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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Evaluation of cytotoxicity, anti-herpes simplex virus type 1 (HSV-1) and antibacterial activities of *Ficus vasta* and phytoconstituents

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ABSTRACT

This study deals with the evaluation of *in vitro* cytotoxicity of the methanol 80% extract of *Ficus vasta* aerial parts in Vero cells, anti-HSV-1 and antibacterial activities and the determination of the phytochemical content of the *F. vasta* methanol extract. The extract was tested for its cytotoxicity in Vero cells and for anti-HSV-1 and against bacterial strains as *S. aureus*, *S. epidermidis*, *E. coli* and *P. aeruginosa* and also phytochemical constituents were detected. The results showed that this extract presented a CC_{50} value of 389 µg/mL, an intermediate value taking into account other researchers reported for some Moraceae species. Regarding the other biological activities, antiviral effect was not demonstrated by a HSV-1 challenge dilution of 100x tissue culture infective dose 50% ($TCID_{50}$) and also by the disk-diffusion method at the higher non-toxic concentration of the extract (119 µg/mL). Phytochemical analysis of the methanol extract proved the presence of carbohydrates, tannins, flavonoids, coumarins, alkaloids and triterpenes. Chromatographic separation of the methanol extract resulted in the isolation and identification of β-sitosterol, stigmasterol, lupeol, ursolic acid, naringenin, luteolin, quercetin, vitexin, quercetin 3-*O*-β-glucoside and rutin. The results indicated that *F. vasta* aerial parts methanol extract is potential candidate for experiments of biological activity screening that are not anti-HSV-1 or antibacterial against the strains evaluated in this study, to be carried on in the future.

***Key Words*:** Plant extract, biological activities, cytotoxicity, anti-HSV-1, antibacterial, phytoconstituents.

INTRODUCTION

Infectious diseases remain as an important worldwide health problem and the control of them is the subject of constant scientific endeavor. Additionally, the appearance of microbial strains resistant to chemotherapeutic agents is an emerging problem. The severe side effects and the emergence of drug-resistance mutants during long-term medication with these drugs have often limited their administration to patients (Bacon *et al.*, 2003; Morfin and Thouvenot, 2003). Herbal remedies used in the traditional folk medicine provide an interesting and still largely unexplored source for the creation a development of potentially new drugs for chemo-therapy which might help to overcome the growing problem of resistance and also the toxicity of the currently available commercial drugs. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries (Awadh *et al.*, 2001). Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents (Awadh *et al.*, 2001) and thus, the evaluation of the cytotoxic potential of novel candidates to antimicrobial agents is an important area of research. In our searching for natural products as potential antimicrobial drugs, *Ficus vasta* is a very large tree from Moraceae family growing over 25 m tall. *F. vasta* is a tree of dry north and eastern Africa, Sudan, Ethiopia, Saudi Arabia and Tanzania (Dawit *et al.*, 2003). In traditional medicine, *F. vasta* used in rheumatism, pains, intestinal worms (Kitessa *et al.*, 2007). It was already studied for the extract from the fruits of *F. vasta* antibacterial (Al-Fatimi *et al.*, 2007), mainly against Gram-positive bacteria, and antihelmintic (Raju *et al.*, 2011) activities. There are no previous reports about phytoconstitutents and the cytotoxicity against Vero cells, anti-HSV-1 and antibacterial activities from the aerial parts of *F. vasta*, which are now described in this research article.

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MATERIALS AND METHODS

Experimental

UV/VIS: Shimadzu UV-visible recording spectrophotometer model-UV 240 (NRC, Egypt). ^{1}H-NMR and ^{13}C-NMR (Varian Unity Inova). MS (Finnigan MAT SSQ 7000, 70 ev). (Silica gel (0.063-0.200 mm for column chromatography) and Sephadex LH-20 (Pharmacia Fine Chemicals). Thin layer chromatography (TLC) F_{254} plates. Solvent mixtures, BAW (*n*-butanol:acetic acid:water 4:1:5 upper phase, 15% acetic acid: water: glacial acetic acid: 85:15). Paper Chromatography (PC) Whatman No.1 (Whatman Led. Maid Stone, Kent, England) sheets for qualitative detection of flavonoids and sugars.

Plant identification and collection

Ficus vasta aerial parts were collected from Al-Zohiriya garden, Giza, Egypt in May 2011. The plant was identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereeza Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen was deposited in the herbarium of Al-Zohiriya garden, Giza, Egypt.

Preparation of *F. vasta* aerial parts methanol 80% extract

Air dried powder of *F. vasta* aerial parts (720 g) was extracted with methanol : water (80 : 20) (v/v) at room temperature several times until exhaustion by maceration

Table 1: Compounds isolation result of methanol extract (36 g) of the *F. vasta* aerial parts. It was subjected to silica gel column chromatography and eluted with different amounts of hexane, dichloromethane, ethyl acetate and methanol.

