IN-VITRO EVALUATION OF ANTI-TRICHOMONAL ACTIVITIES OF *EUGENIA UNIFLORA* LEAF.

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Abstract

Eugenia uniflora, used ethnomedically in some tropical countries as an anti-infective, has shown anti-malarial and anti-trypanocidal activities. Therefore using bioactivity guided fractionation, anti-trichomonal activity of *E. uniflora* leaf was investigated. Anti-trichomonal activities of leaf methanol extract and its fractions against *Trichomonas gallinae* as well as their cytotoxicities using an *in vitro* haemaglutination assay were determined. Anti-trichomonacidal activities of the extract improved on purification up to a stage. Subfractions $\mathbf{E}_{2\text{-}5}$ had LC_{50} and LC_{90} values of 4.77 - 5.28, 18.49 - 25.00 and 4.53 - 5.18, 18.32 - 19.07 µg/ml at 24 and 48 hrs, respectively that were better than those of metronidazole. Further purification of $\mathbf{E}_{2\text{-}5}$ led to loss of activity suggesting that the active components were probably working synergistically and additively. Demonstration of low haemaglutination titre values of 0.00 – 5.33 by methanolic extract and its partition fractions suggested their low toxicity profile. The established safety of the leaf indicated that its anti-trichomonal activity was not due to non-specific cytotoxicity, hence could be used in ethnomedicine as an anti-trichomonal agent.

Key words: *Eugenia uniflora*, leaf extract, *Trichomonas gallinae*, *in vitro.*

Introduction

Trichomoniasis affects humans and animals causing heavy economic losses in poultry and livestock due to high mortality in birds, and morbidity in man (Adebajo et al., 2009). In Nigeria, the prevalence depends on age, profession and location of the citizens (Omisore et al., 2005). Its chemotherapy has been undermined by resistance, variable efficacy between strains or species, toxicity, need for parenteral administration and requirement for long courses of administration (Narcisi and Secor, 1996; Munoz et al., 1998; Camacho et al., 2000, 2003). Since the safe drugs available are limited, investigation of plants with possible anti-trichomonal activities is obligatory (Camacho et al., 2000, 2003; Adebajo et al., 2004, 2006, 2007, 2009; Omisore et al., 2005; Mahdi et al., 2006).

Eugenia uniflora L. (Myrtaceae), native to Surinam, Guyana, Southern Brazil, Uruguay, is widely distributed in other South American countries. It is cultivated as an ornamental hedge and for its edible fruits in tropical and subtropical countries, including Nigeria (Dalziel and Hutchinson, 1966; Adebajo et al., 1989a). Mono- and sesqui-terpenoids from leaf and fruit essential oils (Weyerstahl et al., 1988; Onayade et al., 1999); triterpenoids and their acetates (Rücker et al., 1977), tannins (Consolini and Sarubbio, 2002), macrocyclic hydrolysable tannin dimers (Lee et al., 1997), xanthine oxidase inhibitory flavonoids (Schmeda-Hirschmann et al., 1987) and uniflorines A and B (Matsumura et al., 2000) have been reported. Ethnomedically, various extracts of the whole plant or leaves are used in several countries as remedy for headaches, influenza, bronchitis, chest cold, cough, gout, sore throat, hypertension, hepatic disease, fever, rheumatism, stomach diseases and other gastro-intestinal disorders, as a diuretic, for its astringent and insect repelling properties, or drunk as a tea shortly before child birth (Weyerstahl et al., 1988; Adebajo et al., 1989a,b; Arai et al., 1999; Consolini and Sarubbio, 2002). It is also used to stimulate menstrual flow (Sussman, 1980), against obesity and diabetes (Arai et al., 1999); its volatile oil as a digestive, eupeptical and carminative remedy and in Nigeria, a hot water extract of the fresh leaf and unripe fruit is used as antipyretic, febrifuge and antimalarial drug (Adebajo et al., 1989a, b).

