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COMPARITIVE EVALUATION OF *JATROPHA CURCAS* LEAVES EXTRACT FOR ANTIBACTERIAL, ANTI-AVIAN INFLUENZA A VIRUS (H5N1) PROPERTIES AND GC-MS PROFILE

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ABSTRACT

The potent antibacterial and antiviral activity of petroleum ether, ethyl acetate, successive and crude methanolic extracts of *J. curcas* leaves was evaluated. The antibacterial activity of *J. curcas* extracts was studied against 6 common foods borne bacteria using disc diffusion assay. The antibacterial activity of *J. curcas* extracts showed different degrees of inhibition zone against the pathogens bacteria. Yet, successive methanolic and ethyl acetate extracts were effective against most of the tested strains. Additionally, all *J. curcas* extracts were evaluated against the highly pathogenic H5N1 influenza virus (A/chicken/Egypt/M7217B/2013 (H5N1)) using plaque reduction assay. Crude methanolic extract showed antiviral activity with virucidal effect of 75% at concentration 25 µg/µl. The GC-MS analysis of petroleum ether and ethyl acetate extracts revealed the presence of major active constituents. The present study may suggest the possibility for developing new antibacterial and antiviral compounds from *J. curcas* leaves.

KEY WORDS: Antiviral; H5N1; food borne pathogens; GC-MS; *J. curcas*; phytoconstituents.

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INTRODUCTION

Medicinal plants are the greatest asset to human health and represent a viable treasure for discovering new potential compounds with various therapeutic effects. Infectious diseases or transmissible diseases rising from microorganisms or infectious agents pose a health problem throughout the world [1]. Antibiotics have become so broadly used clinically and at the same time many microorganisms have developed antibiotic resistance. Pharmacological research on the bioactive compounds of medicinal plants became very important for the development of new potential antibiotics. Influenza A virus (IAV), the causative agent of influenza, is a large burden to the economy and public health worldwide. With waterfowl as the primary reservoir, the virus is able to infect a wide variety of birds and mammals, including humans. Due to this trait, zoonotic spillovers occur occasionally and can lead to pandemics with severe consequences for the human population. IAV belongs to the family Orthomyxoviridae and possesses a segmented, negative-sense RNA genome. IAV has two important surface proteins called Hemagglutinin (HA) and Neuraminidase (NA). IAV can be classified into several serotypes ranging from H1'H16 and N1'N9. H1, H2 or H3 of IAV are more common in humans [2]. Unlike most RNA viruses, IAV replicates in the nucleus [3]. IAV causes mortality in humans at a rate of approximately 60% worldwide [4]. Vaccines play a critical role in the prevention of influenza. In spite of the efforts made to produce effective vaccines against influenza, it is not likely that efficient vaccines against different subtypes would be available in time to prevent rapidly-spreading pandemics [5,6]. The efficacy of vaccine could be significantly reduced owing to a mismatch between the seasonal influenza vaccine and the circulating influenza virus and the inability of the host to mount a proper immune response [7]. Consequently, much attention has been focused in the development of new antiviral drugs for the effective treatment and overcoming the resistant viruses. Many researchers are attempting to find new anti-influenza viruses agents from both chemically synthesized and natural compounds [8-10]. *J. curcas* shrub (*Euphorbiaceae*) has received extensive attention of many scientists because of its great economic importance, medicinal significance and the use of its seed oil as a commercial source of fuel [11]. A Previous study has reported that, the plant shows activity against for fever, mouth infections, jaundice, guinea worm sores and joint rheumatism [12]. Also, *J. curcas* crude stem extracts inhibit the growth of bacteria of family Enterobacteriaceae like *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia* [13]. *J. curcas* leaf methanolic and aqueous extracts showed effective anti-viral activity against Human Immunodeficiency Virus (HIV) [14].The GC-Ms analysis of methanolic and hot water extracts of *J. curcas* seeds showed manifold compounds with antibacterial, antioxidant and cytotoxicity activities such as β-sitosterol, furfural (2-furancarboxaldehyde) and acetic acid [15]. Also, the GC-MS of *J. curcas* leaf extracts prepared by supercritical fluid CO2 extraction contained multiple compounds with anti-tumor, anti-virus and antimicrobial activities [16].Therefore, the main objectives of the present work are to evaluate the antibacterial activity of *J. curcas* leaves extracts against some selected pathogenic (gram positive and gram negative bacteria), and to evaluate thier antiviral activity against H5N1 virus, as well as to analysis the volatile compounds of petroleum ether and ethyl acetate extracts by GC-MS.

MATERIALS AND METHODS

(i) Chemicals

L-(tosyl**-**amido**-**2**-**phenyl) ethyl chloromethyl ketone (TPCK) and agarose, were obtained from Merck, Sigma and Himedia (Mumbai, India). All other chemicals and solvents used were of analytical grade.

(ii) Collection and identification of plant material

Fresh leaves of *J. curcas* were collected from the farm of Aromatic and Medicinal Plant Department, Agriculture Research Centre, Egypt during July 2013. The plant was authenticated by Agricultural Engineer Terese Labib, El Orman Botanical Garden, Cairo, Egypt.

(iii)Preparation of the sample

The whole leaves samples of *J. curcas* were washed with tap water twice followed with distilled water and dried under shade at room temperature for 5 days. The dry leaves were ground using a grinder. The whole leaves powder of *J. curcas* was packed in a plastic bottle until further analysis.

