International Journal of Ecotoxicology and Ecobiology
2017; 2(1): 45-51
http://www.sciencepublishinggroup.com/j/ijee
doi: 10.11648/j.ijee.20170201.16

Comparative Study on the Anti-Parasitic Activities of *Securidaca* and *Senna occidentalis* Root Extracts Against *Trichomonas vaginalis*

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To cite this article:

Imam T. S., Abdullahi M. M., Dabo. N. T. Comparative Study on the Anti-Parasitic Activities of *Securidaca* and *Senna occidentalis* Root Extracts Against *Trichomonas vaginalis*. *International Journal of Ecotoxicology and Ecobiology.* Vol. 2, No. 1, 2017, pp. 45-51.
doi: 10.11648/j.ijee.20170201.16

Received: September 26, 2016; **Accepted**: October 13, 2016; **Published**: March 1, 2017

Abstract: *Trichomonas vaginalis* resistance to conventional antibiotics justifies the need to explore alternative remedies from medicinal plants since they represent a rich source of antiparasitic agents. This research was aimed to determine the inhibitory activities of two medicinal plants (*S. longepedunculata* and *S. occidentalis)* used to treat parasitic infections amongst the tribes of northern Nigeria. In the present study, extracts of the plants were obtained using maceration method, and their growth inhibitory activity against *Trichomonas vaginalis* evaluated *in vitro*. The extracts from the two plants revealed varied antiparasitic activities against the test organisms. The aqueous root extracts of the plants generally demonstrated higher growth inhibitory activity at 50 and 25mg/ml concentrations, while the least activity of the aqueous roots extracts were at lower concentration of 3.125mg/ml. The maximum antiparasitic activity was recorded for the aqueous root extract of *S.* synergistically against *T. vaginalis.* (Growth inhibition = 99.15%GI) at 50mg/ml. Findings from the statistical analysis, the result revealed significant difference ($P<0.05$) between *S.* and *S. occidentalis*. However, the result revealed significant difference ($P<0.001$), between the whole group of the aqueous root extracts with the negative control, and no significant difference among the other group of the aqueous roots extracts, The result presents the basis for which these plants have been used for treatment of *Trichomonas vaginalis* infections in traditional medicine. Results obtained suggest potential of the plants in the search for novel antiparasitic agents. Based on the findings from the current studies, the following suggestions are recommended: further researches should focus on other STI of medical importance such as *Gonorrhea*, *Chlamydia*, and Syphilis, and also research should be geared toward isolating active chemical component(s) and elucidate their exact mechanism(s) of action, safety margin and efficacy, Conservation of *S.* and *S. occidentalis* biodiversity should be also an important aspect to ensure sustainable availability of the plant.

Keywords: Anti-parasitic Activities, *S. longepedunculaat, S. occidentalis, T. vaginalis*

1. Introduction

Trichomoniasis is a sexually transmitted disease caused by the parasitic protozoan *Trichomonas vaginalis*. It is the most common nonviral sexually transmitted disease, with an estimated 170 million cases occurring worldwide each year [1]. *Trichomonas vaginalis* was first described by Donne' in 1836, research on this organism was began right from 20th century [2]. It was considered either a harmless vaginal colonizer or simply a minor nuisance. Therefore *Trichomonas vaginalis* resistance to conventional antibiotics justifies the need to explore alternative remedies from medicinal plants since they represent a rich source of antiparasitic agents. This is better supported by the fact that, the plant Family (Polygalaceae) to which *S. longepedunculata* and *J. curcas* belong, and (Caesalpiniodeae) family of *S. occidentalis* were known to be involved in ethnomedicine in the management of sexually transmitted diseases and some other ailments notably epilepsy [3].

Here a comparative evaluation of *in vitro* antiparasitic activities of different extracts from three medicinal plants

used to treat parasitic infections amongst the tribes of northern Nigeria was carried out. This idea of the research was derived from the folkloric utilization of these plant species in the treatment of sexually transmitted infections especially in women in northern Nigeria.

2. Materials And Methods

2.1. Collection and Identification of Plant Material

The fresh roots of *Securidaca ongepedunculata* and *Senna occidentalis* plants were collected from Rano Local Government Area, Kano State. The plants were identified using standard keys and description [4, 5]. The plants were further confirmed and authenticated in the Herbarium of the Department of Biological Sciences, Bayero University Kano, Nigeria.