Anti-parasitic activities such as anti-trypanocidal and anti-malarial (Agbedahunsi and Aladesanmi, 1993; Adewunmi et al., 2001), other biological and pharmacological activities have been reported for the plant (Adebajo et al., 1989a; Schapoval et al., 1994; Gbolade et al., 1996; Arai et al., 1999; Matsumura et al., 2000; Consolini and Sarubbio, 2002). Anti-trichomonal activity of *E. uniflora* has not been reported. We therefore investigated the anti-trichomonal effects of methanolic leaf extract and fractions of *E. uniflora*, using activity-directed fractionation and *Trichomonas gallinae.*

Materials and methods

Plant material, extraction and purification

Eugenia uniflora leaves were collected in January 2005 from the shrubs earlier identified and voucher specimen FHI 102196 was deposited at the Forestry Research Institute of Nigeria, Ibadan, Nigeria (Adebajo et al., 1989a,b). A 5.0 kg of powdered leaf was cold extracted with 5 litres of methanol (MeOH) for 3 days, the extract was filtered and concentrated *in-vacuo*. This process was repeated four times and the combined extracts were further concentrated *in vacuo* to give a 541 g methanolic extract (**A**, 10.82 % w/w yield), which was subjected to anti-trichomonal activity testing.

TLC analyses of methanolic extract, its partition and chromatographic fractions

The Thin Layer Chromatographic (TLC) analyses of the extract (**A**) was done using the solvent systems: **1**: n-hexane-$CHCl_3$ 1:1; **2**: $CHCl_3$ 100%; **3:** $CHCl_3$-MeOH 9:1; **4:** $CHCl_3$-MeOH 7:3; **5:** $CHCl_3$-EtOAc-MeOH 5:2:3 and **6:** $CHCl_3$-MeOH-H_2O 5:3:2 and the spots were detected by UV (366 nm) and 10 % H_2SO_4 followed by heating. Partition, vaccum liquid (VLC) and column chromatographic (CC) fractions were bulked into ($\mathbf{B_{1\text{-}5}}$), $\mathbf{C_{1\text{-}5}}$, $\mathbf{D_{1\text{-}9}}$, $\mathbf{E_{1\text{-}13}}$, $\mathbf{F_{1\text{-}12}}$, $\mathbf{G_{1\text{-}7}}$ and $\mathbf{H_{1\text{-}7}}$ based on similarity of their chromatograms and using solvent systems 1 – 6.

Partition fractions ($\mathbf{B_{1\text{-}5}}$)

520 g methanolic extract (**A**) was suspended in 150 ml water (H_2O), solvent partitioned (5 x 250 ml) and concentrated *in vacuo* to give n-hexane ($\mathbf{B_1}$, 75 g), $CHCl_3$ ($\mathbf{B_2}$, 133 g), EtOAc ($\mathbf{B_3}$, 65 g), BuOH ($\mathbf{B_4}$, 123.4 g) and aqueous ($\mathbf{B_5}$, 106 g) partition fractions that were tested for anti-trichomonal activity.

Vacuum liquid chromatographic (VLC) fractions $\mathbf{C_1}$–$\mathbf{C_5}$

The most active $\mathbf{B_2}$ and $\mathbf{B_3}$ fractions were combined (183 g) and subjected to VLC, gradiently eluted with petroleum ether, $CHCl_3$, MeOH. The fractions, based on TLC similarities in solvent systems (**1**–**3, 5),** were bulked into $\mathbf{C_1}$ (petroleum ether 100 %, 5 x 200 ml, 32.0 g), $\mathbf{C_2}$ (pet. ether-$CHCl_3$ 1:1, 5 x 200 ml, 48.2 g) and $\mathbf{C_3 – C_5}$.

