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PHARMACOLOGICAL EFFECTS OF LEAVES AND FRUITS OF *Solanumerianthum* (Solanaceae) FOR ANTI-TRICHOMONAL ACTIVITY

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ABSTRACT

The treatment of trichomoniasis has been based on the use of metronidazole developed in 1959 and the derivatives but has now been stopped because of its effect on the DNA.The extracts of *Solanumerianthum* D. Don. leaves and fruits were evaluated to determine the phytotherapeutic activities against *Trichomonas gallinarum in vitro.* The leaves and the fruits of *S.erianthum* were extracted with methanol to obtain leaves and fruits extracts coded as SEL and SEF respectively. The SEL and SEF were further purified by partitioning into Petroleum ether (SEL-P & SEF-P), chloroform (SEL-C & SEF-C), ethyl acetate (SEL-E&SEF-E) and aqueous (SEL-A&SEF-A) fractions. The SEL, SEF and their partition fractions were each tested for anti-trichomonal activity. The anti-trichomonal activities of SEL and SEF at 24h and 48 h showed that SEL was more active than SEF with LC_{50} and LC_{90} of 134.70±35.90, 153.64±91.84, 554.0±55.72, 749.61±41.66, (µg/ml) and 45.55±11.57, 56.51±5.52, 132.02±26.20, 247.66±41.66 (µg/ml) respectively. The SEL-Eactivity observed at 48h gave LC_{50} and LC_{90} of 26.98±1.1, 14.04±4.04 and 79.43±2.94, 64.37±4.37 (µg/ml). Comparatively the purified fraction SEL-E had very good rating with the standard drug (metronidazole) at 48 h which had LC_{50} and LC_{90} of, 14.04±4.04 and 64.37 ±4.37 (µg/ml) respectively. The SEL-E fraction and standard drug at 48 h did not show significant difference (P>0.05) in their bioactivities and it is therefore a potential source of drug for the treatment of trichomonasis.

Keywords: Crude methanolicextract, trichomonasis, anti-trichomonal, *Trichomonasgallinarum*, *Solanumerianthum*and Metronidazole.

INTRODUCTION

Trichomoniasis refers to an infection of the genital and urinary tracts. It is the most common sexually transmitted disease, affecting about 180 million people annually worldwide (.Hook Edward W.1999). The estimates for North America alone are between 5 and 8 million new infections each year, with an estimated rate of asymptomatic cases as high as 50%. (HookEdward W.1999). Some of the complications of *Trichomonasvaginalis*in women include: preterm delivery, low birth weight, and increased mortality as well as predisposing to HIV infection, AIDS and cervical cancer (Mulla, *et al*., 2009, Schwebke JR and Burgess D, 2004).Having *T.vaginalis* also may increase the chances of the infected woman passing on the HIV virus to her sex partner(s) (Mavedzenge*, et al*., 2010). Trichomonads are found in the mouth, where they may contribute to gingivitis, in the intestine

where they may be associated with diarrhea conditions and in the urethra and vagina, where they cause an inflammation and purulent discharge (Ibikunle*et al*., 2011, Ibikunle GF and Ogbadoyi, EO, 2011, Omisore*et al*.,2005, Camacho *et al.,* 2003, Pelletier KR, 2002, Meingassner JG and Thurner J, 1979).

Developed in 1959, metronidazole was approved for the treatment of trichomoniasis in the early 1960sand currently the only drug approved for the treatment of trichomoniasis (CosarC andJulouL, 1959).The metronidazole and its derivatives have effect on the DNA (Narcisi EM and Sacor NE, 1996) and unfortunately, metronidazole-resistant *T. vaginalis* has been implicated in an increasing number of refractory cases. Clearly, alternative curative therapies are needed, given the wide spreadoccurrence and impact of the disease.