Fraction No.	Amount (g)	Mobile phase (ratio)	Compound No.
1	1.64	n-hexane: dichloromethane (60:40 v/v)	Comp. 1
		n-hexane: dichloromethane: (80:20 v/v)	Comp. 2
2	1.45	dichloromethane: ethyl acetate (70:30 v/v)	Comp. 3
		dichloromethane: ethyl acetate (50:50 v/v)	Comp. 4
3	1.9	ethyl acetate: methanol (95:5 v/v)	Comp. 5
		ethyl acetate: methanol (90:10 v/v)	Comp. 6
		ethyl acetate : methanol (85:15 v/v)	Comp. 7
4	1.65	ethyl acetate : methanol (75:25 v/v)	Comp. 8
		ethyl acetate: methanol (60:40:10 v/v)	Comp. 9
		Further elution with methanol	Comp. 10

process. The extract was concentrated under reduced pressure to give 39 g of the crude extract and the extract was subjected to preliminary phytochemical analysis to detect the main phytoconstituents and all the phytochemical tests were carried out according to that described by Yadav and Agarwala (2011).

Phytochemical Characterization of *F. vasta* aerial parts methanol 80% extract

All the isolated compounds were purified on Sephadex LH–20 column using methanol and different mixtures of methanol and distilled water.

General method for acid hydrolysis of flavonoid glycosides

5 mg of each flavonoid glycoside 8, 9 and 10 in 5 ml 10% HCl was heated for 5h. The aglycones were extracted with EtOAc and identified by co-TLC with authentic standards. The sugars in the aqueous layer were identified by co-paper chromatography (co-PC) with authentic markers on Whatman No. 1 sheets in solvent system (*n*-BuOH-AcOH-H_2O 4:1:5 upper layer).

Cytotoxicity assay

Vero cells (epithelial cells from kidney of *Cercopithecus aethiops*) were cultured in 96-well microplates, and the monolayers were incubated for 72 h at 37°C and 5% CO_2 with DMEM containing 5% fetal bovine serum (FBS) and 1% dimethyl sulfoxide (DMSO), penicillin G (100 IU/ml), enrofloxacin (10 µg/ml) and amphotericin B (1.25 µg/ml) with 2-fold serial dilutions of the extract at different concentrations, ranging from from 3.8 to 1900 µg/ml to methanol 80% of *F. vasta* methanol extract. For this, 3 repetitions of 8 wells were used for the evaluation of extract dilution. The *in vitro* toxicity of methanol 80% of *F. vasta* extract which was sterilized by filtration through PVDF membranes (pore size 0.22 µm), was determined by quantifying the viable cells using 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), which is converted into a purple formazan by mitochondrial dehydrogenases (Denizot and Lang, 1986). Fifty percent cytotoxic concentration (CC_{50}) was defined as the extract

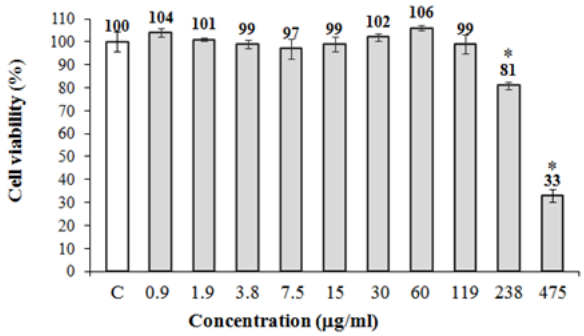


Figure 1: Evaluation of cytotoxicity of F. vasta methanol extract in Vero cells after 72h of incubation at 37°C and 5% CO_2 by the MTT assay. Control: without extract treatment. Bars represent means, with vertical lines indicating standard deviations, n = 3, *P <0.01.

concentration which could reduce by 50% the number of viable cells, when compared with a control without it, and it was calculated by regression analysis of the dose-response curves.

Antiviral assay

The screening of the antiviral activity of *F. vasta* methanol extract against HSV-1 was carried out on confluent monolayers of Vero cells in 96-well microplates by adding the highest non-toxic concentration determined by the MTT method (119 µg/mL) during the HSV-1 (100x$TCID_{50}$) adsorption step and after adsorption in the same concentrations within the maintenance medium (DMEM with 5% FBS and 1% DMSO added by antibiotics and antifungal). The viral replication was performed for 72h at 37°C and 5% CO_2 incubation. The inhibition of the HSV-1 replication was related to the absence of any viral induced cytopathic effect at the highest non-toxic concentration of the extract, evaluated by observation of the monolayers under microscope, when compared with a control without it. Thus this test aimed to verify a decrease in the virus titer obtained by the endpoint titration method (Reed and Müench, 1938) due to the plant extract.

Antibacterial assay

The antibacterial activity of *F. vasta* methanol extract was evaluated against *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) strains by the disk-diffusion method. Briefly, sterilized filter paper disks of 6 mm diameter were impregnated with 10 µL of the control substances (1% DMSO, 0.05% enrofloxacine or 20% chlorhexidine) or with extract solution (119 µg/mL in 1% DMSO) previously sterilized by filtration on polyvinylidene difluoride membranes with pore diameter of 0.22 µm. The impregnated disks (n=3) containing 1.19 µg of the extract were positioned on the top of the Petri dishes containing 25 mL of Mueller-Hinton Agar previously seeded with a bacterial suspension adjusted to the 0.5 degree of the McFarland turbidity scale, which corresponds to a concentration of $1.5x10^8$ CFU/mL. The plates were incubated at 35°C for 24 h and after that, the diameter of the zone of inhibition of the bacterial growth around the discs were determined.

