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# ASSESSMENT OF ANTIBACTERIAL, ANTIAMOEBIC AND SPASMOLYTIC ACTIVITIES OF THE AQUEUSOUS EXTRACTS, THE ETHANOL EXTRACTS AND THEIRS RESPECTIVE FRACTIONS FROM THE SEEDS OF RIPE AND UNRIPE FRUITS OF *CARICA PAPAYA* L. (CARICACEAE) COLLECTED IN KINSHASA, DEMOCRATIC REPUBLIC OF CONGO*

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## ABSTRACT

The aqueous extracts, the ethanol extracts and their respective fractions of the seeds from ripe and unripe fruits of *Carica papaya* were screened for their putative antibacterial, antiamoebic and antispasmodic activities. Results indicate that the aqueous extracts, the ethanol extracts and their respective fractions from the seeds of both states of the fruit exhibited antibacterial activity at different extents. Samples of the seeds from ripe fruits showed higher activity with minimum inhibitory and minimum bactericidal concentrations ranged from 7.81 to 250 µg/ml compared to samples from the seeds of unripe fruits (15.62 to 500 µg/ml) against the majority of selected bacteria. The evaluation of the antiamoebic activity against *Entamoeba histolytica* revealed that the aqueous extracts, the ethanol extracts and their fractions of seeds from ripe and unripe fruits were able to inhibit the growth of this parasite with minimum amoebicidal concentrations (MAC) less than 20 µg/ml. A significant and gradual decrease of vital parasites in the presence of all tested samples was daily observed from Day 1 to Day 6 while in negative control tubes, a significant increase was recorded. The most active samples were

the aqueous, ethanol and total alkaloid extracts from the seeds of ripe fruits with 1 < MAC (minimal amoebicidal concentration) < 5 µg/ml and the ethanol extract from the seeds of unripe fruits with MAC value of 3.12 µg/ml. In antispasmodic activity testing, the aqueous extracts, the ethanol extracts and their respective fractions from both seed fruits state produced more than 60% inhibition of contractions of guinea-pig ileum induced by acetylcholine and depolarized solution rich in KCl at the tested concentration of 40 µg/ml. The most active samples were the aqueous and alkaloid extracts, and ethanol extracts from both seeds states showing more than 70% and 80% inhibition respectively, the ethylacetate fraction from the partition of the ethanol extract of seeds from ripe fruits producing more than 70% inhibition of contractions of guinea pig-ileum induced by both agonists while the same fraction of the seeds from unripe fruits produced more than 65% inhibition, but less than 70%. These reported results can justify and support the use of mainly the seeds from the ripe fruits of *C. papaya* in traditional medicine for the treating of diarrhea since they would act by their antibacterial, antiamoebic and spasmolytic activities proven in the present study.

**KEYWORDS**: *Carica papaya*, Caricaceae, seeds, ripe and unripe fruits, antibacterial, antiamoebic and spasmolytic activities, diarrhea.

## INTRODUCTION

*Carica papaya* L. (Caricaceae) is a widely grown, tropical and subtropical tree cultivated mainly for its edible fruits when it comes in ripe state. It grows up 4 to 10 m tail with an erect and branches trunk. Its leaves are large 50-70 cm in diameter, deeply, palmately with 7 lobes diversified and profoundly divided. Male and female flowers are white and can be found at the same place.

All plant parts contain latex. Its fruit has variable forms, generally ovoid, with yellow or green pulpe containing back seeds, it become yellow at the maturity state (Kerharo and Adam, 1974; Duke, 1984). It is used as a desert or processed into puree and wine while green fruits are cooked as vegetable (Ahmed et al., 2002; OECD, 2005). In Democratic Republic of Congo (DRCongo), the tree in known by local names depending to the local language of the tribe such as papayi in Kiyanzi and Swahili, payipayi in Lingala, dilolo or nlolo in Kiyombe and mutshi wa tshipapayi in Tshiluba cultured in family gardens for it fruit which is eaten at maturity (Kerharo and Adam, 1974; Kambu, 1990).

Each plant part is attributed with different medicinal values in traditional medicine. For example, the root is used as an aqueous decoction to treat syphilis, icteria, dysentery, intestinal affections, oedema; leaves are used to treat rheumatism and panaris; green fruit is employed to prepare remedies against hepatitis and icteria. Bark and leaves are used as lactogens, antivenimous and hemostatic agents while the aqueous decoction from the seeds of ripe fruits is used for the treatment of dysentery, diarrhea and other ailments (Kerharo and Adam, 1974; Kambu, 1990).