Solanumerianthum D Don.(Solanaceae) Synonym S. verbascifoliumauct.non Linnis a shrub or small tree about 6m high, native of Central America, and now pan-tropical, and present in forest areas, coastal areas and throughout the Western part of Nigeria (Bukenya-ZirabaR and Hall JB, 1988).Originally from the West Indies, Central America and Mexico, but now an almost pan tropical weed, although hardly penetrating South America (Blomqvist M M and Nguyen TB, 1999). Ethno medically the leaves act as an anti-virginal discharge, abortifacient and are considered a potent medicine for expelling all impurities through the urine, and in particular to treat leucorrhoea. Pounded leaves are poultice to treat piles, haemorrhoids and scrofula (Blomqvist MM and Nguyen TB, 1999). Heated leaves are applied as a cream to the forehead against headache. A decoction of the leaves is drunk against vertigo; an infusion of the plant is used for a bath after childbirth. Various plant parts are ground with warm water and applied externally to lessen inflammation, burning sensation and pain. In Papua New Guinea, the plant is used internally to treat stomach-ache and is applied externally to skin irritations and rashes. In the Solomon Islands, leaf juice is used as a rinse for sores in the mouth. *S. erianthum* is considered poisonous to livestock. The root bark is poisonous and can be used as an antiphlogistic and against arthritis. The fruits can be eaten when cooked. The velvety leaves are used to remove grease from dishes in Nigeria and Philippines **(**Burkill HM, 2000; Blomqvist MM and Nguyen TB, 1999).

Due to its ethno medicinal uses, the *S. erianthum*as anti-virginal discharge, the present study was conducted on the plant leaf and fruit to evaluate its anti-trichomonas activity by using *in-vitro* techniques.

MATERIALS AND METHODS

1 Materials

Birds and eggs

Local Pigeon *Columba lavia* Anth (Columbidea) and the raw chicken eggs were purchased from Central Market, Minna, Niger State, Nigeria. The parasites *T. gallinarum* were isolate from the throat of local Pigeon *C. lavia* using sterilized swab stick and saline (Ibikunle GF and Ogbadoyi EO, 2011). The trichomonas in saline solution was multiplied by culturing in test tubes containing prepared egg slant and overlay (50ml ringer solution which consist of–NaCl, 6.5g; $NaHPO_4$, 0.01g; KCl, 0.14g; $NaHCO_3$, 0.2g; $CaCl_2$, 0.12g; glucose (2.0g dissolved in 1L with distilled water and sterilized- + 1 $cm^3$of 10% glucose solution + 1 cm^3Cow blood serum) and then incubated at 37^oC for 24 hours (Omisore*etal*. 2005, Ibikunle*et al*. 2011, Ibikunle GF and Ogbadoyi EO, 2011).

2 Plant material

S.erianthum (Solanaceae) leaves and unripe fruits were collected in June 2009 from the shrubs at Iddo-Ekiti, Ekiti State, Nigeria and authenticated at Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria with herbarium number 6868. Immediately after the collection, the leaves and unripe fruits were garbled to remove the unwanted parts and then air dried in the laboratory. They were then powdered and kept in polythene bags until needed.

3 Preparation of Extracts.

3.1 Preparation and Extraction of Plant material.

The powdered plant material 40.0 and 60.0gram of *S. erianthum* leaves and *S. erianthum* fruits were cold extracted five times for six days with 100% methanol by maceration (250 cm^3 x 6 days). The crude extracts were bulked and concentrated *in-vacuo* using a rotatory evaporator and coded SEL and SEF respectively. The weight and dry weight yield of the crude methanolic extracts were SEL(3.91g & 9.78%) and SEF(7.03g & 28.78%), yield respectively.

3.2 Partition Of Crudemethanolic extracts (SEL &SEF)

Methanolic extracts of $SEL_{2.5g}$and $SEF_{6.1g}$ were suspended in 50 cm^3water each. The two of them were separately solvent partitioned (5 x 50 cm^3) using separating funnel, concentrated *in vacuo*and coded Petroleum ether (SEL-P & SEF-P), chloroform (SEL-C & SEF-C), ethyl acetate (SEL-E & SEF-E) and aqueous (SEL-A & SEF-A). The partitionfractions were each tested for anti-trichomonal activity.

