# *In vitro* antioxidant and inhibitory activity of enzyme HMG-CoA reductase from the methanol extract of *Jatropha Gossypifolia*

*Actividad antioxidante e inhibidora in vitro de la enzima HMG-CoA reductasa del extracto metanólico de Jatropha Gossypifolia.*

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ACCESO ABIERTO

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## ABSTRACT

**Introduction:** *Jatropha gossypifolia* is a plant traditionally used as an antidiabetic and hypolipidemic agent. Therefore, it is important to evaluate the antioxidant and inhibitory activity of the enzyme HMG CoA reductase, to validate its traditional use.

**Objective:** to determine the in vitro inhibitory activity of HMG-CoA reductase and the antioxidant activity of the methanol extract of *Jatropha gossypifolia*.

**Methodology:** inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA) was evaluated using the HMG-CoA reductase (HMGR) assay kit sigma Aldrich, the antioxidant activity was assessed by the inhibition of hemolysis of red blood cells exposed to H2O2. The results were analyzed by analysis of variance, accepting statistical significance with P < 0, 05.

**Results:** total methanol extract of *Jatropha gossypifolia* at different concentrations inhibited the enzyme HMG-CoA reductase, specially at the concentration of 0.001 ppm, where it inhibited around 76.5%, is pointed out, as it could be associated with secondary high polarity metabolites. Besides, it was evidenced that a concentration of 0.1 ppm of extract inhibited 96.6% of the hemolysis in human erythrocytes induced by H2O2, (p <0.001).

**Conclusions:** total methanol extract of *Jatropha gossypifolia* at different concentrations inhibitr HMG-CoA reductase, and ha antioxidant activity at low concentrations.

**Keywords:** HMG-CoA reductase; antidiabetic; antioxidants; hypolipemiant; *Jatropha Gossypifolia.*

## RESUMEN

**Introducción:** la *Jatropha gossypifolia* es una planta tradicionalmente utilizada como agente antidiabético e hipolipemiante. Por tanto, es importante evaluar la actividad antioxidante e inhibidora de la enzima HMG CoA reductasa, para validar su uso tradicional.

**Objetivo:** determinar la actividad inhibidora in vitro de la enzima reductasa HMG-CoA y la actividad antioxidante del extracto metanólico de *Jatropha gossypifolia*.

**Metodología:** se evaluó la inhibición de la 3-hidroxi-3-metilglutaril-coenzima A reductasa (HMG-CoA) usando el kit de ensayo HMG-CoA reductasa (HMGR) de sigma Aldrich, la actividad antioxidante se realizó mediante la inhibición de hemólisis de los glóbulos rojos expuestos a H2O2. Los resultados se analizaron mediante análisis de varianza, aceptando significancia estadística con $p < 0,05$.

**Resultados:** el extracto metanólico total de *Jatropha gossypifolia* a diferentes concentraciones inhibió la enzima HMG-coA reductasa, especialmente a la concentración de 0.001 ppm, donde inhibió alrededor del 76.5% podría estar asociado a metabolitos secundarios de alta polaridad. Además, se evidenció que una concentración de 0.1 ppm de extracto inhibió el 96.6% de la hemólisis en eritrocitos humanos inducida por H2O2 ($p < 0.001$).

**Conclusiones:** el extracto de metanol total de *Jatropha gossypifolia* a diferentes concentraciones inhibe la enzima HMG-coA reductasa y tiene actividad antioxidante a bajas concentraciones.

**Palabras clave:** HMG-CoA reductasa, antidiabético, antioxidantes, hipolipemiante, *Jatropha Gossypifolia*.

## INTRODUCTION

Cardiovascular diseases are considered as the leading cause of death worldwide. According to the World Health Organization (WHO) in 2018, 17.7 million people died, representing 31% of all deaths recorded in the world, and representing 82% of deaths in low and middle-income countries (1,2). With hypertension and hypercholesterolemia being the main factors related to coronary heart disease (3), for which about 1.6 million people annually die in Latin America of which, half a million pass away before the age of 70 years (4,5).

