Antiviral Activities of *Jatropha curcas*: A Review

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Authors’ contributions

This work was carried out in collaboration among all authors. Author ALU and EMK designed the study and wrote the first draft of the manuscript. Authors CTK, ROO, DOO and BMM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: The Plant of *Jatropha curcas* is known for their many biological activities including antiviral, antifungal and antibacterial.

Aim: To provide update knowledge on the phytochemistry, toxicology and antiviral activity of some medically interesting *Jatropha curcas*.

Study Design: Multidisciplinary advanced bibliographic surveys and dissemination of the resulted knowledge.

Results: The literature review shows that *Jatropha curcas* have antiviral properties on different types of viruses. Different parts of the plant such as leaves, roots, latex, seeds, fruits and other parts have been reported to have different compounds which have interesting bioactivities and antiviral properties.

Conclusion: The present mini-review can therefore help inform future scientific research towards the development of anti-Hepatitis B virus and antiCovid-19 herbal drugs of relevance as well as nutraceuticals from *J. curcas* for the improvement of human health and wellbeing using reverse pharmacology approach. Molecular docking of some naturally occurring isolate compounds against anti-Hepatitis B virus protease is in progress.

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1. INTRODUCTION

“The human being and their Animals depend on the plants for their daily life. The main source of drugs remains plants products since ancient time. Tropical medicinal plant species are known for their richness in biologically active secondary metabolites of therapeutic relevance. These products are claimed to be safe, economic, effective and available. Because of these advantages, the medicinal plants are widely used by the traditional healers in their day-to-day practice” [1].

The World Health Organization (WHO) recognizes that traditional and complementary medicines are a vital part of the global health care system. In Africa, it is estimated that over 80% of the population rely on medicinal plant species to meet their basic health care needs.

The Democratic Republic of the Congo (DRC) is one of the most plant diversity rich countries in Africa, and contains 47% plant diversity of the African rain forests [2] Jatropha curcas Linn is a part of this richful flora among which many pharmaceutical plants.

J. curcas Linn., called Purghère, Médicinier de Barbades, Fève d’enfer, Gros pignon d’Inde in french, or in DRC languages Mukadi pemba, Nzo bamputu (Kiyanzi), Mpulungu (Kyombe) Mpluluka (Kikongo), Lundimba, mutshi au mvidie (Tshiluba), Ngbozinga (Ngbaka), Kada (Mono, Ngbandi), Efiteza (Ngombe), Lubonobono, Lw’ebufuliro (Mashi), Laky-akya (Bembe), Kivurahinda (Fuliro), Mutanzania (Nande) is a well known medicinal plant in DRC. J. curcas Linn has some known medicinal properties include antiviral activities.

“Viruses are a large group of submicroscopic infectious agents that can infect animals, plants, fungi and even bacteria. Once infected in living cell, it produces more viruses using host machinery. They consist of genetic material, RNA or DNA surrounded by a coat of protein, lipid or glycolipoids. They are capable of growth and multiplication only in living cells and can cause various important diseases in humans, animals, and plants. Most Commonly Occurring Viral Diseases are AIDS, Hepatitis, Small Pox, Polio, Measles, Rubella, Severe Acute Respiratory Syndrome (SARS) etc. New Severe Acute Respiratory Syndrome caused by coronavirus was reported, in December 2019, in Wuhan, Hubei Province, China and became a global pandemic, killing millions of people” [3].

The occurrence of the covid-19 pandemic has led many researchers to examine plants that may have activity on viruses. J. curcas being a plant widely used against several diseases including hepatitis B in DRC, this mini review aims to review the antiviral activity of this multiple-use plant.

2. METHODOLOGY

A literature search was conducted to obtain updated information about the phytochemistry and virucidal activity of J. curcas from various electronic databases like PubMed, Science Direct, Sci-hub and Google scholar. The scientific name of this plant was used as a keyword for the search, along with the terms biological activities, antiviral activities and phytochemistry, toxicology and virucidal/antiviral activity. We used "Mendeley" software for Bibliographical references.