4 Anti-trichomonal assay

The assay was done according to the method of Omisore*et al*. (2005) and Ibikunle*et al*. (2011). 5mg of the Crude Methanolic extract (SEL and SEF) and their partition fractions from petroleum ether (SEL-P & SEF-P), chloroform (SEL-C & SEF-C), ethyl acetate (SEL-E & SEF-E) and aqueous (SEL-A & SEF-A) were tested for anti-trichomonal activity respectively with each dissolved in 1 cm^3 of dimethylsulphoxide (DMSO) and diluted serially to give 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.812 and 3.906 µg/ml concentrations. The same serial dilutions were prepared for the positive control using metronidazole as the standard drug. The inoculum (150 µL) was added into each well of the 96-well flat-bottom microwell plates and 50 µL of the test substance or metronidazole in its appropriate concentration was then added. The plates were there after incubated at 37^0C and the cell growth was monitored at 24 and 48 hours by counting their numbers with the microscope. For the negative control, 50 µL of overlay with DMSO was added to the 150 µL inoculum instead of the test substance (Narcisi EM and Secor NE, 1996). The tests were repeated six times (N = 6) for the test agents and the controls while the lethal doses (LC_{50} and LC_{90}) were determined at 24 and 48 hours using the Finney Probit analysis and Minitab14 computer statistical software (McLauglinJL. 1988).

RESULTS

The percentage yields of the crude methanolic extract were 9.78% and 28.75% for sample SEL and SEF respectively. While their partition fractions in petroleum ether, chloroform, Ethyl acetate and aqueous were 19.6% -SEL-P, 5.6% -SEL-C, 4.0% - SEL-E, 48.4% - SEL-A and 20.16% - SEF-P, 6.72% - SEF-C, 6.88% - SEF-E and 62.3% - SEF – Arespectively (see Table 1).

The antitrichomonas activities from the crude methanolic extract of the leaves SEL and the fruit SEF at 24h and 48h showed that SEL is more active than SEF with LC_{50} and LC_{90}134.70±35.90, 153.64±91.84, 554.0±55.72, 749.61±41.66, .(µg/ml) and 45.55±11.57, 56.51±5.52, 132.02±26.20, 247.66±41.66 (µg/ml) respectively. The partition fractions of SEL indicated that the highest cidalactivities reside in ethyl acetate fraction of the leaf SEL-E at 48h with LC_{50} and LC_{90} as 26.98±1.1 and 79.43±2.94 (µg/ml) respectively. The partition fractions of SEF indicated that cidalconstituents was dispersed in all the fractions at 48 h with LC_{50} ranging from47.34^{de}±19.49 to 77.53^{bc}±8.76and LC_{90} as 147.83^{cd}±14.05 to 164.82^{cd}±21.94 (µg/ml) respectively (table II).

Table 1: ***The table of crude methanolic extract of Solanumerianthum leaf(SEL) and fruits (SEF)and their partition fractions***

The code for the fractions	SEL-P	SEL-C	SEL-E	SEL-A	SEF-P	SEF-C	SEF-E	SEF-A
Weight of the crude partitioned (g)	2.5				6.1			
Weight of each fraction	0.49	0.14	0.10	1.21	1.23	0.41	0.42	3.8
% of the fraction	19.6	5.6	4.0	48.4	20.16	6.72	6.88	62.3

Key: S. erianthumleaves crude methanolic extract = SEL, S. erianthum fruits crude methanolic extract = SEF, petroleum Partition fraction of SEL =SEL-P, Chloroform Partition fraction of SEL =SEL-C, Ethyl acetate Partition fraction of SEL =SEL-E, Aqueous fraction of SEL = SEL-A, petroleum Partition fraction of SEF =SEF-P, Chloroform Partition fraction of SEF=SEF-C, Ethyl acetate Partition fraction of SEF =SEF-E, Aqueous fraction of SEF =SEF-A.

Table2:*Anti-trichomonal Activity of crude methanolic extract of the leaves (SEL) and fruits(SEF) with their partition fractions into petroleum, Chloroform Ethyl acetate and Aqueous respectively.*