VLC subfractions $\mathbf{D_{1\text{-}9}}$ and $\mathbf{E_{1\text{-}13}}$

Most active $\mathbf{C_1}$ and $\mathbf{C_2}$ were separately subjected to VLC. VLC of $\mathbf{C_1}$ (31.0 g) was repeated with a more gradient elution of petroleum ether, $CHCl_3$, MeOH and similarly bulked to give $\mathbf{D_1}$ **-** $\mathbf{D_6}$, $\mathbf{D_7}$ ($CHCl_3$ 100 %, 450 ml, 1.6 g) and $\mathbf{D_8}$ **-** $\mathbf{D_9}$. A 40.9 g of $\mathbf{C_2}$ was similarly treated to obtain $\mathbf{E_1}$, $\mathbf{E_2}$ (pet. ether–$CHCl_3$ 45:55, 450 ml, 1.9 g), $\mathbf{E_3}$ (pet. ether-$CHCl_3$ 45:55, 600 ml, 2.3 g), $\mathbf{E_4}$ (pet. ether-$CHCl_3$ 45:55, 600 ml; pet. ether-$CHCl_3$ 30:70, 700 ml, 7.1 g), $\mathbf{E_5}$ ($CHCl_3$ 100 %, 900 ml, 2.7 g) and $\mathbf{E_6}$ **-** $\mathbf{E_{13}}$.

CC subfractions $\mathbf{F_{1\text{-}12}}$

The most active $\mathbf{D_7}$ and $\mathbf{E_{2–5}}$ showed great similar TLC characteristics and due to their low yields, they were combined and further purified using CC to obtain CC subfractions $\mathbf{F_1}$ **-** $\mathbf{F_{12}}$.

Anti-trichomonal bioassay

Trichomonas gallinae were collected from local Pigeon *Columba lavia* (Anth) Columbidea by inserting a sterile cotton swab into its crop (Narcisi and Secor, 1996; Omisore et al., 2005). The swab was dipped into a test tube of normal saline, distributed into test tubes of Ringers-egg-serum culture for enteric protozoan, prepared according to the modified method of Levine (1961) and incubated at 37^o C for growth. Stock solutions of Metronidazole (positive control drug), the extract, fractions and subfractions obtained above, in DMSO at the concentration of 20 mg/ml were made. Their serial dilutions to 0.00, 3.906, 7.8125, 15.625, 31.25, 62.5, 125, 250, 500 and 1,000 µg/ml with the fluid nutrient solution were used as the test agents. A 50 µl of each test agent and 150 µl of the nutrient solution were pipetted into the 96-microwells and incubated in the steam incubator at 37^o C for 24 and 48 hr; 50 µL of DMSO served as a negative control. The number of surviving trichomonads per millilitre in each well for 0, 24 and 48 hr were counted using the microscope. The experiments were replicated nine times (Adebajo et al., 2009). The lethal doses (LC_{50} and LC_{90}) at these times were determined using the Microsoft Excel 2008 and subjected to statistical analysis using ANNOVA followed by Dunnett post-hoc test. $P < 0.05$ was considered as significant.

Cytotoxicity Assay

Cytotoxicity of the extract and its fractions were determined by haemagglutination activity using formaldehyde fixed bovine erythrocytes. 100 µL of phosphate buffer saline (PBS) was pipetted into 96-well microtitre plates. The first row was without the extract/fractions and used as control. The extract and fractions (100 µL) were added into the first well of the second row, and two-fold serial dilutions were made until the last well in row three. Thereafter, 50 µL of bovine red blood cell was added to all the wells and incubated for one hour. The presence of buttons at the centre of the well indicated no agglutination and

the heamagglutination titre values of the extract/fractions were read as the reciprocal of the last dilution showing agglutination (Peumans, et al, 1982; Sadique et al., 1989; Wang et al, 1995).