In Colombia, cardiovascular diseases continue to be the main cause of death, by the year 2015 196,933 individuals died in the country, and of these 24,570 (12.48%) correspond to deaths by acute myocardial infarction, and 3,184 (1.62%) due to hypertensive heart disease, thus becoming the leading cause of death in the country (6-8).

Another predisposing factor for the development of CVD has been evidenced, which is the oxidative stress generated by the metabolic processes of the organism, these resulting cytotoxic and mainly affecting the vascular endothelium, leading to its dysfunction, and the activation of various inflammatory pathways (9).

The excess of free radicals, inhibition of antioxidant enzymes such as superoxide dismutase, catalase and peroxides could occur, resulting in toxic effects in the cells caused by oxidation of macromolecules (lipids, proteins, and DNA), and triggering diseases like atherosclerosis, a progressive disease described as the accumulation of cholesterol deposits in large and medium arteries, therefore generating a decrease in the vessel´s lumen light of the vessel, preventing blood from flowing freely (9-11).

Oxidative stress alters endothelial function by is the mechanism of nitric oxide (NO) inactivation by superoxide anions and oxidized low-density lipoproteins (LDLs-ox), which leads to inadequate smooth muscle cells´ relaxation, therefore becoming a risk factor for the formation of the atheroma plaque (12).

In the treatment of hypercholesterolemia, drugs whose mechanism of action is mainly based on the inhibition of the activity of the enzyme HMG-CoA

reductase are used, converting HMG-CoA to mevalonate, a key metabolite in the cholesterol synthesis (12, 14). These drugs are known as statins and are the treatment of choice for hypercholesterolemia due to their proven efficacy and safety profile, it has also been shown that these produce a substantial reduction in cardiovascular morbidity and mortality in patients with and without heart disease. However, some patients do not achieve the recommended objectives of low-density lipoprotein (LDL) cholesterol <80 mg/dl and high-density lipoprotein (HDL) 40 to 60 mg/dl (14,15).

It is important to note that the use of these drugs is contraindicated in approximately 40% of patients, due to the appearance of side effects, such as myalgia, myopathy, liver disease, and rhabdomyolysis as a more serious consequence. This limits the use of statins and motivates the research for new natural molecules to combat hypercholesterolemia, as well as the induced oxidative stress that leads to the formation of atherosclerosis (15).

Considering the foregoing, natural products are an economical, safe, and easily accessible alternative, it is important to determine the potential promising biological activity of *Jatropha gossypifolia* extracts as antioxidant activity, and the possible inhibitory activity of HMG-CoA enzyme reductase to validate the traditional use of these extracts for the management of such pathologies. *Jatropha gossypifolia*, this is a tropical plant that grows approximately up to 1.5 m, which is worldwide distributed, counting with approximately 175 species, different secondary metabolites group have been evidenced such as flavonoids, coumarins, triterpenes, mucilages, resins, Tannins which may be responsible for most of their pharmacological actions (16,17).

Pharmacological studies have demonstrated some properties of these species, among which are antimicrobial effects, anti-inflammatory, antidiarrheal, diuretic, cytostatic, and antiseptic (18, 19). We highlight the study of Felix et al., On the local and systemic anti-inflammatory activity of *Jatropha gossypifolia* extract in animal models of mice, finding a reduction of induced edema in rats´ legs, which probably attributed these effects to flavonoids which are is the main secondary metabolite of the plant (20). Another very important property described is the regulation of blood pressure, in the study conducted by Abreu et al in which it was shown that the administration of *J. Gossypifolia* extract produced blockage in the calcium channels, leading to the decrease of blood pressure (18).