(iv)Successive and crude methanolic extracts preparation

Successive extracts preparation [17]

The successive extracts were prepared using three solvents in increasing order of polarity. Petroleum ether 40-60°C (referred as E1) followed by extraction with ethyl acetate (referred as E2) and methanol (referred as E3) the ratio was 1:3 w/v. Briefly, 2.5 kg of powdered leaves of *J. curcas* were soaked in petroleum ether (E1) and shaked using shaker (Heidolph UNIMAX 2010) at 150 rpm for 48 h. The extract was filtered using a Buchner funnel and Whatman No. 4 filter paper. The plant residue was re-extracted with the addition of fresh petroleum ether 40-60°C for another two times. Combined filtrates were dried using rotary evaporator (Heidolph-Germany) at 40°C under vacuum. The remaining plant residue was air-dried and soaked in ethyl acetate (E2) as described earlier followed by extraction with methanol (E3). The resulting dry extracts were weighted and then stored at 4°C in the refrigerator for further investigation.

Crude methanolic extract preparation

About 300 g of *J. curcas* powdered leaves were extracted by soaking with methanol (referred as E4) and shaked on shaker at 150 rpm for 48 h. The extract was filtered by using a Buchner funnel and Whatman No. 4 filter paper. The filtrate was dried at 40°C under reduced pressure using a rotary evaporator till complete dryness. The resulting dry crude extract was weighted and then stored at 4°C in the refrigerator for further investigation.

(v) Antimicrobial activity of different J. curcas extracts

Microbial strains and culture medium

The microorganisms used for the evaluation of antibacterial activity were obtained from the American type culture collection (ATCC; Rockville- MD-USA) and the culture collection of the Agricultural Microbiology Dept., National Research Centre, Egypt. The Gram-positive bacteria were; *Bacillus cereus* (ATCC-4513), *Staphylococcus aureus* (ATCC-25923) and *Listeria monocytogenes* (ATCC- 35152). Where, Gram-negative bacteria were; *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-29212) and *Pasterilla hemogensis* (ATCC- 33396). The stock cultures of microorganisms used in this study were maintained on plate count agar slants at 4°C. Inoculums were prepared by suspending a loop full of bacterial cultures into 10 ml of nutrient agar broth and were incubated at 37°C for 24 h. About 60 µl of bacterial suspensions, adjusted to 10^6-10^7 colony forming unit (CFU)/ml were taken and poured into Petri plates containing 6 ml sterilized nutrient agar medium. Bacterial suspensions were spread to get a uniform lawn culture.

Antimicrobial bioassay [18]

Antimicrobial activity of *J. curcas* extracts was determined by using agar-well diffusion method. The tested bacteria were grown on nutrient agar. Wells of 6 mm diameter were dug on the inoculated nutrient agar medium and 50 µl (0.2 mg/ml) of successive extracts (petroleum ether, ethyl acetate and successive methanolic) were re-dissolved in its solvent as well as crude methanolic extract (re-dissolved in chloroform). The plates were allowed to stand at 4°C for 2 h before incubation with the tested extracts. Then the plates were incubated at 37°C overnight and then examined for the zone of inhibition. An extract was classified as active when the diameter of the inhibition was larger than 6 mm. All the assays were performed in triplicate and expressed as average values ± S.D. Negative controls were set up in parallel using the same solvents that were used to reconstitute the extract and the control activity was deducted from the test.

(vi)Antiviral bioassay

Cells and virus

Madin-Darby Canine Kidney (MDCK) cells were maintained in the Center of Scientific Excellence for influenza viruses at the National Research Center. The cells were propagated till confluence in

multiwell plates. The highly pathogenic avian influenza (HPAI) virus A/Chicken/Egypt/M7217B/2013 (H5N1) used in this study was isolated from the infected chickens in Egypt in 2013 and characterized at immunologic and molecular levels.

Preparation of extracts for bioassay

Stock solutions of the tested *J. curcas* extracts were dissolved as 0.1 g in 1 ml of 10% Dimethyl Sulfoxide (DMSO) in deionized water. The prepared extract solutions were used for both cytotoxicity and antiviral bioassays.

Cytotoxicity by MTT assay

Different dilutions of the tested extracts (50, 100, 200 and 400 µg/µl) were conducted by using Dulbecco's Modified Eagle's Medium (DMEM) infection media (4%, BSA, 1% antibiotic). Briefly, the cells were seeded in 96 well-plates (100 µl/well at a density of 3×10^5 cells/ml) and incubated for 24 hrs at 37°C in 5% CO_2. After 24 hrs, cells were treated with various concentrations of the tested extracts in triplicates. After further 24 hrs, the supernatant was discarded and the cell monolayers were washed with sterile phosphate buffer saline (PBS) 3 times and MTT solution (20 µl of 5 mg/ml stock solution) was added to each well and incubated at 37°C for 4 hrs followed by medium aspiration. In each well, the formed formazan crystals were dissolved with 200 µl of acidified isopropanol (0.04 M HCl in absolute isopropanol). Absorbance of formazan solutions were measured at λmax 540 nm with 620 nm as a reference wavelength using a multi-well plate reader. The percentage of extracts cytotoxicity compared to the untreated cells was determined with the following equation:

$$\text{Cytotoxicity \%} = \frac{[\text{Absorbance of cell without treatment} - \text{Absorbance of cell with treatment}] \times 100}{[\text{Absorbance of cell without treatment}]}.$$

The plot of cytotoxicity % versus sample concentration was used to calculate the concentration which exhibited 50% tissue cytotoxicity (TC_{50}).

