British Journal of Pharmacology and Toxicology 7(2): 20-25, 2016
DOI:10.19026/bjpt.7.2859
ISSN: 2044-2459; e-ISSN: 2044-2467

Submitted: October 10, 2014 Accepted: April 7, 2015 Published: May 25, 2016

Research Article

In Vitro Evaluation of Extracts of Solanum torvum (Solanaceae) Fruits for Anti-Trichomonal Activity

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Abstract: Trichomonasis is curable when treated. However there is increasing incidence of resistance to therapeutic drugs. *Solanum torvum* has several ethnomedicinal applications and therefore a potential source of antiparasitic drugs. The objective of this study was to assess the therapeutic potentials of extracts of fruits of *Solanum torvum* against *Trichomonas gallinarum*. Crude methanol extract of *Solanum torvum* was partitioned into petroleum ether (STF_1), chloroform (STF_2), ethyl acetate (STF_3), aqueous (STF_4) and STF_5 ($_{precipitate}$) fractions. STF_1, STF_2 and STF_3 were each subjected to vacuum liquid chromatography to obtain STF_{11-17}, STF_{21-28} and STF_{31-37}. The crude extract STF_0, partition fractions STF_{1-5} and sub fractions STF_{11-17}, STF_{21-28} STF_{31-37} were assayed for anti-trichomonal activity. The crude extract, STF_0, at 24 h and 48 h showed LC_{50} and LC_{90} of 386.34±17.96, 2168.31±119.43, 85.81±20.10, 324.72±17.73 (µg/mL), respectively. STF_5 ($_{precipitate}$) had LD_{50} and LD_{90} at 24 and 48 h as 25.84±5.12, 70.36±15.64 and 13.87±1.61, 42.94±6.20 (µg/mL), respectively. STF_1, STF_2 and STF_3 had LD90 at 48h as 72.76±1.72, 119.39±3.90 and 114.54±36.08 (µg/mL) while STF_{13}, STF_{14}, STF_{23} and STF_{34} had corresponding values of LD_{90} at 48h as 67.18±14.90, 88.07±4.78, 89.95±7.99 and 84.03±8.03 (µg/mL). The partition fractions STF_1, STF_2 and STF_3, the VLC sub fractions STF_{13}, STF_{14}, STF_{23}, STF_{34} and the standard drug did not show significant difference ($p>0.05$) in their bioactivities. It is concluded that methanol extracts of the fruits of *Solanum torvum* are a potential source of drug (s) for the treatment of trichomoniasis.

Keywords: Solanum, torvum, trichomonas vaginalis, trichomoniasis, trichomonas gallinarum

INTRODUCTION

Trichomoniasis is a genitourinary tract, largely nonreported, sexually transmitted disease caused by the multiflagellated protozoan parasite, *Trichomonas vaginalis*. In males, infection. is restricted to the urethra and prostate and in females, to the lower part of the genital tract. the vagina, vulva urethra (Eckert, 2005). The only known trichomonas host to date are humans and the parasite is transmitted from one person to the other mainly through sexual intercourse (Petrin *et al*., 1998). Although very rare, transmission through means other than sexual contact involves contaminated douche nozzles, moist wash-clothes, specula or toilet seats (Petrin *et al*., 1998; Burch *et al*., 1959; Peterson and Drame, 2010). Infection of newborn by the mother during vaginal delivery is also possible and has been reported in 2-17% of cases (Petrin *et al*., 1998).

In about 70% of people infected with trichomoniasis, there are no signs or symptoms of the disease http://www.cdc.gov/std/Trichomonas/STDFact-Trichomoniasis.htm). Although the disease is generally asymptomatic, especially in men in women in the first six months of infection, symptoms may include scanty vaginal secretion mixed with mucus, a copious discharge that is foamy, may be clear, white, yellowish or greenish with offensive odour (Cudmore *et al*., 2004). Other symptoms in women are itching, burning, redness, or soreness of the genitals discomfort while urinating. In men, symptoms may include itching or irritation inside the penis, burning after urination or ejaculation, or some discharge from the penis (http://www.cdc.gov/std/Trichomonas/ STD Fact-Trichomoniasis.htm).

