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THE SUSCEPTIBILITY OF TRICHOMONAS VAGINALIS TO THE LEAF AND BARK EXTRACTS OF MANGIFERA INDICA PLANT

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ABSTRACT

Trichomoniasis is the most prevalent non-viral sexually-transmitted infection in the world. A wet-mount smear to detect possible trichomonads from the specimens was prepared by placing each inoculated swab stick in vials containing 1ml of normal saline. The mango plant's leaves and barks were cut out and washed with sterile distilled water. Cold extraction method was employed. The phytochemical screening was carried out in other to determine the active ingredients in the plant's extracts which could be responsible for their anti-protozoal effect. The inocula for the anti-protozoal efficacy assay were prepared using less than 24hr-old trichomonads. At the end of the study, both the trophozoite and amoeboid forms of *T. vaginalis* were positively identified from the vaginal swab samples. 26 out of the 30 volunteers tested positive for the presence of *T. vaginalis* parasite while the remaining four tested negative. Both extracts possess just tannins in common. In addition, the leaf extract possesses phlobatannins, anthraquinone glycosides and alkaloids while the bark extract possesses terpenoids. The trichomonads isolated were all susceptible, as demonstrated by their immobilization to both crude extracts of the plant's leaves and barks at 100mg/ml only. The minimum immobilization concentration of the leaf extract is 90mg/ml while that of the bark extract still stood at 100mg/ml. It could be deduced that the *Mangifera indica* leaf and bark extracts used possess anti-protozoal potency against *T. vaginalis* but the leaf extract was found to be more efficacious.

Keywords: Smear, cold extraction, assay, immobilization, efficacious

INTRODUCTION

Trichomoniasis is the most prevalent non-viral sexually-transmitted infection in the world (Herbt *et al.*, 2016). *Trichomonas vaginalis*, the causative agent, is a protozoan parasite infecting the urinogenital tract of both females and males (Mao and Liu, 2015). In general, the infection is asymptomatic in men, although, it can be associated with urethral discharge and dysuria (Arbabi *et al.*, 2018) while infected women can have different symptoms consisting of yellowish-green frothy-discharge, dysuria and cervical

inflammation, which is recognized by punctuates haemorrhagic lesions. Trichomoniasis, while not a reportable disease, the World Health Organization estimated that there were 276.4 million cases in 2008 and nearly 90% of these infections occurred among people living in resource-limited settings (WHO, 2008). Trichomoniasis is more prevalent than *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and syphilis combined. The global prevalence of trichomoniasis has been estimated at 8.1% for women and 1.0% for men (WHO, 2001). These rates may be underestimates as they are derived from studies that use microscopy, rather than the more sensitive nucleic acid amplification tests (NAAT) and no formal surveillance system exists.

There has been a tremendous pressure on medicinal plants for their extensive utilization as sources of raw materials for pharmaceutical industries. Demands for medicinal plants are rapidly increasing not only in developing countries but also in the developed ones. Medicinal plants have various effects on living systems. Some are sedatives, analgesics, antipyretics, cardio-protective, anti-inflammatory, antioxidants, antispasmodics and immunomodulatory functions.

Some factors could be responsible or induce a high prevalence and transmission of trichomononiasis caused by *Trichomonas vaginalis* in potentially endemic areas and highly sexually-active individuals, particularly the females. Those factors may include; poverty harsh economic conditions, poor sanitation and personal hygiene. 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 or therapy, by the use of potent plant extracts, is now being considered as an effective and more-friendly alternative.

The aim of this investigation is to determine the susceptibility of *Trichomonas vaginalis* to the leaf and bark extracts of *Mangifera indica* plant.

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 volunteer female students of the institution at the institution's Medical Centre. The assistance of a female medical staff was sought in obtaining the vaginal fluid from the participants under a strict condition of confidentiality and the use of pseudonyms. The plant samples used were obtained from a local farm after its identification with the help of a local farmer. They were 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 samples were kept in a moist-free area in the Microbiology Laboratory until required.

Identification of Trichomonads

Motile trichomonads from the vaginal fluid of the female-students-volunteer 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 their screw caps gently tightened for onward transit to the Microbiology Laboratory for microscopy.

A wet-mount smear to detect possible trichomonads from the specimens was 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 x40 magnification to detect *T. vaginalis*. The presence of the trichomonads was confirmed by the detection of visible large motile parasites with flagella and undulating membranes on a side of the organisms.

Preparation of Extracts

The mango plant's leaves and barks were cut out and washed with sterile distilled water. They were then air-dried for some days until they were considered brittle enough. A warring industrial blender was used to crush the leaves and barks 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 a growth medium (nutrient agar).