For its part, the hypoglycemic and lipid-lowering activity of 95% chloroform fraction of ethanolic extract and the butanol fraction of the aqueous extract of *J. gossypifolia* L. stem) in hyperlipidemic and diabetic mice were evaluated by Rahuja in India. in which the extract and blood parameters were evaluated before and after 15 days of treatment, at the end of the treatment a significantly decreased blood glucose levels of diabetic rats were observed. The elevated blood glucose profile was restored, levels of serum lipids, renal and hepatic markers were at almost normal level compared to the untreated group, there was a decrease in serum triglyceride, cholesterol, and LDL levels of around 27.7%, 17.9%, and 16, 2% respectively, and increased the HDL level around 28.5% on day 15 post-treatment (21).

Therefore, in this study, the in vitro inhibitory activity on the HMG-CoA reductase enzyme and antioxidant activity of *Jatropha Gossypifolia* methanolic extract was determined to validate the popular use of the plant.

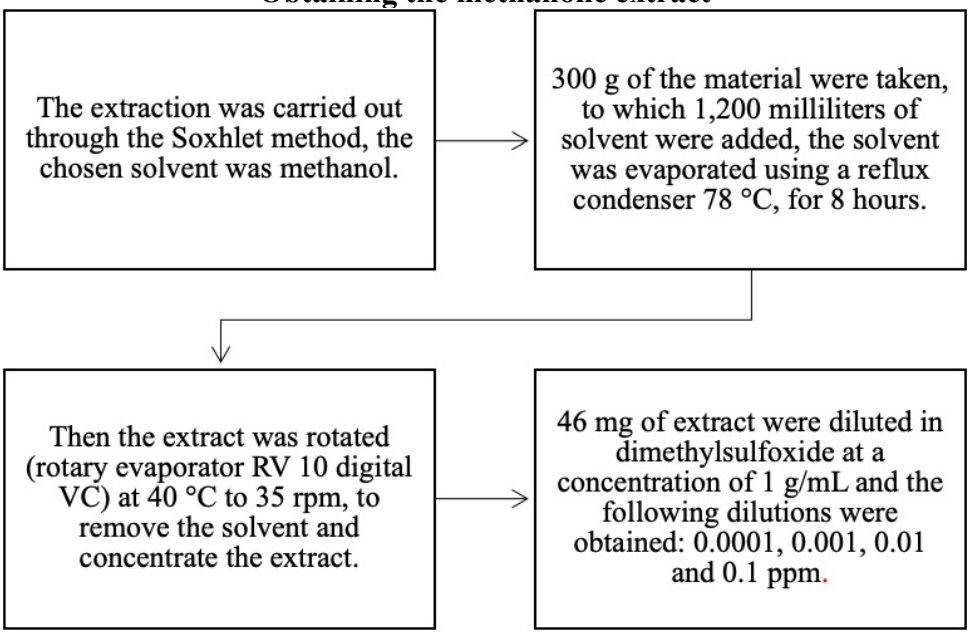
## METHODS

### Obtaining plant material

The green leaves of *Jatropha Gossypifolia* L. were collected during morning hours in on the rural areas of Cartagena located at sea level, the collected material was transferred to paper bags, and sent to the pharmacology laboratory of the University of Cartagena. The plants were washed with abundant deionized water and 2 specimens with flowers were used to perform the botanical identification.

The material was dried using artificial heat using an oven at 50 ° C for 6 hours, the sheets were spread in metal trays and were frequently removed to avoid microbiological contamination, after drying the material, and they were crushed in a mortar to reduce it to fine dust.

**Obtaining the methanolic extract**




**Inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) enzyme**

The HMG-CoA reductase test kit was used (Sigma, St. Louis, USA; Catalog Number CS1090), which contains the human catalytic domain of the enzyme HMGR (concentration 0.6 mg protein/mL) and pravastatin as a positive control (22). The spectrophotometric measurement of the decrease in absorbance at 340 nm was then performed, representing the oxidation of NADPH by the catalytic subunit of HMGR in the presence of the substrate of HMG-CoA at a temperature of 37 degrees Celsius for a period of 10 minutes, making measurements every 20 s using a spectrophotometer (Infinite™ 200 series).