Although the disease does not kill, it is a serious public health problem largely because of associated complications and its implication as a predisposing factor for HIV infection these complications and associated hazards include: Development of prostate cancer, preterm delivery, prenatal morbidity, delivery of low-birth-weight, cervical cancer, sterility increased risk of infection with other sexually transmitted diseases such as gonorrhoea, chlamydiasis and yeast infections (http://en.wikipedia.org/wiki/Trichomoniasis downloaded on 16/07/2012).

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The high prevalence rate of the disease also contributes to its being of very important public health problem. With a global incidence of over 180 million people infected annually (Weinstock *et al*., 2004) is no consensus figure for the prevalence of trichomoniasis in Nigeria but reports of prevalence rates of 9.7, 10.2, 11.1, 23.53, 24.7% up to 74.5% have been documented (Prevalence of trichomonas vaginalis in patients with vaginal discharge in Lagos, Nigeria (Ogunbanjo *et al*., 1989; Anosike *et al*., 1993; Woken, 2006; Ulogu *et al*., 2007).

Chemotherapy is yet the main method of control although preventive measures of safe sex and avoidance of having multiple sex partners can significantly limit infection. Trichomoniasis is completely curable but the chemotherapeutic armoury is extremely inadequate as only either metronidazole or tinidazole is currently used for the treatment of the disease. This is unacceptable because of the increasing incidence of resistance to therapeutic drugs by infective agents.

Pants provide excellent sources for antiparasitic drugs and the use of herbal preparations for the treatment of trichomoniasis has been reported and such herbs include garlic, Echinacea, goldenseal, barberry, milk thistle, tea tree oil and Chinese gentian root (Long Dan Chao) tea (http://herbs.lovetoknow.com/ Natural_Cures_for_Trichomonas; http://www.ehow. com/how_542 7023_heal-trichomoniasis-herbs.html downloaded 18/07/2012). It is believed that these natural remedies act by boosting the immune system and subsequent clearance of the parasites. Antitrichomonal activity of several other plants have also been documented in the literature (Hakizamungu *et al*., 1992; Calzada *et al*., 2007; Desrivot *et al*., 2007; Fernandes *et al*., 2008; Ibikunle and Ogbadoyi, 2011a).

The use of metronidazole and its derivatives for the treatment of trichomoniasis, apart from the resistance problem, is limited by the effects on the DNA (Narcisi and Sacor, 1996) Clearly, alternative curative therapies are needed, given the wide spread occurrence and impact of the disease.

Solanum torvum Solanaceae plants produce small perfectly spherical fruits and are commonly grown in the tropical regions of Nigeria. The fruits are used in some areas of the world as sauces, condiments, spices food flavorings (Burkill, 1985).

The ethno medicinal applications includes treatment of arteries, naso-pharyngeal affections, veins, cutaneous, subcutaneous parasitic-infections, diarrhoea, dysentery, insanity, pain killer, gout, epilepsy, dropsy, swelling, oedema, stomach troubles, sedatives, diuretic tonic for liver and pulmonary troubles (Burkill,1985). Antiviral and rodenticidal agents from extracts of *Solanum torvum* fruits have also been documented in the literature (Chah *et al*., 2000; Arthan *et al*., 2002; Muhammud *et al*., 2008).

Methanolic extract of *S. torvum* is known to reduce blood pressure, vascular reactivity changes to catecholamines and reverse metabolic alterations induced by fructose (Muhammud *et al*., 2008).