Table 2: Spectroscopic analyses of the isolated compounds of *F. vasta* methanol extract.

Compound (No.)	Amount recovered (mg)	Physical state	^{1}H-NMR data	^{13}C-NMR data	UV data	MS data
***β*-sitosterol (1)**	12	White needles	^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.37 (IH, m, H-6), 3.52 (IH, m, H-3) , 1.09 (3H, s, CH3-19), 0.98 (3H, d, J= 6.5, CH3-21), 0.92 (3H, t, J= 7.4, CH3-29), 0.85 (3H, d, J= 6.7Hz, CH3-26), 0.81 (3H, d, J= 6.7Hz, CH3-27), 0.75 (3H, s, CH3-18)	^{13}C-NMR(100 MHz, $CDCl_3$): δ 140.4 (C-5), 121.5 (C-6), 71.6 (C-3), 57.2 (C-17), 56.4 (C-14), 50.3 (C-9), 46.3 (C-24), 42,8 (C-13, 4), 39.8 (C-12), 37.6 (C-1), 36.7 (C-10), 35.9 (C-20), 34.2 (C-22), 31.7 (C-8, 7), 31.4 (C-2), 29.2 (C-25), 28.4 (C-16), 26.2 (C-23), 24.5 (C-15), 23.4 (C-28), 21.1 (C-11), 19.8 (C-26), 19.5 (C-19), 19.2 (C-27), 18.6 (C-21).		
Stigmasterol (2)	15	white needle crystals	^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.32 (IH, m, H-6), 5.11 (1H,dd, *J*= 14.2, 8.2 Hz, H-22), 5.04 (1H,dd, *J*= 14.2, 8.2 Hz, H-23), 3.54 (IH, m, H-3), 1.04 (3H, s, CH_3-10), 0.9 (3H, d, *J*= 6.5, CH_3-20), 0.84 (3H, d, J= 7.4, CH_3-27), 0.82 (3H, d, *J*= 7.4, CH_3-26), 0.68 (3H, s, CH_3-13).	^{13}C-NMR(100 MHz, $CDCl_3$): δ 140.4(C-5), 121.5(C-6), 71.6 (C-3), 57.2 (C-17), 56.4 (C-14), 50.3 (C-9), 46.3 (C-24), 42,8 (C-13, 4), 39.8 (C-12), 37.6 (C-1), 36.7(C-10), 35.9 (C-20), 34.2 (C-22), 31.7 (C-8, 7), 31.4 (C-2), 29.2(C-25), 28.4 (C-16), 26.2 (C-23), 24.5 (C-15), 23.4(C-28), 21.1 (C-11), 19.8 (C-26), 19.5 (C-19), 19.2 (C-27), 18.6 (C-21)		
Lupeol (3)	14	White powder	^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.75, 0.8, 0.85, 0.96, 0.98, 1.08, 1.75 (each 3H, s), 3.25 (1H, dd, *J* = 5.6, 10.8 Hz, H-3), 4.58 (1H, s, H-29a), 4.68 (1H, s, H-29b).	^{13}CNMR($CDCl_3$, 100MHz): δ 151.4 (C-20), 108.7 (C-29), 78.6(C-3), 55.8 (C-5), 50.7 (C-9), 48.7 (C-18), 48.4 (C-19), 43.2 (C-17), 43.2 (C-14), 40.8 (C-8), 39.7 (C-22), 38.7 (C-4), 38.5 (C-1), 38.7 (C-13), 37.6 (C- 10), 35.7 (C-16), 34.5 (C-7), 29.4 (C-21), 28.4 (C-23), 27.6 (C-2), 27.6 (C-15), 25.4 (C-12), 21.4 (C-11), 19.4 (C-30), 18.7 (C-6), 18.4 (C-28), 16.5(C-25), 16.2 (C-26), 15.7(C-24), 15.2 (C-27).		
Ursolic acid (4)	10	White powder	^{1}H-NMR ($CDCl_3$, 400 MHz):δ 5.26 (IH, t, *J*=3.5, H-12), 3.17 (1H, dd, *J*=10, 4.2 Hz, H-3), 2.15 (1H, d, *J*=11.5 Hz, H-18), 1.92 (1H, dd, *J*=12.8, 4.2 Hz, H_b-22), 1.12 (1H, m, H_a-22), 1. 22 (3H, s, Me-23), 0.94 (3H, s, Me-24), 0.75 (3H, s, Me-25), 1.04 (3H, s, Me-26), 1.12 (3H, s, Me-27), 0.92 (3H, d, *J*= 6.4 Hz, Me-29), 0.89 (3H, d, *J*=5.8Hz, Me-30)			(+)ESI-MS:m/z 455[M-H]$^+$.
Naringnin (5)	14	Yellow needles	^{1}H-NMR (300 MHz, CD_3OD) δ 2.95 (1H, dd, J= 1.7, 9.5 Hz, H-3α), 3.02 (1 H, dd, J= 9.5, 12.4 Hz, H-3β), 5.15 (1H, dd, J= 1.7, 9.5 Hz, H-2α), 5.78 (2H, s, H-6, 8), 6.72 (2H, d, J= 6.5 Hz, H-3', 5'), 7.28 (2H, d, J= 6.5 Hz, H-2', 6').		UV λmax (MeOH): 289, 324sh, (NaOMe):246, 265sh, 328 (AlCl3):312, 374 ($AlCl_3$/HCl) 312, 375, (NaOAc): 294 sh, 325 (NaOAc/H_3BO_3): 290, 324sh, 332 sh.	EI-MS: m/z 272.
Luteolin (6)	8	Yellow powder	^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 12.9 (1H, s, 5-OH), 7.4 (1H, d, *J* = 8 Hz, , H-6´), 7.38 (1H, d, *J* = 2 Hz, H-2´), 6.85 (1H, d, *J* = 8 Hz, H-5´), 6.6 (1H s, H-3), 6.4 (1H, d, *J* = 2 Hz, H-8), 6.15 (1H, d, *J* = 2 Hz, H-6).			EI-MS: m/z 286