The chemical study on the seed revealed the presence of ash, fat, protein, lipids, sugars (carbohydrates), crude fibers, lignin, volatile oil, mineral elements and vitamins (Kerharo and Adam, 1990, Marfo et al, 1986, Kambu, 1990, Nwofia et al., 2012). and saturated and unsaturated fatty acids (Kerharo and Adam, 1974). Important principles were represented by an the alkaloids carpasemine and carpaine (carposide) and benzylsenevol, and the enzyme myrosine in traces (Perrot, 1943; Karrer, 1958).

On the other hand, some interesting biological activities were previously reported on various extracts of the seeds from ripe and unripe of *C. papaya* fruits. They include the male antifertility (contraceptive properties) of crude aqueous seed extracts (Lohiya et al., 1994a,b, 2000, 2001; Udoh and Kehinde, 1994; Ekamen and Bassey, 2003: Abdulazeez et al., 2009; Hamman al., 2011) and different fractions (Pathak et al., 2000; Lohiya et al., 1999a, 2002) or crude organic extracts such chloroform (Lohiya and Goyal, 1992; Lohiya et al., 1999b) attributed to the presence of alkaloids in crude extract obtained from the seeds of ripe fruits administered at oral doses of 50 and 150 mg/kg body weight in Wistar rats (Udoh et al., 2005) and female contraceptive properties (Abdulazeez et al., 2009), antiparasitic effects demonstrated in Nigerian children with human intestinal parasites receiving immediate doses of 20 ml of an elixir prepared with air-dried *C. papaya* seeds showing a stool clearance rate of parasites encountered between 71.4 to 100% compared with 0-15% produced by honey used as a placebo. Air-dried *C. papaya* seeds were found to be efficacious in treating these parasitosis without no significant side effects (Ohengi et al,(2002), it showed a hepatoprotection in carbon tetrachloride test (Adeneye et al., 2009; Eze, 2102), anthelmintic properties (Ameen et al., 2010), antibacterial activity against some pathogen bacteria by diffusion disc method (Ocloo et al, 2012) and antidiabetic properties in rats (Venkateshwarlu et al., 2012). In most biological activities, the state of the fruit was not determined,

particularly in the evaluation of the antibacterial activity of the seeds and this state give some difficults for comparison the results.

In Democratic Republic of Congo (DRCongo), the seeds from ripe *Carica papaya* fruits are used as an aqueous decoction to treat amoebiasis, diarrhea and other diseases in traditional medicine and the treatment is effective according to the informations from practioners and literature data (Kerharo and Adam, 1974; Kambu, 1990). Taking account of its current use against these tropical ailments, the present study was designed to evaluate the antibacterial activity against some bacteria implicated in diarrhea, antiamoebic activity against *Entamoeba histolytica*, and spasmolytic activity on isolated guinea-pig ileum of aqueous extracts (aqueous decoction), ethanol extracts and their respective fractions obtained from the seeds of ripe and unripe *C. papaya* fruits, activities which can partly explain their use as antidiarrheal agents.

## 2. MATERIALS AND METHODS

### *2..1. Plant material*

Five ripe and unripe fruits of *C. papaya* L. (Caricaceae) each, were bought in a market in Kinshasa, DR Congo. The plant was authentified by Mr. Nlandu Lukebiabo Boniface of the Institut National d'Etudes et de Recherches an Agronomie (INERA), Department of Biology, Faculty of Sciences, University of Kinshasa. A voucher specimen NLB-032011CPS was deposited in the herbarium of this institute. The seeds from both fruits were removed, dried at room temperature end reduced to powder kept in brun bottles hermetically closed

### *2.2. Preparation of the aqueous extract (decoction) and fractionation*

20 g of powdered seeds of ripe fruits were mixed with 200 ml distilled water and boiled for 15min. After cooling and filtration, the filtrate was evaporated *in vacuo* yielding corresponding dried extract denoted as ARF-1 (8.56 g)

On the other hand, 50 g of powdered of the same plant material were macerated with 300 ml ethanol for 24 h. The marc was exhaustively percolated with the same solvent. The macerate et percolate were mixed, filtered and evaporated in *vacuo* yielding dried extract denoted as ERF-2 (12.58 g). An amount ERF-2 (5 g) was dissolved in 50 ml distilled water and filtered. The filtrate was successively and exhaustively extracted with chloroform, ethyl acetate and *n*-butanol and further treated as described above yielding corresponding dried extracts denoted as ERF-1.1 (0.225g); ERF-1.2 (0.486 g), ERF-1.3 (0.315g) and the residual aqueous phase

was also treated in the same way giving dried extract denoted as ERF-1.4 (3.945 g). The total alkaloids were obtained using the classical acid/base procedure described in the literature (Harborne, 1998) yielding dried extract denoted as RF-3 (0.35g).