Antiviral activity by plaque reduction assay [19]

Anti-H5N1 activity of successive extracts (E1, E2 and E3) and crude methanolic extract (E4) investigated by plaque reduction assay with confluent 24 h old monolayer of MDCk cells. Assay was carried out in a six well plate where MDCK cells (10^5 cells / ml) were cultivated for 24 hrs at 37°C. A/Chicken/Egypt/M7217B/2013 (H5N1) virus was diluted to give 10^4 PFU/well and mixed with the safe concentrations of the tested compounds and incubated for 1 hour at 37°C before being added to the cells. Growth medium was removed from the cell culture plates and virus-Cpd or virus-extract and Virus-Zanamivir mixtures were inoculated (100 µl / well). After 1 hour contact time for virus adsorption, 3 ml of DMEM supplemented with 2% agarose was added into the cell monolayer, plates were left to solidify and incubated at 37°C till formation of viral plaques (3 to 4 days). Formalin (10%) was added for two hours then plates were stained with 0.1% crystal violet in distilled water. Control wells were included where untreated virus was incubated with MDCK cells and finally plaques were counted and percentage reduction in plaques formation in comparison to control wells was recorded as following: % of inhibition= [viral count (untreated) - viral count (treated)/viral count (untreated)] x 100.

(vii) Gas Chromatography/Mass Spectrometry (GC-MS) analysis

The GC-MS analysis of *J. curcas* petroleum ether and ethyl acetate extracts was performed using a Thermo Scientific capillary gas chromatography (model Trace GC ULTRA) directly coupled to ISQ Single Quadruple MS and equipped with TG-5MS non polar 5% phenyl methylpolysiloxane capillary column (30 m x 0.25 mm ID x 0.25 µm). The operating condition of GC oven temperature was maintained as: initial temperature 40°C for 3 min, programmed rate 5°C/min up to final temperature 280°C with isotherm for 5 min. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium was used as a carrier gas at a constant flow rate

of 1.0 ml/min. 1µl solution of petroleum ether and ethyl acetate extracts dissolved in diethyl ether was injected automatically in the splitless mode. Detection was performed in the full scan mode from 40 to 500 m/z. The identification of the chemical components was carried out based on the retention time of each component (Rt) compared with those of the Wiley 9 and NIST08 mass spectra libraries (NIST, 2010).

RESULTS

1. Antibacterial activity

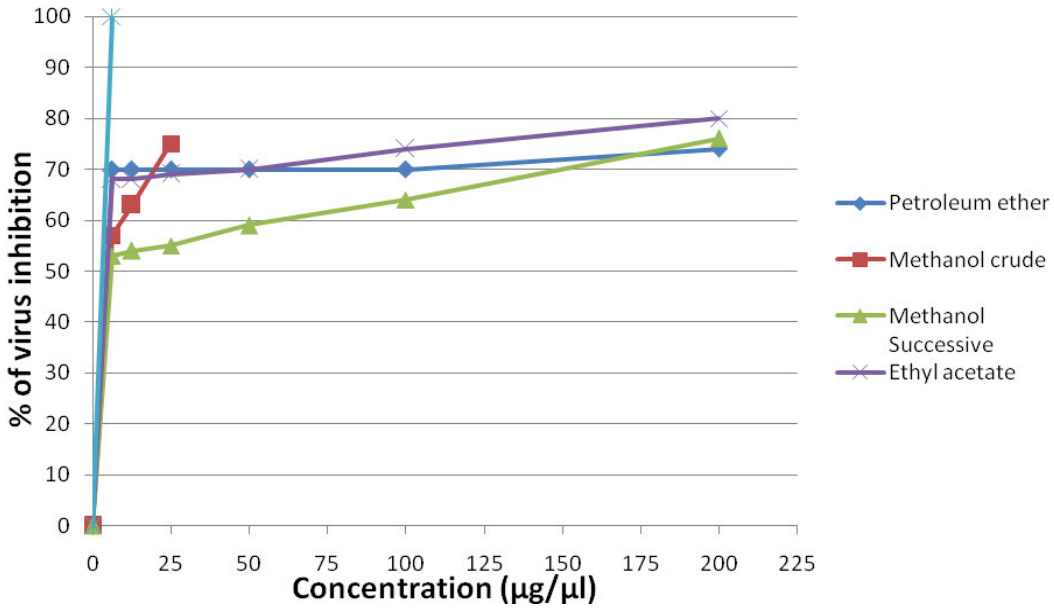
Table (1) showed the *in vitro* antibacterial activity of different *J. curcas* leaves extracts. The results indicated that *J. curcas* extracts have different inhibitory effects on Gram-positive and Gram-negative bacteria. Ethyl acetate (E2) showed promising effects against Gram-positive bacteria *(B. cereus* and *S. aureus)* with maximum inhibition zone 14.33±0.58 and 21.33±3.21 mm. While successive methanolic extract (E3) showed the highest antibacterial activity against *L. monocytogenes* with maximum inhibition zone 15.00±1.73 mm at concentration of 0.2 mg/ml. In addition, successive methanolic extract (E3) showed the highest depression of Gram-negative bacteria (*E. coli* and *P. aeruginosa)* with maximum inhibition zone 8.33±2.52 and 14.00±2.65, respectively. Yet, ethyl acetate extract (E2) and crude methanolic extract (E4) exhibited the highest antibacterial activity against *P. hemogensis* with maximum inhibition zone 15.00±4.36 and 15.00±1.00 mm, respectively.