Table 2: Cont.

Compound (No.)	Amount recovered (mg)	Physical state	^{1}H-NMR data	^{13}C-NMR data	UV data	MS data
Quercetin (7)	11	Yellow powder			UV λmax (MeOH): 255, 267, 371; (NaOMe): 270, 320, 420; ($AlCl_3$): 270, 455; ($AlCl_3$/HCl): 264, 303sh, 315sh, 428; (NaOAc): 257, 274, 318, 383; (NaOAc/H_3BO_3): 259, 387	EI–MS: m/z 302
Vitexin (8)	15	yellow amor-phous powder	^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.04 (d, *J* = 8.5 Hz, 2H, H-2′,6′), 6.88 (d, *J* = 8.5 Hz, 2H, H-3′,5′), 6.42 (s, 1H, H-3), 6.74 (s, 1H, H-6), 4.65 (d, *J* = 9.6 Hz,1H, H-1").		UV λmax (MeOH): 269, 331; (NaOMe): 279, 325 (sh), 391; ($AlCl_3$): 276, 303 (sh), 346, 382; ($AlCl_3$/HCl): 277; 303, 343, 380 (NaOAc): 278, 387 (NaOAc/H_3BO_3): 270, 319, 346	ESI-MS *m/z*: 433 $[M+H]^+$
Quercetin 3-*O*-*β*-glucoside (9)	12	Yellow crystals	^{1}H-NMR(DMSO-d_6, 400 MHz): δ 7.78 (1H, dd, *J*=2, 8.5 Hz, H-6'), 7.54 (1H, d, *J*=2 Hz, H-2'), 6.82 (1H, d, *J*=8.5 Hz, H-5'), d 6.42 (1H, d, *J*= 2 Hz, H-8), 6.24 (1 H,d, *J*= 2 Hz, H-6), 5.5 (1H, d, *J*=7.5 Hz, H-1").			(-) ESI-MS: m/z 463 $[M-H]^-$.
Quercetin 3-O-rutinoside (Rutin) (10)	18	Yellow powder	^{1}H-NMR (400 MHz, DMSO-d_6): δ ppm 7.54 (2H, m, H-2′/6′), 6.85 (1H, d, *J* = 9 Hz, H-5′), 6.38 (1H, d, *J* = 2.5Hz, H-8), 6.19 (1H, *J* = 2.5 Hz, H-6), 5.35 (1H, d, *J* = 7.5 Hz, H-1''), 4.39 (1H, s, H-1'''), 3.90-3.20 (m, remaining sugar protons), 0.99 (3H, d, J = 6 Hz, H-6′′′).	^{13}C-NMR(DMSO-d6, 100 MHz): δ ppm 177.85(C-4), 164.70(C-7), 161.68(C-5), 157.14(C-2), 156.95(C-9), 148.92(C-4′), 145.25(C-3′), 133.76(C-3), 122.12(C-6′), 121.66(C-1′),116.73(C-2′), 115.72(C-5′), 104.41(C-10), 101.66(C-1′′′), 101.23(C-1′′), 99.24 (C-6), 94.16 (C-8), 74.58 (C-3′′), 72.33 (C-5′′), 72.2 (C-4′′′), 71.05(C-2′′), 70.8 (C-2′′′),70.87 (C-3′′′),70.49 (C-4′′),63.74 (C-6′′),18.19(C-6′′′).	UV λmax (MeOH): 258, 269, 361; (NaOMe): 276, 322, 416; ($AlCl_3$): 232, 276, 302, 366; ($AlCl_3$/HCl): 232, 276, 302, 366; (NaOAc): 284, 306, 381; (NaOAc/H_3BO_3): 261, 312, 376	

Table 3: Phytochemical Analysis of F. vasta aerial parts methanol 80% extract.

