

Antioxidant Potential and Acute Toxicity of Aqueous Extracts to A Plant from Caricaceae's Family: *Carica papaya* L., Used in Traditional Medecine in Burkina Faso

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Abstract: The leaves of *Carica papaya* L. (*Caricaceae*) are used in traditional medicine in Burkina Faso for the treatment of malaria or to treat the dengue, also called "tropical flu", a viral disease. Aqueous extracts of the leaves, which is the form of use recommended by traditional healers, was used for the various tests. We studied the antioxidant potential, acute toxicity, antibacterial activity and phytochemical composition of this plant, used everywhere in traditional medicine at Burkina Faso. The extracts showed an antioxidant activity with an $IC_{50} = 23\mu\text{g/ml}$, by the method of reduction of DPPH° radical. Acute toxicity was studied in NMRI strain mice. The aqueous extracts were not toxic at the maximum dose of 2000mg/kg body weight. The extracts showed no bacterial activity on three strains of bacteria tested: *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The phytochemicals which found in aqueous extracts of the leaves are alkaloids, saponosides and triterpenes.

Key words: *Carica papaya* L. • Antioxidant Activity • Acute toxicity • DPPH • Antibacterial Activity • Phytochemical compounds

INTRODUCTION

Carica papaya L., is not a native plant to Burkina Faso. It is native to Mexico and grown for its fruits which are called papaya. Although an introduced plant, the leaves are widely used throughout the country to treat malaria and to treat dengue, a viral disease; its seeds are used to treat stomach upset and intestinal parasites.

According to Nacoulma [1] various parts (Leaves, latex, seeds) are used for several therapeutic purposes. Leaves are used to treat: asthma, fever, beriberi, intestinal worms, gonorrhoea, rheumatism, dysentery, hepatitis, bloody stools, pneumonia, etc.

Traditional cures and plant-based remedies remain the main solution to health problems in many developing countries, Azaizeh *et al.* [2]. According to World Health Organization, OMS [3], medicinal plants usefulness was estimated that over 80% of developing countries populations have resorted to traditional medicine.

The use of antioxidants is related to their ability to reduce tissue damage from free radicals in several diseases such as cardiovascular diseases, cancers, inflammatory diseases, skin, malaria, immune deficiency diseases, etc. Scientific research of secondary plant metabolites should be encouraged for their antioxidant effect to combat the effects of free radicals in several diseases: [4-7]. Extracts of some medicinal plants have antioxidant properties, [8]. Flavonoids, tannins, vitamins E, C are known for them antioxidant activities, [9].

The antibacterial activity of several plant species is studied by several authors: [10, 11]. It is also to find extracts with antibacterial activity.

The aim of this work to study the acute toxicity of *Carica papaya* leaves aqueous extracts to make available the toxicity studies of plants that are still predominantly used in Burkina Faso or in Africa.

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MATERIALS AND METHODS

Materials: The studies were conducted at University Ouaga 1 Pr Joseph KI-ZERBO (Burkina Faso), UFR/SVT, Department of Biochemistry-Microbiology, in the Laboratory of Biochemistry and Applied Chemistry, specializing in medicinal plants. The leaves of *Carica papaya* were harvested in Ouagadougou.

Aqueous Extraction: About 50g of vegetable powdered was extracted with 500 ml of distilled water during one hour at 100°C. Then the mixture was filtered on Wattman paper after cooling. The decoction is lyophilized and kept in a box, for studies.

Antioxidant Activity by the Reduction of the DPPH°: The antioxidant activity of the extracts was evaluated *in vitro* by the capacity of reduction of the radical DPPH (1, 1Diphenyl- 2-PyridylHydrazil) according to the method of Sharma and Bhat [12]. The extracts to be tested were diluted in methanol from 100µg/ml by the limit dilution technique. In an Eppendorf tube, take 250 µl of extract diluted in methanol and then 500 µl of the DPPH solution (2 mg / ml). The white consists of 250 µl of methanol and 500 µl of DPPH (2 mg / l). Zero was made up of 750 ml of methanol. The absorbance was read every 15 minutes at 517nm. Each test was realised three times.

Evaluation of Acute Toxicity of Carica Papaya: The method was that described by Lompo *et al.* [13] and Done [14]. Female NMRI strain mice, approximately 10 weeks old, weighing between 25-35g were used for testing. A concentration of dry extracts diluted in water (200mg/ml) was prepared for the dose of 2000 mg/kg to be administered to each mouse. The test mice and the control group of mice were fasted 12 hours before the test. Two batches of mice were made as homogeneous as possible. The administration of the extracts is done by gavage according to the dose of 2000mg/Kg. The evaluation of the LD₅₀ lethal dose was done at 72hours. Mice were observed during 14 days. A curve drawn of dose-mortality regression help to know if the extracts were an extremely toxic substance, a very toxic substance or a weakly toxic substance.