3. RESULTS AND DISCUSSION

3.1 Botany and Propagation

J. curcus L. is large shrub of 3-4m high (Fig.1), formed by juicy multi-branched stem Leaves alternately arranged, 10-15cm x 7.5-12.5 cm, they are broadly ovate, conate, acute usually palmately 3 or 5 lobed, glabrous; flowers in loose panicles of the cymes, yellowish green, fruits are 2.5cm, long ovoid, black, seeds are ovoid – oblong, dull brownish black. The plant is monoecious and the terminal inflorescences contain unisexual flowers on the same inflorescence. The inflorescence is a panicle, with the female flowers (about 10–20%) at the apices of the main stem and branches of the inflorescence. Male flowers are more numerous (about 80–90%) and occupy subordinate positions on the inflorescence The period of the male inflorescence is 8-10 days for the male flowers while the female flowers take only 2-4 days. The continuous flowering results in a sequence of reproductive development stages from yellow mature fruits at the base of the branch, to green fruits in the middle, and flowers at the top. After pollination, the inflorescences form grapes of 10 green fruits, 2–3 cm long with an ovoid shape. Each fruit typically has three
carpels and the potential for two seeds per carpel. The seed resembles castor seed in shape but are smaller in size and dark brown in color.

The plant root system proceeds through the development of a main taproot and four shallow lateral roots [4,5].

J. curcas belongs to the Jatropha genus which is composed of 170 species. The genus Jatropha belongs to the tribe Jatrophae in the Euphorbiaceae family [6]. The Euphorbiaceae family, composed of more than 8000 species, contains distinct representatives in commercial exploitation, such as the Hevea brasiliensis, Manihot esculenta (cassava) and Ricinus communis (castor oil).

J. curcas can easily be propagated by both cuttings or seed. But the seed propagation is recommended for establishment of long-lived plantations. When J. curcas plants develop from cuttings, they may produce many branches but yield fewer seeds and do not have enough time to develop their taproot, which makes them sensitive to wind erosion. The seeds of J.curcas exhibit orthodox storage behaviour and under normal treatment and storage will maintain viability at high percentages for eight months to a year. Furthermore, propagation through seed or sexual propagation leads to a lot of genetic variability in terms of growth, biomass, seed yield and oil content

3.2 Ecological and Geographic Distribution

J. curcas is native to Central America but now grows naturally in most tropical areas of the world (Fig. 2) [7,8,9,10]. It is widely distributed in the wild and cultivated tropical areas of Central America, South America, Africa, India, South Eastern Asia, and Australia. Therefore, it typically grows between 15 and 40°C with rainfall between 250 and 3000 mm. The optimum water regime is around 700 mm/year.

Although is an open-field plant species, it requires intense sun such as found in the savanna or desert periphery. It is not adapted to grow under the shadow of forest canopy and does not compete with fast-growing species of the rain forest. It is well adapted to arid and semi-arid climates, and can resist to adverse environmental conditions. J. curcas can grow on a large range of soils provided they are well-drained and aerated.

3.3 Uses of Jatrophas

"J. curcas is a multiple purpose plant (Fig.3). Apart from its medicinal uses, the most widespread use for which large plantations of J. curcas are made is the production of oil from its seeds. The oil content of the seeds varies from 20 to 30% and can even reach 80% depending on the type of extraction. On a hectare 400 to 600 liters of oil can be obtained for a soil of average quality J. curcas oil is not suitable for human consumption, it can induce diarrhea and vomiting. This oil can but uses directly as biofuel for combustion engines or may be subjected to transesterification to produce biodiesel. Several tests have also been carried out to use Jatropha oil in aviation as jet fuel mixed in different proportions. This is the case of Air New Zealand 50/50 of Jatropha oil and A-1 fuel, Continental Airlines (USA) 50/50 mixture of algae/jatropha-oil-derived biofuel and Jet A and Indian airline SpiceJet (India) which used jatropha based biofuel in the ratio of conventional jet fuel to jatropha oil of 25:75" [12].

J. curcas is also an important plant in relation to climate change and sustainability. It has also been reported that it contributes to carbon sequestration. This plant of Jatropha genus is very resistant to aridity so it can be planted in hot and dry land in soil unsuitable for food production. This plant can also be used in erosion control, soil stabilization and water saving.