Results

The anti-trichomonal activities of *E. uniflora* leaf methanolic extract (**A**), its five partition fractions ($\mathbf{B_{1-5}}$) and the VLC subfractions ($\mathbf{C_{1-5}}$) of the most active $\mathbf{B_2}$ and $\mathbf{B_3}$ fractions were given in Table 1. Those of the subfractions $\mathbf{D_{1-9}}$, $\mathbf{E_{1-13}}$ and $\mathbf{F_{1-12}}$ were presented in Tables 2 – 4, respectively. Haemagglutination values, as indices of cytotoxicities of the leaf extract and partition fractions, were also given in Table 5.

Discussion

Leaf methanolic extract of *E. uniflora* was less active than Metronidazole (Table 1). The $CHCl_3$ and EtOAc partition fractions were the most active and had lower LC_{50} and LC_{90} values at 48 than 24 hrs indicating, that their activities were trichomonacidal. Hence, the main active constituents were likely to be moderately non-polar to mediumly polar in nature. N-hexane fraction had higher LC_{50} and LC_{90} values at 48 than 24 hrs, indicating that the trichomonads were able to recover after 24 hrs and the activity was trichomonastatic (Table 1). Similar anti-parasitic activities of antimalarial (Agbedahunsi and Aladesanmi, 1993) and anti-trypanocidal (Adewunmi et al., 2001) have been reported for the leaf extract and fractions. However, anti-trichomonal activity of the plant is hereby reported for the first time.

Table 1: Anti-trichomonal activities of *Eugenia uniflora* leaf methanolic extract, its partition and VLC fractions.

Extract/Fractions/	**24 hrs**		**48 hrs**	
Drug	**LC_{50} (µg/ml)**	**LC_{90} (µg/ml)**	**LC_{50} (µg/ml)**	**LC_{90} (µg/ml)**
MeOH (A)	61.70±1.65†	386.84±4.32†	55.15± 2.17†	302.93±9.54†
n-Hexane (B_1)	40.00±2.24*,†	271.10±5.72*,†	70.52± 3.41†	551.69±2.37*,†
$CHCl_3$ (B_2)	27.60± 3.76*	126.95±2.82*,†	18.67± 3.29*	68.82± 5.41*
EtOAc (B_3)	41.51± 4.48†	194.79±7.57*,†	24.18± 1.37*	86.39±2.04*
BuOH (B_4)	58.62± 2.56†	353.79±10.75†	52.31±3.71†	427.39±9.65*,†
Aqueous (B_5)	103.19±0.96*,†	704.65±2.66*,†	59.10± 8.34†	592.06± 2.01*,†
C_1	35.08±2.89*	238.50± 9.17*,†	18.05± 5.09*	106.90± 5.21*,†
C_2	36.43± 2.73*,†	265.34± 9.43*,†	32.69± 4.66*,†	156.95± 5.68*
C_3	222.83± 9.26*	1063.57±5.81*,†	40.88± 4.95†	263.50± 4.76†
C_4	75.10± 4.64*,†	747.77± 3.14*,†	206.08± 9.86*	1010.40±10.56*,†
C_5	125.69±1.30*,†	1274.49±4.89*,†	27.12± 7.00	168.75±11.11*
Metronidazole	13.67± 0.87*	42.86± 2.60*	14.04± 5.85*	61.37±8.69*

A: Methanolic extract; $\mathbf{B_1}$: N-hexane, $\mathbf{B_2}$: Chloroform, $\mathbf{B_3}$: Ethylacetate, $\mathbf{B_4}$: Butanol, $\mathbf{B_5}$: Aqueous partition fractions; $\mathbf{C_{1-5}}$: VLC subfractions of the combined most active partition fractions $\mathbf{B_2}$ and $\mathbf{B_3}$. N = 9; $p < 0.05$; *: LC_{50} and LC_{90} vs MeOH, †: LC_{50} and LC_{90} vs metronidazole. LC_{50} and LC_{90}: Values ± SEM.

