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Therapeutic potentials of *Vachellia nilotica (L.)* extracts in Hepatitis C infection: A review ☆

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ABSTRACT

Hepatitis C Virus (HCV) infection represents a huge burden on healthcare systems worldwide. *Vachellia nilotica* (*V. nilotica*) is a widely used plant specie in folk medicine for viral diseases and in some communities for HCV infection. However, little is known regarding its role and possible mechanisms in the prevention and treatment of this viral infection. This review presents ethno-pharmacological, *in vitro* and *in vivo* shreds of evidence of the role and underlying mode of action of *V. nilotica* and its implication in treatment and complication management of HCV infection. PubMed, Library of Congress, SCOPUS, Science Direct and Google scholar databases were searched. Twenty-eight articles of which 15 were *in vitro* while remaining were *in vivo* studies were reviewed. *V. nilotica's* modes of antiviral activity are direct inactivation of HCV and inhibition of HCV NS3 protease. Its immunomodulatory activity showed by immune cell proliferation and inhibition of immunosuppressive cytokine. *V. nilotica's* anticancer activity through inhibition of oxidative stress, inhibition of chromosomal aberrations and enhancement of antioxidant enzymes could be beneficial in treating HCV infection and delaying its progression to cancer. It can be inferred that *V. nilotica* could be a promising source of anti-hepatitis C virus drug leads with the ability to prevent its long-term sequelae while promoting immune competence. Further studies are needed to explore the applicability of the herb to clinical settings.

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Introduction

Hepatitis C virus is an enveloped positive-sense Ribonucleic acid (RNA) virus in the genus Hepacivirus of the Flaviviridae family. It has a 9.6 kb single-strand genome that encodes a long polyprotein precursor of ~3,000 amino acids [40]. This polyprotein is processed proteolytically upon a translation by viral proteases and cellular proteases. The HCV genome is composed of structural proteins namely core protein, envelop protein (E1, E2) and non-structural (P7, NS2, NS3, NS4 and NS5) proteins. The non-structural protein act majorly as proteases and polymerase in the replication process. The HCV, endowed with a high rate of replication coupled with a high rate of error of its polymerase, expands increasing rate of mutation during replication and thus drug resistance [15,60]. The virus is transmitted through needle sharing among drug users or in poor hygienic conditions in medical practice but also sexually and from mother to child. About 60-80% of infected patients develop chronicity without any symptoms over the decades, while the disease progresses to cancer stage.

Hepatitis C infection represents a huge burden in public health in the world. The last prevalence reported by the World Health Organization indicates that 71 million persons in the world were chronically infected, with 1.75 million of new cases of HCV infection occurring each year (World Health Organization, 2019). The mortality also increased from 350,000 in 2010 to 399,000 in 2016, predominantly due to the complications of HCV infection which are liver cancer (cirrhosis and hepatocellular carcinoma), non-Hodgkin lymphoma and recently reported prostate and renal cancers [48].

Though there are lots of effort to make the conventional drugs widely accessible, the cost of direct-acting antivirals conventionally used for treatment, in low and middle-income countries, is estimated at US $ 600-900 for one set of 12 weeks course of treatment [28,82]. This amount is far beyond the means of most people in this region, so very few can afford the treatment. It is estimated that only 1.1 million patients in 2015 were able to receive the treatment [99]. Although these drugs have shown dominant inhibitory activity with 90% of viral clearance [41], they are challenged by increasing viral resistance and associated incidence of hepatocellular carcinoma [26,77,81].

Therefore, an increasing number of patients are seeking alternative or complementary medicines [86], particularly in developing countries where 80% of infected people reside [82]. The use of alternative medicines and the evidence thereof is not well documented, especially in Africa. Some plant isolated compounds such as gallic acid [42], chebulagic acid and punicalagin [63], Epigallocatechin-3-gallate [25], delphinidin [21], saikosaponins [64] have been proved in vitro to inhibit earlier steps of HCV infection, and considered as HCV entry inhibitors. Natural products appear thus to be a promising source of antiviral compounds against HCV [19]. *Vachellia nilotica* also called *Acacia nilotica* or *Acacia arabica* is a plant species belonging to the Fabaceae family [87].

The species is present throughout tropical and sub-tropical regions of Africa, India and Australia. It is widely used in folk medicine in the treatment of respiratory infections (cold, cough, tuberculosis) [31], skin diseases (leprosy, leukoderma, burning sensation) (Atif [8]), digestive diseases (constipation, dysentery, diarrhea, and ulcer) [1], and gynecologic concerns (postpartum wound healing, female fertility, leucorrhea, and sexually transmitted diseases) [73]. *V. nilotica* is one of the antiviral medicinal plants used in traditional medicine for the management of HCV infection in Sudan [7] and Zimbabwe for the management of sexually transmitted diseases "*njovhera* " including HCV infection in the Guruve district [50].

Pharmacologic studies revealed that *V. nilotica* contains several secondary metabolites [53], and is proven as an antibiotic [54], antidiabetic [6,10], antifungal [75], antimalarial [4], anthelmintic [12], gastro-protective [14], anti-inflammatory [22], anticancer [71] as well as antiviral and anti-hepatitis C virus [80]. The mechanisms supporting its relevance in HCV infection management is not well documented. Reviewing the pharmacologic properties of this plant with regard to HCV infection will contribute to a better understanding of the use of the plant, to encourage clinical trials and offer reliable alternative or complementary medicine in the management of the infection. Therefore, this review aimed at synthesizing shreds of evidence on mechanisms of action of *V. nilotica* applicable in hepatitis C treatment and relevance for HCV complications care.

Plant taxonomy

The name *Acacia* was deducted from the Greek word 'akis', meaning point or barb, considering the thorns found on African species. Among the 1350 species of acacia 1000 are Australians species where the local name is wattle and represent an icon. In 1986, it was suggested based on morphological, palynological and biochemical characteristics that the genus Acacia could be divided into three genera: *Acacia, Senegalia*, and *Racosperma* which encompass the largest number of species (960) [76]. More recently Maslin et al [70] based on classical taxonomy and chemotaxonomy attributes proposed the re-classification of the genus acacia into five genera: *Vachellia, Senegalia, Mariosousa, Acaciella* and *Acacia* [70]. The nomenclature session at the 17th International Botanical Congress, approved the proposition of Maslin and decided that the name *Acacia* is appropriate to the 'Australian group' and *Racosperma* is a synonym, and *Vachellia* is the correct generic name for species included in the former *Acacia* subgenus *Acacia* [69].