The isolated methyl caffeate (1) from the extract inhibited sucrase and maltase in rat intestinal. Also caffeoyl derivatives have sucrase-and maltase-inhibitory activities. Methyl caffeate moderate inhibitory action against α-glucosidase provides a prospect for antidiabetic usage of *S. torvum* fruit.

In an *in vitro* study of *S. torvum* against human pathogenic strains and animal clinical isolates, the water, methanolic and ethanol extracts were found effective against all bacterial strains with an inhibition comparable to that of commercial antibiotics (Takahashi *et al*., 2009; Chah *et al*., 2000).

In this study, crude and partially purified extracts of *S. torvum* were screened for *in vitro* activity against *Trichomonas gallinarum*. We report here that crude extract of *S. torvum* and fractions thereof possess remarkable anti-trichomonas activity against *T. gallinarum*. fractions there of possess remarkable anti-trichomonas activity against *T. gallinarum*.

MATERIALS AND METHODS

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 isolated from the throat of local Pigeon *C. lavia* as previously described. (Ibikunle and Ogbadoyi, 2011a).

Plant material: *Solanum torvum* (Solanaceae) 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 14 Immediately after the collection, the 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.

Methods:

Preparation of extracts: The powdered plant material 100 g of *S. torvum* fruits was cold extracted in 250 cm^3 five times for six days with 100% methanol by maceration. The crude extracts were bulked and concentrated *in-vacuo* using a rotatory evaporator and coded STF. The weight and % dry weight yield of the crude methanolic extract were 7.03 g and 7.03%, respectively.

Partitioning of crude methanolic extracts (STF): Methanolic extracts of STF was suspended in 50 cm^3 water. It was solvent partitioned (5×130. 50 cm^3) using separating funnel, concentrated *in vacuo* and coded

Petroleum ether (STF_1), chloroform (STF_2), ethyl acetate (STF_3), aqueous (STF_4) and aqueous precipitate (STF_5). The partition fractions were each tested for anti-trichomonal activity.

Vacuum Liquid Chromatography (VLC) of STF_1, STF_2 and STF_3: The three active fractions (STF_1, STF_2 and STF_3) were separately subjected to VLC, gradient eluted with petroleum ether, $CHCl_3$ and MeOH. Thin Layer Chromatography (TLC) was used to determine the bulking order of gradient fractions collected and the resulting subfractions were coded as STF_{11-17}, STF_{21-28} STF_{31-37}.

Anti-trichomonal assay: The assay was done according to the method of Omisore *et al*. (2005) and Ibikunle *et al*. (2011b). Briefly, 5 mg each of the crude methanolic extract STF and the partition fractions from Petroleum ether (STF_1), chloroform (STF_2), ethyl acetate (STF_3), aqueous (STF_4) aqueous precipitate (STF_5), sub fractions STF_{11-17}, STF_{21-28} STF_{31-37}, was dissolved in 1 cm^3 of Dimethylsulphoxide (DMSO), diluted serially to give 1000, 500, 250, 125, 62.5, 144. 31.25, 15.625, 7.812 and 3.906 µg/mL concentrations and each was assayed for anti- trichomonal activity. 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 thereafter incubated at 37°C and the cell growth was monitored at 24 and 48 h 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 and Sacor, 1996). The tests were repeated three times (N = 6) for the test agents and the controls while the Lethal Doses (LD_{50} 153. and LD_{90}) were determined at 24 and 48 h using the Finney Probit analysis and Minitab14 computer statistical software (Finney, 1971).

RESULTS

The percentage yield of the crude methanolic extract (STF_0) was 7.03%. While their partition fractions in Petroleum ether (STF_1), chloroform (STF_2), ethyl acetate (STF_3), aqueous (STF_4) aqueous precipitate (STF_5),were 31.4%-STF_1, 1.6%-STF_2, 9.20% STF_3, 34.4%-STF_4* and 12. 6%-STF_5, respectively (Table 1).