Phytochemical Screening

The phytochemical screening was carried out in other to determine the active ingredients in the plant's extracts which could be responsible for their anti-protozoal effect. The method of Ogbobe 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 2 minutes.

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% H_2SO_4. The pH of the filtrate was adjusted to between 6 and 7 and few drops of Hoger'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 of 100% concentration of the mango's leaf and bark aqueous extracts was placed on wet-mount smears containing confirmed *T. vaginalis* respectively 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 trichomonad, *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.

RESULTS

The study was conducted on a total of 30 female-students-volunteer participants of The Federal Polytechnic, Ilaro, Ogun State. Vaginal fluid samples were collected from them at the institution's medical centre, 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. 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 shown in table 1, as the trichomonads were present in some samples while absent in the others. In the samples where they were present, they showed varied population when microscopically examined. From the table, 26 out of the 30 volunteers tested positive for the presence of *T. vaginalis* parasite while the remaining four tested negative (i.e. samples F_3, F_5, F_9 and F_{10}). The parasitic load ranged between 1 and 10 parasites.

Table 1: The Parasitic Load of the Volunteers' Swab Samples

Sample Code	Inference	Quantity
F_1	+	2
F_2	+	3
F_3	-	-
F_4	+	10
F_5	-	-
F_6	+	9
F_7	+	5
F_8	+	8
F_9	-	-
F_{10}	-	-
F_{11}	+	7
F_{12}	+	5
F_{13}	+	4
F_{14}	+	1
F_{15}	+	3
F_{16}	+	8
F_{17}	+	4
F_{18}	+	9
F_{19}	+	6
F_{20}	+	5
F_{21}	+	8
F_{22}	+	6
F_{23}	+	9
F_{24}	+	7
F_{25}	+	3
F_{26}	+	6
F_{27}	+	4
F_{28}	+	2
F_{29}	+	2
F_{30}	+	1

Table 2: The Phytochemical Constituents of *Mangifera indica* Plant's Extracts

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

Keys: + = Positive, - = Negative

After the plant's extracts were screened to ascertain their purity, there was visible and clear absence of microbial growth which confirmed the purity of the two extracts.

Table 2 shows the result of the phytochemical screening carried out on the leaf and bark extracts of *Mangifera indica* (mango) plant in other to determine their active constituents with supposedly anti-parasitic efficacy. Both extracts possess just tannins in common. In addition, the leaf extract possesses phlobatannins, anthraquinone glycosides and alkaloids while the bark extract possesses terpenoids. The table also shows that the leaf extract lacks saponins and terpenoids while the bark extract lacks saponins, phlobatannins, anthraquinone glycosides and alkaloids. 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 bark extracts of *Mangifera indica* 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 extracts 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 *Mangifera indica* Extracts

	n-Hexane Extract (mg/ml)					
Extract	**60.0**	**70.0**	**80.0**	**90.0**	**100.0**	**MIC**
Leaf extract	+	+	+	-	-	90
Bark extract	+	+	+	+	-	100

Keys: + = Active movement, - = Immobilization

MIC = Minimum Immobilization Concentration

The trichomonads isolated were all susceptible, as demonstrated by their immobilization to both crude extracts of the plant's leaves and barks at 100mg/ml (100%) only. They were susceptible to only the leaf extract at 90mg/ml (90%) but not the bark 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 bark 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 bark extract.

DISCUSSION

At the conclusion of the research, it was discovered that the phytochemical constituents of both leaf extract and bark extract of *Mangifera indica* plant possess some of phytochemica' 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 concentration. This confirms the potency of both the leaf and bark extracts of *Mangifera indica* plant against *T. vaginalis* but the leaf extract appeared to be more potent than the bark extract, especially that the crude bark extract showed no activity at 60mg/ml, 70mg/ml, 80mg/ml and 90md/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 the insufficient 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.

The presence of the active components of this plant may be due to its high non-polar compounds. This is similar to the findings of Chidozie *et al*. (2014) who also documented the absence of saponnins, glycosides and alkaloids in the bark extract of *Mangifera indica* but slightly at variance with it, as tannins were also found to be present in its bark extract. Chidozie *et al*. (2014) did not report the presence of tannins in their findings.

CONCLUSION

By this investigation, it could be deduced that this study showed that the *Mangifera indica* leaf and bark extracts used possess anti-protozoal potency against *T. vaginalis* but the leaf extract was found to be more efficacious than the bark extract. Indeed, the former is a promising pharmaceutical!

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