Extract/Fractions	24hours		48hours	
	LC50(µg/ml)	LC90(µg/ml)	LC50(µg/ml)	LC90(µg/ml)
SEL	134.70^{bc}±35.90	554.0^{c} ± 55.72	45.55^{de}±11.57	132.02^{d}±26.20
SEF	153.64^{b}±91.84	749.61^{b}±41.66	56.51^{cd}±5.52	247.66^{c}±41.66
SEL-P	37.97^{d}±3.14	113.82^{de}±14.49	83.99^{b}±14.96	360.91^{b}±39.54
SEL-C	357.52^{a}±49.20	1297.05^{a}±169.85	49.43^{de}±4.50	159.26^{cd}±10.8
SEL-E	120.20^{bc}±15.80	534.51^{c}±109.7	26.98^{ef}±1.1	79.43^{d}±2.94
SEL-A	50.70^{d}±10.98	114.34^{de}±3.84	154.45^{a}±40.04	581.68^{a}±149.46
SEF-P	127.2^{bc}±9.30	231.31^{d}±26.08	48.56^{de}±5.00	158.79^{cd}±16.95
SEF-C	76.46^{cd}±4.26	180.99^{d}±5.77	48.07^{de}±6.62	164.82^{cd}±21.94
SEF-E	56.58^{d}±0.41	702.32^{b}±78.02	77.53^{bc}±8.76	160.07^{cd}±59.99
SEF-A	135.22^{bc}±21.78	497.49^{c}±79.59	47.34^{de}±19.49	147.83^{cd}±14.05
Metronidazole	16.67^{d}±6.67	45.86^{e}±5.86	14.04^{f}±4.04	64.37^{d}±4.37

Mean values on the same column with different letters as superscripts were significantly different from each other (p < 0.05). The values are ± standard deviation.

Key: *S. erianthum* leaves crude methanolicextract= SEL, *S. erianthum* fruits crude methanolic extract = SEF, petroleum Partition fraction of SEL = SEL-P, Chloroform Partition fraction of SEL = SEL-C, Ethyl acetate Partition fraction of SEL =SEL-E, Aqueous fraction of SEL = SEL-A, petroleum Partition fraction of SEF = SEF-P, Chloroform Partition fraction of SEF= SEF-C, Ethyl acetate Partition fraction of SEF = SEF-E, Aqueous fraction of SEF = SEF-A.

DISCUSSIONS

The cidal effect of the crude methanolic extract of *S. erianthum*leaves (SEL) and *S. erianthum*fruits (SEF) at 48 h indicated that both could be used to treat chronic trichomonasis. Meanwhile SEL was more active both at 24 h and 48 h indicating that it is potentially better than SEF for acute and chronic trichomonasis.The partition fractions of (SEL) further revealed that fractions SEL-P and SEL-A have cidal effect at 24h only, while fractions SEL-C and SEL-E showed efficacy for chronic at 48h. The activity of fractions SEL-C and SEL-E at 24 h and cidal activity at 48 h suggest their potential use for acute and chronic trichomonasis (IbikunleGF and Ogbadoyi EO, 2011). Furthermore the fruit partition fractions(SEF) indicated slight improvement over the crude at 24 h and 48h with fractions SEF-C and SEF-E, but the SEF-A was the best at 48h. Although fractions SEF-P, SEF-C,SEF-E and SEF-Acan perform the function of the standard drug at 48 h as well serve as prophylaxis and therapeutic controls but only SEF-C had the pharmacological properties to perform the acute and chronic like the Metronidazole on trichomonasis. Comparatively the

purified fraction SEL-E had very good rating with standard drug, Metronidazole at 48 h which had LC_{50} and LC_{90} of 26.98±1.1, 14.04 and 79.43±2.94, 64.37(µg/ml) respectively. The standard drugdid not have significant difference ($p > 0.05$) with fraction SEL-E at 48 h, meaning that it has acute and chronic potency against trichomonasis like the Metronidazole. Meanwhile fraction SEL-P was more efficacious at 24h and lost its efficacy at 48h this probably means that the fraction could be for acute infection. The fractions from fruit did not showed distinct pharmacological and statistical difference ($p>0.05$) at 48 h from each other implicating that the crude SEF effect could be due to synergetic effect or the active constituents were dispersed in all the fractions. Furthermore, despite the fact that SEF fractions were all active and did not show significant difference ((p >0.05) from each other, they could statistically perform the same pharmacological functions of the standard drug.

CONCLUSIONS

Generally, the people of Nigeria still have strong belief in the efficacy and success of herbal medicine. The results of the present study therefore provide evidence that medicinal plants *S. erianthum,* continue to play an important role in the treatment of trichomonasis of this tribal community. Anti-trichomonal activity directed purification of *S. erianthum* leaf extract made partition fraction from SEL-E comparable with metronidazole in activity. This result has justified the ethno medicinal importance and the application of the plant for trichomonasis. Therefore the anti-trichomonal and its chemotherapeutic potential may need to be further exploited.

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