Antibacterial Activity: Aqueous extracts of *Carica papaya* leaves were used to determine their antibacterial activity. Reference strains from ATCC (American Type Culture Collection, Rockville): *Staphylococcus aureus*

ATCC 6538, *Escherichia coli* ATCC 25922 and a wild strain of *Pseudomonas aeruginosa*. The following reference antibiotics were used: Ampicillin, Bactrim, Erythromycin and Penicillin.

Research of the Bacterial Inhibiting Activity:
Preparation of inoculate: Inoculates of bacterial strains were adjusted to 10⁶ bacteria/ml, according to Ezoubeiri *et al.* [15]. In each Petri plate containing solid medium, put 3mL of the suspension. Eliminate excess from inoculate. Incubate 24 hours. Make wells and put it 50 µL of the extracts (50, 100, 200, 500 µg/ml) or antibiotics of reference. Incubate during 24 hours. Measure the diameters of inhibition. Each test is carried out three times.

Minimal Inhibition Concentration (MIC)

Micro-Well Dilution Assay: Minimum inhibition concentration (MIC) was determined by the micro dilution method in culture broth as recommended by Ellof [16] and the National Committee for Clinical Laboratory Standard (NCCLS, 2001). The 96-well micro-plate (NUNC, Denmark) containing 100µL of Mueller Hinton (MH) broth were used. For each bacteria strain, three columns of eight wells to the micro plate were used. Each well has getting: the culture medium + extract + inoculums (10µL of inoculate) and INT (50 µl; 0, 2mg/mL). The plate were covered and incubated overnight at 37°C and at 44°C for *Escherichia coli* for 24 h. Each MIC experiment was repeated three times. Inhibition of bacterial growth was judged by rose or yellow colour. The MIC is defined as a lowest concentration of the extract at which the bacteria does not demonstrate the visible growth.

Phytochemical Studies: Methods of Ciulei [17] were used. Alkaloids were revealed with Dragendorff's reagent: Appearance of a yellowish-white precipitate shows the presence of alkaloid bases or salts depending on the type of extract used. Flavonoids can be revealed with ammonia (NH₄OH). The observation of a yellow color indicates the presence of flavonoids. Polyphenols and tannins are revealed by ferric chloride (FeCl₃). The appearance of a blue-black or blackish-green color respectively indicates the presence of gallic tannins and catechin tannins.

The property of saponosides is their foaming power. They are soluble in water.

Then, poured 2ml of extract (Dissolved in water) into a test tube that was vigorously stirred. The appearance and persistence of a foam column of at least 1 cm for 15 minutes indicates the presence of saponosides.

Triterpenes and / or free steroids were revealed with concentrated sulfuric acid. Slowly poured 2 ml of on the tube wall. The appearance of a purplish red ring at the interface of the two liquids indicates the presence of terpenes. While the appearance of a blue-green color indicates the presence of steroids.

Statistical Analyses: All experiments are performed in triplicate and the results were expressed in means +/- standard deviation using Microsoft excel 2013.

RESULTS AND DISCUSSIONS

Antioxidant Activity: The ability of extracts to reduce DPPH° has been tested. The reduction of DPPH° by the extracts reduces the initial violet coloration. The first parameter determined was the percentage reduction (Pr) of the DPPH by the extracts, which was calculated according to the formula:

$$Pr = \frac{(Absorbance\ of\ Control - Absorbance\ Extract)}{Absorbance\ control} \times 100$$

These Pr values, Table 1, allowed us to determine the IC₅₀. IC₅₀ was the concentration of antioxidant required to inhibit or reduce the initial concentration of DPPH° by 50%. The aqueous extracts of *Carica papaya* have an IC₅₀ which was 23µg/ml, determined from the Pr=f(Extracted concentration).

Some phytochemical compounds such vitamins, are destabilized with the boiling, during 6 minutes, [18]. Our extracts contain phytochemical compounds which antioxidant activity is not destroyed in spite of the

conditions in which extracts were obtained: boiling in 100°C, during 30 minutes. We did not determine the compound responsible of antioxidant activity, but it will be the object of our next studies.

