white precipitate for alkaloids. A red and pink colouration is a negative result for the presence



of phlobatannins and anthraquinone glycosides respectively.

Table 3 shows the result of the anti-protozoal activity of the leaf and stem extracts of *Jatropha curcas* plant on the isolated trichomonads. Wet mounts were prepared to determine the resistance or susceptibility of the trichomonads to the extracts at 100% concentration. At 100% concentration, the microscopic evaluation showed that the

inhibitory property of both extracts was not disputable as the trichomonads were all sensitive to the extracts, shortly after their introduction. The control; without either extract but instead distilled water, showed no anti-protozoal activity. In other words, the motility of the trichomonad parasites was not in any way impaired by the presence of the distilled water.

Table 3: The Minimum Immobilization Concentrations of the *Jatropha curcas*' Extracts

Extract	60.0	70.0	80.0	90.0	100.0	MIC
Leaf extract	+	+	+	-	-	90
Stem extract	+	+	+	+	-	100

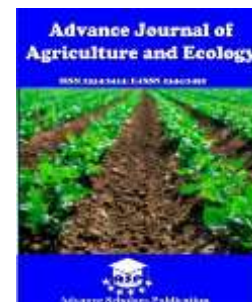
Keys: + = Active movement, - = Immobilization

MIC = Minimum Immobilization Concentration

The trichomonads used were all susceptible, as demonstrated by their immobilization to both crude extracts of the plant's leaves and stems at 100mg/ml (100%) only. They were susceptible to only the leaf extract at 90mg/ml (90%) but not the stem extract, as the spectrum of their anti-protozoal activity is slightly significantly varied, as depicted in table 3. Hence, the minimum immobilization concentration of the leaf extract is 90mg/ml while that of the stem extract still stood at 100mg/ml. At 60mg/ml (60%), 70mg/ml (70%) and 80mg/ml (80%) for the leaf extract, active movement was still noticeable, which is an indication that the

trichomonads are resistant at those concentrations. In contrast, active movement was noticeable by the trichomonads at 60mg/ml (60%), 70mg/ml (70%), 80mg/ml (80%) and 90mg/ml, for the stem extract.

The result of this investigation shows that the phytochemical constituents of both leaf extract and stem extract of *Jatropha curcas* plant possess some of phytochemical agents tested for. In the end, it was revealed that the two extracts showed inhibitory property, as the trichomonads were all sensitive to both extracts, being that they were clearly immobilized by them shortly after the



introduction of the extracts, though at varied minimum immobilization concentrations. This confirms the potency of both the leaf and stem extracts of *Jatropha curcas* plant against *T. vaginalis* but the leaf extract appeared to be more potent than the stem extract, especially that the crude stem extract showed no activity at 60mg/ml, 70mg/ml, 80mg/ml and 90mg/ml. This could suggest that the diminished strength of the extract is not capable of immobilizing the trichomonads at those concentrations and also may likely be due to its limited phytochemicals. The leaf extract showed a better anti-protozoal capability which makes it potentially promising as a pharmaceutical agent. The distilled water used as a control displayed no anti-protozoal ability, as none of the test organisms was inhibited by it; for its lack of anti-protozoal compound.

A previous investigation by Oduwobi and Adeniji (2024) has equally evaluated the susceptibility of *Trichomonas vaginalis* parasite but to the leaf and bark extracts of *Mangifera indica* plant, using n-hexane solvent instead.

Trichomonas vaginalis is one of the most common STI in the world but its prevalence is

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very heterogeneous across countries (Kissinger, 2015). The use of medicinal plants in the treatment of infection and diseases is very common. In Nigeria, rural and urban communities use medicinal plants as food and traditional medicine. The presence of the active components of medicinal plant may be due to their high non-polar compounds (Chidozie *et al.*, 2014).

CONCLUSION

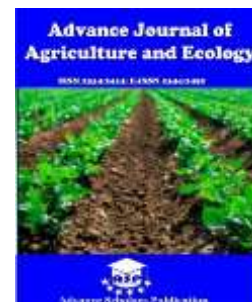
This study shows that the *Jatropha curcas* plant's leaf and stem extracts investigated possess anti-protozoal potency against *T. vaginalis* but the leaf extract was found to be more effective than the stem extract. Indeed, the former is a more promising pharmaceutical!

The observed anti-protozoal effect of these *Jatropha curcas* plant's extracts on the trichomonads, by the *in-vitro* assessment, potentially implies that the extracts could indeed be effective *in-vivo*, if processed and approved for commercial usage after clinical trials. To increase parasite-detection rate, additional procedures such as polymerase chain reaction (PCR) could be employed.

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