3.4 Ethnnopharmacology

Different parts of J. curcas (leaves, roots, fruits, latex, grains, whole young plants, oil ...) are used in traditional medicine across the tropics and in particular in Africa against various ailments. According to [14] in some african countries, a few drops of diluted water solution of twig sap are given by mouth to new-born babies affected by tetanus; leaves have been used as haemostatic agent when applied to cuts and bleeding wounds; seed oil has been used as ingredient in the treatment of rheumatism, seeds are chewed when in need of a laxative and used for treating ascites, gout, paralysis, skin diseases and as a purgative, anthelmintic and abortifacient; etc.

In DRC, decocted from the leaves, is used against malaria; decocted roots (as a gargle) against gingivitis and stomatitis; latex from leafy stem (in instillation ocular) against eye disorder (conjunctivitis, etc.), but also as remedies for several other ailments.
Fig. 1. *J. curcas*. (A) inflorescence; (B) Fruits and leaves

Fig. 2. Jatropha distribution [11]
Several scientific studies have confirmed the different pharmacological activities which would be responsible for the effects observed in traditional medicine.

“For instance Acetone, chloroform, ethanol and methanol extracts of root bark of this plant has been reported to inhibit the growth of both gram-positive (Staphylococcus aureus) and gram negative bacteria like Salmonella typhimurium, Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumonia” [17,18,19,20].

Various plant parts of J. curcas have shown antifungal activity against Candida albicans, Bacillus subtilis, Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Phymatotrichopsis omnivora, etc [21,22,17,18,19] Application of Jatropha curcas root powder in paste form in mice and rats has been reported to possess anti-inflammatory activity [23]. Root bark extract showed also a very good scavenging activity when compared with ascorbic acid [19]. The polar (using methanol) and non-polar (using petroleum ether) extracts of J. curcas roots have shown antidiarrhea activity in various species of albino mice [24]. “Various plant parts of J. curcas have been reported to possess insecticidal and larvicidal and anthelmintic activity” [25].

3.5 Phytochemistry

Due to their medical value, “Diterpenes have been dominating the research area in J. curcas. Diterpenes have a range of in vitro biological activities such as antihypertensive, anticancer, antiretroviral, anti-inflammatory, analgesic, antimicrobial, insecticidal and molluscicidal activities” [26,15].

On the below Table 2, we have noted chemicals compounds reported by [26,27,28,29] as identified in J. curcas.
Table 1. Summarizes the main biological activities of *J. curcas* [15,16]

| Bioactivity and ethopharmaceutical use                  | Part of the plant        | Form, extract or molecules                                                                 |
|--------------------------------------------------------|--------------------------|------------------------------------------------------------------------------------------|
| Anti-bacterial activity                                | Leaves, roots            | Terpenes                                                                                  |
| Antifungal activity                                    | Various plant parts      | Various extracts                                                                           |
| Antiinflammatory activity                              | Roots                    | Methanolic extract                                                                         |
| Antidiabetic activity                                  | Leaves                   | Ethanolic extract                                                                          |
| Anthelmintic activity                                  | Leaves                   | Aqueous extract                                                                           |
| Antidiarrea activity                                   | Roots                    | Methanolic extract                                                                         |
| Anticancer activity                                    | Leaves                   | Various extracts                                                                           |
| Antilucer activity                                     | Leaves                   | Methanolic extract                                                                         |
| Larval and insecticidal activity                       | Various plant parts      | Methanol and ethanol extracts                                                              |
| Antioxydant activity                                   | Shoots                   | Glutathione reductase, ascorbate peroxidase, guiacol peroxidase                           |
| Pregnancy terminating effect                           | Fruits, seeds            | Methanol, petroleum ether, dichloromethane extract; jatraphone                           |
| Wound healing activity                                 | Leaves                   |                                                                                           |
| Anti toothache effect                                  | Tender stem               |                                                                                           |
| Coagulant and anticoagulant activities                 | Latex                    | Whole latex                                                                               |
| Hepatoprotective effect                                | Shrup                     |                                                                                           |
| Dermatomucosal effect                                  | Latex                    |                                                                                           |
| Anti arthritis effect                                  | Seeds                    |                                                                                           |
| Anti-jaundice and liver trouble effects                | Seeds; leaves            |                                                                                           |
| Cytotixicity                                           | Seeds,                   | Jatrapone                                                                                 |
| Antimalarial                                           | Young leaves             | Aqueous infusion                                                                           |
| Anti sickle cell disease                               |                          |                                                                                           |
| Antihemorrhoids effect                                 | Fresh leaves             | Aqueous decoction                                                                         |
| Antihypertension effect                                | Leaves                   |                                                                                           |
| Antirheumatism effect                                  | Roots                    | Aqueous infusion                                                                           |
| Anticoconjestion, laxative effect                      | Fruit powder             | Aqueous infusion                                                                           |
| Anticonvulsant activity                                | Aeral parts              | Ethanol/water (1:1) extract                                                               |
| Analgesic activity                                     | Leaves                   | Ethanolic and aqueous extracts                                                            |
Table 2. Chemicals Compounds