Acute Toxicity: Results of the toxicity tests for *Carica papaya* are shown in Table 2, for the two batches of mice: controls and 2000mg/Kg. The results indicate that there were no dead animals in any group of mice: controls or 2000mg/kg. The mortality rate was 0%. For body weight, controls were increased from 34g to 36g. Mice receiving 2000 mg/kg aqueous extracts leaves from *Carica papaya* increased from 34g to 35g during 14 days. This result indicates that the aqueous extracts of *Carica papaya* were not toxic.

Medicinal plants can be toxic. A study led to Morocco showed that on 123 listed healing plants, 83 were known by the populations as being toxic, [19]. It turns out to be necessary to lead scientific studies to classify a plant medicinal as being toxic or not. In our case, the aqueous extracts of the leaves from *C. papaya* were not toxic, by scientific tests.

Antibacterial Activity: Two different tests were performed to determine whether the extracts inhibit bacterial growth or not. In the first test, in Petri dishes where a bacterial strain was seeded, the extracts were distributed in the wells. Compared with the positive controls in which observed a growth inhibition, the wells where there were the extracts at 500µg/ml did not inhibit the growth of the bacteria. The inhibition diameters that we measured around the wells for each bacterial strain were of the order

Table 1: Percentage Reduction of DPPH° obtained with Aqueous Extracts of Leaves from *C. papaya*

Concentrations g/ml	3, 125	6, 25	12, 5	25	50	100
Percentage of DPPH° reduction (%)	8, 1±0.72	15.41 ±1, 25	24.20 ± 2, 55	53, 86 ±0, 66	57, 51 ± , 55	61, 42 ±1, 10

Table 2: Acute toxicity tests performed with aqueous extracts of leaves from *C. papaya*

Mice	Weight (g)	Administered volume	Number of dead animals				Weight (g)			
			D1	D2	D3	D14	D1	D2	D3	D14
Controles mice										
1	34, 8	0	0	0	0	0	34, 47	34, 92	35, 51	36, 89
2	35, 62	0	0	0	0	0	36, 48	36, 30	37, 10	37, 95
3	32, 11	0	0	0	0	0	33, 33	33, 10	33, 42	34, 25
Averages	34, 10±1, 80		0	0	0	0	34, 76±1, 59	34, 77±1, 61	35, 34±1, 85	36, 36± 1, 76
Results after 14 days for 2000mg/Kg										
1	33, 71	0, 34	0	0	0	0	33, 43	34, 58	35, 44	36, 01
2	33, 07	0, 33	0	0	0	0	34, 69	34, 54	35, 04	35, 14
3	34, 08	0, 34	0	0	0	0	33, 32	32, 84	33, 91	34, 04
Averages	33, 62±0, 51		0	0	0	0	33, 81±0, 76	33, 99±0, 99	34, 80±0, 79	35, 06±0, 99

of 11 mm, which was very insignificant. In the 2nd test, it can be used the 96-well plates where distributed the extracts at different concentrations, then the bacterial strains. After incubation and addition of INT, all wells were stained purple, regardless of the concentrations of extracts used, ranging from 62.5 µg/ml to 500 µg/ml. The pink color indicating the presence of the bacteria, that means that their growth was not inhibited in the presence of extracts. In the wells where the study used conventional antibiotics, there was no pink staining, depending on the bacterial strain and the antibiotic used. The aqueous extracts of *Carica papaya* did not have any antibacterial activity.

Phytochemical Studies: The phytochemicals identified by the simple characterization tests were: alkaloids, triterpenic saponosides and triterpenes. According to Nacoulma's work [1], the leaves of *Carica papaya* contain the following phytochemicals: carpaine (Pyridinic alkaloid), pseudocarpain 4 alkaloids (Nicotine 0.1%, cotinine 0.0278%, myosmine 0.0014%), tocopherol (vit E) 36 mg/100g, vit C 286mg/100g, Choline, glucocapparin, triterpenesaponosides, triterpenes.

CONCLUSION

The main objective of our work was to know if the two species used in traditional medicine in Burkina Faso were toxic or not. According to the obtained data, the aqueous extracts of leaves of *Carica papaya* recommended for use by patients, which found to be no toxic. Also this research evaluated other potentialities of these extracts by determination of antioxidant activity. Aqueous extracts of *Carica papaya* leaves showed no anti-bacterial activity on three strains of bacteria which used. The IC₅₀ is 23 µg/ml for *Carica papaya*. The phytochemicals which identified in *Carica papaya* extracts were alkaloids, triterpenes and saponosides.

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