**Antioxidant activity**

**Preparation of erythrocyte suspension.** Human erythrocytes of heparinized peripheral blood were obtained by centrifugation at 1500 rpm per 5 min (Centrifugeuse pour PRP - Plasma 22 - Ortoalresa) at room temperature from two healthy individuals, with prior informed consent.

The erythrocytes were separated from the plasma and washed four times with a saline phosphate buffer (PBS) at pH 7.3 and suspended in the same solution at a 2% hematocrit and suspended in Phosphate-Buffered Saline (PBS) supplemented with glucose (23).

**Evaluation of the antioxidant activity of *Jatropha Gossypifolia* extracts**

To evaluate the antioxidant activity, the method proposed by Grinberg was used (24) 100 µL of human erythrocyte content / mL were incubated and *Jatropha Gossypifolia* Plasma 22 - Orthoresa extract was administered), the supernatant was used to measure the released hemoglobin.

A 100% of blank of erythrocyte hemolysis was taken as a negative control at different concentrations at a temperature of 37 ° C under constant stirring for 30 minutes in the presence of 5% H2O2 (v / v); after

this treatment, they were centrifuged at 10,000 rpm for 5 minutes (Centrifugeuse pour PRP- treated with 5% H2O2 (v / v).

**Statistical analysis**

The experimental design used for the research was a factorial design with three repetitions. The factor was *Jatropha Gossypifolia* methanol extract at a concentration in ppm, with four levels (0.0001, 0.001, 0.01, and 0.1 PPM). The response variables were enzymatic inhibition and antioxidant activity. Enzymatic inhibition was performed using the assumptions of Michaelis and Menten. The results are shown as the mean ± standard deviation (EE), they were processed using GraphPad Prism (version 5.00 for Windows). The differences between the concentrations of the analyzed extracts were evaluated by employing the ANOVA test and for all analyses, the criterion of significance was established at $p < 0.05$.

## RESULTS

The chosen concentrations to perform the tests were: 0.0001, 0.001, 0.01, and 0.1 ppm, this was determined prior to a leukocyte cytotoxicity test performed by the trypan blue exclusion method, which showed that at these concentrations the cell death percentage was not higher than 20%.

**Inhibition of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase)**

The spectrophotometric measurement of the decrease in absorbance at 340 nm, which represents the oxidation of NADPH by the catalytic subunit of HMGR in the presence of the HMG-CoA substrate, is shown below. All the tests were performed in triplicate to which the average was calculated with which the initial velocities were subsequently calculated. An initial speed of the positive control (Pravastatin) and negative control that is given by enzyme activity without inhibitor is below, (Figure 1).