With the exception of the crude extract (STF_0), all the partition fractions, including the precipitate, had LC_{50} of below 100 µg/mL at 24 h post treatment (25.84±0.51 µg/mL in STF_4 to 88.39±2.35 µg/mL in STF_1). The LC_{90} was however above 200 µg/mL except for STF_4 which had an LC_{90} of 70.36±1.56 µg/mL (Table 2). At 48 h, the LC_{50} ranged from 13.87±1.61 µg/mL in STF_4 to 85.81±2.0 µg/mL in the crude. The corresponding LC_{90} values were 42.94±6.20 µg/mL for STF_4 to 72.76±1.72 µg/mL in STF_1.

The petroleum ether Vacuum Liquid Chromatography (VLC) sub fractions STF_{11-17} all had LC_{50} values below 100 µg/mL except for sub fraction 7 at 24 h (Table 3). The values ranged from 37.86±2.35 µg/mL for sub fraction 3 to 87.14±1.38 µg/mL for sub fraction 4. The LC_{90} were all above 100 µg/mL. At

Table 1: Percentage yield of the crude extract of solanum torvum fruits methanolic extract STF_0 and hexane, chloroform, ethyl acetate and aqueous fractions

The code for the partition fractions	STF_1	STF_2	STF_3	STF_4	STF_5
The weight of sample extracted (g)	100.00				
The initial volume of MeOH added (mL)	500				
Consecutive addition of MeOH (mL)	100x5				
Weight of the extract (g)	7.03				
% yield of the extract STF_0	7.03				
Weight of the crude partitioned STF_0 (g)	5.0				
Weight of each partition fraction (g)	1.57	0.08	0.46	1.74	0.63
% of the partition fraction	31.4	1.60	9.2	34.8	12.6

Key: Solanum torvum fruits crude methanolic extract STF_0 and its partition fractions into Hex = STF_1, $CHCl_3$ = STF_2, EtOAc = STF_3, precipitate = STF_4 and Aqueous = STF_5

Table 2: Anti-trichomonal Activity of methanolic crude extract STF_0 and partition fractions (STF_1, STF_2, STF_3, STF_4 and STF_5) from Solanum torvum crude methanolic extract showing the LC_{50} and LC_{90} (µg/mL) at 24 and 48 h

	24 h		48 h	
Fraction codes	LC_{50} (µg/mL)	LC_{90} (µg/mL)	LC_{50} (µg/mL)	LC_{90} (µg/mL)
STF_0	386.34^{a}±17.96	2168.31^{a}±119.43	85.81^{a}±2.01	324.72^{a}±17.73
STF_1	88.39^{b}±2.357	289.97^{b}±7.91	28.41^{d}±0.60	72.76^{d}±1.72
STF_2	80.03^{b}±1.63	217.84^{b}±3.033	44.52^{b}±3.86	119.39^{b}±3.90
STF_3	75.86^{b}±2.11	208.09^{b}±5.86	38.61^{c}±1.27	114.54^{b}±3.61
STF_4	25.84^{c}±0.51	70.36^{c}±1.56	13.87^{e}±1.61	42.94^{e}±6.20
STF_5	81.35^{b}±1.70	262.67^{b}±5.72	39.03^{c}±5.40	100.45^{c}±1.40
Metronidazole	16.67^{c}±0.67	45.86^{c}±0.59	14.04^{e}±0.40	64.37^{d}±4.37

Values on the same column with different superscript are significantly different ($p \leq 0.05$) While those with the same superscript are not significantly different ($p>0.05$)

Table 3: Anti-trichomonal Activity of VLC sub-fractions (STF_{11}, STF_{12}, STF_{13}, STF_{14}, STF_{15}, STF_{16}, STF_{17},) from the partition fraction of Solanum torvum STF1 showing the LC_{50} and LC_{90} (µg/mL) at 24 and 48 h