The same amount of plant material of the powdered unripe fruit seeds were treated with the same solvents in the same way as described above yielding corresponding aqueous dried extract denoted as AUF-4 (6.95 g), the ethanol extract EUF-5 (6.48 g) and its fractions EUF-4.1 (0.190 g), EUF-4.2 (0.387 g), EUF-4.3 (0.301 g) and EUF-4.4 (3.84 g), and the total alkaloids extract denoted as and UF-6 (0.23g) respectively.

### *2.2. Phytochemical screening*

The phytochemical screening was carried out by TLC on precoated silica gel plates (60 $GF_{250}$, thickness layer 0.25 mm, Merck) using different mobile phases and chemical reagents described in the literature for the identification of major phytochemical groups such as alkaloids, flavonoids, anthraquinones, terpenes, steroids, coumarins and proanthocyanidins. The froth test, 2N hydrochloric acid/iso-amylic alcohol, Stiasny's reagent (conc. HCl + formol) were used for the identification of saponins, anthocyanins and tannins respectively (Harborne, 1998)

### *2.3. Antibacterial testing*

The following microorganisms were used: *Bacillus cereus***,** *Escherichia coli*, *Klebsiella pneumonia*, *Klebsiella oxytoxica*, *Proteus mirabilis*, *Pseudomonas areuginosa*, *Salmonella thyphimurium*, *Salmonella enteretidis*, *Staphylococcus aureus*, *Shigella dysenteria and Shigella flexneri*. These microorganisms were clinical isolates obtained from patients at the Laboratory of Microbiology, Cliniques Universitaires du Mont Amba, University of Kinshasa diagnosed with infections, mainly diarrhea.

The antibacterial activity of samples was evaluated by the dilution method as previously described by Vanden-Berghe and Vlietinck (1991), Cimanga et al., 1998) and Tona et al. (1999). Briefly, microorganisms were cultured overnight at 37°C in agar base (Difco) in sterile tubes. Colonies were directly suspended into a small volume 0.9% saline. 5 ml of this suspension was added to 100 µl Muller-Hinton medium (Difco) together with the separated sample dilution tests (0.1-100 µg/ml) from the seeds of *C. papaya* ripe and unripe fruits. On the other hand, a sterile tube containing only bacterial suspension and distilled water was

used as a negative control, an other tube containing bacterial suspension and ampicillin and chloramphenicol (0.1 to 25 µg/ml) was considered as a positive control.

The lowest concentration of the test sample that inhibited the bacterial growth after incubation was taken as the MIC value. A volume of 10 µl from each test tube was placed on new culture medium in order to determine the MBC which was defined as the lowest concentration yielding negative subcultures or only one colony. All samples were tested in triplicate.

***2.4. In vitro antiamoebic testing***

*Entamoeba histolytic* used in the present study is a laboratory isolated strain from patients with acute dysentery diagnosed in the Tropical Medicine Institute, Faculty of Medicine, University of Kinshasa. The evaluation of activity was performed using the method previously described by Ridet and Chartol (1964); O'Ndir and Pousset (1981) with same modifications (Tona et al., 1998, 2000).

Briefly, the parasite was grown and cultured in sterile tubes containing 9 ml of diphasic medium (medium N of Pasteur Institute) called Dobbel and Laidlaw medium. The mixture was stirred and incubated for one week at 37°C The daily examination and counting of amoebae through a optic microscope with the aid of Neubauer's cells were performed in order to monitor the parasitic growth and to detect possible contamination.

Uncontaminated tubes containing an average number of $2.5.x10^6$ amoebae/ml culture medium were selected as test tubes. 10 mg of each test sample was dissolved in 10 ml hydroethanol solution (eau-ethanol :9:1) to have corresponding stock solutions of 1 mg/ml. These last solutions were submitted to two fold dilutions to give a series of test solutions of 500 to 0.1 µg/ml. Next, 1 ml of the test solution with a known concentration was added to a separated test tubes containing parasites. On the other hand, two tubes were used as controls, one containing parasites in hydroethanol (1:9) without test sample as negative control and an other containing test tubes with parasites and metronidazole or dehydroemetine as positive controls tested at concentrations from 10 to 0.31 µg/ml.

All tubes were plugged with sterile cotton, vigorously stirred and incubated at 37°C for 6 days. The daily counting of dead and living amoebae was done described above. The test was considered as positive when the vegetative or kystic forms of amoebae was not

microscopically observed. The minimum amoebicidal concentration (MAC) was determined by using linear-courbes doses-responses. The test for each sample was done in duplicate.