2. Antiviral activity

The toxicity of *J. curcas* extracts were determined by using MTT assay (Table 2). In the present study, *J. curcas* crude methanolic leaves extract shows a potential activity against influenza A virus (H5N1) infection up to 75% virus inhibition activity at concentration 25 µg/µl. The polar extracts (crude methanolic and ethyl acetate) showed the highest antiviral activity (Fig. 1) as compared to the low polar one (petroleum ether). This is may be due to the high ability of polar solvents to extract bioactive compounds.

Figure 1

Percentage of virus inhibition of J. curcas leaves extract at different concentrations.



3. Chemical composition of J. curcas extracts by GC-MS

The (GC-MS) study of petroleum ether and ethyl acetate extracts of *J. curcas* leaves has shown many phytochemicals (Tables 3 and 4). Petroleum ether extract gave 31 compounds while ethyl acetate extract gave 39 compounds. The major phytoconstituents presented in the petroleum

ether extract were phytol (11.95%) and squalene (10.29%). However, 2-Pentadecanone, 6, 10, 14-trimethyl10 and -Dodecyn-1-ol; are the major phytoconstituents in ethyl acetate extract of *J. curcas* leaves with area percent 6.89 and 6.85%, respectively.

Figure 2

The GC-MS chromatogram of petroleum ether extract of J. curcas leaves.

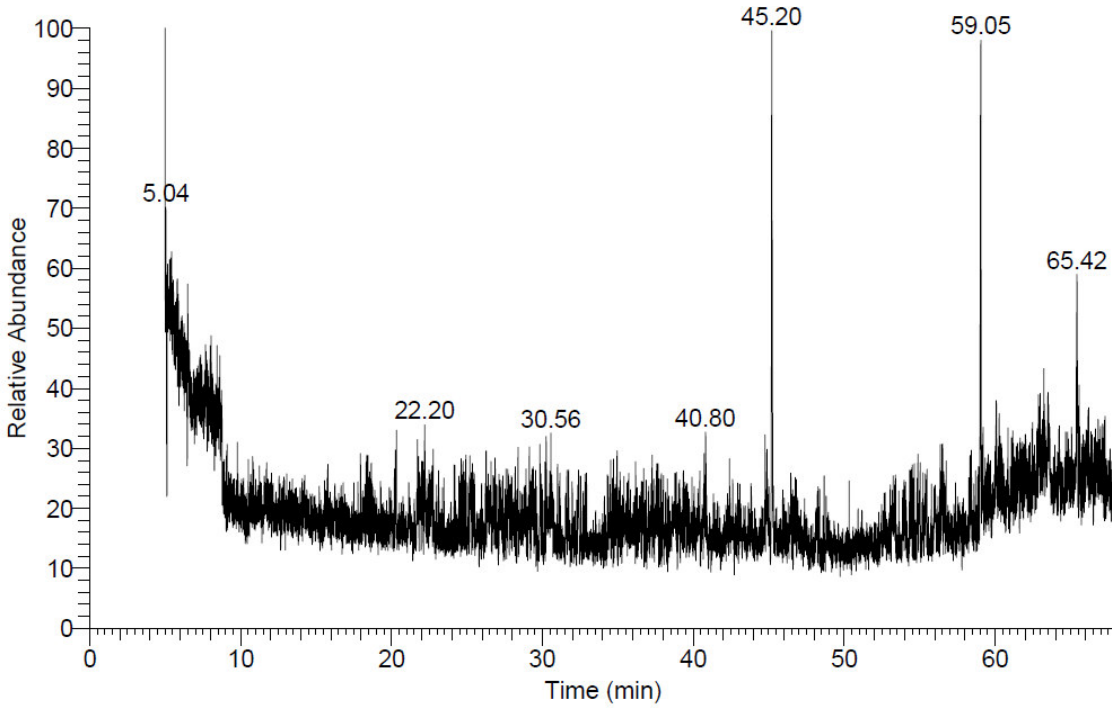


Figure 3

The GC Chromatogram of ethyl acetate extract of J. curcas leaves.