| No | Chemical compounds                           | Type       | Sources     |
|----|---------------------------------------------|------------|-------------|
| 1  | 2α-hydroxy-epiisojatrogrossidione            | Diterpenes | Aerial part |
| 2  | Spirocursacone                              | Diterpenes | Root        |
| 3  | Curculathyran-A                             | Diterpenes | Root        |
| 4  | Curculathyran-B                             | Diterpenes | Root        |
| 5  | Curcusone-A                                 | Diterpenes | Root        |
| 6  | Curcusone-B                                 | Diterpenes | Root        |
| 7  | Curcusone-C                                 | Diterpenes | Root        |
| 8  | Curcusone-D                                 | Diterpenes | Root        |
| 9  | Curcusone-E                                 | Diterpenes | Root        |
| 10 | Jatrophone                                   | Diterpenes | Root        |
| 11 | Jatrohalactam                               | Diterpenes | Root        |
| 12 | Palmarumycin CP1                            | Diterpenes | Stem        |
| 13 | Palmarumycin JC1                            | Diterpenes | Stem        |
| 14 | Palmarumycin JC2                            | Diterpenes | Stem        |
| 15 | 5-OH-pyrrolidin-2-one                       | Alkaloid   | Leaf        |
| 16 | Pyrimidine-2,4-dione                        | Alkaloid   | Leaf        |
| 17 | 2-methylantraquinone                        | Anthraquinone | Aerial part |
| 18 | Marmesin                                    | Coumarin   | Root        |
| 19 | Tomentin                                    | Coumarin   | Root        |
| 20 | Propacin                                    | Coumarino-Lignane | Root  |
| 21 | Jatrophin                                   | Coumarino-Lignane | Root  |
| 22 | Curcacyline-A                               | Cyclic peptide | Latex     |
| 23 | Curcacycline-B                              | Cyclic peptide | Latex     |
| 24 | 4E)-15-O-Acetyl-15-epijatrogrossidentadione | Diterpenes | Aerial part |
| 25 | (14E)-14-O-Acetyl-5,6-epoxygrossidentadione | Diterpenes | Aerial part |
| 26 | (4E)-15-epijatrogrossidentadione             | Diterpenes | Aerial part |
| 27 | 3β-acetoxy-12-methoxy-13-methylpodocarpa-8,11,13-trien-7-one | Diterpenes | Aerial part |
| 28 | 3β, 12-Dihydroxy-13-methylpodocarpane 8,10,13-triene | Diterpenes | Aerial part |
| 29 | Heudelotinone                               | Diterpenes | Aerial part |
| 30 | Epi-isojatrogrossidione                     | Diterpenes | Aerial part |
| 31 | Apigenin                                    | Flavonoid  | Leaf        |
| 32 | Vitexin                                     | Flavonoid  | Leaf        |
| No | Chemical compounds                                      | Type        | Sources   |
|----|--------------------------------------------------------|-------------|-----------|
| 33 | Isovitexin                                             | Flavonoid   | Leaf      |
| 34 | Curcin                                                 | Lectin      | Seed      |
| 35 | Tetradecyl-E-ferulate                                  | Lignane     | Aerial part |
| 36 | 12-deoxy-16-hydroxy phorbol-C13-C16 diesters           | Phorbol ester | Seed      |
| 37 | Factor 1                                               | Phorbol ester | Seed      |
| 38 | Factor 2                                               | Phorbol ester | Seed      |
| 39 | Factor 3                                               | Phorbol ester | Seed      |
| 40 | Factor 4                                               | Phorbol ester | Seed      |
| 41 | Factor 5                                               | Phorbol ester | Seed      |
| 42 | Factor 6                                               | Phorbol ester | Seed      |
| 43 | B-sitosterol                                           | Phytosterol | Seed      |
| 44 | Curcain                                                | Protease    |           |
| 45 | Stigmasterol                                           | Triterpenes | Leaf      |
| 46 | 3-O-(Z)-coumaroyl oleanolic acid                       | Triterpenes | Aerial part |
| 47 | Acetoxyjatropholone                                    | Diterpenes  | Root      |
| 48 | Multidione                                             | Diterpenes  | Root      |
| 49 | Myristic acid                                          | Fatty acid  | Seed      |
A part from which roles have been enlighten above, [30] noted that flavonoids from *J. curcas* have antioxidant activities. [31] pointed out the role in the free radical scavenging after he quantified various flavonoids of the leaf extract.