However, this re-classification is yet to be accepted by the entire scientific community, because some African and Asian species have not yet been combined into the new genera. This explains the reason, from The Plant List online database, the name *Vachellia nilotica* (L) Del is recognised with a full confidence level, whereas the name *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb is accepted with partial confidence level www.theplantlist.org. According to the Royal Botanical Garden, Kew science-Plants of the world online, the recognized name is *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. http://mpns.kew.org/mpns-portal/

Ethnopharmocological uses of *Vachellia nilotica*

Vachellia nilotica is a scented, thorny and nitrogen-fixing tree, used in the management of a wide range of diseases. Its indication in folk medicine covers common and frequent ailments like cough, cold and fever to serious disease like diabetes, cancer, central nervous system disorders etc [30]. In some Asian countries like India, Pakistan, Bangladesh, as well as in Egypt and Nigeria, it's been used traditionally for diabetes and its complications [34,36]. It has been suggested and proved by a scientific investigation that the efficacy of *V. nilotica* in diabetes treatment relies on its antioxidant activity [84]. In line with this *V. nilotica* is used in West Africa against tumour and cancers, liver induration and liver-related diseases in Peninsula, Egypt [30]. *V. nilotica* is one of the medicinal plant used by traditional healers in Zimbabwe to treat sexually transmitted infections including HCV infection, and HIV/AIDs, as well as syphilis and herpes simplex virus [50]. The leaves of *V. nilotica* was reported to treat patients with "blood in the urine" in Uttar Pradesh, India [59]. The indication against sexually transmitted infections was also reported in Zambia and Sudan in addition to gonorrhoea infection [23,24,94]. *V. nilotica* is reputed in the Massai community in Kenya as a strengthener and aphrodisiac medicinal plant [56]. During a survey among different ethnic groups (Ngakarimajong, Bakiga, Bagwere and Baganda) in Uganda, *V. nilotica* was among the plants used to treat traditionally meningitis, scabies, snakebite, malaria, uterus infection, diarrhea, wounds, cough, headache, fever, abdominal pain, common cold, worms, and viral infections like measles and yellow fever [96]. In the Karamoja region (Uganda), the common use of *V. nilotica* applies in disease prevention [47]. This prophylactic use indicates that the plant must have compounds that confer an immunostimulant activity.

Search strategy

We searched articles from PubMed, Cochrane database of systematic reviews, Scopus, Science Direct, and Google scholar databases. Articles were retrieved without language restrictions from inception to 14th of December 2018. The following search terms were used in the search: *Vachellia nilotica, Acacia Arabica*, gum Arabic tree, thorn mimosa, Egyptian acacia, thorny acacia, antiviral activity, antimutagenic activity, immunomodulatory activity, immunostimulant activity, hepatitis C, hepatitis C virus, HCV. Search terms were combined with the Boolean logic terms "OR", "AND" and searched the same way across all the databases. Retrieved articles were exported in Mendeley and duplicates were removed. The journal articles with any of the above-cited search terms either in the title, abstract, keywords were considered for the initial screening. Review papers, book chapters, encyclopedias were removed in the first step. The titles and abstracts of research articles were checked for relevance; for titles with non-available manuscript or abstract, efforts were made to search missing information by contacting authors through research gate and direct email especially.

In vitro and *in vivo* studies screening antiviral, antioxidant, antimutagenic, immunomodulatory or immunostimulatory activity of *Vachellia nilotica* (*Acacia nilotica* or *Acacia arabica*) were included in the review. Antioxidant activity testing *in vitro* are weakly relevant for human diseases therapeutic, hence for the antioxidant and antimutagenic activities, *in vitro* studies were excluded, except *in vitro* studies which supplement *in vivo* testing of the activity. The full text or abstracts of these selected articles were thoroughly scrutinized for the methodology, results and discussion regarding the relevance for the present review.

The relevant available papers for the antiviral, antimutagenic and immunomodulatory activity of *V. nilotica* were retained for the review. A total of 1002 was recorded after retrieval, of which 28 articles were finally studied (Fig. 1).

Considering these above pharmacological activities, we assessed the information reported in each paper. The assessment was based on guidelines for manuscript submission in the peer-reviewed pharmacological literature http://dx.doi.org/10.1016/j.bcp.2015.06.023. Seven criteria domain were adopted as follows: Plant identification; Study design (cells lines characteristics, passage number, population doubling time. For *in vivo* studies, animals characteristics, including sex, age and weight); controls and/reference standards used to validate experiment; range of concentration tested for toxicity evaluation and/or activity testing; reporting of maximal nontoxic concentration/dose (MNTC/MNTD), or minimal active concentration (MAC), or toxic concentration for 50% of cells or animals (CC50/LD50); reporting after the MIQE guidelines (https://www.ncbi.nlm.nih.gov/pubmed/19246619) for studies including RT-PCR, specifically the PCR protocol, primers sequences, oligonucleotides concentrations, buffer composition and the manufacturers of the previous; reporting of quantified results like effective or inhibition concentrations for 50% the initial infective concentration (EC50/IC50), inhibition percentage; appropriate statistical measures, including threshold of statistical significance, number of experiment replicates>= 3. Studies with full information reporting were categorized A, with partial information categorized B and studies with inadequate or none information categorized C.

Results-discussion

Among the 28 studies included under review, 13 studies were *in vivo* and the remaining were *in vitro*. Following the defined criterion, the quality of the studies has been assessed as presented inTable 1 and summarized inTable 2. Overall, 32.14% of the studies provided full information, and 17.86% had inadequate or no information. The antioxidant studies were classified only as fully reported and partially reported.

Table 1
Assessment of quality of included studies reporting.

Authors and rating	Plant identification			Animals or cells characteristic	reference standard/ control	MNTC/MNTD/ CC50 MAC	Range of concentrations tested	MIQE guidelines	Inclusion of quantified results (IC50, EC50 values)	Approach for statistical significance
	Local name	Taxonomical name (family)	Voucher number							
[17] B	Babul	*Acacia arabica* (Mimosaceae)	NP	Vero cells (ATCC) grown in cell culture p late for 48h Attenuated goatpox virus (Passage 60)	Infected treated and uninfected treated cells	MNTC: 99.93±0.38µg/mL CC	NP	Manufacturers NP	EC50 : 3.75µg/mL	Mean value with standard deviation of 3 replicates
[5] B	Babool	*Vachellia nilotica* (Fabaceae)	NP	MDBKcell line> Madine-darby bovine kidney cells procured from national institute of virology BHV-1 virus	Set of virus control and cells control	MNTD: 0.156 mg/mL	0.039mg/mL-20mg/mL	NA	NP	NP
[37] A	NP	*Vachellia nilotica*	NP	MDBK (Madine-darby Bovine kidney cells)	Virus control and cell control without sample	Non toxic concentrations : 0.156 and 0.039 mg/mL	0.039 – 20 mg/mL	NA	EC=0.156mg/mL Inhibition %: 61.1%	results are the mean_SD (n = 3)
Balamurgan *et al*., 2008 B	Babul	*Acacia arabica* var. *indica* (Mimosaceae)	NP	Vero cells (CCL-81) between the 140th and 150th pas-sages -The PPR vaccine virus (attenuated Sungri 96 isolate, 60th passage) adapted to Vero cells	Set of virus, extract and cells controls	MNTC: 200µg/mL MAC: 75µg/mL	Various nontoxic concentrations (50, 75, 100, 150, 200, and 400 μg/mL) of extracts	PCR was carried out for 30 cycles: denaturation at 94°C for 30s, primer annealing at 55°C for 60s, and extension at 72°C for 60s with an initial activation of enzyme at 95°C for 15 min. All the reagents provided in the kit using a PTC 200 Thermal cycler (MJ Research Inc., USA)	EC50: 75µg/mL (moi: 0.01)-EC50: 150µg/mL (moi: 0.1)	NP

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Table 1 (*continued*)