### *2.5. Antispasmodic testing*

The spasmolytic activity of the aqueous extracts, ethanol extracts and their respective fractions from the seeds of *C. papaya* ripe and unripe fruits was evaluated according to the procedure previously described by Kambu et al. (1990) and Cimanga et al. (2010). For this, male guinea-pig (200-300 g body weight) were killed by blow to the head. The ileum was removed and washed before with distilled water and after with Tyrode solution composed with: (mM): KCl:2.2, $MgCl_2$: 0.11, $NaH_2PO_4.2H_2O$: 0.42, $CaCl_2$: 1.8, NaCl: 137, $NaHCO_3$: 11 and glucose: 5.6. Lengths of approximately 4 cm of the guinea-pig ileum were cut from the middle region of the ileum and transferred into an organ bath containing 50 ml of Tyrode's solution maintained at 37°C under carbogen (95% O2 + 5% CO2) bubbling and connected to a Palmer kymograph transducer to record the longitudinal muscle contraction of the ileum.

The ileum strips was allowed to stabilize during 30 min. 2 ml of Tyrode's solution was removed in organ bath and the ileum stimulated every 5 min either with 2 ml of acetylcholine solution ($5 \times 10^{-5}$g/l) or by 2 ml of the depolarized solution ((mM): NaCl: 2.7, KCl: 100,$NaHCO_3$: 15, $CaCl_2$: 1.25, $MgCl_2$: 12.5 and glucose: 11) to obtain maximal contraction of the ileum. After obtaining three consecutive maximal contractions of the ileum in the presence the agonist, the ileum was washed with Tyrode's solution, 2 ml of the Tyrode's solution was removed and then 2 ml of the respective stock solution in distilled water (1 mg/ml) was added in the organ (representing 40 µg/ml of the test sample in organ bath). The ileum was maintained in contact with the test sample for 30 min before the next stimulation with the respective agonist. Atropine and papaverine (5 µg/ml) were used as a reference antispasmodic products (n = 3).

The inhibition of ileum contraction by sample in the presence of agonist was expressed as percentage of mean ± S.D from three experiments and was calculated using the following formula: % Inhibition = (A-B) x 100/A were A is the amplitude of the ileum concentration (cm) induced by the agonist and B the amplitude of the ileum contraction induced by the test sample in the presence of the agonist.

### 3. Statistical analysis

All data collected were summarized as mean ± sem. Significant differences were determined using Student-*t* test and the difference were considered as significant at $p < 0.05$.

## 4. RESULTS AND DISCUSSION

An extensive biological evaluation including antibacterial, antiamoebic and antispasmodic activities of the aqueous extracts, the ethanol extracts and their respective fractions of the seeds from the ripe and unripe fruits of *C. payaya* was conducted in the present study. In the present study, and for antibacterial activity, following criteria were adopted to appreciate the level of the antibacterial activity of tested samples: MIC and MBC ≤ 20 µg/ml: strong activity, 20 < MIC or MBC ≤ 100 µg/ml: good activity, 100 < MIC or MBC ≤ 250 µg/ml: moderate activity, 250 < MIC or MBC ≤ 500 µg/ml: weak activity, MIC, MBC > 500 µg/ml: inactive.

In the antibacterial testing, results indicated that the aqueous extract (ARF-1) from the seeds of *C. papaya* ripe fruits exhibited strong antibacterial activity against *Bacillus cereus, Escherichia coli, Shigella flexneri* and *Staphyloccocus aureus (*MIC = 15.62 µg/ml, good activity against *Proteus mirabilis, Pseudomonas aeruginosa, Salmonella epidermis* and *Salmonella thyphimurium* with MIC values ranging from 31.25 to 62.5 µg/ml (Table 2) (Vanden Berghe and Vletienck, 1991). It showed moderate activity against *Klebsiella oxytocica* (MIC = 250 µg/ml) /and was inactive against *Klebsiella pneumonia* (MIC > 500 µg/ml). Concerning its bactericidal action, the extract exerted moderate bactericidal activity against *K. oxytocica*, without effect against *K. pneumonia* and exhibited good bactericidal action against the remaining bacteria with MBC values ranging from 31.25 to. 62.55 µg/ml (Table 3) (Vanden Berghe and Vlietinck, 1991).

The ethanol extract (ERF-2) from the seeds of *C. papaya* ripe fruits displayed strong antibacterial activity against *B. cereus,. E. coli, P. mirabilis, P. areuginosa, , S. aureus* and *S. flexneri* with MIC values ranging from 7.81 to 15.62 µg/ml, good and moderate activity against *K. oxytocica* and *S. enteritidis,* and *K. pneumonia* respectively (Table 2). In addition, this extract exerted strong bactericidal effect against *B. cereus, P. mirabilis, S. aureus* and *S. flexneri* with minimal bactericidal concentration (MBC) values from 7.81 to 15.62 µg/ml, good activity against *E. coli, P. aeruginosa, S. enteritis* and *S. thyphimurium* (MBC = 31.25 µg/ml) (Vanden Berghe and Vlietinck; 1991).