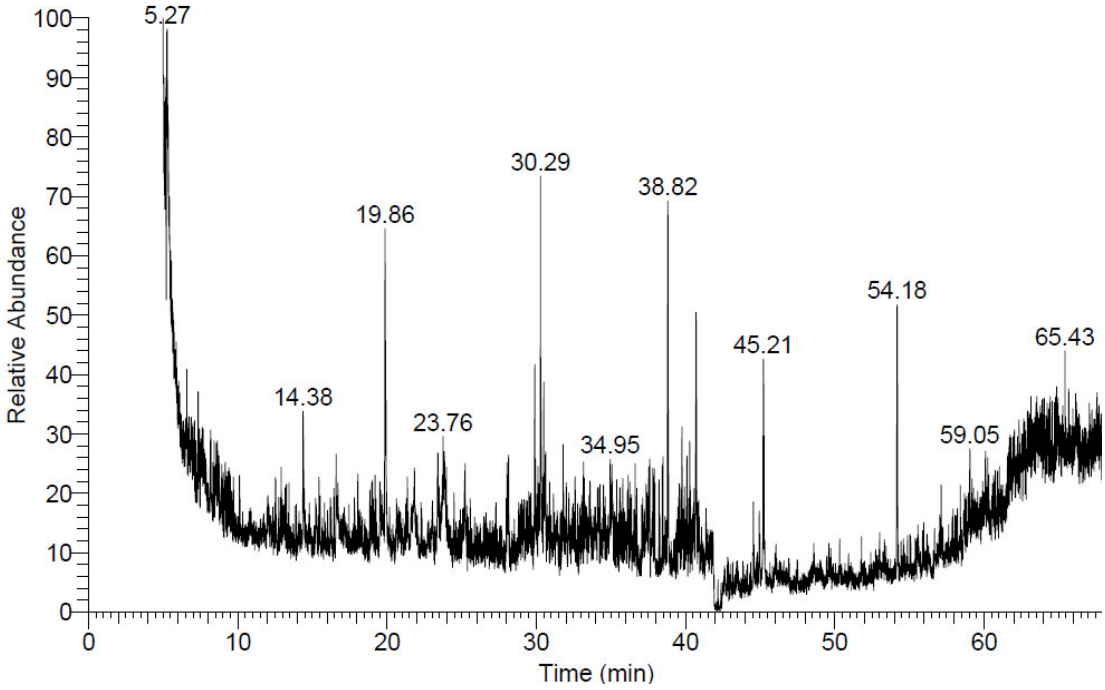


Table 1

The antibacterial activity of J. curcas leaves extracts at 0.2 mg/ml concentration.

Microorganisms	Inhibition zone (mm)			
	(E1)	(E2)	(E3)	(E4)
Gram-positive				
B. cereus	NI	14.33±0.58	10.33±0.58	NI
S. aureus	NI	21.33±3.21	20.17±2.36	NI
L. monocytogenes	NI	14.00±0.40	15.00±1.73	12.00±1.73
Gram-negative				
E. coli	NI	7.66±0.58	8.33±2.52	NI
P. aeruginosa	NI	10.00±2.00	14.00±2.65	8.00±3.46
P. hemogensis	NI	15.00±4.36	14.00±3.00	15.00±1.00

Values are mean of inhibition zone (mm) ± S.D of three replicates.
The diameter of the well (6 mm) is included. NI: No inhibition

Table 2

Tissue culture infectious dose for 50 percent (TC50) of J. curcas leaves extract.

J. curcas extracts	Tissue culture infectious dose 50% (TC_{50})
Petroleum ether (E1)	225 µg/µl
Ethyl acetate (E2)	274 µg/µl
Successive methanol (E3)	481.5 µg/µl
Crude methanol (E4)	44.5 µg/µl

Table 3

Compounds detected in petroleum ether leaves extract.

No.	Name of compound	RT (min)	Area %	Molecular formula
1	3-Trifluoroacetoxypentadecane	5.08	1.82	$C_{17}H_31F_3O_2$
2	2-Trifluoroacetoxypentadecane	5.16	5.15	$C_{17}H_31F_3O_2$
3	4-Trifluoroacetoxypentadecane	6.50	2.44	$C_{17}H_31F_3O_2$
4	2,2'Dibromo5,5'di(4-methoxyphenyl)4,4' ditertbutyl biphenyl	8.44	1.59	$C_{34}H_{36}Br_2O_2$
5	Trans-2-Phenyl-1,3-Dioxolane-4-Methyl Octadec 9, 12,15-Trienoate	20.27	1.74	$C_{28}H_{40}O_4$
6	N,N'Bis-[3-methoxy-4-hydroxy-5-bromobenzylidene (cyano)acetyl]-1,4-butanediamine	22.00	1.74	$C_{26}H_{24}Br_2N_4O_6$
7	5,10-bis-(3aminophenyl)15,20-diphenyl porphyrin	22.20	1.92	$C_{44}H_{32}N_6$
8	l-Gala-l-idooctose	27.18	2.27	$C_8H_{16}O_8$
9	N,N'Dicyclohexyl1cyano-7 pyrrolidinyl perylene-3,4:9,10-tetracarboxylic acid Bisimide	29.28	1.51	$C_{41}H_{36}N_4O_4$
10	Acetic acid, (2,4,5-trichlorophenoxy), isooctyl ester	29.83	1.81	$C_{16}H_{21}C_{l3}O_3$
11	5,11,17,23-Tetratbutyl-25,26,27,28-tetra hydroxyl- calix-4-arene	30.63	1.42	$C_{44}H_{56}O_4$
12	Hematoporphyrin dimethyl ester	31.61	2.00	$C_{36}H_{42}N_4O_6$
13	1[2,4,6tris(trimethylsiloxy)phenyl]-3-[3-methoxy-4-(trimethylsiloxy)phenyl]2-propen1one	31.72	1.56	$C_{28}H_{46}O_6Si_4$
14	Tetratertbutyl-2,6-di(3propenyl)-3,7-dimethoxybi-cycle[3.3.0]octa3,7-diene 2,4, 6,8-dicarboxylate	32.41	1.43	$C_{36}H_{54}O_{10}$
15	2,9-Bis(5tertbutyl-2-hydroxy-3-pyridyl phenyl)-1,10-phenanthroline	34.49	1.70	$C_{42}H_{38}N_4O_2$
16	1,3,7,9-tetra(methoxymethoxy)-2-(4 (tbutoxycarbon-yl)hex-1-en-5-only) anthraquinone	34.92	1.57	$C_{33}H_{40}O_{14}$
17	15,11metheno11Htribenzo[c,g,n]-[1,6] dioxacyclo pentadecin-7-carbox aldehydes,22-ethoxy	36.49	2.13	$C_{42}H_{40}O_6$
18	Hexadecane, 1,1-bis-(dodecyloxy)	37.55	2.42	$C_{40}H_{82}O_2$
19	Pentadecanoic acid,1,4-methyl,methylester	40.81	2.48	$C_{17}H_{34}O_2$
20	2,2-Dideuterooctadecanal	4.88	1.54	$C_{18}H_{34}D_2O$
21	Phytol	45.20	11.95	$C_{20}H_{40}O$
22	4a,5a,6,11,11a,12aHexahydro-19-methyl1,4-di phenyl6,11[1',2']benzene	53.23	1.45	$C_{36}H_{27}NO_4$