$\mathbf{C_1}$ and $\mathbf{C_2}$ with activities comparable to their mother fractions $\mathbf{B_{2-3}}$, were the most active VLC fractions obtained by purification of the combined fractions $\mathbf{B_{2-3}}$. They were however less active than metronidazole (Table 1) and since their TLC characteristics were slightly different, they were separately further purified. Subfractions $\mathbf{D_1}$, $\mathbf{D_3}$, $\mathbf{D_5}$ and $\mathbf{D_7}$ gave similar activity at 24 and 48 hr. Trichomonacidal activity of $\mathbf{D_7}$ was better than that of $\mathbf{C_1}$, showing that it should contain more of the active compounds (Tables 1, 2). $\mathbf{D_7}$ with LC_{50} values of 13.81 and 12.38 µg/ml at 24 and 48 hr, respectively was as potent as metronidazole (Table 2). Similarly, $\mathbf{E_2}$-$\mathbf{E_5}$ had higher activities and lower LC_{50} and LC_{90} values than $\mathbf{C_2}$ and metronidazole (Tables 1, 3).

Up to this stage, purification led to better activities than the mother fractions, suggesting that chemical compounds in the extract are responsible for its anti-trichomocidal activity. Hence, the most active $\mathbf{D_7}$ and $\mathbf{E_2}$-$\mathbf{E_5}$ fractions were combined and subjected to CC. The resulting fractions $\mathbf{F_{1-12}}$ had higher LC_{50} and LC_{90} values than $\mathbf{D_7}$ and $\mathbf{E_2}$-$\mathbf{E_5}$ subfractions (Tables 2 - 4), indicating that further purification did not result in improved activity. Only $\mathbf{F_6}$ and $\mathbf{F_7}$ had activities that were comparable to that of metronidazole and $\mathbf{D_7}$ (Table 4).

Table 2: Anti-trichomonal activities of subfractions $\mathbf{D_{1-9}}$.

Subfractions / Drug	24 hrs		48 hrs	
	$\mathbf{LC_{50}}$ (μg/ml)	$\mathbf{LC_{90}}$ (μg/ml)	$\mathbf{LC_{50}}$ (μg/ml)	$\mathbf{LC_{90}}$ (μg/ml)
$\mathbf{C_1}$	35.08±2.89†	238.50± 9.17†	32.69± 4.66†	106.90± 5.21†
$\mathbf{D_1}$	34.91 ± 3.14†	246.76±14.86†	49.11± 1.98†	218.26±13.16*,†
$\mathbf{D_2}$	61.99± 2.11*,†	300.82± 16.88†	39.90± 1.77†	130.90±10.69†
$\mathbf{D_3}$	37.83± 1.54†	188.24± 12.36*,†	34.68±3.05*,†	131.35±10.89†
$\mathbf{D_4}$	26.76± 0.99†	204.09±13.25†	56.23± 2.32†	235.11±7.60*,†
$\mathbf{D_5}$	33.34± 1.46†	149.20±17.74*,†	44.82± 3.09†	181.85± 7.36†
$\mathbf{D_6}$	25.95± 0.78†	112.01±11.56*,†	46.56±4.26†	135.10± 5.33†
$\mathbf{D_7}$	13.81± 1.27*	41.26± 8.10*,†	12.38±4.34*	37.71± 2.79*,†
$\mathbf{D_8}$	30.40± 3.30†	127.47± 8.77*,†	22.03± 0.30*	72.21± 2.97*
$\mathbf{D_9}$	55.24±0.30*,†	318.27±19.95*,†	31.97±1.95†	118.22±4.99†
Metronidazole	13.67± 0.90*	42.86± 0.87*	14.04± 5.85*	61.37±8.69*

$\mathbf{D_{1-9}}$: VLC subfractions of an active VLC fraction $\mathbf{C_1}$. N = 9; $p < 0.05$; *: LC_{50} and LC_{90} vs $\mathbf{C_1}$; †: LC_{50} and LC_{90} vs metronidazole. LC_{50} and LC_{90}: Values ± SEM.