Fractions from the partition of the ethanol extract from the seeds of *C. papaya* ripe fruits (ERF-2) also exhibited antibacterial activity at different extents.

Briefly, the chloroform soluble fraction (ERF-2.1) rich in steroids and terpenes showed pronounced activity against *B. cereus, E. coli, S. aureus* and *S. flexneri* (MIC < 20 µg/ml), good activity against *K. oxytocica* and *K. pneumonia*, and moderate activity against *P. mirabilis* and *P. aeruginosa* (Table 2) (Vanden Berghe and Vlietinck, 1991). This fraction showed pronounced bactericidal action against *E. coli, S. enteritidis, S. aureus* and *S. flexneri* (MBC < 20 µg/ml), moderate activity against *P. mirabilis* and *P. aeruginosa*, and good activity (MBC < 100 µg/ml) against the remaining tested bacteria (Table 3) (Vanden Berghe and Vlietinck, 1991).

The ethylacetate soluble fraction (ERF-2.2) rich en flavonoids displayed strong antibacterial activity against *E. coli, P. aeruginosa, S. enteritidis* and *S. flexneri* (MIC < 20 µg/ml) and good activity against the remaining tested bacteria (Table 2). It showed pronounced bactericidal action against *S. aureus* and *S. flexneri*, was without bactericidal effect against *P. mirabilis* (MBC > 500 µg/ml) and exhibited nevertheless, good bactericidal action against the remaining tested bacteria with MBC values < 100 µg/ml (Table 3).

The *n*-butanol soluble fraction (ERF-2.3) rich in saponins showed strong activity against *S. flexneri* ( MIC = 15.62 µg/ml), good or moderate activity according to the tested bacteria with MIC values < 100 µg/ml (Table 2). It displayed moderate bactericidal action against *P. mirabilis*, and good action (MBC < 100 µg/ml) against the remaining tested bacteria (Table 3) (Vanden Berghe and Vlietinck, 1991).

The aqueous residual phase (ERF-2.4) rich in phenolic compounds exhibited good antibacterial activity *B. cereus, S. enteritis, S. aureus* and *S. flexneri* (MIC < 100 µg/ml), was inactive against *P. mirabilis* and *P. aeruginosa* (MIC > 500 µg/ml) and displayed moderate activity against the remaining tested bacteria (Table 2). This fraction showed good bactericidal action against *E. coli, S. enteritidis* and *S. flexneri*, and was without effect against *P. mirabilis* and *P. areruginosa* (MBC > 500 µg/ml). It however exhibited good bactericidal action (MBC < 100 µg/ml) against the remaining tested bacteria (Table 3).

The total alkaloids extract (RF-3) was found to be the most active sample since it inhibited the growth of most selected bacteria with MIC and MBC values from 3.90 to 62.5 µg/ml, its

antibacterial and bactericidal activity was considered as strong or good according to the case, excepted against *P. mirabilis* and *P. aeruginosa* for witch its MBC value was 125 µg/ml (moderated activity).

Tested against *Shigella dysenteria*, the aqueous extract, the ethanol extract and its chloroform and ethylacetate soluble fractions from seeds of *C. papaya* ripe fruits exhibited pronounced antibacterial activity with MIC < 20 µg/ml while the remaining samples showed good activity with MIC < 100 µg/ml (Table 2). In addition the aqueous, ethanol and total alkaloid extracts showed pronounced bactericidal activity against this bacteria with MBC of 15. 62 µg/ml. The bactericidal effect presented by the remaining samples were considerate as good since they inhibited the growth of this bacteria with MBC < 100 µg/ml (Vanden Berghe and Vlietinck, 1991).

For samples of the seeds from *C. papaya* unripe fruits (UF), the aqueous extract (AUF-4) displayed good antibacterial activity against *E. coli, P. mirabilis, S. enteritidis* and *S. flexneri* with MIC values from 31.25 to 62.5 µg/ml and moderate activity against the remaining bacteria (Table 2). It only exhibited good bactericidal activity against *B. cereus* , *S. aureus* and *S .flexner*i (MIC < 100 µg/ml) (Vanden Berghe and Vlietinck, 1991) while this bactericidal action was moderate against the remaining selected bacteria (Table 3).