Authors and rating	Plant identification Local name	Taxonomical name (family)	Voucher number	Animals or cells characteristic	reference standard/ control	MNTC/MNTD/ CC50 MAC	Range of concentrations tested	MIQE guidelines	Inclusion of quantified results (IC50, EC50 values)	Approach for statistical significance
[27] A	NP	*Vachellia nilotica (Fabaceae)*	Identified -voucher no, SM-5	cells (Vero) (ATCC CCL-81) cervical carcinoma cell lines HeLa (ATCC CCL-2) The 293TT cell line - HSV-2 strain (ATCC VR-540) HPV-16 PsVs	Acyclovir (Sigma-Aldrich) heparin (Laboratori Derivati Organici S.p.A., Milan, Italy).	NP	0, 0.025, 0,25, 2,5, 25 µg/mL	NA	EC50: CI (confidence interval at 95%) HSV: 4.71µg/mL (3.11-7.12) HPV: 1.80µg/mL (1.42-2.27) EC90: HSV: 8.07 (2.66-24.4) HPV : 5.48 (3.63-8.28)	Significance was set at the 95% level.
[44] C	NP	*V. nilotica* (L.) Del. (Mimosaceae	NP	HCV-PR was prepared as a fused form of NS3 serine protease (MBP-NS345Ps) and maltose-binding protein (MBP) according to Kakiuchi *et al.*, 1995.	Positive control:Compound K-1, 5-2-[(4-chlorophenyl) thio]-5-nitrobenzylidene-2-thioxo-1,3-thiazolan-4-one, was purchased from Maybridge Chem. Co. Ltd, UK and used as a control substance of HCV-PR inhibition. Negetive control: Solvent instead of sample in reaction mixture	NP	NP	NA	IC50: 40.5 µg/mL	Results are the mean_SD (n = 3).
[45] A	Garad	*Vachellia nilotica* (L.) Del. (Mimosaceae)	NP	MT-4 cells	Control assays were adopted in the absence of plant extract with HIV-infected and uninfected cultures. Modified cyclodextrin sulphate as positive control	Minimum cytotoxic concentration: 125µg/mL	0.49- 1000 mg/mL	NA	Inhibition percentage : 63.1 ±2.5	Results are the mean_SD (n = 3).

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Table 1 (*continued*)

Authors and rating	Plant identification Local name	Taxonomical name (family)	Voucher number	Animals or cells characteristic	reference standard/ control	MNTC/MNTD/ CC50 MAC	Range of concentrations tested	MIQE guidelines	Inclusion of quantified results (IC50, EC50 values)	Approach for statistical significance
[55] C	Babool	*Vachellia nilotica* Var. Benth, Family: Mimocaceae	H9 cells	NP	Azidothymidine was used as a positive control that inhibited the enzyme activity at 600 μg/mL by 36.94%. Phosphate buffered saline (PBS) was used as a negative control.	NP	The water and methanol extracts were used in the concentration of 50 mg/mL, 100 mg/mL, 150 mg/mL, 200 mg/mL and 400 mg/mL.	NA	EC50: 200mg/mL (methanol, water) ; µg/mL	NP
Mahmoud *et al.*, 2007 C	Garad	*(Vachellia nilotica)* sub species *tomentosa*	NP	Vero cells -Freeze-dried, live, chick- embryo adapted vaccine containing komarov strain (K) of Newcastle disease virus	Control cells were incubated without test samples	MNTC: 40μg/mL	0.32μg/mL - 200μg/mL	NA	NP	NP
[78] B	Kikar	*Vachellia nilotica* (Linn) Delile indica leguminosae	Identified-voucher no NP	Vero cell line-Purified PPRV	Vero cells with M199 media was considered as positive control, Vero cells and PPRV with M199 media was considered as negative control	MNTC: 100 µg/mL	1.56-200µg/mL	NA	NP	The results were evaluated as CSP and expressed in terms of means ± S.D Result were subjected for significance at $P<0.05$
Rahmasari *et al.*, 2017 B	NP	*Vachellia nilotica* (fabaceae)	NP	MDCK cells-influenza virus A/WSN/33	Uninfected treated cells	CC50: 0.14 µg/mL	NP	NA	IC50: 0.3 µg/mL C50:0.3	NP
[80] B	Babul, Scented-pod Acacia	*Vachellia nilotica* (fabaceae)	Identified-no voucher no	Huh-7 cell line HCV-3a patient's serum	Set of treated and untreated experiment	MNTC: 100µg/mL	1-200µg/mL	Primers sequences, buffer composition, PCR protocol NP,	NP	NP

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Table 1 (*continued*)

Authors and rating	Plant identification Local name	Taxonomical name (family)	Voucher number	Animals or cells characteristic	reference standard/ control	MNTC/MNTD/ CC50 MAC	Range of concentrations tested	MIQE guidelines	Inclusion of quantified results (IC50, EC50 values)	Approach for statistical significance
[33] A	Babul	*Acacia arabica*	Specimen#: vi p-174629	Avian influenza A/chicken/CL/15–12/103075 (H9N2)-in ovo testing (pathogen free embryonated chicken eggs)	Amantadine hydrochloride and Oseltamivir as drug control and hydro-methanol as vehicle control group	MNTC: 135mg/0.1mL	5 -200 mg/0.1 mL	referenced	IC100: 33mg/0.1mL	The data is Presented as mean ± standard deviation. P values equal to or less than 0.05 were considered statistically significant
Saha *et al.*, 2017 A	NP	*Vachellia nilotica (Mimosaceae)*	Identified-voucher specimen (NBU/MLD/433)	Male Swiss albino mice (7-8 wk)	glibenclamide (standard) on alloxan induced diabetic mice- group of non-diabetic mice (non-alloxanized	lethal-dose higher than 2000 mg/kg	0-2000 mg/kg	NA	Serum MDA decreased by 32%-200mg/kg	All data are reported as the mean ± SD of six measurements $P<0.05$ was considered significant
[39] B	NP	*Acacia Arabica*	NP	female albino rats weighing 160-170 g and averaging 16 weeks old	The control animals were given the citrate buffer (pH-4.5) instead of extract	NP	NP	NA	significant decrease in MDA (p=0.003), with 100 mg/kg	Data are expressed as means ± standard deviation (SD) and minimum – maximum- Values of $p<0.05$ (2-tailed) were considered statistically significant
[74] B	NP	*Vachellia nilotica*	Identified-no voucher no	streptozotocin-induced diabetic rats(Male mature Sprague Dawley rats, weighing: 120–150 g)	reference drug glibenclamide and negative control receiving equivalent volume of saline	NP	NP	NA	significant reduction in MDA level (369.67±4.5; 352.78±3.16 nmol/g tissue in 150 and 300 mg/kg	Result subjected to significant difference of the means (LSD) at p < 0.05
[58] B	Not provide	*Vachellia nilotica*	Identified-voucher no NP	Cadmium chloride induced toxicity female albino rats	one group of animal non induced of cadmium chloride toxicity receiving same volume of distilled water, one untreated group	NP	NP	NA	significant increase ($p < 0.05$) in blood SOD and GPx; significant decrease ($p < 0.05$) was observed in MDA and NO	calculation of the mean, standard deviation, three levels of statistical significance ($p < 0.05$, $p < 0.01$, $p > 0.05$)