Table 3: Anti-trichomonal activities of subfractions $\mathbf{E_{1-13}}$.

Subfractions/ Drug	24 hrs		48 hrs	
	$\mathbf{LC_{50}}$ (μg/ml)	$\mathbf{LC_{90}}$ (μg/ml)	$\mathbf{LC_{50}}$ (μg/ml)	$\mathbf{LC_{90}}$ (μg/ml)
$\mathbf{C_2}$	36.43± 2.73*,†	265.34± 9.43†	18.05± 5.09	156.95± 5.68†
$\mathbf{E_1}$	131.71 ± 1.38*,†	2363.73 ± 9.33*,†	133.37 ±2.04*,†	2124.73 ±12.72*,†
$\mathbf{E_2}$	4.95 ± 0.78*,†	20.93 ± 1.92*	3.61 ± 2.54*,†	18.69 ± 2.96*,†
$\mathbf{E_3}$	5.61± 1.30*,†	20.72 ± 3.29*	3.24 ± 0.63*,†	16.28 ± 2.03*,†
$\mathbf{E_4}$	4.77 ± 0.83*,†	18.49 ± 2.94*	4.53 ± 1.23*,†	18.38 ± 3.70*,†
$\mathbf{E_5}$	7.28 ± 0.87*,†	25.00 ± 2.95*	4.95 ± 0.81*,†	19.07 ± 2.20*,†
$\mathbf{E_6}$	40.28 ± 0.85†	168.04 ± 8.27*,†	22.53 ± 1.38†	157.95 ± 6.31†
$\mathbf{E_7}$	32.78 ± 1.28†	182.67 ± 4.63*,†	33.38 ± 0.85*,†	207.89 ± 7.64†
$\mathbf{E_8}$	84.27 ± 0.75*,†	935.05 ± 11.11*,†	58.11± 0.99*,†	565.03 ± 9.30*,†
$\mathbf{E_9}$	61.55 ± 0.93*,†	828.32 ± 4.06*,†	63.40 ± 0.66*,†	551.64 ± 7.22*,†
$\mathbf{E_{10}}$	60.73 ± 0.79*,†	694.85 ± 5.53*,†	58.87 ± 1.45*,†	390.53 ± 8.14*,†
$\mathbf{E_{11}}$	69.15 ± 0.09*,†	725.31 ± 9.33*,†	78.63 ± 0.64*,†	639.83 ± 3.57*,†
$\mathbf{E_{12}}$	69.28 ± 1.22*,†	507.34 ± 3.27*,†	89.16 ± 0.44*,†	680.21 ± 6.99*,†
$\mathbf{E_{13}}$	70.46 ± 0.37*,†	735.55 ± 9.27*,†	124.46 ± 0.87*,†	959.10 ± 6.63*,†
Metronidazole	13.67 ± 0.03*	42.86 ± 0.87*	14.04 ± 1.95	61.37 ± 8.69*

$\mathbf{E_{1-13}}$: VLC subfractions of an active VLC fraction $\mathbf{C_2}$. N = 9; $p < 0.05$; *: LC_{50} and LC_{90} vs $\mathbf{C_2}$; †: LC_{50} and LC_{90} vs metronidazole. LC_{50} and LC_{90}: Values ± SEM.

The decreased activity as purification progressed showed that the active trichomocidal components of the leaf were probably working synergistically. Comparing the TLC characteristics of the various active fraction/subfractions showed that spots with R_f 0.12 and 0.06 (silica gel, 100% $CHCl_3$) fluorescencing blue under UV and red colour with H_2SO_4 were common to all of them, suggesting that the active compounds are likely to be moderately polar. Since flavonoids and phenolics have been detected during phytochemical screening of the combined active $\mathbf{B_2}$ and $\mathbf{B_3}$ fractions of *E. uniflora* leaf (Adebajo et al., 1989a) and xanthine oxidase inhibitory flavonoids have been isolated from equivalent leaf fraction (Schmeda-Hirschmann et al., 1987), the active constituents are therefore suspected to be flavonoids. Due to this demonstrated synergism of the constituents of this plant, further purification was deemed unnecessary.