2.2. Extraction of Plant Material

The protocol used by Adoum *et al.* [6] was adopted. The roots of *S. longepedunculata* and *S. occidentalis* plants were separately plucked and washed thoroughly, but gently with distilled water, at room temperature, and cut into small bits to facilitate drying. The pieces of plant material were dried under shade for three weeks. These were pounded using an electric blender and obtained a fine powder.

One hundred grams (100) of each plant root were percolated in 1000ml of sterile distilled water for 2 weeks with constant shaking at regular intervals. The percolates were then filtered, and the solvent evaporated using Rotor evaporator (R 110) at 40°C, and the extracts were stored in a refrigerator at 4°C for the analysis.

2.3. Phytochemical Screening of Aqueous Roots Extracts of the Selected Plants

The crude extracts of the samples were screened for phytochemicals in order to determine their chemical constituents using standard methods described by Evans and Trease [7] and Sofowora [8]. The tannins were determined by placing 1 g of each extract in 2cm^3 of distilled water after which it was filtered and iron (III) chloride reagent was added the alkaloids were tested by taking 0.5 g of the aqueous roots extract in 5 cm^3 of 1% HCl. This was then boiled, filtered and Mayer's reagent was added [6]. While for saponins, the extracts were subjected to frothing test. Anthraquinones were tested for, by treating 1.0g of each extract with 2 cm^3 of benzene, filtered and ammonia solutions were added [8]. The presence of flavonoids were determined using Shinoda's test for flavonoids by dissolving 0.5 g of each extract in 5 cm^3 of ethanol it which then was warmed and filtered. This was followed by the addition of magnesium chips to the filtrate and few drops of concentrated HCl [6]. The presence of reducing sugar was established by Fehling's test. For each extract, 0.5g were dissolved in distilled water and filtered. The filtrate was heated with 5cm^3 of equal volumes of Fehling's solutions A and B [8].

2.4. Collection of Test Organism

The protocol used by Donné [9] was adopted. Test microorganisms were obtained from Parasitological laboratory of Murtala Muhammad Specialist Hosipital, Kano and Sir. Muhammad Sunusi Specialist Hospital, which were then isolated from vaginal discharge of female patients attending Obstetrics and Gynecology unit. Informed consents of the subject were obtained prior to collection of sample, after explaining the procedure and justification of the research accordingly. Two vaginal swabs were obtained from Trichomoniasis infected women by sterile vaginal swab. The first swabs was obtained from the lateral walls of the vagina and were used to make a wet mount preparation on a glass slide with a drop of normal saline and looking for motile trichomonads. The second swab was obtained from the posterior fornix of the vagina and inoculated immediately after collection on TV-media at 32^{o}C it was then examined for motile trichomonades at 24, 48, and 96 hrs of incubation.

2.5. Preparation of Culture Medium

Trichomonas medium was prepared by dissolving the powder (Nutrient broth- 1.3gm, Glucose- 1.0gm, and L-cystieine hydrochloride- 0.2gm, powder) in 90ml of distilled water which was then boiled in order to obtain homogenized solution. This was sterilized by autoclaving at 121°C for 15minutes. It was then allowed to cool to 50°C. Inactivated pooled human serum (80ml) was added to it. Chloramphenicol (10gm/L of medium) was also added aseptically to the sterile medium, while pH was adjusted to 6.4 with 1mol/l NaOH. The medium was dispensed in sterile Bijou bottles in 2ml volumes and stored in the fridge [10, 11].

2.6. Bioassay of the Extracts on T. vaginalis

Assay of *J. curcas, S.* and *S. occidentalis* root extracts: 0.05g of the aquous root extracts each were diluted with 1ml dimethyl sulfoxide (DMSO). This gave a 50mg/ml concentration. The mixture was shaken gently in Eppendorf, tubes. Doubling dilution was cried out to give 25, 12.5, 6.25, 3.125 mg/ml concentrations. These various concentrations of the extracts each were tested against 50µl of the test organisms' (*T. vaginalis)* suspension. Metronidazole at the concentration of 100µg/ml was used as positive control against 50µl of the tests organisms' suspension and 100 mg/ml DMSO and phosphate buffer were used as the negative control accordingly. All tubes were incubated at 37°C after 24, 48, and 72 hours intervals. A drop of samples suspension prepared was placed on the heamocytometre slide and covered with a cover slip. The cover slip was pressed slightly until a rainbow ring forms at the either sides of or edges of the cover slip [12]. The heamocytometre slide was viewed under microscope (Hundmetzlar 640, Germany). At the centre of the slide, sixteen (16) squares were observed. Complete active and flagella active parasites were considered as viable [13].