### 3.6 Antiviral Activity

The water extract of the branches of *J. curcas* strongly inhibit the HIV-induced cytopathic effects with low cytotoxicity [32]. Latex of *J. curcas* possesses inhibitory property against Water melon mosaic virus [33].

The chromatography on Diaion HP-20 has been use to fraction the water extract of the branches of *J. curcas* (eluted with water and increasing concentrations of MeOH). The bioassay-guided fractionation afforded corilagin and ferulic acid from 50% MeOH-eluted fraction, and 5,7-dimethoxycoumarin and 6,7-dimethoxycoumarin from MeOH-eluted fraction. Comparing their 1 H and 13 CNMR data with those of authentic samples has conducted to the identification of the compounds. Of the compounds isolated, only 5,7-dimethoxycoumarin and 6,7-dimethoxycoumarin showed moderate inhibition on HIV-1-induced cytopathic effect but with considerable cytotoxicity.

“Liver disease: *J. curcas* L., Euphorbiaceae Piñon blanco (yura piñon), 033695 L The sap is expressed from the leave, then diluted in water and drunk 3 times a day, with dietary restrictions” [34].

“It has been reported that a moderate cycloprotective activity against HIV in cultured human lymphoblastoid CEM-SS cells for the methanol extract from *J. curcas*” [35]. On the other side, [32] “after investigation, mentionned the effects of aqueous and methanolic extracts from *J. curcas* branches for the inhibition of HIV-induced cytopathic effects in cultured cells, HIV reverse transcriptase and HIV-protease enzymes. The water extract of *J. curcas* branches showed potent inhibition (IC₅₀ 24 μg ml⁻¹) of the HIV-induced cytopathic effects with low cytotoxicity (CC₅₀ N 1000) and selectivity index CC₅₀/IC₅₀ (N41.7)”. [36] reported the possibility of synthesing prostratin and DPP from phorbol esters from *J. curcas*. A high clinical candidate that could be used in the treatment of HIV has been identified after this synthesis.

A ribosome-inactivating protein (curcin 2) induced from *J. curcas* can reduce viral and fungal infection in transgenic tobacco [37].

“From various medicinal uses that known for *J. curcas*, the antimicrobial, anti-cancer and anti-HIV activity has been well classified. Because of its broadspectrum activity, some investigations using aqueous and methanol leaf extracts for cytotoxicity and its potential to inhibit hemagglutinin protein of influenza virus. The bioactive compounds from leaf extracts were characterized by high performance thin layer chromatography which confirmed the presence of major phytochemicals groups including flavonoids, saponins and other tannins. The cytotoxic concentration 50 for aqueous and methanol extracts were determined using trypan blue dye exclusion assay. Inhibition of hemagglutinin protein was assessed using minimal cytotoxic concentrations of the extracts and 102.5 TCID₅₀ (64 HA titre) of the Influenza A (H1N1) virus with different exposure studies using hemagglutination assay. Aqueous and methanol extracts were found to be non-toxic to Madin darby canine kidney cells below concentration of 15.57 and 33.62 mg/mL for respectively. Inhibition of hemagglutinin was studied using reducing hemagglutination titre which confirmed that the *J. curcas* extracts have direct effect on the process of virus adsorption leading to its inhibition. Results provide the information which shows the potential of Jatropha extracts in the treatment of influenza A (H1N1) virus infection. With an established reduced toxicity and prevention of infection by inhibiting hemagglutinin protein, these extracts and its derivatives may be further developed as broad-spectrum anti-influenza drugs for prevention and treatment of infections by different types of influenza viruses with further mechanistic studies on anti-influenza” [38].