	24 h		48 h	
Fraction codes	LC_{50} (µg/mL)	LC_{90} (µg/mL)	LC_{50} (µg/mL)	LC_{90} (µg/mL)
STF_{11}	77.69^{c}±8.41	222.14^{c}±3.82	45.19^{c}±9.26	117.52^{d}±2.25
STF_{12}	66.49^{d}±6.05	187.80^{e}±2.53	77.17^{b}±2.42	186.87^{c}±5.42
STF_{13}	37.86^{e}±2.35	105.05^{f}±8.63	25.25^{e}±5.94	67.18^{f}±1.49
STF_{14}	87.14^{b}±1.38	275.19^{b}±4.29	$34,11^{d}$±1.42	88.07^{e}±4.78
STF_{15}	64.44^{d}±1.27	209.59^{d}±63.24	38.23^{cd}±1.93	110.80^{d}±1.26
STF_{16}	80.77^{bc}±1.47	280.11^{b}±5.99	84.15^{a}±2.12	273.45^{b}±1.74
STF_{17}	120.82^{a}±1.04	388.15^{a}±1.62	96.37^{a}±1.58	324.08^{a}±7.77
Metronidazole	16.67^{f}±6.67	46.86^{g}±5.86	14.04^{f}±4.04	67.37^{f}±4.37

Values on the same column with different superscript are significantly different ($p \leq 0.05$) while those with the same superscript are not significantly different ($p > 0.05$), Means are±standard deviation

Table 4: Anti-trichomonal Activity of VLC sub-fractions (STF_{21-28}) from the partition fraction of Solanum torvum STF_2 showing the LD_{50} and LD_{90} (µg/mL) at 24 and 48 h

	24 h		48 h	
Fraction codes	LC_{50} (µg/mL)	LC_{90} (µg/mL)	LC_{50} (µg/mL)	LC_{90} (µg/mL)
STF_{21}	197.72^{a}±7.06	731.67^{b}±31.39	161.76^{b}±1.35	813.99^{b}±20.52
STF_{22}	63.41^{e}±5.97	183.28^{e}±21.70	46.53^{d}±3.13	139.26^{d}±7.51
STF_{23}	34.03^{g}±3.29	85.60^{g}±7.62	40.77^{d}±3.55	105.95^{e}±9.55
STF_{24}	31.69^{g}±2.08	83.63^{g}±4.79	31.61^{e}±4.84	89.95^{e}±7.99
STF_{25}	155.31^{b}±2.96	830.97^{a}±9.61	249.53^{a}±2.15	1397.42^{a}±13.95
STF_{26}	$87.44b^{d}$±3.37	302.55^{d}±6.50	82.45^{c}±3.24	277.70^{c}±1.051
STF_{27}	47.91^{f}±3.21	121.89^{f}±7.38	46.75^{d}±2.54	147.84^{d}±1.49
STF_{28}	108.01^{c}±4.46	389.99^{c}±1.65	79.47^{c}±4.45	282.79^{c}±1.13
Metronidazole	16.67^{h}±6.67	45.86^{h}±5.86	14.04^{f}±4.04	64.37^{f}±4.37

Values on the same column with different superscript are significantly different ($p \leq 0.05$) while those with the same superscript are not significantly different ($p > 0.05$), Means are±standard deviation

Table 5: Anti-trichomonal Activity of VLC sub-fractions (STF_{31-37}) from the partition fraction of Solanum torvum STF_3 showing the LD_{50} and LD_{90} (µg/mL) at 24 and 48 h