2.7. Statistical Analysis

Percentage mortality of the parasites (PM %) was calculated using the following equation:

$$PM = \frac{a-b}{a} + 100 \quad (1)$$

Where "a" stands for mean number of viable parasites in negative control tube and "b" stands for mean number of viable parasites in test tube [14]. For comparing the Percentage mortality, Analysis of Variance (ANOVA) were applied using Statistical software (GraphPadInStat), to determine the significant difference between control and plant extracts at level of $p \leq 0.05$.

3. Results and Discussion

Table 1, below showed the result yield for the aqueous extraction of the root powder of the two selected plants evaluated. From 100g of each of the root powder of two selected plants tested, the crude root extract of *S.* weighted after extraction was found to be highest of 4.3g and the lest extract gained was for the root powder of *S. occidentalis* where it was found to be 3.1g.

3.1. Phytochemical Screening of Aqueous Roots Extracts of the Two Selected Plants

Table 2; presents the results of the phytochemical screening of *Securidaca* and *Senna occidentalis.* The phytochemical were given positive sign for easier identification of presence and negative sign for absent as presented in Table 2.

In the present investigation, the preliminary phytochemical screening of *S.* showed the presence of saponins, reducing sugar, tannins, triterpenoids, and alkaloids (Table 2), while the amino acid, flavonoids and anthraquinones was found to be absent.

Phytochemical screening of the aqueous root extracs of *S. occidentalis*shawed the presence of saponins, reducing sugar, flavonoids, tannins, anthraquinones, triterpenoids, and alkaloids while the amino acid was found to be absent (Table 2).

The result shawed that saponins, reducing sugar, tannins, triterpenoids and alkaloids, are all present in the aqueous root extracts of *S.* and *S. occidentalis*while amino acid, anthraquinones and flavonoids was found to be absent in the aqueous root extracts of *S.* and only amino acid was found absent in the aqueous root extract of *S. occidentalis* (Table 3).

Table 1. *Extract Yield for the Three Selected Plants Evaluated.*

S/N	Plant/ Root Powder	weight of Powder	Extract Yield	% Yield
1	SL	100g	4.3	4.3%
2	SO	100g	3.1	3.1%

Key: SL = Securidaca longepedunculata; SO = Senna occidentalis

Table 2. *Phytochemical constituents of aqueous extracts from roots of Securidaca longepedunculata and Senna occidentalis.*

Chemical compound	Aqueous extract	
	SL	SO
Saponins	+	+
Reducing sugar	+	+
Amino acids	-	-
Flavonoids	-	+
Tannins	+	+
Anthraquinones	-	+
Triterpenoids	+	+
Alkaloids	+	+

Key: + = Presence; - = Absence; SL = Securidaca; SO = Sennaoccidentalis

3.2. Effect of Aqueous Root Extract of S. and S.occidentalis on T. vaginalis

Findings from the statistical analysis, the result shows significant difference ($P<0.05$) between *S.* and *S. occidentalis* andbetween the whole group of the aqueous root extracts with the negative control, ($P<0.001$) (Table, 3).

In contrast, with the effect of aqueous roots extracts of *S. longepedunculata,* the percentage mortality (99.01% and 98.01%) were observed at the concentration of 50mg/ml and 25mg/ml respectively, while the least Mortality (89.50%) at the concentrations of 3.125mg/ml was observed in culture media, following 24, 48 and 72hours incubation (Table 3)

The higher percentage mortality (95.89%) of the parasite (*T. vaginalis*) due to the exposure of the aqueous root extracts of *S. occidentalis*, was observed at the highest concentrations of 50mg/ml while the least mortality (68.51 and 74.18%) were observe at the concentration of 6.25 and 3.125mg/ml respectively following 24, 48 and 72hours of incubation, (Table 3).