The ethanol extract (EUF-5) exhibited strong antibacterial activity against *B. cereus, S. aureus* and *S. flexneri* with MIC < 20 µg/ml, good activity against *E. coli, P. mirabilis* and *P. aeruginosa* (MIC < 100 µg/ml) and weak activity against *K. oxytoxica* and *K. pneumonia* (MIC = 500 µg/ml). Its fractions also displayed antibacterial activity at different extents.

The chloroform soluble fraction (EUF-5.1) rich in steroids and terpenes exhibited good antibacterial activity against *B. cereus, E.coli, K. aeruginosa, S. aureus* and *S. flexneri* (MIC < 100 µg/ml) (Vanden Berghe and Vlietinck, 1991) and showed moderate activity against *K. pneumonia, P. mirabilis, P. aeruginosa* and *S. enteritidis* (125 ≤ MIC ≤ 250 µg/ml) (Table 2). It showed strong bactericidal action against *S. flexneri* (MBC = 15.62 µg/ml), good action against *B. cereus, E.coli, K. oxytocica* and *S. aureus* (MBC < 100 µg/ml) (Table 3) (Vanden Berghe and Vlietinck, 1991) and moderate effect against the remaining selected bacteria (Table 3).

The ethylacetate soluble (EUF-5.2) displayed good antibacterial activity *against B. cereus, E. coli, K. oxytocica S. aureus* and *S. flexneri* with MIC values < 100 µg/ml (Table 2) and moderate activity against *P. mirabilis* and *S. thyphimurium* and was inactive against *K. pneumonia* (Table 2). Its bactericidal action was good against *S. flexneri, B. cereus, K. oxytocica, S. aureus* and *S. flexneri* (MBC < 100 µg/ml,) weak against *P. aeruginosa* and was devoid with bactericidal effect against *S. thyhimurium* (Table 3).

The *n*-butanol soluble fraction (EUF-5.3) exhibited good antibacterial activity against *E. coli, S. aureus* and *S. flexneri* and moderate activity against the remaining selected bacteria (Table 2). In addition, this fraction only showed good bactericidal effect against *S. flexneri* and was devoid with bactericidal action against *S. thyphimurium*, but displayed moderate bactericidal action against the remaining tested bacteria (Table 3).

The residual aqueous soluble fraction (EUF-5.4) was inactive against *K. pneumonia, P. mirabilis* and *P. aruginosa*, showed weak activity against *S. thyphimurium* (MIC = 500 µg/ml) and moderate activity against the remaining tested bacteria (Table 2). Its bactericidal action against all tested bacteria was considerate as moderate since its MBC values were ranging from 125 to 250 µg/ml (Table 3).

The total alkaloids extract (UF-6) from the unripe fruits exerted strong antibacterial activity against *B. cereus, S. aureus* and *S. flexneri* (MIC < 20 µg/ml), good activity against *E. coli, K. oxytocica, K. pneumonia* and *S. thyphimurium* (MIC < 100 µg/ml) and moderate activity against *P. mirabilis* and *P. aeruginosa* (Table 2). Moreover, this extract showed strong bactericidal effect when tested against *B. cereus* and *S. flexneri* with MBC value of 15.62 µg/ml, good and moderate bactericidal activity against the remaining tested bacteria according to the case (Table 3).

Against *Shigella dysenteria*, it was observed that the ethanol and total alkaloid extracts from the seeds of *C. papaya* unripe fruits exhibited pronounced antibacterial activity against this bacteria with MIC values of 15.62 and 7.81 respectively while the remaining samples showed good activity with MIC values < 100 µg/ml (Table 2) (Vanden Berghe and Vlietinck, 1991). Excepted the total alkaloids extract which showed pronounced bactericidal effect against this bacteria with MBC value of 15.62 µg/ml and the residual aqueous soluble fraction with moderate activity (MBC = 125 µg/ml), the remaining samples from this kind of fruits

exhibited good bactericidal activity with MBC < 100 µg/ml (Table 3) (Vanden Berghe and Vlietinck, 1991).

But by disc diffusion method, Thomas (1989) had previously reported the ineffective effect of an aqueous extract from the seeds (macerate) against some pathogen bacteria and yeasts such *S. aureus*, *S. pyogenes*, *K. pneumonia*, *E. coli* and *C. albicans* and unfortunately, the state of the fruit was not defined. In a recent study, Ocloo et al. (2012) had also shown that the cold and hot water extracts respectively, of the seeds from unripe fruits were devoid with antibacterial activity against *S. flexneri*, *S. aureus* and *E. coli* by disc diffusion method while the acetone and methanol extracts produced a slightly inhibition against these bacteria with inhibition diameters from 10 to 13 mm.