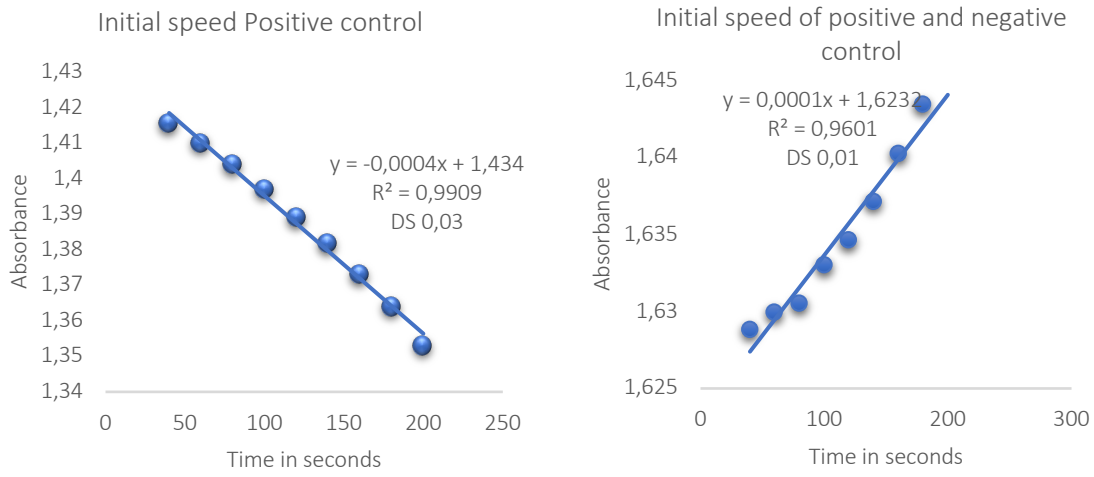



**Figure 1. Initial speed of positive and negative control.** The initial speeds of the positive and negative controls are described in the Inhibition of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase.

Like the positive and negative controls on the extract tests, three replications were made to which the average was calculated, and the initial velocities were subsequently calculated. The different concentrations of the extract show an activity like that reported by the positive control, (Figure 2).