Constituents	Methanol extract
Triterpenes and /or Sterols	+
Carbohydrates and/or glycosides	+
Flavonoids	+
Coumarins	+
Alkaloids and/or nitrogenous compounds	+
Tannins	+
Saponins	-

(+) presence of constituents, (-) absence of constituents

Statistical analysis

All biological experiments were statistically expressed as mean ± standard deviation, and analyzed by Student´s *t* test with $P<0.01$. Variables exceeding the upper quantification limit were considered statistically significant.

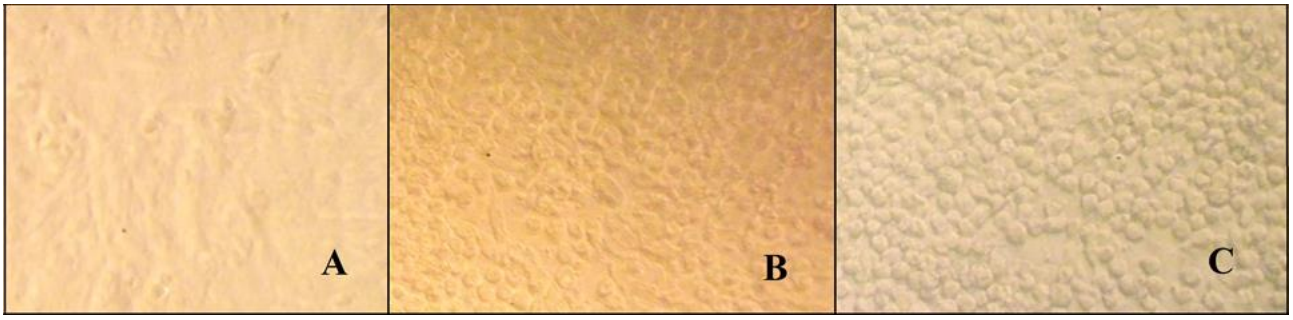


Figure 2: Screening of the anti-HSV-1 activity in Vero cells after 72h of incubation at 37°C and 5% CO_2 in 96-well microplates. (A): negative control, DMEM 5% FBS; (B): positive control, 100xTCID$_{50}$ of HSV-1; (C): 100xTCID$_{50}$ of HSV-1 + methanol 80% extract of *F. vasta* aerial parts at 119 µg/mL. Magnification of 200x.

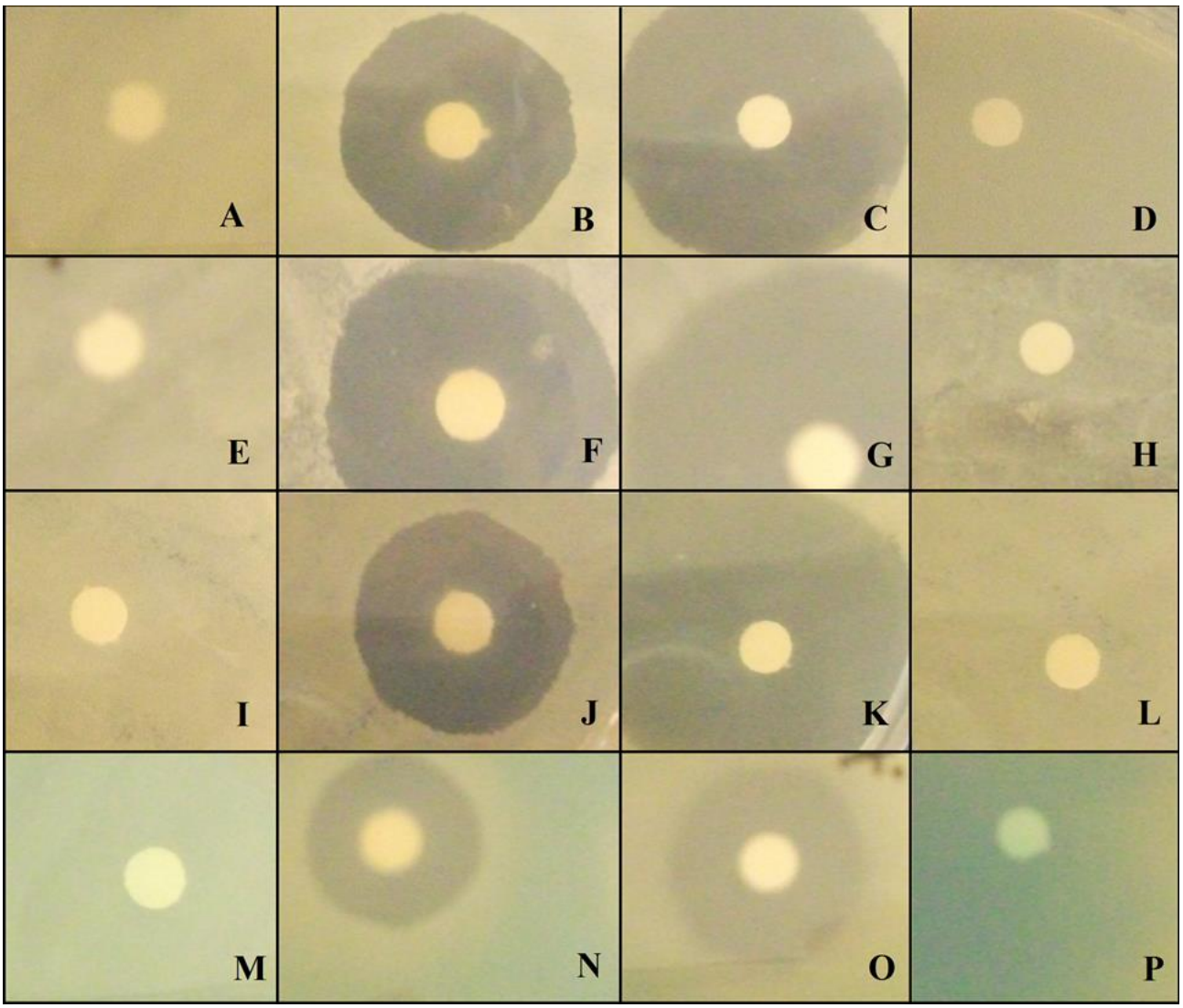
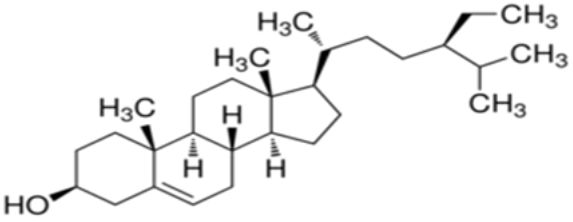
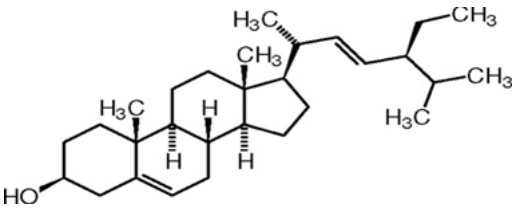