The phytochemical analysis of these extracts revealed the presence of different constituents likely to be due the varying degrees of solubility of the active constituents that influence the level of the activity. The antibacterial activity of seed extracts of *C. papaya* fruits was attributed to the presence of the alkaloid carpaine (Kerharo and Adam t1974), but results from the present study suggested also that there are also other constituents belonging to phytochemical groups such as terpenes, steroids, saponins and phenolic compounds presents ripe and unripe fruits with antibacterial activity since it was clearly demonstrated the chloroform, ethylacetate, *n*-butanol and residual aqueous phase from the partition of the ethanol extracts containing these phytochemical groups, exhibited antibacterial activity at different extents. On this point, our observations are in good agreement with Ocloo et al. (2012).

For the antiamboebic activity, all samples from the seeds of both states of *C. papaya* were assessed for their potency to reduce *Entamoeba histolytica* growth. Results showed that all samples possessed the capacity to significantly reduce *E. histolytica* growth as summarized in Tables 4 and 5 showed by the effect of both aqueous (ARF-1 and AUF-4 respectively) and ethanol extracts (ERF-2 and EUF-5 respectively) from the seeds of *C. papaya* ripe and unripe fruit respectively on the growth of this parasite. By a daily microscopic examination, at tested concentrations from 50 to 0.78 µg/ml, it was observed for all tested extracts, significant and gradual reduction in number of viable parasites from Day 1 to Day 6 or a drastic increase of these kind of parasites according to the case, compared to negative control and this effect was dose-dependent (Tables 4 and 5).

On Day-6, the minimal amoebicidal concentration (MAC) of each sample was deduced and was defined as a concentration at which no viable parasite and kystic forms were microscopically observed (Table 6). To appreciate more the level of antiamoebic activity of the tested samples, following criteria were adopted: MAC ≤ 5 µg/ml: strong activity, 5 < MAC ≤ 10 µg/ml: good activity, 10 < MAC ≤ 30 µg/ml: moderate activity, 30 < MAC ≤ 50 µg/ml: weak activity, 50 < MAC ≤ 100 µg/ml very weak activity, MAC > 100 µg/ml: inactive. Results showed that the aqueous extract (ARF-1), the ethanol extract (ERF-2), and its ethylacetate (ERF-1.2) soluble fraction rich in flavonoids, and the total alkaloids extract (RF-3) from the seeds of *C. papaya* ripe fruits exhibited strong antiamoebic activity with MAC values of 3.12, 1.56 and 3.12. µg/ml respectively. Its chloroform soluble fraction (ERF-1.1) rich in steroids and terpenes displayed good antiamoebic activity with MAC value of 6.25 µg/ml while the *n*-butanol (ERF-1.3) rich in saponins and aqueous residual phase (ERF-1.4) rich in phenolic compounds soluble fractions showed moderate antiamoebic activity (Table 5). These results clearly indicated that the total alkaloids extract RF-3 was the most active sample followed by the ethanol extracts (ERF-2) and the aqueous (ARF-1)

Concerning samples for the seeds from *C. papaya* unripe fruits (UF), it was observed that the ethanol (EUF-5) and the total alkaloid (UF-6) extracts showed strong antiamoebic activity with MAC values of 3.12 and 0.78 µg/ml respectively and were the most active samples in this group. The aqueous extract (AUF-4), the chloroform (EUF-5.1), *n*-butanol (EUF-1.3) and residual aqueous (EUF-1.4) soluble fractions from the partition of the ethanol extract (EUF-5) displayed moderate activity (Table 6) while the ethylacetate (EUF-5.2) exhibited good antiamoebic with MAC value of 6.12 µg/ml. The comparison of the antiamoebic level of these extracts remained valid to that deduced before for the same samples from the seeds of ripe fruits.

This antiamoebic effect was strong with the aqueous and ethanol extracts from the seeds of the ripe fruits compared to the same extracts of the unripe fruits. In fact, at a concentration of 50 to 3.12 µg/ml, it was observed an absence of viable parasites at Day 3 to Day 6, Day 4 to Day 6 and Day 5 to Day 6 respectively with the testing of the aqueous extract (ARF-1) of the seeds from *C. papaya* ripe fruit. In addition this extract produced significant decrease of the number of vital amoeba at concentrations of 1.56 and 0.78 µg/ml compared to negative control (Table 4). The same effect was also observed for the ethanol extract (ERF-2) until 0.78 µg/ml compared to negative control (Table 5). On the other hand, the aqueous extract