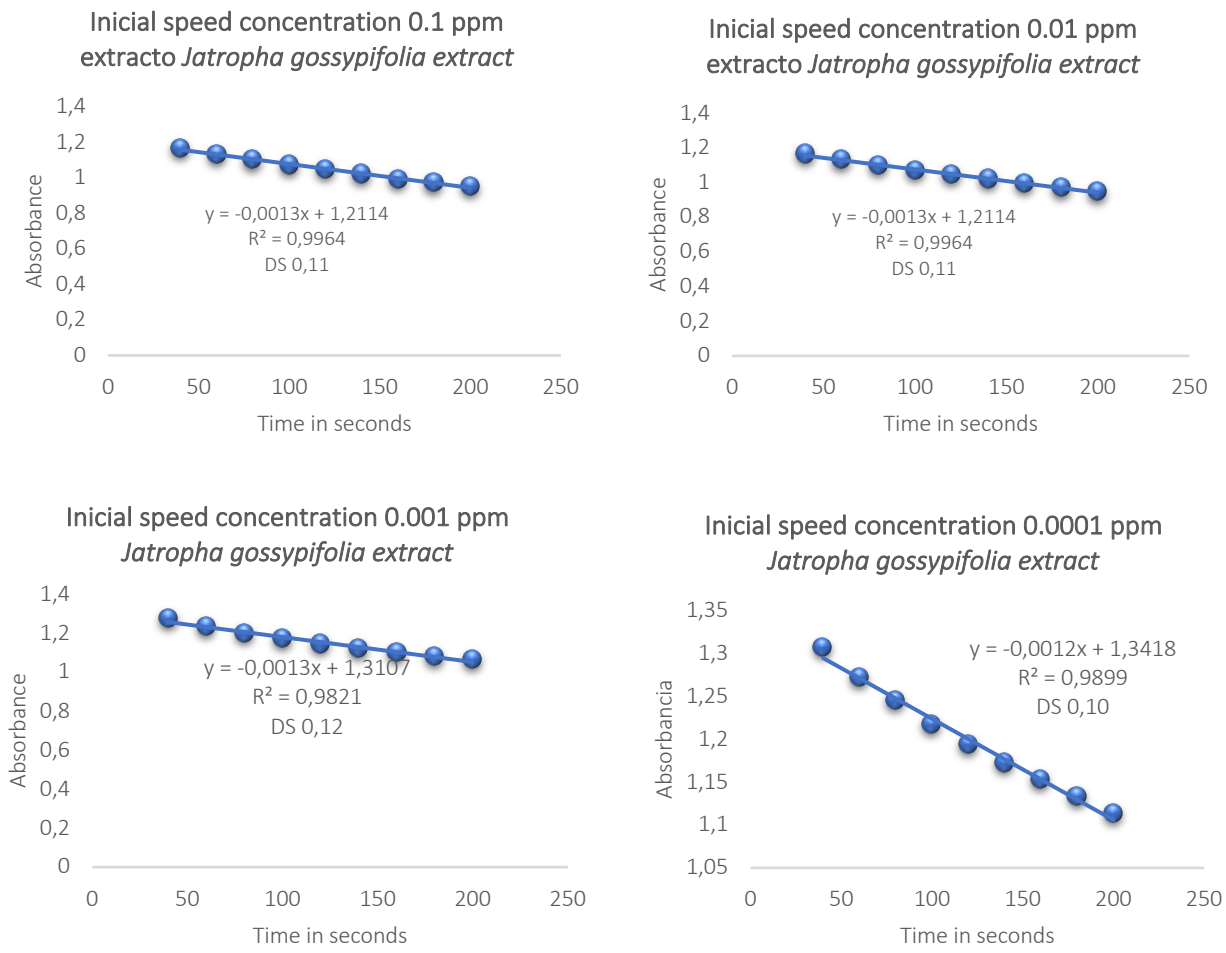



**Figure 2. Initial speed of the different concentrations, in parts per million, of the *Jatropha gossypifolia* extract.** The initial speed of the different concentrations of the *Jatropha gossypifolia* extract are described in the Inhibition of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase.

The percentages of inhibition of HMG-CoA reductase, were 69.2; 50.0; 76.5 and 73.3% at concentrations of 0.1; 0.01; 0.001; 0.0001 ppm respectively of the total methanolic extract. The analysis of variance (ANOVA test) presented significant statistical differences ($p < 0.001$).

The concentrations evaluated in the extract showed inhibition of the enzyme independently of the concentration; the activity of the concentration of 0.001 ppm is mainly highlighted, it is identified that at a higher concentration there is a lower activity, which could be associated with the components of medium to high polarity found in the extract.

Controls were made to determine if the components of the extract could be interfering with the kit, by performing an extract control. The equipment was brought to 0 before starting the readings of the extract. This was calculated in the presence of a control (Pravastatin), this represented 100% enzymatic inhibition, (Figure 3).

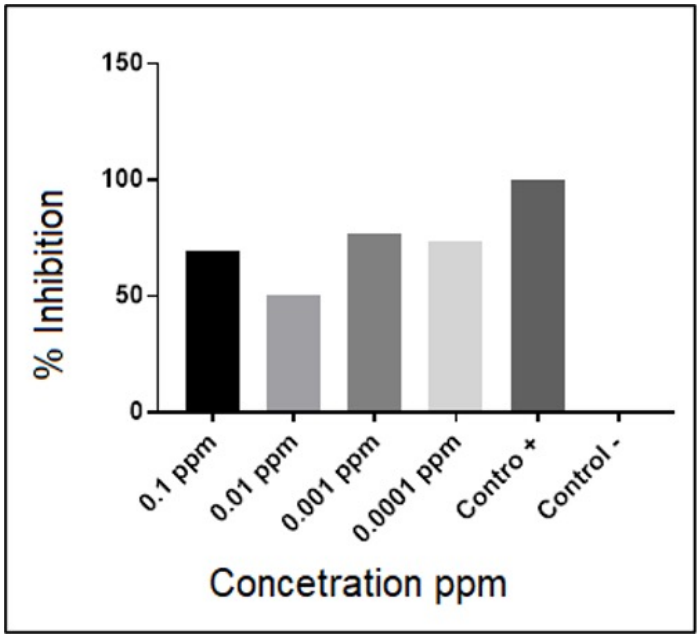



**Figure 3. 3-hydrohi-3-methylglutaryl coenzyme reductase enzyme (HMG-CoA reductase) inhibition.** Percentage of inhibition of the 3-hydrohi-3-methylglutaryl coenzyme reductase enzyme (HMG-CoA reductase at different concentrations of *Jatropha gossypifolia* extract.