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Table 1 (*continued*)

Authors and rating	Plant identification			Animals or cells characteristic	reference standard/ control	MNTC/MNTD/ CC50 MAC	Range of concentrations tested	MIQE guidelines	Inclusion of quantified results (IC50, EC50 values)	Approach for statistical significance
	Local name	Taxonomical name (family)	Voucher number							
Singh[b] *et al*., 2009 B	NP	*Vachellia nilotica* (mimosace)	NP	male Wistar rats weighing 140 ± 20 g.	one group of animal non induced of carbone tetrachloride toxicity receiving dose of liquid paraffin, one group treated with α-tocopherol	NP	NP	NA	highly significant activities ($p < 0.001$) of CAT and SOD, decrease in the level of MDA ($p < 0.001$) at 150mg/kg	values are mean ±standard deviation (SD) of at least three determinations, and judged significant, if $p < 0.01$
Sundaram *et al*, 2007 B	NP	*Acacia arabica* (Mimosaceae)	NP	Inbred Wistar male rats (220-250 gram)	one group unintoxicated with carbone tetrachloride and a group intoxicated untreated	NP	NP	NA	Signifiant increase of superoxide dismutase, catalase and decrease of TBARS (100 mg/kg [$P < 0.05$] and 150 mg/kg [$P < 0.01$])	the values were expressed as mean ± SEM The minimum level of significance was fixed at 95% confidence limit
kanan *et al*., 2013 A	NP	*A.nilotica* Subsp. *indica* Mimosoidae	NP	MaleWistar Albino rats (150 - 160 g)	groups of unintoxicated untreated, acetaminophen intoxicated untreated and intoxicated treated with sylmarin, Liv52	MNTD: 3000 mg/kg	1-3000mg/kg	NA	At 250mg/kg, highly significant decrease of LPO level and increase of Glutathione	Values are expressed as mean (±SD) values ($P < 0.05$, $P < 0.01$) were considered Statistically significant.

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Table 1 (*continued*)

Authors and rating	Plant identification			Animals or cells characteristic	reference standard/ control	MNTC/MNTD/ CC50 MAC	Range of concentrations tested	MIQE guidelines	Inclusion of quantified results (IC50, EC50 values)	Approach for statistical significance
	Local name	Taxonomical name (family)	Voucher number							
Kannan *et al.*, 2012 A	NP	*Vachellia nilotica*	NP	Male BALB/c mice(22-25g)	tumor control group receiving vehicle (PBS), positive control group treated with methotrexate	MNTD: 2000mg/mL	1-2000mg/kg	NA	At 10mg/kg lifespan percentage increased (56%) glutathione reductase significantly reduced ($p<0.01$)	Values are expressed as mean (±SD). p-Values ($p<0.05$,$p<0.01$) were considered statistically significant
Singh[a] et al., 2009 A	NP	*Vachellia nilotica*	NP	Swiss albino rats (160±20 g)	one group untreated, intoxicated with carbone tetrachloride, one group intoxicated treated with sylmarin	MNTD: 2000mg/kg	300mg/kg-2000mg/kg	NA	At 200mg/kg, significantly increased the levels of reduced glutathione, Catalase and Superoxide dismutase, glutathione-*S*-transferase ($P < 0.001$); increased the level of glutathione peroxidase $P < 0.01$	Values were represented as mean±S.E.M, and results were judged significant, if $P < 0.01$
[52] C	NP	*V. nilotica* (L.) Willd. Ex Del.	NP	Balb/C female mice, 3–4weeks old, intoxicated by carcinogen 7,12-dimethylbenz[a]-anthracene (DMBA)	NP	NP	NP	NA	10 mg/1 mL, 100% inhibition of mutagenicity	The results presented as the average and standard error of three experiments, A level of probability <0.05 was taken as indicating statistical significance.

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Table 1 (*continued*)

Authors and rating	Plant identification Local name	Taxonomical name (family)	Voucher number	Animals or cells characteristic	reference standard/ control	MNTC/MNTD/ CC50 MAC	Range of concentrations tested	MIQE guidelines	Inclusion of quantified results (IC50, EC50 values)	Approach for statistical significance
[71] B	NP	*Vachellia nilotica* Linn.(leguminosae)	NP	Random bred male Swiss albino mice (8-9 weeks old)	Only Croton oil treatment for control group (DMBA induced skin papillomagenesis)	NP	NP	NA	At 800mg/kg, significant reduction in the tumor incidence ($p<0.001$), a significant reduction in the frequency of micronuclei, chromosomal aberrations, hepatic LPO levels ($p<0.005$)	Values are expressed as mean (±SD) values ($P < 0.05$, $P<0.01$) were considered Statistically significant.
[38] B	NP	*Vachellia nilotica* (L.) Willd Mimosaceae	NP	Swiss male albino mice (6–8 weeks of age), weight(25-30g)	Control group with water instead of treatment. Positive control with sylmarin and Butoxyethanol	NP	NP	NA	At 20mg/kg, reduced glutathione level were significantly increased ($p<0.05$) in co-administration with standards	Data presented as mean ± standard error, Significant differences evaluated at P **<0.05**.
[57] A	NP	*Vachellia nilotica* L. (Mimosaceae)	NP	human polymorphoneutrophils (PMNs) and mononuclear cells (MNCs)	the cells incubated with activator (SOZ) with no extract	MNTC: 100µg/mL	6.25–100µg/mL)	NA	96% inhibition at 100µg/mL	The differences were considered to be significant at levels of $P\leq0.05$
[88] A	NP	*Vachellia nilotica* (Leguminosae)	NP	Wistar Albino Rats (Av wt-120 gm) The spleen cells were collected aseptically from the rats	only spleen cells as negative control and wells containing spleen cells with Con-A as positive control	MNTC: 250µg/mL	18-500µg/mL	NA	9.61% increase in the proliferation of spleen cells at 250µg/mL	Mean values were calculated in comparison to control and taken positive if ratio was of significant difference
[2] B	NP	*Vachellia nilotica* L. (Mimosaceae)	NP	Mice	Group of animals unintoxicated with cyclophosphamide and a group intoxicated untreated	NP	NP	NA	6800 ± 733.9 cells/cm² increase of white blood cells	Data presented as mean ± standard deviation

NA: Not applicable; NP: Not provided; MNTC/MNTD: Maximal nontoxic concentration/dose; CC50: toxic concentration for 50% of cells; EC50/ED50: Effective concentration/dose against 50% of infectivity; IC50: Inhibitory concentration for 50% of infectivity. A: more 90% of information is given; B: 60-90% information given; C: Less than 60% information given.