For the exposure of aqueous roots extracts of *S.* and *S. occidentalis* synergistically on *T. vaginalis* showed percentage mortality 99.01%at the highest concentration of 50mg/ml after 72 hours of incubation and 78.72% was revealed at the concentration of 3.125mg/m after 24, 48 and 72 hours of incubation in Culture medium (Table 3).

The negative control of the culture medium showed zero percentage (0%) mortality after 74 hours of incubation, while the positive control of the culture medium showed the percentage mortality (99.57%) at the concentration of 100µg/ml after 72 hours of incubation, (Tabele 3).

Table 3. *Percentage Mortality of T. vaginalis after Exposure to Various Concentrations of S. longepedunculata and S. Occidentalis in Comparison to Normal Control.*

Concentrations (mg/ml)	Percentage Mortality (%) according to extract				
	SL	SO	SLSO	Control (-ve)	Control (+ve)
50	99.01	95.89	99.01	0	99.57
25	98.01	91.49	96.03	0	
12.5	96.17	78.01	91.63	0	
6.25	92.19	68.51	84.97	0	
3.125	89.50	74.18	78.72	0	

Keys: SL = S. longepedunculata; SO = S. occidentalis; SLSO = S. longepedunculata & S. occidentalis

From the statistical analysis of the results, the mean count for the viable number of parasite (*T. vaginalis)* after the exposure of the aqueous root extracts of *S.* following 24, 48 and 72 hours of incubation at the highest concentration of 50 mg/ml was 2.33±2.52 and at the lowest concentration of 3.125 the mean standard deviation was found to be 24.67±17.79 after 24, 48 and 72 hours of incubation. (Table 4).

The Aqueous root extract of S. occidentalis in a concentration of 50mg/ml showed the least mean standard deviation (9.67±8.62) and the highest Mean standard deviation (74.00±53.69) was at the concentration of 6.5mg/ml after 24, 48 and 72 hours of incubation, (Table 4).

The highest Mean standard deviation (50.00±37.47) of the parasite (*T. vaginalis)* counted due to the exposure of aqueous root extracts of *S.* and *S. occidentalis* after 72hours of incubation was at the concentration of 3.125mg/ml while the least mean standard deviation (2.33±2.52) counted after 24, 48 and 72 hours of incubation was observed at concentrations of 50mg/ml (Table 4).

The activity of the aqueous roots extracts for the two selected plants individually and synergistically showed Mean Standard deviation was highest at the concentrations of 3.125mg/ml, and the lowest Mean standard deviation was observed at the concentration of 50mg/ml (Table 4). The Mean Standard deviation (1.00 ± 1.00) of the positive control was observed at the concentration of 100µl/ml (Table 4)

Table 4. *Mean Count for the Viable Parasite in the Various Concentrations of the Aqueous Roots Extracts of the Three Selected Plants on T. vaginalis in Culture Media after 72hours.*

Concentrations (mg/ml)	SL	SO	SLSO	Control (+ve)/100µg	Control (+ve)/0.00µg
50	2.33±2.52	9.67±8.62	2.33±2.52	1.00±1.00	235.00±25.00
25	4.67±3.51	47.33±46.44	9.33±4.51		
12.5	9.00±6.24	51.67±51.73	19.67±11.59		
6.25	18.33±14.98	74.00±53.69	35.33±31.97		
3.125	24.67±17.79	60.67±60.58	50.00±37.47		

LSD = 1.992

Figure 1: showed the percentage mortality of the parasite due to the exposure of the aqueous root extract of *Securidaca longepedunculata* after 72 hours of incubation. The Y-axes of the graph showed the number of the viable parasite par 50µl of the liquid culture medium and the X-axes showed the different level of mortality of the parasite (*T. vaginalis*) at the different concentration (mg/ml) of the aqueous root extracts used after 24, 48 and 72 hours of incubation.

Figure 2 showed the percentage mortality of the parasite due to the exposure of the aqueous root extract of *Senna occidentalis* after 72 hours of incubation. The Y-axes of the graph showed the number of the viable parasite par 50µl of the liquid culture medium and the X-axes showed the different level of mortality of the parasite (*T. vaginalis*) at the different concentration (mg/ml) of the aqueous root extracts used after 24, 48 and 72 hours of incubation.