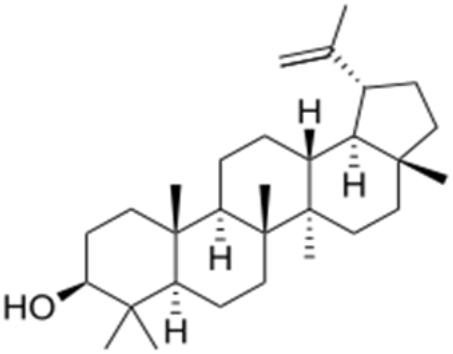
Figure 3: Screening of the antibacterial activity of the methanol extract of *F. vasta* by the disk-diffusion method after 24h of incubation at 37°C. (A): *S. aureus* and 1% DMSO; (B): *S. aureus* and 20% chlorhexidine; (C): *S. aureus* and 0.05% enrofloxacine; (D): *S. aureus* and extract at 119 µg/mL; (E): *S. epidermidis* and 1% DMSO; (F): *S. epidermidis* and 20% chlorhexidine; (G): *S. epidermidis* and 0.05% enrofloxacine; (H): *S. epidermidis* and extract at 119 µg/mL;(I): *E. coli* and 1% DMSO; (J): *E. coli* and 20% chlorhexidine; (K): *E. coli* and 0.05% enrofloxacine; (L): *E. coli* and extract at 119 µg/mL; (M): *P. aeruginosa* and 1% DMSO; (N): *P. aeruginosa* and 20% chlorhexidine; (O): *P. aeruginosa* and 0.05% enrofloxacine; (P): *P. aeruginosa* and extract at 119 µg/mL.