	24 h		48 h	
Fraction codes	LC_{50} (µg/mL)	LC_{90} (µg/mL)	LC_{50} (µg/mL)	LC_{90} (µg/mL)
STF_{31}	199.26^{c}±2.65	1108.85^{c}±1.98	277.75^{b}±5.38	1553.48^{b}±19.72
STF_{32}	310.64^{b}±10.61	1585.18^{b}±2.56	279.22^{b}±8.19	1335.09^{c}±4.73
STF_{33}	78.33^{d}±4.98	259.70^{d}±2.59	69.21^{d}±1.15	262.53^{e}±6.48
STF_{34}	37.39^{e}±1.23	96.01^{g}±7.29	32.61^{f}±2.65	84.03^{g}±8.03
STF_{35}	40.50^{e}±1.38	107.20^{f}±2.89	44.89^{e}±3.36	119.0^{f} ±1.01
STF_{36}	398.13^{a}±9.420	2050.47^{a}±3.97	328.43^{a}±9.56	1722.68^{a}±45.967
STF_{37}	$71.65c^{d}$±8.89	194.05^{e}±1.55	98.28^{c}±1.25	442.55^{d}±7.10
Metronidazole	16.67^{f}±6.67	45.86^{h}±5.86	14.04^{g}±4.04	64.37^{g}±4.37

Values on the same column with different superscript are significantly different ($p \leq 0.05$) while those with the same superscript are not significantly different ($p > 0.05$), Means are±standard deviation

48h, the LC_{50} values were all below 100 µg/mL and only STF_{13} and STF_{14} had LC_{90} values below 100 µg/mL, the value for STF_{13} even slightly lower than that of the standard drug.

At 24 h, the chloroform VLC sub fractions all had remarkable activity except for sub fractions 1, 5 and 8 which had LC_{50} values above 100 µg/mL (Table 4).

The methanol VLC sub fractions 3, 4, 5 and 7 all had LC_{50} below 100 µg/mL with only sub fraction 4 having LC_{90} below 100 µg/mL at 24 and 48 h post treatment (Table 5).

DISCUSSION

Analysis of data obtained clearly demonstrates the remarkable antitrichomonal activity of extracts of *Solanum torvum* with the aqueous partition fraction (STF_4) having significantly higher bioactivity than the standard drug metronidazole. With a much better bioactivity at 48 h post treatment than the standard drug, it may well be that this fraction has better metabolic stability and therefore cleared much slower than the standard drug. The better activity of the standard drug at 24 h post treatment is however worthy of note in order to adequately assess fast acting and faster metabolic clearance versus slow acting but slower metabolic clearance.

Impressed by the bioactivity of the petroleum ether, chloroform and methanol partition fractions, it was thought that further purification of the fractions using a combination of Vacuum Liquid Chromatography (VLC) and thin layer chromatography, the bioactivity may be significantly improved. It turned out that this was not necessarily so in all the cases. The petroleum ether VLC sub fraction 3 (STF_{13}) had an improved bioactivity with its LC_{90} value at 48 h post

treatment not significantly different from that of the standard drug. In fact the absolute value for STF_{13} was slightly lower therefore, better than that of the standard drug. The methanol VLC sub fraction 4 (STF_{34}) also had an LC_{90} value that was not significantly different from that of the standard drug though the absolute value for the standard drug was much lower than that of STF_{34}. Taken together, these results clearly show the huge potential for the development of an effective antitrichomoniasis drug from *Solanum torvum*. The exciting aspect of all this is the bioactivity being found in aqueous and organic fractions, suggesting that the bioactive compounds are different and might be acting on different targets in the parasite. If this turns out to be the case, the prospects for the parasite developing resistance to any new drug that may be developed will be very low. This is a very important consideration in antiparasitic drug development generally. It is hoped that further studies on the bioactive fractions, particularly, the aqueous partition fraction will lead to the isolation of a pure compound with further improved bioactivity. Bioactive compound (s) so isolated can be standardized and packaged as herbal preparation for use in the treatment of trichomonal infections or serve as template (s) for chemical modifications in the production of efficacious synthetic drugs. These studies are currently on going in our laboratory.

CONCLUSION

It is concluded that extracts of the fruits of *S. torvum* contain bioactive antitrichomanal phytochemicals that form the basis for the development and production of drugs for the effective treatment of trichomonal infections.

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