Figure 3 showed the percentage mortality of the parasite due to the exposure of the aqueous root extract of *Securidaca longepedunculata* and *Senna occidentalis* synergistically after 72 hours of incubation. The Y-axes of the graph showed the number of the viable parasite par 50µl of the liquid culture medium and the X-axes showed the different level of mortality of the parasite (*T. vaginalis*) at the different concentration (mg/ml) of the aqueous root extracts used after 24, 48 and 72 hours of incubation.

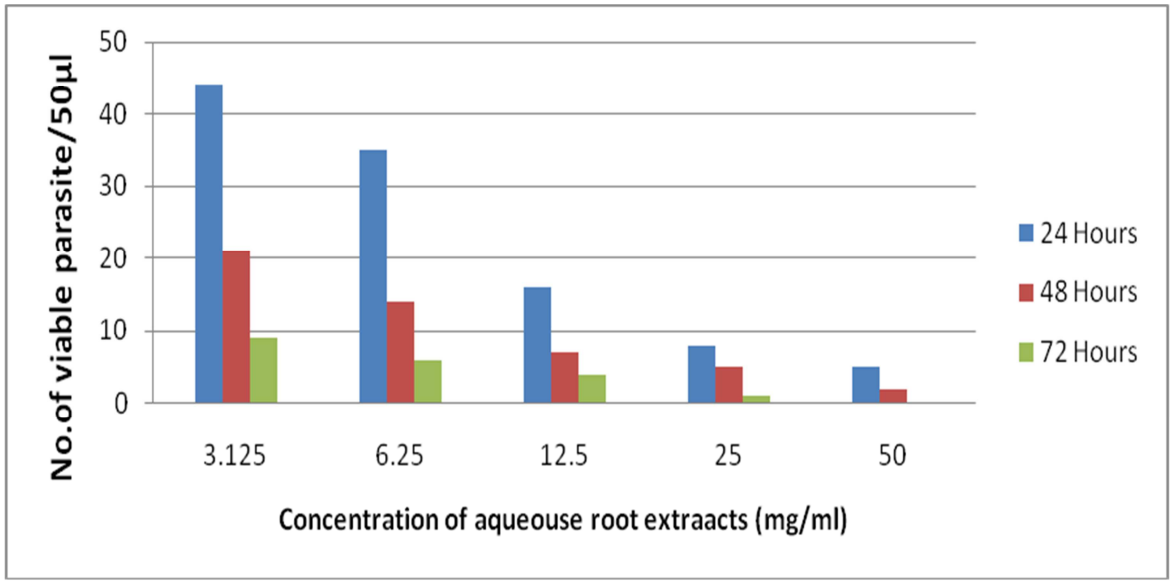

Figure 1. *Effect of aqueous root extracts of S. longepedunculata on T. vaginalis culture medium following 24, 48, and 72 h.*