23	9,10-Secochola5,7,10(19)-triene3,24-diol,(3á,5Z,7E)	54.87	1.54	$C_{24}H_{38}O_2$
24	Squalene	59.04	10.29	$C_{30}H_{50}$
25	D-Nerolidol	60.07	1.63	$C_{15}H_{26}O$
26	2(6[1-Ethyl4[4-(1-Hpyrrole-2-Carbonyl)-2,3, 3A, 4,5 ,7-Ahexahydro-1-Hinden-5-YL]-Buta-1,3-Dienyl]-5 Methyltetrahydropyran-2-YL)	61.65	1.51	$C_{29}H_{39}NO_4$
27	Desulphosinigrin	62.01	2.12	$C_{10}H_{17}NO_6S$
28	Thiosulfuric acid ($H_2S_2O_3$), S-(-2-aminoethyl) ester	62.87	2.38	$C_2H_7NO_3S_2$
29	9-Octadecenoicacid (Z)	63.50	3.07	$C_{18}H_{34}O_2$
30	2-Myristynoylpantetheine	64.26	1.50	$C_{25}H_{44}N_2O_5S$
31	Erucic acid	65.42	4.41	$C_{22}H_{42}O_2$

Table 4
Compounds detected in ethyl acetate leaves extract.

No.	Name of the compound	RT (min)	Area %	Molecular formula
1	Trimethylsilyl DerivativeOf 2-Monoolein	5.15	1.96	$C_{27}H_{56}O_4Si_2$
2	4-H-Cyclopropa[5',6']benz[1',2':7,8]-azuleno [5,6b] oxiren-4-one, 8,8 abis (acetyloxy) 2a [(acetyloxy) methyl]1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8adodecahydro3,3a,6-btrihydroxy-1,1,5,7-tetramethyl	5.25	6.69	$C_{26}H_{34}O_{11}$
3	3,5,9-Trioxa-5-phosphaheptacos-18-en-1-aminium,4-hydroxyN,N, Ntrimethyl-10-oxo7-[(1-oxo-9-octa-decenyl)oxy],hydroxide,inner salt,4-oxide,(R)	6.58	1.50	$C_{44}H_{84}NO_8P$
4	9-Desoxo-9-xihydroxy3,7,8,9,12-pentaacetateIngol	12.03	1.39	$C_{30}H_{42}O_{11}$
5	Penitrem A	12.52	1.45	$C_{37}H_{44}C_lNO_6$
6	5-Hepten1ol,2,6-dimethyl	14.38	2.69	$C_9H_{18}O$
7	Ethyl iso allocholate	16.58	1.52	$C_{26}H_{44}O_5$
8	3-Cyclopentyl-1-propyne	19.53	2.13	C_8H_{12}
9	10-Dodecyn-1-ol	19.87	6.85	$C_{12}H_{22}O$
10	2-Decenal,(Z)	21.82	1.41	$C_{10}H_{18}O$
11	2-MethylE,E3,13-octadecadien-1-ol	23.40	2.25	$C_{19}H_{36}O$
12	I-B-A-TMS	23.76	1.69	$C_{32}H_{41}IO_3Si$
13	2,2-Bis[4[(4,6dichloro1,3,5-triazin 2yl)oxy]phenyl] 1,1 ,1,3,3,3-hexafluoropropane	23.83	2.11	$C_{21}H_8C_{l4}F_6N6O_2$
14	9-Octadecenoic acid (Z)	25.23	2.04	$C_{18}H_{34}O_2$
15	6,7-Bis(3bromopropyl)1,3,5,8-tetramethyl2,4-divinyl porphyrin	28.04	1.50	$C_{34}H_{36}Br_2N_4$
16	Tetratetracontane	28.14	1.81	$C_{44}H_{90}$
17	Ethanone,1(5,6,7,8tetrahydro2,8,8-trimethyl-4-Hcyclo-hepta[b]furan5yl)-	29.89	3.36	$C_{14}H_{20}O_2$
18	(E,E)7,11,15-Trimethyl-3-methylene hexadeca1 ,6,10, 14-tetraene	30.29	4.34	$C_{20}H_{32}$
19	Chlorocadmium(II)(19-S)-1-Methylden-2,2,7,7,12,12-Hexamethyl-15-Cyano-19-Methoxycarbonnyl1,19-Secocorrinate	30.50	1.85	$C_{29}H_{36}CdClN5O_2$
20	Milbemycinb,13-chloro-5-demethoxy -28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1methylethyl) ,(6R,13R, 25R)-Rhodopin	30.60	1.74	$C_{33}H_{46}ClNO_7$
21	7{4'[4"(5"'Chloro2"'methoxybenzoyl)-amino]phenyl}-2-(thienylmethylene)-3-oxo-5-(pfluorophenyl)2,3-Dihydro-5-Hthiazolo[3,2a]pyrimidine	31.80	1.74	$C_{31}H_{21}ClFN_3O_3S_2$
22	psi.,.psi.Carotene,3,4didehydro1,2,7',8'tetrahydro1methoxy-2-oxo-Spherodenon	34.11	1.66	$C_{41}H_{58}O_2$
23	1,4-Bis(3,5dibromo-2-thienyl) benzene	35.07	2.39	$C_{14}H_6Br_4S_2$
24	1"'Trimethylsilyl-3-bromo[1[4 (2phenyl1,4dihexy-l-phenyl)phenyl]]benzene	37.59	2.00	$C_{39}H_{49}BrSi$
25	Hahnfett	37.81	2.59	N/A
26	Lycopene	38.44	1.41	$C_{40}H_{56}$
27	2-Pentadecanone,6,10,14-trimethyl	38.82	6.89	$C_{18}H_{36}O$
28	N,N'Dicyclohexyl1cyano-7-pyrrolidinylperylene 3,4 :9, 10-tetra carboxylicacid Bisimide	39.62	1.98	$C_{41}H_{36}N_4O_4$