Metronidazole is the drug of choice in the treatment of human and animal trichomoniasis all over the world. Side effects of metronidazole include allergic reaction, nausea, diarrhoea, dryness of the mouth, leucopoenia and intolerance to alcohol. It is also not recommended during the first trimester of pregnancy or breast feeding while increasing resistance against metronidazole is common (Narcisi and Secor, 1996; Munoz et al., 1998). Acceptable drugs, particularly from plant sources, against trichomoniasis are therefore of a high priority (Adebajo et al., 2006, 2007; Mahdi et al., 2006). Hence, any of the purified fractions of $\mathbf{D_7}$, $\mathbf{E_{2-5}}$, $\mathbf{F_6}$ and $\mathbf{F_7}$ that were more active or comparable in activity to metronidazole, may be relevant as a readily available alternative drug for the treatment of animal and human trichomoniasis.

Table 4: Anti-trichomonal activities of subfractions $\mathbf{F_{1-12}}$.

CC Subfractions	24 hrs		48 hrs	
	$\mathbf{LC_{50}}$ **(µg/ml)**	$\mathbf{LC_{90}}$ **(µg/ml)**	$\mathbf{LC_{50}}$ **(µg/ml)**	$\mathbf{LC_{90}}$ **(µg/ml)**
$\mathbf{D_7}$	13.81± 1.27	41.26± 8.10	12.38±4.34	37.71± 2.79†
$\mathbf{F_1}$	100.21± 0.83*,†	1347.09±5.14*,†	179.59 ±1.04*,†	4692.0 ± 6.19*,†
$\mathbf{F_2}$	26.63 ± 0.71*,†	78.43 ± 8.83*,†	23.56 ± 0.99*,†	128.14±11.24*,†
$\mathbf{F_3}$	22.28 ± 0.64*,†	91.00 ± 3.94*,†	24.76 ± 0.72*,†	96.63 ± 5.33*,†
$\mathbf{F_4}$	23.96 ± 0.70*,†	117.04 ±4.18*,†	19.46 ± 0.72*,†	99.32 ±7.26*,†
$\mathbf{F_5}$	21.30 ± 1.09*,†	97.23 ± 6.13*,†	21.09 ± 0.52*,†	98.49 ± 2.64*,†
$\mathbf{F_6}$	18.49 ± 0.89*,†	69.85 ± 5.75*,†	16.88 ± 0.35	64.71 ± 6.42*
$\mathbf{F_7}$	16.79 ± 0.92	60.60 ± 4.11	14.17 ± 0.95	63.59 ± 2.63*
$\mathbf{F_8}$	26.41 ± 0.59*,†	138.84 ± 6.68*,†	20.80 ± 0.73*,†	104.57 ± 6.26*,†
$\mathbf{F_9}$	47.21 ± 0.83*,†	203.96 ± 7.91*,†	42.95 ± 0.78*,†	432.16 ± 2.14*,†
$\mathbf{F_{10}}$	36.29 ± 1.02*,†	177.24 ± 7.32*,†	60.34 ± 0.70*,†	571.35 ± 5.49*,†
$\mathbf{F_{11}}$	62.13 ± 0.70*,†	382.18 ± 8.47*,†	33.00 ± 0.62*,†	198.15 ± 7.59*,†
$\mathbf{F_{12}}$	257.80 ±0.89*,†	11960.92±8.12*,†	137.74 ±1.07*,†	2114.56±5.86*,†
Metronidazole	13.67± 0.30	42.86 ± 0.87	14.04 ± 1.95	61.37 ± 8.69*

$\mathbf{F_{1–12}}$: CC subfractions of the combined most active VLC fractions $\mathbf{D_7}$ and $\mathbf{E_{2-5}}$. N = 9; $p < 0.05$; *: LC_{50} and LC_{90} vs $\mathbf{D_7}$; †: LC_{50} and LC_{90} vs metronidazole. LC_{50} and LC_{90}: Values ± SEM.