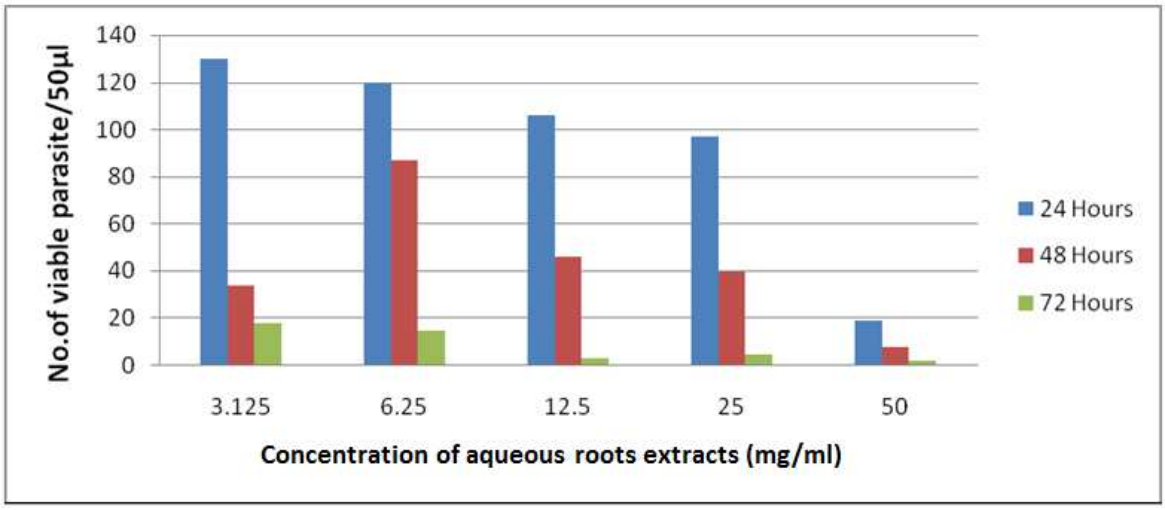

Figure 2. *Effect of aqueous root extracts of S. occidentalis on T. vaginalis in culture medium following 24, 48, and 72 h.*

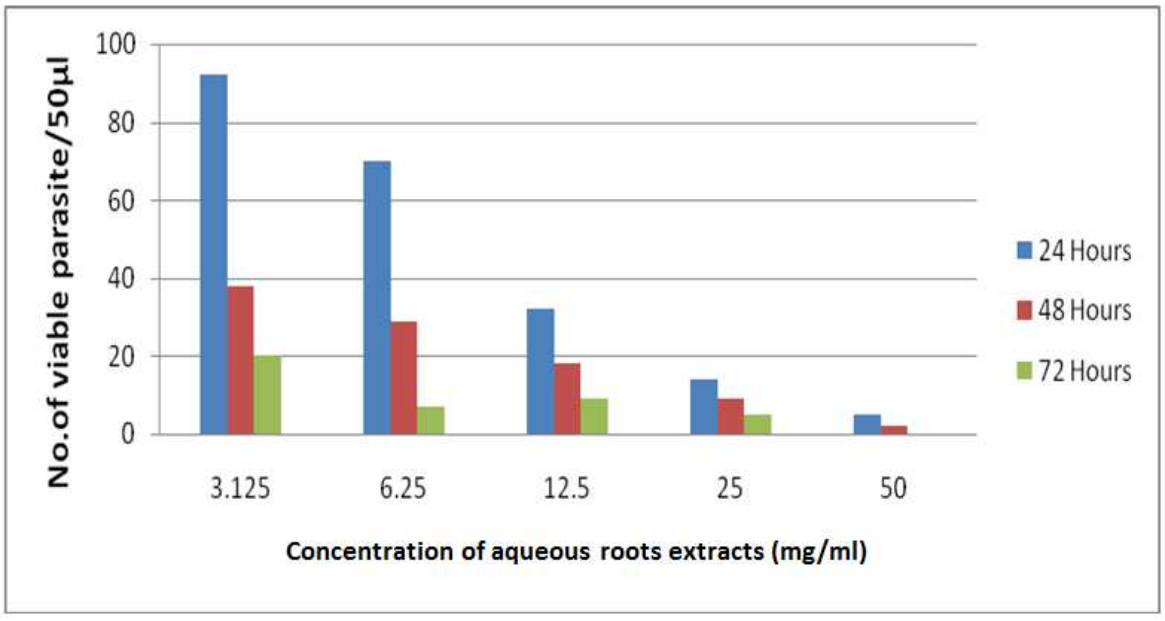

Figure 3. *Number of Viable T. vaginalis (Parasites) in 50µl Challenged with Various Concentrations of the Combined Aqueous Root Extracts of Securidaca longepedunculata and Senna occidentalis on T. vaginalis in culture medium following 24, 48, and 72 h.*

4. Discussion

Among the plausible explanations of these results is that successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure, properties of a good solvent in plant extraction that induces ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, a preservative action and inability to cause the extract to complex or dissociate [15]. The choice will also depend on targeted compounds. The most commonly used solvents for investigation of microbial activity in plants are methanol, ethanol, and water [16]. In this study water extract was used for the extractions of bioactive compound.

Previous studies on aqueous root extracts of *Securidaca* reported the presence of flavonoids alkaloids, tannins, and saponins, [17]. The present study also correlated with the aforesaid studies. Presence of varieties of chemical compounds impact significant amount of biological activities of *S.* extracts.