29	N,N'Bis[3methoxy4hydroxy-5-bromo benzylidene (cyano)acetyl]1,4-butanediamine	39.76	2.07	$C_{26}H_{24}Br_2N_4O_6$
30	Grcchpacgknys C/3	40.09	1.59	N/A
31	Isochiapin B	40.71	4.56	$C_{19}H_{22}O_6$
32	Pregn4ene3,11,20-trione, 6,17, 21 tris [(trimethyl silyl) oxy],3,2,0bis (Omethyloxime) ,(6á) (CAS)	41.83	1.57	$C_{32}H_{58}N_2O_6Si_3$
33	1-Octadecanol(CAS)	44.55	1.49	$C_{18}H_{38}O$
34	Docosane(CAS)	44.95	1.29	$C_{22}H_{46}$
35	2-Hexadecen1ol,3,7,11,15-tetramethyl,[R[R*,R*(E)]]	45.22	5.61	$C_{20}H_{40}O$
36	1,2-Benzenedicarboxylicacid,bis(2-ethylhexyl)ester	54.18	4.93	$C_{24}H_{38}O_4$
37	2,4,6,8,10-Tetradecapentaenoic acid, 9a-(acetyloxy)1a, 1b,4,4a,5, 7a,7b, 8,9,9a-decahydro4a,7-bdihydroxy-3-(hydroxymethyl) 1,1,6,8-tetramethyl-5-oxo-1-Hcyclo propa [3,4] benz [1,2e]azulen-9-ylester	59.06	1.62	$C_{36}H_{46}O_8$
38	Dehydrocancentrinea	61.72	1.40	$C_{36}H_{32}N_2O_7$
39	Stearic acid, 3-(octadecyloxy)propyl ester	62.03	1.33	$C_{39}H_{78}O_3$

DISCUSSION

Microbial pathogens are developing resistance against existing antibiotics therefore; the discovery of new pharmaceutical compounds is considered as a great stress [20]. Previous studies have reported that aqueous, methanolic and hexane extracts of *J. curcas* leaves, stem bark, root bark, root wood and kernel seeds exhibits different antibacterial activity against *B. cereus, E. coli, K. pneumonia, P. aeruginosa, S. aureus and Methicillin-resistant S. aureus (MRSA)* [21]. The present results (Table 1) are run in harmony with those obtained by Nyembo et al. [22]. They found that, crude methanolic extract of *J. curcas* leaves showed antibacterial activity against *P. aeruginosa* with 8 mm inhibition zone. Also, the crude methanolic extract of *J. curcas* leaves showed antibacterial activity against *E. coli* and *S. aureus* with inhibition zone 8 and 11 mm, respectively [23]. In the present results, *J. curcas* extracts (E2 and E3) show higher antibacterial activity against Gram-positive bacteria more than against Gram-negative bacteria. This is may be due to that the Gram-negative bacteria are more resistant than the Gram-positive bacteria because of their containing lipopolysaccharide in their outer membranes which handled them resistant to dyes, detergent and antibiotics [24]. Additionally, in the present study, polar solvents (E2, E3 and E4) extracts exhibited the highest antibacterial activity against the tested bacteria as compared to the low polar one, E1 which has no effect on the tested bacteria. This may be attributed to the highly ability of high polar solvents to extract higher concentrations of bioactive molecules such as phenolics and terpenoids [25]. In this concern, saponins, tannins, flavonoids, alkaloids and glycosides in the methanolic extract of *J. curcas* leaves were detected [26]. Phytochemicals such as alkaloids, terpenoids, saponins and phenolics are considered as strong antimicrobial agents that can aid in solving the problem of antibiotic resistance [27,28]. The antibacterial activity of phenolics compounds extracted from *Hippophae salicifolia* may be due to their ability to form complex with extra cellular soluble proteins and bacterial cell walls throughout the formation of nonspecific forces like hydrogen bonding, hydrophobic effects and covalent bond [29]. In accordance with the present work, the GC-MS analysis of ethyl acetate extract (Table 4) showed the presence of several compounds such as ethyl iso allocholate that has antimicrobial activity as was found by Muthulakshmi et al. [30]. 3-cyclopentyl-1-propyne, lycopene, 2-pentadecanone, 6, 10, 14-trimethyl and docosane as they have antimicrobial activity [31-34]. Hence, the potential antibacterial activity of *J. curcas* against Gram-positive and Gram-negative bacteria may be explained on the basis of, the presence of these bioactive phytoconstituents. The probable antimicrobial mode of action of *J. curcas* plant may rely on the explanation of [35,36]. The authors postulated that, the antimicrobial activity of *J. curcas* may be related to the inhibition of the enzymes catalytic action *via* formation of stoichiometric complex with the target enzymes resulting in the blocking or altering their active site. The antiviral activity against H5N1 virus was determined by plaque reduction assay. E1 showed moderate inhibition against the tested strain of H5N1 influenza virus, nevertheless it cannot be recognized as a promising one since on doubling the concentration of the extract, the percentage of virus inhibition remains steady, whereas the reference antiviral Zanamivir shows