The MeOH extracts of *Murrya koenigii* leaf and stem were reported less active than metronidazole, and its carbazole constituents, especially girinimbine, girinimbilol and girinimbiyl acetate with comparable activity with metronidazole, were the active compounds (Adebajo et al., 2004, 2006). MeOH extracts of the furocoumarin-producing *M. koenigii* seeds and pericarp also showed comparable anti-trichomonal activities with metronidazole. Their active constituents were identified as the furocoumarins isoimperatorin, isogosferol and indicolatone. Since none of the furocoumarins isolated gave a significantly higher activity than the MeOH extracts of the seeds or pericarps, it has been suggested that active compounds were probably working synergistically (Adebajo et al 2007). Antitrichomal activities of extracts of *Dorstenia barteri* and *D. convexa* (Omisore et al., 2005) and *Harungana madagascariensis* have also been reported (Iwalewa et al., 2008). Imperatorin and 3-formylcarbazole that had better activities than the dichloromethane extract were the most active compounds of *Clausena lansium* stem bark (Adebajo et al., 2009).

Table 5: Agglutination and haemagglutination values of the leaf extract and partition fractions.

Extract/fractions	Agglutination concentrations (mg/ml)	Haemagglutination titre value
MeOH **(A)**	0.26 ± 0.05	5.33 ± 2.93
n-Hexane **(B_1)**	3.00 ± 0.00	0.33 ± 0.00
$CHCl_3$ **(B_2)**	0.23 ± 0.05	4.67 ± 0.67
EtOAc **(B_3)**	0.28 ± 0.05	4.00 ± 0.77
BuOH **(B_4)**	0.47 ± 0.09	2.34 ± 0.34
Aqueous **(B_5)**	0.53 ± 0.02	1.89 ± 2.17

A: Methanol extract; $\mathbf{B_1}$: N-hexane, $\mathbf{B_2}$: Chloroform, $\mathbf{B_3}$: Ethylacetate, $\mathbf{B_4}$: Butanol, $\mathbf{B_5}$: Aqueous partition fractions. LC_{50} and LC_{90}: Values ± SEM.

The *E. uniflora* leaf extract and partition fractions showed low haemaglutination titre values (Table 5), suggesting their low toxicity profile and safe internal use in medicine (Sadique et al., 1989). Since the plant is therefore considered safe, its anti-trichomonal activity was not due to non-specific cytotoxicity. A hydro-alcoholic extract of the leaves, when administered orally to mice in doses up to 4,200 mg/kg, lacked acute or subacute toxicity. This extract also gave an LD_{50} of 220 mg/kg when given *i.p.* to mice. Based on these results, the plant was declared safe for internal use in treating gout by herbalists in Paraguay (Schmeda-Hirschmann et al., 1987). Our present results of no toxicity therefore agreed closely with the earlier report (Schmeda-Hirschmann et al., 1987).

Conclusion

Anti-trichomonal activity directed purification of *E. uniflora* leaf extract gave subfractions **D_7, E_{2-5}, F_6** and **F_7** with higher or comparable activity to that of metronidazole. Since the folkloric use of the plant as a safe anti-parasitic agent was confirmed, *E. uniflora* leaf could be used in ethnomedicine as an anti-trichomonal agent and its chemotherapeutic potential may need to be further exploited.

Acknowledgements

The authors are grateful to the Obafemi Awolowo University Research Committee, OAU, Ile-Ife, Nigeria for the research grant, 11-813-ACZ, for the study and to Mr. K.A. Olaniyan, DRPU, Ile-Ife.

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