**In vitro evaluation of antioxidant activity *Jatropha gossypifolia* methanolic extract**

The hemolytic activity of the extracts was 12.6; 11.2; 4.4; 23.6 % at the concentrations of 0.001 ppm, 0.01 ppm, 0.1 ppm, and 0.5 respectively, with the highest concentration being the one with the highest hemolysis induction, (Figure 4).

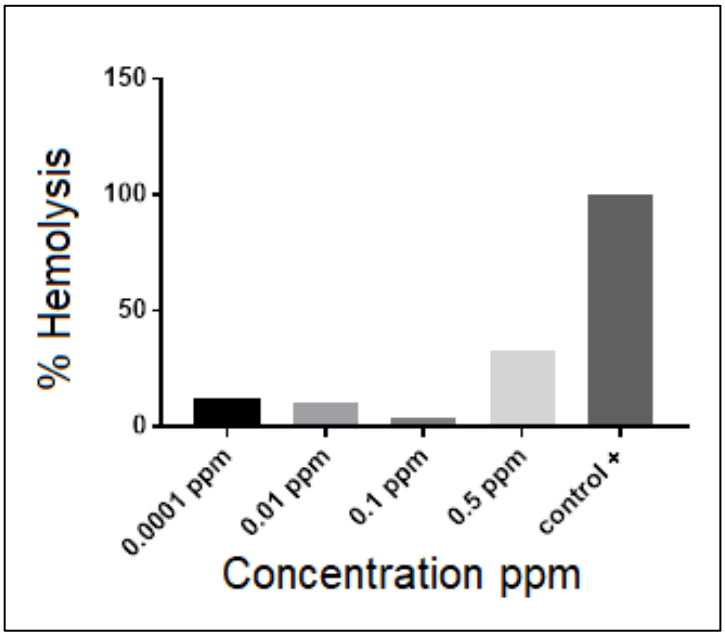



**Figure 4. Hemolytic activity in the presence of hydrogen peroxide at the different concentrations of *Jatropha gossypifolia* extracts.** Percentage of Hemolytic activity in the presence of hydrogen peroxide at the different concentrations of *Jatropha gossypifolia* extracts

The antioxidant activity was evaluated by the inhibition of hemolysis in the erythrocytes induced by H2O2, observing that *Jatropha gossypifolia* extracts inhibited the induction of hemolysis in a proportion of 87.4; 89.8; 96.6; 67.4% at the concentrations of 0.001 ppm, 0.01 ppm, 0.1 ppm, and 0.5 respectively and with a p <0.001. (Figure 5)

These tests were performed with a positive control, represented by 100% of hemolysis of erythrocytes treated only with H2O2 and negative hemolysis control (erythrocytes without any component).

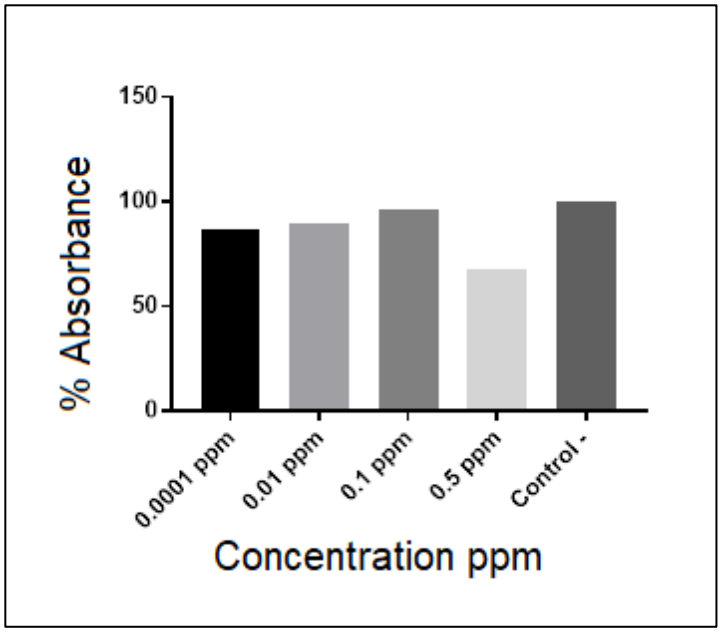



**Figure 5. Antioxidant activity in the presence of hydrogen peroxide at different concentrations of *Jatropha gossypifolia* extracts.** Percentage of Antioxidant activity in the presence of hydrogen peroxide at the different concentrations of *Jatropha gossypifolia* extracts.