complete inhibition at concentration 10µg/µl. E3 showed also, moderate inhibition potency (Fig.1) and this may be due to the solvent used. In addition, in this study E4 and E2 exhibited a moderate virus inhibition percentages i.e. as the concentration of the extracts increase their inhibition potential against the H5N1 virus strain increase (dose-dependent relationship), thus they are considered as good candidates for further studies. Flavonoids were detected in methanolic extract of *J. curcas* leaves by Dahake et al. [37]. In this concern, flavonoids produce various clinical characters such as antibacterial, antifungal and antiviral effects [38]. In addition, the *in vitro* anti-H5N1 molecular mode of action of flavonoids is by their direct binding to H5N1 virus particles resulting in the H5N1 viruses' inability to enter host cells, consequently effectively preventing H5N1 infection. So, *J. curcas* leaves may support antiviral activity due to the presence of flavonoids [39]. The GC-MS analysis of *J. curcas* kernel meal was studied [15]. The current study detected the presence of 2-(Hydroxymethyl)-2-nitro-1, 3-propanediol in methanolic extract with area percent (23.10% w/w) and 2-furancarboxaldehyde, 5-(hydroxymethy) (29.70% w/w) as major compounds. Moreover, the GC-MS analysis of *J. curcas* leaves was carried out in concomitant with Wang et al. [16]. The authors have declared the presence of 43 compounds and the four most abundant components were 22,23-dihydro-stigmasterol (16.14%), alpha-tocopherol (15.18%), beta-amylin (7.73%) and dotriacontanol (7.02%) as they have anti-tumor, anti-virus and antimicrobial activities. Also, reported the presence of phytol in *Jatropha gossypifolia* ethanolic extract with area percent 2.11% was reported [40].The GC-MS analysis of E1 revealed the presence of different compounds with different biological activities such as phytol (11.95%) diterpene, which is considered as effective vaccine and squalene (10.29%) which has antitumor properties, chemo preventive and immune stimulant [41,42]. Thus, the antiviral activity of E1 may be due to the presence of phytol and squalene compounds. In the present study, the GC-MS analysis of E2 of *J. curcas* demonstrates the presence of bioactive compounds such as; ethyl iso allocholate (1.52%) which has antibacterial and antifungal activities [43]. Moreover, lycopene (1.41%) which has antimicrobial activity as reported by Lingen et al. [31]. 3-cyclopentyl-1-propyne (2.13%) which has antimicrobial activity as was studied by Robert et al. [44]. As well as 2-pentadecanone 6, 10, 14-trimethyl (6.89%) and 2-hexadecen1ol,3,7,11,15-tetramethyl,[R[R*,R*(E)]] (5.61%) as they have antibacterial activity [32,45]. Hence, the potential antibacterial activity of E2 against Gram-negative and Gram-positive bacteria may be relayed to the presence of these compounds.

CONCLUSION

In the present study, different extracts (petroleum ether, ethyl acetate, successive methanolic and crude methanolic extracts) of *J. curcas* leaves possessed antibacterial and anti-H5N1 activities. The successive methanolic extract exhibited the highest antibacterial activity. This is may be due to the high ability of methanol to extract higher concentrations of antimicrobial molecules. While, crude methanolic extract showed the highest antiviral activity. This is may be attributed to the presence of antiviral molecules such as flavonoids and saponins. In addition, 31 compounds and 39 compounds were identified in petroleum ether and ethyl acetate extracts of *J. curcas* respectively by GC-MS analysis. The presence of different compounds with several biological activities enhances the possibility to use *J. curcas* plant as antimicrobial and antiviral agent in pharmaceutical and medicinal applications. This is the first report of anti-H5N1 of *J. curcas* leaves extract. Further studies for isolation and purification of the compounds responsible for antimicrobial and anti-H5N1 activities are needed.

CONFLICT OF INTEREST

Conflict of interest declared none.

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