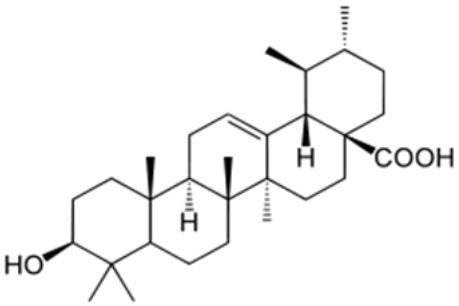
β-sitosterol **(1)**



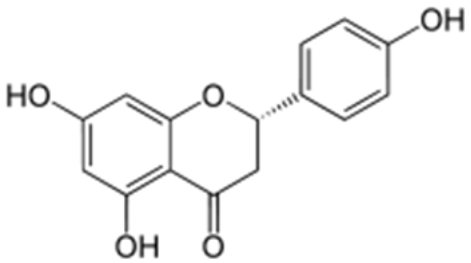
Stigmasterol **(2)**



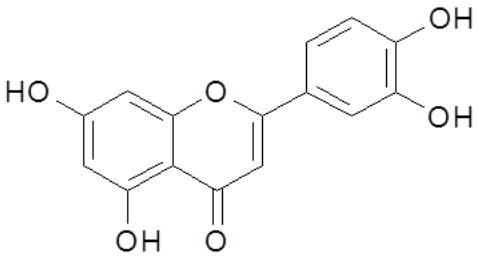
Lupeol **(3)**



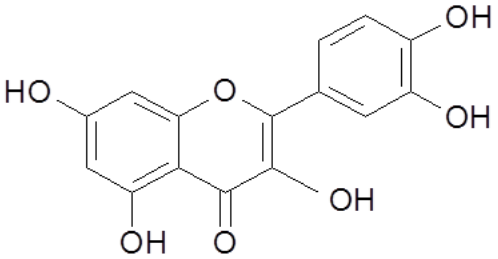
Ursolic acid **(4)**



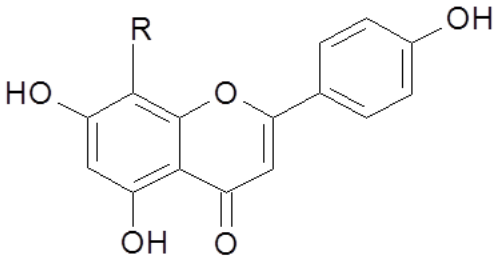
Naringenin (**5**)



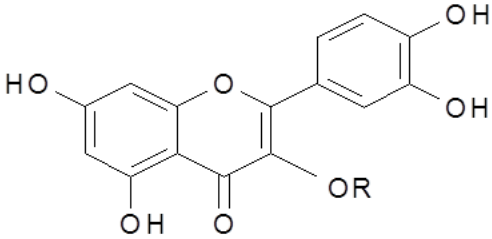
Luteolin (**6**)



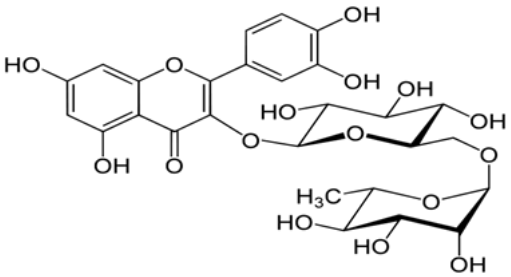
Quercetin (**7**)



Vitexin (R= glucose) (**8**)



Quercetin 3-O-β-glucoside (R=glucose) (**9**)



Rutin (**10**)

Figure 4: Chemical structures of compounds isolated from *F. vasta* methanol extract.

RESULTS AND DISCUSSION

The present investigation evaluated the cytotoxicity, antiviral and antibacterial activities of *F. vasta* aerial parts methanol extract *in vitro,* determined the main phytoconstituents of the extract which are carbohydrates, tannins, flavonoids, coumarins, alkaloids and triterpenes and also detected the bioactive phytoconstituents of *F. vasta* methanol extract which are *β*-sitosterol, stigmasterol, lupeol, ursolic acid, naringenin, luteolin, quercetin, vitexin, quercetin 3-*O*-*β*-glucoside and rutin.

Evaluation of cytotoxicity, antiviral and antibacterial activities

F. vasta methanol extract did not decrease the percentage of viable cells up to 119 µg/mL ($P > 0.01$), presenting a CC_{50} = 389µg/mL in Vero cells. This value is below that reported for the methanol extract of the fruits of *F. vasta* for FL-cells (Al-Fatimi *et al.*, 2007), which is a tumoral cell line derived from human amniotic epithelial cells, presenting a CC_{50} = 980µg/mL. This indicates higher toxicity of the methanol extract of the aerial parts of *F. vasta* for Vero cells by the MTT method in relation to the toxicity of the methanol extract of its fruits for FL-cells by the neutral red method. On the other hand, the CC_{50} of 389µg/mL in Vero cells observed in this study is higher than those reported for other extracts from plants with anticancer potential, such as *Rheum ribes*, *Ficus bengalensis*, *Morus alba*, *Musa sapientum*, *Arnebia decumbens*, *Citrus limon*, *Fraxinus excelsior*, *Rumex acetosella* and *Arnebia echioides* against 7 tumoral cell lines (Sardari *et al.*, 2009), some of them from the Moraceae family. It is already reported that the cytotoxicity potential of compounds varies with the type of cell line and the method employed to evaluate it (Nogueira *et al.* 2011), also with the type of substance which is investigated and its time of exposition (Van de Sandt, 1992). The screening of anti-HSV-1 activity of the extract was carried on at the highest non-toxic concentration (119 µg/mL) in Vero cells, because being the viruses intracellular parasites, the death of the cells could lead to a false antiviral effect. The extract did not inhibit the development of the HSV-1 induced cytopathic effect (rounded cells and production of syncytium) at this concentration, as observed in figure 2. Regarding the compounds identified in the phytochemical characterization, there are reports of antiviral activity against HSV-1 for purified flavonoids quercetin and naningerin (Cotin *et al.*, 2012) evaluated by the ortho-nitrophenyl-β-D-galactopyranoside (ONPG) method, which measures the percentage of surviving cells in relation to an infected control. Differences in the methods employed to evaluate the antiviral activity as well as possible lower concentrations of these compounds in the methanol extract of *F. vasta* or even interactions of the different molecules present in the extract with them could perhaps explain the absence of anti-HSV-1 activity observed in this study. The screening of the antibacterial activity of the methanol extract of *F. vasta* aerial parts showed no inhibition of the growth of Gram-positive (*S. aureus*, *S. epidermidis*) or Gram-negative (*E. coli*, *P. aeruginosa*) bacteria, unlike the controls 20% chlorhexidine and 0.05% enrofloxacine (figure 3). Concentrations above 119 µg/mL (highest non-toxic concentration in Vero cells) could be tested further aiming non-parenteral applications of the extract as antiseptic or disinfectant. This is supported by a report of antibacterial activity mainly against Gram-positive bacteria for an methanol extract from the fruits of *F. vasta* (Al-Fatimi *et al.*, 2007), which was evaluated by the disk-diffusion method employing disks impregnated with 2 mg of the extract, despite the 1.19 µg employed in this study.