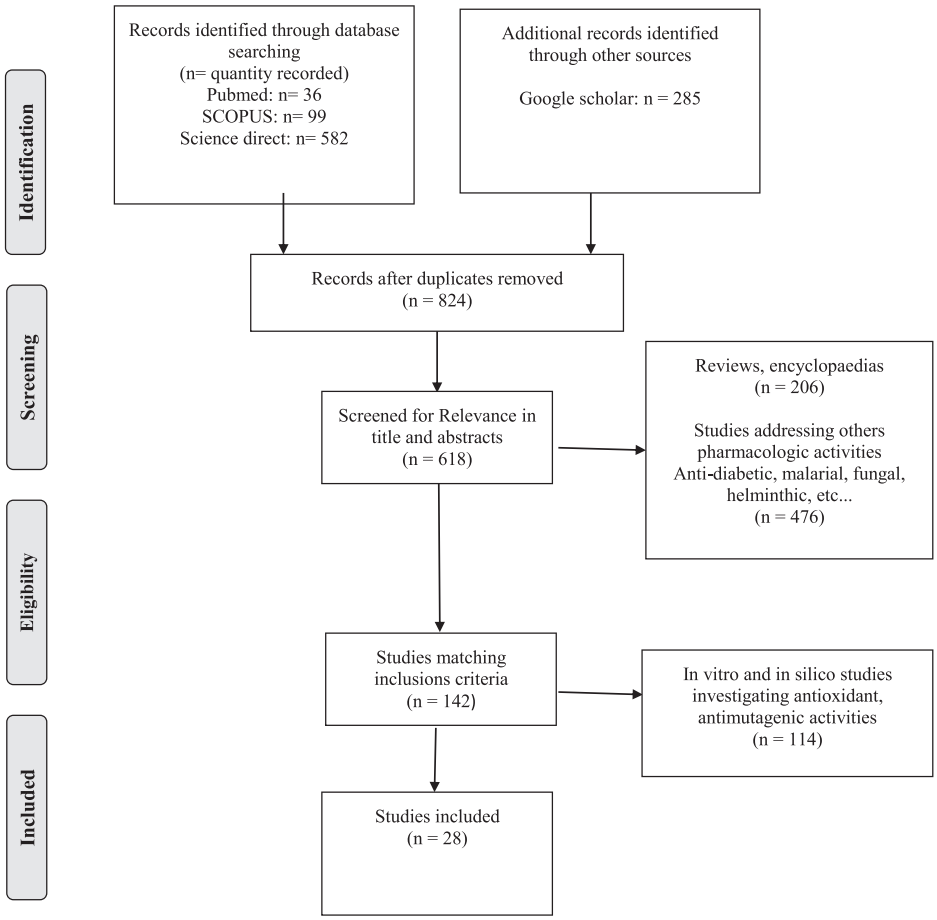

Fig. 1. PRISMA flow diagram for study inclusion.

Table 2
Summary of overall quality assessment.

Studies Grade	Grade A	Grade B	Grade C	Total
Overall	9 (32.14)	14 (50%)	5 (17.86%)	28
Antiviral activity	3 (23.08)	7 (53.85%)	3 (23.07)	13
Antioxidant activity	3 (37.5%)	5 (62.5%)	O	8
Antimutagenic activity	1 (25%)	2 (50%)	1 (25%)	4
Immunomodulatory activity	2 (66.66%)	0	1 (33.34%)	3

Various antiviral activity

All studies reporting antiviral activity of *V. nilotica* have been conducted *in vitro*. The pods, barks, leaves and fruits have been used in the form of crude extract but also as aqueous and organic solvents extracts (Table 3). The organic solvents used were n-hexane, chloroform, ethanol, methanol, ethyl acetate and acetone. Where several solvents were analyzed, methanol and acetone extracts were reported to have a higher antiviral effect. Among DNA viruses, four viruses belonging to Poxviridae, Herpesviridae and Papillomaviridae family were targeted using *V. nilotica*, and resulted in direct virucidal activity against goatpox virus [17], anti-bovine herpes virus activity(Anjana *et al.*, 2011), attachment inhibitory activity against herpes simplex virus and virucidal activity against herpes papillomavirus [27]. Among RNA virus, *V. nilotica* displayed inhibitory activity on *"peste des petits ruminants"* infectivity, inhibited new castle disease virus's replication, inhibited influenza virus [67]. Concerning the positive sense virus, *V. nilotica* inhibited HIV reverse transcription and HIV-1 protease. It also inhibited HCV NS3 protease and showed direct inactivation of HCV [80].

The whole aim of drug development, specifically anti-HCV drug development is to discover more effective, safe and affordable drug with fewer side effects. Antiviral agents are expected to stop only virus progression and not to cause damage

Table 3
Diverse antiviral activities with *Vachellia nilotica* extracts *In vitro*.

Category	Family	Genera	Species	Plant parts	Solvents	Effects of the extracts	References	Effective concentration/Percentage of inhibition/Inhibition concentration, selectivity index (SI)
DNA virus	Poxviridae	capripoxvirus	Goatpox virus	Leaves	Water	Direct virucidal activity	Banukaprash *et al*., 2008	Inhibition%: 99.70% SI: 127.1
	Herpesviridae	Varicellovirus	Bovine herpes virus1	Leaves Pods	Hot water	Anti-BHV-1 activity	[37]	EC=0.156mg/mL Inhibition %: 61.1%
				Leaves Fruits	Water	Anti-BHV-1 activity More effective antiviral activity with leaves	[5]	Percentage of inhibition: 61.1%
	Herpesviridae	simplexvirus	Herpes simplex virus-2	Bark	Water Methanol Chloroform	Inhibition of virus attachment Inactivation extracellulaire of virus particles	[27]	EC50=4.71µg/mL (3.11-7.12) SI HSV: 30.6
	Papillomaviridae	Alphapapillomavirus	Herpes papillomavirus-16	Bark	Methanol	Virucidal activity		EC50=1.80µg/mL (1.42-2.27) SI HPV: 32.6
Negative sense RNA virus	Paramyxo-viridae	Morbillivirus	Peste des Petits Ruminants Virus (PPRV)	Leaves Pods Bark	Water	Significant anti-PPRV activity with the leaves and high toxicity with the barks	[78]	EC=6.25 µg/mL, Cell survival percentage: 53±2.65
				Leaves	Water	Inhibition of PPRV infectivity, virucidal activity of extract	Balamurgan *et al*., 2008	EC50= 150µg/mL
	Paramyxo-viridae	Avulavirus	New Castle Disease virus	Fruits	Methanol Chloroform, water	Inhibition of replication	Mahmoud *et al*., 2007	EC=200μg/mL
	Orthomyxoviridae	Influenzavirus	Influenza virus	Pods	Hot water Ethanol Ethyl acetate	Potent anti-influenza activity	Rahmasari *et al*., 2017	IC50:0.3 SI: 4.6
		Influenza virus	avian influenza	Leaves	Methanol	effective inhibition of H9N2 replication and preventive effect	[33]	EC=33mg/0.1mL 100% virus inhibition
Positive sense RNA virus	orthoretrovirinae	lentivirus	Human immunodeficiency virus	Pods Bark	Water	Anti-HIV-1 protease	[45]	Inhibition percentage : 63.1 ±2.5
					Water	Inhibition of reverse transcriptase	[55]	EC50: 200mg/mL
	Flaviridae	Hepacivirus	Hepatitis C virus	Bark Pods	Methanol Water	Anti-HCV protease, over 90% inhibition of virus	[44]	HCV-Pr inhibition: 91.0 ±0.0 (Bark) IC50: 40.5 µg/mL (Bark)
				Leaves	Methanol Chloroform Acetone n-hexane	Anti-HCV activity by direct inactivation of virus	[80]	73% inhibition at 100µg/mL

of host tissue [67], thus drug with HCV protease as the target must be very specific because of the presence of other serine-type proteases in human body [44].