The result of the phytochemical screening of the aqueous root extract of and *S. occidentalis* showed the presence of saponins, reducing sugar, flavonoid, tannins, anthraquinone, triterpenoids and alkaloids, and for the *S. longepedunculata* the result showed the presence of saponnins, reducing sugar, tannins, triterpenoids and alkaloids. The presence of tannins and saponins in aqueous root extracts of *S.,* agrees with the previous work on phytochemical constituents of the aqueous root bark extract of *Securidaca longepedunculata* [17]. This may be due to the greater solubility of saponins and tannins in aqueous solution [18].

The presence of these chemical constituents in the aqueous root extract of *S. longepedunculata* and *S. occidentalis* was indicating that these plants if properly screened would yield

drugs of plant origin with pharmacological significance. This is better supported by the fact that, the plant Family (Polygalaceae) to which *S. longepedunculata* and *J. curcas* belong, and (Caesalpiniodeae) family of *S. occidentalis* were known to be involved in ethnomedicine in the management of sexually transmitted diseases and some other ailments notably epilepsy [3].

The interesting point to note is that from the result all the extracts showed response to the mortality of *T. vaginalis*, in culture media. As it has been observed from Table 3 and from Figures 1 to 3 the percentage mortality (%PM) of the combined extract (SLSO) and single extracts of SL at 50mg/ml concentrations was 99%, while for the single extract of SO, at the same concentrations was found to be 95%GI. Aiyelaagbe *et al.* [19]; and Sebua *et al.* [20] showed that the roots of these plants used were effective against venereal diseases, such as Vagina itches, which is causes by sexual infections, although, they did not mentioned the types of sexual disease, this results prove the efficacy of *S.* and *S. occidentalis* against *T. vaginalis* [20, 21].

The results indicated that the extracts are more effective against *T. vaginalis* at higher concentrations of 50 and 25mg/ml after 72 h. respectively, and in comparison to positive control. It seems the difference between the activities of the extracts can be due to the presence of different bioactive compounds which are of greater solubility in aqueous solution like saponins and tannins [18].

In all of the extracts the statistical analysis of the result, revealed that the higher the concentrations of the aqueous root extract the higher the mortality and the lower the concentrations of the aqueous root extract the less mortality. And statistically it is the same that the Mean standard deviation of the viable parasites (*T. vaginalis*) counted after 24, 48 and 72 hours of incubation revealed that the higher the concentration the more mortality of the parasites (*T. vaginalis*) and the lower the concentration of the aqueous root extract the least mortality of the parasite (*T. vaginalis*). This indicated that the mortality of the parasites was base on concentration dependant. This effect was observed in the previous studies that explored the use of plants crude extracts against *T. vaginalis in vitro* [22, 23, and 24].

5. Conclusion and Recommendations

5.1. Conclusion

Among the extracted plants, *S. longepedunculata* aqueous root extract (4.3g) was found to have more extracted bioactive components. The mortality of *T. vaginalis* was shown very high at the highest concentration of 50 mg/ml of aqueous root extracts. The potency was compared among the extracts as well with the standard antiparasitic metronidazole. There was no significant difference between the two in terms of activities, which could be due to the presence of different bioactive compounds like flavonoids, triterpenoids, saponins, and tannins [17]. Conclusively, the result obtained in this work has buttressed the efficacy of these crude extracts as has been used popularly in the northern Nigeria.

5.2. Recommendations

Based on the findings from the current studies, the following suggestions are recommended:

(1) Research should be geared to isolate active chemical component(s) and elucidate their exact mechanism(s) of action, safety margin and efficacy.

(2) Both acute and sub acute as well as chronic toxicity test should also be conducted in order to acess fully the impact of toxicity effect of the extract to the tissue damage of the *Rattus norvegicus* (Albino rats)

(3) Further research should be focus on other STD of medical importance such as Gonorrhea, Chlamydia, and Syphilis, etc.

(4) Research should be geared to isolate active chemical component(s) and elucidate their exact mechanism(s) of action, safety margin and efficacy.

(5) Conservation of *S. longepedunculata* and *S. occidentalis* biodiversity should be an important aspect to ensure sustainable availability of the plant.

(6) Government should set a program that should provide public enlightment about the risk and danger of sexual transmitted diseases not only HIV awareness.

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