“*J. curcus* activity against virus: *J. curcas* is known for various medicinal uses. Its antimicrobial, anti-cancer and anti-HIV activity has been well recognized. Because of its broadspectrum activity, investigation has been conducted on aqueous and methanol leaf extracts for cytotoxicity and its potential to inhibit hemagglutinin protein of influenza virus. The bioactive compounds from leaf extracts were characterized by high performance thin layer chromatography which revealed the presence of major phytochemicals including flavonoids, saponins and tannins. The cytotoxic concentration 50 for aqueous and methanol extracts were determined using trypan blue dye exclusion assay. Inhibition of hemagglutinin protein was assessed using minimal cytotoxic concentrations of the extracts and 102.5 TCID₅₀
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3.7 Toxicity Studies

3.7.1 Toxic and antinutritional components

“The major toxic and antinutritional components from J. curcas and their molecular mechanisms are in the Phorbol esters and curcin which are the most toxic phytochemicals of J. curcas” [41]. Major toxic components as the phorbol esters, the antinutritional phytate and other trypsin inhibitor factors are contained in the seeds. As pointed out by [42] J. curcas kernels were separated into cotyledons, hypocotyls, kernel coat and endosperm to determine the location of the antinutrients. From this reseach, it has been proved that majority of phorbol esters (85.7%), phytate (96.5%) and trypsin inhibitor (95.3%) are localised in the endosperm.

3.7.2 Toxicological effects

In the *in vitro* and *in vivo* models J. curcas exhibited toxicity to a wide variety of species i.e., microorganisms, animals including humans. All parts of J. curcas are toxic but the degree of toxicity varies with the extract types, nature of test substances, dose, mode of administration, and sensitivit [41]. The seeds are toxic to humans [9,43] with symptoms of giddiness, vomiting, delirium, muscle shock, decrease of visual capacity, high pulse rate and diarrhoea [44,45,46]. In animals toxic symptoms were reported when raw or defatted seeds were force-fed to chicks [47,48], pigs [49,50], sheep and goats [51,52], rabbits [43], mice and rats [53,15,51], carp Cyprinus carpio [44]. In rabbits, [43] observed symptoms of diarrhea, hemorrhagic eyes and inflammation of the g-tract at 6-, 9- and 13.5-ml kg−1 BW. In mice, [54] reported induction of tumors by topical application of a methanol fraction from J. curcas oil with 36% of the animals having skin tumors in 30 weeks. It has also been reported toxicity for jatrophol-1 (LD50 82.2 mg kg−1 BW) when administrated orally [55] reported toxic activity for phorbol esters (LD50 27.34 mg kg−1 BW) given by intragastric administration to Swiss Hauschka mice. A dead mice on which some histopathological studies on the organs were done showed prominent lesions mainly found in lungs and kidneys, with diffused haemorrhages in lung, and glomerular sclerosis and atrophy in kidney at doses 32.40 mg kg−1 BW. In rats, [56] reported irritant activity from J. curcas seed oil (LD50 25 μg/ear), hydrophilic fraction (LD501.8 μg/ear) and the neutral fraction (LD501.5 μg/ear). For caniojane, [57] reported antiplasmodial activity against *Plasmodium falciparum*.

J. curcas L. is known as two genotypes, the toxic and non-toxic. The presence of phorbol esters in the seed is making the difference between them. No differences were found in the level of amino acids, trypsin inhibitor, lectin, phytate curcin and saponin between these two genotypes.

4. CONCLUSION

At the end of this work, which consisted in making an inventory of data on the phytochemistry or antiviral actions of J. curcas. The large spectrum of biological activities and antiviral properties of this plant lead us to consider that it can play a role against viruses. Therefore J. curcas can be a good candidate to fight against Hepatitis B considering the antiviral properties of this plant lead us to consider that it can play a role against viruses. This mini-review aims to guide and inform future scientific research towards the development of Hepatitis B herbal drugs of relevance as well as nutraceuticals from these three plant species for the improvement of human health. A molecular docking using components isolated from J. curcas against Hepatitis B is ongoing.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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