Medicinal plants have been reported to be a reliable source of antiviral drug with the advantages to have minimum side effects, easily accessible and less potential to be subject to resistance [78]. *Vachellia nilotica* is a medicinal plant which has effective antiviral activity against many viruses in animal and human, both RNA viruses and DNA viruses as shown in Table 3. This multiplicity and diversity of action might be due to the several bioactive metabolites identified in its extracts. Some plant metabolites have been proven to possess antiviral effect such as tannins, flavonoids, saponins, lignans, proteins and

peptides [27]. Interestingly these metabolites are present in *V. nilotica* and include; alkaloids, terpenoids, anthraquinones and reducing sugars ([9], 2013). Of note is that, phytochemical analysis of *V. nilotica* leaves done by Balamurgan *et al*., revealed the absence of alkaloids, anthraquinones, flavonoids and reducing sugars [13]. This contrasting result might be due to the season of plant collection, the geographical diversity or the age of the plant. *Vachellia nilotica* showed important antiviral activity against Paramyxoviridae virus such as new castle disease virus and "peste des petits ruminants virus" [13,67,78]. Leaves and pods of *V. nilotica* showed an inhibitory activity while extract of bark didn't show any antiviral activity against *"peste des petits ruminants virus*" revealing that there is variation in metabolites distribution across plants parts. Through multistep growth experience, Balamurgan *et al*., suggested two mechanisms of action against *"peste des petits remuninants virus*", namely: inactivation of virus and inhibition of virions release. Methanolic extract of fruits of *V. nilotica* also showed potent antiviral activity against new castle disease virus. Significant antiviral effects of *V. nilotica* against bovine herpes virus have been reported with leaves aqueous extract (Anjana *et al*., 2011; [37,67]). Bhanuprakash *et al*., tested aqueous extract of *V. nilotica* leaves against goatpox virus and reported a direct virucidal activity, revealing that *V. nilotica* extract may target several replication steps [17]. In human, *V. nilotica* has been proven to be effective against herpes simplex virus, influenza virus, herpes papillomavirus, HIV and HCV [27,44,55,79]. More than one mechanism of action have been identified against herpes simplex virus. The extract may inhibit virus particles infectivity or inhibit virus attachment to cell. Against herpes papillomavirus, a sexually transmitted virus, methanol extract of the bark of *V. nilotica* showed a virucidal activity [27].

Although the rate of sexual transmission of HCV infection is little, the potency of *V. nilotica* against herpes papillomavirus may have a significance of its use in hepatitis C care. Kaempferol, a flavonoid isolated from *V. nilotica* might be responsible for its antiviral activity against influenza virus, HIV and PPRV [33,55,78,79]. Human immunodeficiency virus protease is known to share many characteristics with hepatitis C protease and there is a high rate of co-infection HIV/HCV. Hussein *et al*. [45] proved the effectiveness of *V. nilotica* bark extract as anti-HIV-1 protease, and inhibition of reverse transcription of HIV was also reported [45]. As far as HCV is concerned antiviral activity of *V. nilotica* has also been proven against its protease with over 90% of inhibition by methanol extract of *V. nilotica* bark [44]. More recently an *in vitro* direct inactivation of HCV genotype3a by acetonic leaves extract have been reported [80]. Of note is that, methanolic extract gives a high anti-HCV activity but after fractionalization in acetone further inhibition has been observed.

From the above, it can be concluded that *V. nilotica* displays a variety of mechanisms as antiviral medicinal agents and potent activity as anti-HCV medicinal plant. This multiplicity of activity may indicate that it contains several antiviral compounds and a synergic action might be possible among these compounds.

Immuno-modulatory activity

Studies investigating immunomodulatory/immunostimulatory activity of *V. nilotica* have been conducted both *in vitro* and *in vivo*. Barks, leaves and fruits have been used in aqueous and organic solvents. Phytochemicals screening of leaves aqueous extract revealed: carbohydrates, glycosides, flavonoids, saponins, phenolics, phytosterols (Table 4). *In vitro* studies revealed overall improved immune response. Specifically, *V. nilotica* extract inhibits induced oxidation in peripheral immune cells, by interacting with protein kinase C (PKC) activation pathway, impeding intracellular and extracellular generation of reactive oxygen species. *Vachellia nilotica* extracts improved splenocytes proliferation and down-regulate interleukin-10 secretion. *In vivo, V. nilotica* provoked an increase of bone marrow cells, total white blood cells and α-esterase positive cells. Lymphoid organs such as spleen and thymus's weights were enhanced.

Though the mechanisms used by HCV to escape the immunity is not well established, investigators suggest that specific HCV gene products target different steps of the immune response. Some studies indicate that some form of latent infection may enable HCV to persist in immune cells for many years after it has been cleared from the serum by successful antiviral therapy [29]. The comprehension of immunologic events that happen and modulate the progression either to spontaneous viral clearance (acute infection) or to chronic infection is essential for the discovery of successful immunotherapy. The body defense against the HCV virus is displayed through innate immunity and adaptative immunity [95]. The adaptative arm of the immunity is performed by HCV-specific cell (CD4+, CD8+). The T-cell HCV-specific CD8+ acts as an effector of the immune adaptive response through a cytolytic and non-cytolytic mechanism. The CD4+ T-cell involved in the immune response are CD4+ helper T-cell and the CD4+ regulatory T-cell. They are involved in induction, maintenance and control of the effector function of the HCV-specific CD8+ T-cell [35,61,89,97].

Studies have been done with immunologic cell or organ with different extract of *V. nilotica* and have been proven effective. Using the methanolic extract of *V. nilotica*, Ahmad at el., [2] observed that bone marrow cell in mice was significantly increased, as well as α-esterase positive cells and white blood cells [2]. Spleen and thymus under treatment with methanolic extract of *V. nilotica* increased in weight, indicating cell proliferation. This finding is in accordance with another one *in vitro* using splenocytes, where the proliferation of splenocytes was reported with a lower concentration of hot aqueous extract of *V. nilotica* [88]. Further experience on cytokines secretion allows researchers to conclude that hot aqueous extract of *V. nilotica* could improve immune response and fight microorganisms that causes immune suppression. An immuno-inhibitory effect of extract of *V. nilotica* on splenocytes was also reported, but only at higher concentration most probably due to the accumulation of toxic substances [88]. As much as CD8+ T-cell are crucial for viral control, they are also critical for liver injury if not regulated. Regulatory CD4+ T-cell in the liver protects it from overwhelming destruction of its tissue due to excessive activity of CD8+ T-cell by secretion of downregulating cytokines: Interleukin-10 (IL-10) and Transforming Growth Factor (TGF). This regulatory activity is altered and overexpressed in chronically infected patients with HCV, possibly due

Table 4
Relevant pharmacological properties of *V. nilotica* in HCV infection management and keys findings.