Phytochemical analysis

Phytochemical analysis of methanol extract of *F. vasta* aerial parts revealed that it contained carbohydrates, tannins, flavonoids, coumarins, alkaloids and triterpenes (table 3). Chromatographic separation and purification of methanol extract of *F. vasta* allowed the identification of *β*-sitosterol, stigmasterol, lupeol, ursolic acid, naringenin, luteolin, quercetin, vitexin, quercetin 3-*O*-*β*-glucoside and rutin (figure 4). Their structures were elucidated on the basis of UV, ^{1}H-NMR, ^{13}C-NMR and MS analyses.

Structure elucidation of the isolated compounds

Data is given in table 2.

Identification of the active compounds of *F. vasta* methanol extract

Chromatographic separation and purification of the methanol 80% extract of *F. vasta* methanol extract resulted in the isolation and identification of compound 1 (*β*-sitosterol) and compound 2 (stigmasterol) which gave dark spot under short UV light that changed to violet colour on spraying with vanillin sulphuric and heating in an oven at 110°C for 5 min. NMR spectral data has shown signals very close to compound 1 (*β*-sitosterol) and compound 2 (stigmasterol) (Pateh *et al.* 2009). Compound 3 (lupeol) gave a dark spot under short UV light and changed to pink to violet upon spraying with vanillin-sulphuric acid and heating in an oven at 110°C for 5 min. NMR spectral data showed signals very similar to lupeol (Abdullahi *et al.*, 2013). Compound 4 (ursolic acid), was detected through spraying with vanillin sulphuric and heating in an oven at 110°C for 5 min and it gave violet spot at visible light, also NMR and MS spectral data are in accordance with published literature (Zuhal *et al.*, 2006). Compound 5 (naringenin) was obtain as deep purple spot under UV light and changed to yellow when subjected to ammonia and $AlCl_3$. Spectral data are very close to that described by Ibrahim *et al.* (2003). Compound 6 (luteolin) showed a deep purple spot under UV light which changed to yellow with ammonia vapor indicating that a flavone with free 5-OH and 4'-OH and spectral data of compound 6 is very close to that of (Owen *et al.* 2003**).** Compound 7 (quercetin) yellow spot and gave fluorescence yellow colour after spraying with $AlCl_3$ and its spectral data are very similar to Manguro *et al.*, 2005. Compound 8 (vitexin) gave deep purple spot under UV light and changed to yellow when subjected to ammonia and $AlCl_3$. With complete acid hydrolysis, there is no change for compound 8 and thus, it was subjected to ferric chloride degradation, the products being chromatographed with authentic flavonoid aglycone and sugar samples, where apigenin as an aglycone and glucose moiety were detected and all spectral data were very close to that of (Yun-Lina *et al.*, 2000). Compound 9 (quercetin 3-*O*-*β*-glucoside) is obtained as deep purple spot and the compound gave yellow colour when exposed to ammonia vapour and gave a bright yellow colour when spraying with $AlCl_3$. Complete acid hydrolysis of the compound gave quercetin as an aglycone and glucose as sugar moiety. Spectral data of this compound is very close to spectra of Song *et al.* (2007). Compound 10 (Rutin) gave a deep purple spot under UV light and changed to yellow when subjected to ammonia and $AlCl_3$ and complete acid hydrolysis gave quercetin as an aglycone and glucose and

rhamnose as sugar moieties and its spectral data was very similar to that of (Sintayehu *et al*., 2012).

CONCLUSION

This study evaluated the cytotoxicity of the methanol extract of the aerial parts of *F. vasta* in Vero cells, presenting a CC_{50} value which is intermediate among those already reported for *F. vasta* methanol extract of the fruits or extracts of some Moraceae species in different types of cell lines. This cytotoxicity determination will be important to be analyzed in the future if biological activities were found for this extract, because effective doses smaller than cytotoxic doses are desirable. There were not observed anti-HSV-1 activity and antibacterial activity against *S. aureus*, *S. epidermidis*, *E. coli* and *P. aeruginosa* at the highest non-toxic concentration of the extract in Vero cells by the methods employed in this study. Also this study gave a light on the main constituents of the extract, carbohydrates, tannins, flavonoids, coumarins, alkaloids and triterpenes and also identifies the bioactive compounds, *β*-sitosterol, stigmasterol, lupeol, ursolic acid, naringenin, luteolin, quercetin, vitexin, quercetin 3-*O*-*β*-glucoside and rutin from *F. vasta* methanol extract.

CONFLICT OF INTEREST

There is no conflict of interest associated with the authors of this paper.

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