## DISCUSSION

HMG-CoA reductase is an enzyme involved in the biosynthetic pathway of cholesterol. When changes occur in the enzymatic activity of this enzyme it affects the overall rate of cholesterol synthesis, so it is inferred that HMG-CoA reductase inhibition reduces cholesterol levels in plasma. (25). In the present in vitro study, inhibition of HMG-CoA reductase by the methanol extract of *Jatropha gosypifolia* was evidenced, which presented different inhibition intervals to those produced by pravastatin, which produced 100% inhibition. The concentration of 0.001 ppm stands out, which presented an inhibition percentage of 76.5%. These results are similar to those obtained by Manolino in his study where he evidenced an enzymatic inhibition of 40% of the HMG-CoA reductase with Amaranth extract (Amaranthus cruentus), levels lower than those of the positive control (Pravastatin 90%) (26).

Rahman et al. 2017 found in vitro inhibitory effect of shiitake mushroom fractions (L. edodes) on HMG Co-A reductase (HMGR) at a concentration of 1mg/ml, the inhibitory effect was 44.26 % compared to pravastatin (87.64%) (27). In the same sense, Hafez evaluated the inhibitory activity of HMG Co-A reductase (HMGR) of spinach leaf extracts (Spinacia oleracea, family Chenopodiaceae), finding that ethanol root extracts registered inhibition percentages of 78.19 % and 72.68 % respectively comparable with the reference drug (90.75 %) (28). The results presented in this study agree with those obtained by Okoh, who found that the leaves and stems of *J. gossypifolia*, have good activity as free

radical scavengers, which indicates that they are good donors of electrons in DPPH (29). On the other hand, Duran evaluated the antioxidant activity of methanolic extracts of guava bark (Psidium guajava L.) and mango (Mangifera indica L.) demonstrating inhibition of hemolysis of 54.6 and 52% in erythrocytes respectively, induced by H2O2 at a concentration of 20µg/mL (30).

The antioxidant activity in the erythrocyte model is based on the composition of the plasma membrane of these cells, which are rich in polyunsaturated fatty acids chains, making them susceptible to oxidation, this membrane consists of two domains, cytoskeleton, and bi-layer phospholipids, which are responsible for maintaining the cell form, when there are attacks of oxidizing substances at the level of erythrocytes as a result there is a lipid peroxidation that leads to the alteration of the integrity of the membrane causing its rupture (31). Another important factor in this oxidative process is iron release from hemoglobin in the red blood cells, this helps to produce oxidation from the reaction of lipid oxygen mediated by free radicals. (32).

According to the above, the antioxidant activity of these extracts can be attributed to the metabolites described in the present literature in the green leaves of *Jatropha gossypifolia* L. (33) which could protect the erythrocyte cell membrane, thus preventing the interaction of structural components such as phospholipids with oxidizing substances, in this case, H2O2 inhibiting their oxidation, as a consequence of this is the protection of the membrane from damage caused by oxidizing compounds (34).

The enzymatic inhibition of HMG-CoA reductase and antioxidant activity of the methanol extract of *Jatropha gossypifolia* L. was independent of its concentration, possibly this is due to the multiple compounds that make up the extract, which allowed it to validate its traditional use in dyslipidemia, diabetes, and other chronic diseases. However, it is necessary to carry out identification studies of the total methanolic extract compounds in order to know what the possible secondary metabolites are related to enzymatic inhibition and antioxidant activity.

## ACKNOWLEDGMENTS

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## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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