Pharmacologic activity	Reference	Keys findings with *Vachellia nilotica* extract	Plant parts	solvent	Compounds	Study model	Effective concentration/Percentage of inhibition/Inhibition concentration (*p value*)
Immuno stimulatory activity	[88]	Proliferation of splenocytes, improved cellular immune response -down regulation of IL-10 secretion by splenocytes	Leaves	water	Carbohydrates Glycosides Flavonoids Saponins Phenoilics phytosterols	*In vitro* (splenocytes)	EC=250µg/mL Proliferation percentage: 9.61%
	[2]	-increase of bone marrow cells, α-esterase positive cells, total WBC -lymphoid organ (spleen, thymus) weight enhanced	Leaves	Methanol	None	*In vivo* (mice)	
	[57]	-Irreversible inhibitory effect on oxidative burst -interaction with PKC activation pathways inhibiting superoxide generation -inhibitory activity on intracellular and extracellular generation of reactive oxygen -cytotoxic activity	Bark Fruits	Ethanol	None	*In vitro* (PMNs, MNCs)	EC= 100µg/mL inhibition percentage=96%
Antimutagenic activity	[38]	-*Vachellia nilotica* and sylmarine significantly decrease chromosomal aberrations and micronuclei frequency - *Vachellia nilotica* and sylmarine increase mitotic index and liver reduced glutathione content	Leaves	Water	none	*In vivo* (mice)	ED= 20mg/kg p<0.05
	Singh *et al*., 2009	-extract prevents malondehylde formation and glutathione reduction -liver cancer markers restored with reduced liver injury -promotion of enzymatic and non-enzymatic oxidation defense system -inhibition of lipid peroxidation	Bark	Ethanol	Gallic acid Protocatechuic Caffeic acid Ellagic acid Quercetin	*In vivo* (mice)	ED=200mg/kg $P < 0.001$-0.01
	[71]	-reduction of tumor incidence, tumor burden, -reduction of chromosomal aberrations (chromatid breaks, chromosome breaks, centric rings, dicentrics, acentric fragments), micronuclei frequency, -lipid peroxidation reduction -increase of reduced glutathione content	Gum Flower Leaves	Water	None	*In vivo* (mice)	-tumor incidence reduction: 66.7% (p<0.001). -Percentage of chromosomal aberrations reduction: 85.5±1.05 p<0.005) - lipid peroxidation reduction p<0.005 - reduced glutathione content (p<0.005)
	[52]	-protection of DNA site against electrophilic attack by reactive carcinogenic moieties -inhibition direct acting mutagens(4-nitro-o-phenylenediamine; sodium azide) as well as indirect acting mutagens(2AF)	Bark	Chloroform Acetone	None	*In vivo* (mice) salmonella strains	ED=10 mg/1 mL, 100% inhibition of mutagenicity

(*continued on next page*)

Table 4 (*continued*)

Pharmacologic activity	Reference	Keys findings with *Vachellia nilotica* extract	Plant parts	solvent	Compounds	Study model	Effective concentration/Percentage of inhibition/Inhibition concentration (*p value*)
Antioxidant activity	Saha *et al*., 2017	- *V. nilotica* mediated improvement of antioxidant status by lowering oxidative stress -Serum MDA decreased with increased activities peroxidase and catalase in liver, kidney and skeletal muscle	Leaves	Ethanol	Phenol, flavonoid, quercetin,catechol, pyrogallol, phytol, squalene, γ-tocopherol, α-tocopherol, stigmasterol, β-sitosterol	*In vivo* (mice)	(P<0.01 to 0.001)
	[39]	-significant decrease in serum level of MDA in concentration dependant manner - significant increase in Coenzyme-Q10	Bark	Chloroform	None	*In vivo* (mice)	*P*(0.0001-0.003)
	[51]	- Treatment with extract restored the decreased level of reduced gluthatione up to normal range. -*V. nilotica* markedly decreased the level of LPO up to normal range - effect of *V. nilotica* extract was comparable to standard drugs silymarin and Liv52	Aerial parts	Methanol	None	*In vivo* (Rats)	- Reduced glutathione (36.68 ± 3.18 nmole/mg protein) -LPO (1.98±0.15 nmole/mg protein)
	[74]	- *V. nilotica* extract attenuated the adverse effect of diabetes on LPO, SOD and GSH activity -*V. nilotica* extract caused significant reduction in MDA level	Pods	n-Butanol	catechin; catechin-7-O-gallate; catechin-4_-O-gallate; catechin-3_-O-gallate; gallic acid; quercetin 3-O-glucoside, quercetin	*In vivo* (rat)	ED= 150 and 300 mg/kg
	Kannan *et al*., 2012	-animals lifespan increase compared to control group - treatment reduced significantly the cellular glutathione and nitric oxide levels -Histopathological studies confirmed protective influence of extract	Aerial parts	methanol	None	*In vivo* (mice)	Lifespan percentage= 56% p<0.01
	[58]	-*Vachellia nilotica* induced a significant increase in blood SOD and glutathione peroxidase when compared with cadmium chloride intoxicated group -significant decrease was observed in MDA and NO	Seeds	Methanol	catechin, catechin 7-*O*-gallate, catechin 3'-*O*-gallate, catechin 4'-*O*-gallate, catechin, 7,3'-di-*O*-gallate, and catechin 7,4'-di-*O*-gallate	*In vivo* (rat)	($p < 0.05$)
	Singh *et al*., 2009	-inhibition of lipid peroxidation by iron chelation and free radicals trapping -inhibition of deoxyribose degradation -increase of superoxide dismutase and catalase -decreased level of malondehylde in liver, lung, kidney and blood	Pods	Ethyl acetate	Phenolics (Acid caffeic Acid ellagic Acid ferrulic Acid epicatechin Acid gallic) Flavonoids (rutin) Gallotanins	*In vivo* (rats) *In vitro*	ED=100 and 200mg/kg $P < 0.001$-0.01
	Sundaram *et al*., 2007	-Treatment with *V. nilotica* significantly prevented the increase in TBARS -significantly ameliorated changes in SOD and catalase. -GSH-Px level increased significantly with extract	Bark	methanol	None	*In vivo* (rat)	-ED:100 mg/kg;150 mg/kg) $P < 0.05$ $P < 0.01$ -($P < 0.01$) -150 mg/kg ($P < 0.05$).

to oxidative stress induced by the virus [3,68,72]. Interestingly, Koko *et al*. in 2008 studied the immune-modulating properties of *V. nilotica* after induction of oxidative burst in polymorphonuclear cells and mononuclear cells, which are composed of lymphocytes T, lymphocytes B, natural killer cells, monocytes, dendritic cells and progenitors' populations. Their result indicates that barks and fruits extracts of *V. nilotica* has an irreversible inhibitory effect on oxidative burst in these cells, regarding both intracellular and extracellular reactive oxygen species [57]. The immune-suppressive cytokine interleukin-10 is known to be associated with progression of chronic HCV infection [20,32]. Additionally, this cytokine released by regulatory CD4+ T-cell has been proven, downregulated with a lower concentration of hot aqueous extract of *V. nilotica* suggesting that the plant extract through inhibition of oxidative stress within regulatory CD4+ T-cells control their activity [88]. Other *Acacia* specie, *Acacia catechu* is reported for its immunomodulatory activity on the humoral arm of the immune system by increasing serum immunoglobulin levels and hemagglutination titer in mice after oral feeding [46].

Anti-mutagenic activity

The anti-mutagenic pharmacologic effect of *V. nilotica* has been majorly investigated in mice model but also *in vitro*, using as plant material: barks, leaves, flower and gum. Phytochemicals screening revealed phenols, gallic acid, protocatechuic, caffeic, ellagic acid, and quercetin. According to studies in mice model, *V. nilotica* protect DNA against electrophilic attack by reactive carcinogenic moieties, decrease significantly chromosomal aberrations and micronuclei formation [52,71]. It has been reported that *V. nilotica* promotes enzymatic and non-enzymatic pathways against oxidative stress in the body. *Vachellia nilotica* restored liver cancer markers and reduced liver injury, tumour incidence and burden. *V. nilotica* reduces hepatic lipid peroxidation and increases reduced glutathione content in the liver [91]. *In vitro* study was conducted in Salmonella strains, mouse breast cancer cells, human sarcoma cells, and human prostate cancer cells. Findings revealed the protective effect of *V. nilotica* against metabolically activated mutagens. *Vachellia nilotica's* barks acetone extract demonstrated chemopreventive activity, high cytotoxic and apoptotic effect *in vivo* [91]. Hepatitis C virus protein NS5A is known to induce intracellular and extracellular accumulation of DNA damaging factor leading to DNA mutation by chromosomal aberrations [100]. Thus HCV protein NS5A promote tumor cells proliferation and metastasis [66]. Fortunately, *V. nilotica's* extract has been proven to inhibit direct-acting mutagen, to restore liver cancer markers and thus reduce liver injury. Furthermore, *V. nilotica's* extracts significantly decrease the frequency of chromosomal aberrations [38] (chromatid breakage, chromosome breaks, centric rings, dicentrics, acentric fragments, fragments exchange appearance and micronuclei formation) on 7, 12-Dimethylbenz(a)anthracene-induced skin papilloma-genesis in Swiss Albino Mice [71]. Of note, hepatocellular carcinoma in HCV infection shares important features with carcinogenesis induced by human papillomavirus. The above reveal *V. nilotica* as antimutagenic medicinal plants with an emphasis on its relevance in hepatitis C treatment to prevent and to stop cancer development [43]. Its pharmacologic activity as antioxidant and mutagenic have been supported by some group of compounds isolated such as polyphenols, tannins, flavonoids, saponins.

Antioxidant activity

The antioxidant activity of *V. nilotica* has been proven against reactive oxygen species as well as reactive nitric species. The identified metabolites through these studies are polyphenols, saponins, flavonoids, anthocyanins, carbohydrates, tannins, gallotannins, lignin, phytol, protein, phosphorous, calcium [84]. Some purified compounds were reported: acid caffeic, acid ellagic, acid ferulic, acid epicatechin, acid gallic, rutin, Kaempferol and ethyl gallate [74]. Apart from the others mentioned in plant material above, the seeds and woods of *V. nilotica* were also used to investigate *V. nilotica* antioxidant effect. (Table 4)

Oxidative stress is one of the major events in multifactorial human diseases in their expression and development [98]. Hepatitis C infection is one of these diseases considering its chronic form. Oxidative stress, defined as an unbalanced increase of reactive oxygen species with prooxidant activity, is recognized to involve both initiation and continuation of liver fibrosis [18,62]. The development of chronic hepatitis C is the result of the development of a progressive process of liver fibrosis that leads to liver cirrhosis with hepatic insufficiency and hepatocellular carcinoma. Thus oxidative stress induced by reactive oxygen species is part of the first events in HCV infection and markers of oxidative stress are positive all along in chronic hepatitis [101]. Several studies investigated antioxidants ability of medicinal plants; among those plants, *V. nilotica* showed strong radicals scavenging activity [49]. Reducing power of a compound or extract is highly related to its radical scavenging activity as it shows the potential of the compound or extracts to stabilize a free radical by hydrogen donating [93]. It has been proven that the reducing power of *V. nilotica's* extract was comparable to that of vitamin C; other study reported that the reducing power of *V. nilotica's* extract was higher than that of this control [83,103]. Ethyl gallate, a polyphenol from *V. nilotica* leaves was more effective not only than acid ascorbic but also than tocopherol, catechin and quercetin. This very compound has been proven to inhibit cancer cells proliferation without harming normal cells, and thus present an advantage compared to direct-acting antiviral used for hepatitis C treatment which is associated with a high incidence of hepatocellular carcinoma development [26,58]. Fibrosis process in chronic hepatitis C is a result not only of chronic inflammation induced by virus antigen but also a result of iron accumulation in hepatic tissue [90]. This accumulation of iron is thought to be linked to HCV presence. This metal iron accumulation in the liver leads to a significant increase of serum indexes of oxidative stress which are malondialdehylde (MDA) and protein carbonyl [51]. As an antioxidant medicinal plant, extract of *V. nilotica* proved its metal iron chelating potential *in vivo* and a significant decrease in level of malondialdehyde have been reported in the blood, kidneys, lungs and liver [51,85,92] Crude extract, aqueous extract, as well as

ethyl acetate extract, revealed good iron-chelating potential though ethyl acetate extract is most effective. Kaempferol, isolated from *V. nilotica* strongly interfered with ferrous and ferrozine complex formation confirming *V. nilotica* potential of iron chelating activity [91]. Hepato-carcinogenesis mechanism in chronic hepatitis C involves HCV core protein which has been associated with increased content of reactive oxygen species, increased level of lipid peroxidation, decreased content of intracellular and mitochondrial glutathione contents [65,102]. Cellular redox environment is regulated by couples of antioxidants/reductants enzymes. Glutathione is the most important redox-regulatory molecule in redox balance [11,16]. *V. nilotica* has been proven to increase reduced glutathione content and to inhibit glutathione oxidation. Several studies reported that different extracts from different parts of *V. nilotica* highly inhibit lipid peroxidation or decrease the level of lipid peroxidation revealing *V. nilotica*'s potential reduction of lipid peroxidation [39]. Antioxidants enzymes such as superoxide dismutase, glutathione peroxidase, glutathione s-transferase and catalase have been proven to increase as well with *V. nilotica*'s extract [92].

Conclusion

Vachellia nilotica showed a large variety of mechanisms as antiviral medicinal agent and as anti-HCV considering HCV genotype 3a but this reported effect needs to be confirmed by other scientific methods and to be extended to other genotypes of HCV. Studies on the immunomodulatory or immunostimulatory effect of *Acacia* species are few, especially with *V. nilotica*, and even more as far as hepatitis C infection is concerned. However, *V. nilotica*'s extract was able to inhibit an inhibitory cytokine (IL10) secretion which is associated with the development of chronic HCV infection, and it would be interesting to investigate the effect of *V. nilotica* on the specific human immune response to HCV. Like many other medicinal plants, *V. nilotica* showed important antioxidant activity, but besides this, it showed anti-mutagenic activity both *in vitro* and *in vivo* studies. *Vachellia nilotica* may thus serve for hepatitis C treatment and prevention of its complications but urgent, critical and scientific investigations regarding the relevant pharmacological activities needed in HCV infection is mandatory.

Author's contributions

The study was designed and led by L A, A M and E P. The rest of the authors reviewed and edited the draft. All authors contributed to and commented on the drafting of the final manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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