(AUF-4) of the seeds from the unripe fruits did not show viable parasites at Day 6 at tested concentrations of 50 and 25 µg/ml while at tested concentrations from 12.5 to 6.25 µg/ml, a slight decrease of vital amoeba was observed, but at 3.12 µg/ml of testing, a slightly increase of the number of vital parasites was noted compared to negative control (Table 4). The ethanol extract (EUF-5) showed absence of parasites at Day 4 to Day 6, Day 5 to D 6 and only at Day 6 when tested at concentration of 50 and 25 µg/ml, 12.5, 6.25 and 3.12 µg/ml respectively. For this extract, a significant decrease of vital parasites was noted at a tested concentration of 1.56 µg/ml while with 0.78 µg/ml, a drastic increase of vital parasites was observed compared to negative control (Table 5). The positive effects of these extracts were dose-dependent. In general, the antiamoebic effects of the aqueous extract, ethanol extract and its fractions from the seeds of unripe fruits were weak compared that of the same samples from the seeds of *C. papaya* ripe fruits as already mentioned above.

Our results indicated that samples from the seeds of ripe fruits (RF) are twice more active than samples from the seeds of unripe fruits (UF) suggesting that this could be due the difference in the amount of active constituents particularly carpaine previously reported as responsible of the antiamoebic activity of *C. papaya* seeds in both states (Watt, 1962; Kerharo and Adam 1994) or to other active principles as suggested by the present study since fractions from the partition of the ethanol extracts containing terpenes, steroids, saponins and phenolic compounds have also shown good antiamoebic activity according to the case. (Table 6). Moreover, Okenyi et al. (2007) had determined the effectiveness of air-dried *C. papaya* seeds (state of the fruits not determined) on human intestinal parasitosis on 80 asymptomatic Nigerian children with stool microscopic evidence of intestinal parasites. They had received immediate an oral dose of 20 mL of either an elixir composed with air-dried *C. papaya* seeds and honey. After 7 days of treatment, more subjects given this preparation had their stools significantly cleared of parasites (76.7%) compared to the treatment of honey alone (16.7%). There were no harmful effects. These authors concluded that air-dried *C. papaya* seeds are efficacious in treating human intestinal parasites without significant side effects.

In the present study, results from the evaluation of spasmolytic activity of samples from the seeds of *C. papaya* ripe and unripe fruits showed that all samples were found to be able to inhibit the contractions of guinea-pig ileum induced by ACh (acetylcholine) and the depolarized solution rich in KCl at different extents (Table 7).

For samples from the seeds of *C. papaya* ripe fruits, it was observed that the ethanol extract (ERF-2) produced 85.64 ± 0.42 and 81.37 ± 0.80 % inhibition of contractions induced by ACh and the depolarization solution rich in KCl respectively. All fractions from the partition of ERF-2 showed antispasmodic activity since they significantly reduced the contractions induced by both agonists. The most active fraction was the ethylacetate faction (ERF-2.2) producing 72.30 ± 0.27 and 70.80 ± 0.44% inhibition of contractions of guinea-ileum induced by ACh and the depolarized solution rich in KCl respectively. The remaining fractions showed a significant inhibition higher than 60% against both agonists ($p < 0.05$) (Table 7). The aqueous (ARF-1) and the total alkaloids (RF-3) extracts produced more than 70% inhibition and the activity of the total alkaloids extract was higher (79.6 ± 1.3 and 76.1 ± 1.5% inhibition) compared to that of the aqueous extract ARF-1 (75.41 ± 1.21 and 73.34 ± 0.50% inhibition) against ACh and depolarized solution rich in KCl respectively, but lower ($p < 0.05$) than that of the ethanol extract (ERF-2) showing 85.64 ± 0.42 and 81.37 ±0.80% inhibition of contractions of ileum-pig induced by both agonsits ($p < 0.05$).

The samples from the seeds of *C. papaya* unripe fruits (UF), also exhibited spasmolytic activity at different extents. Results indicated that the aqueous (AUF-4), ethanol (EUF-2) and the total alkaloid (UF-6) extracts produced more than 70% inhibition of contractions induced by both agonists on guinea-pig ileum with the antispasmodic activity of the ethanol extract being higher with 80.47 ± 0.72 and 78.72 ± 0.31% inhibition of contractions of guinea-pig ileum induced by ACh et the depolarized solution rich in KCl respectively compared to that the aqueous extract (72.33 ± 0.75 and 71.80 ± 0.24% ) and total alkaloids extract (76.32 ± 0.23 and 74.84 ± 0.52%) against both agonists respectively. All fractions from the partition of EUF-5 showed significant inhibition of contractions induced by both agonists more than 60% with the ethyl acetate fraction rich in flavonoids (EUF-5.2) (69.27 ± 1.24 and 67.55 ± 0.94%) as the most active samples compared to other fractions ($p < 0.05$) (Table 7). The spasmolytic activity of all samples from seeds of *C. papaya* ripe and unripe fruits of *C. papaya* was considerate to be the type papaverinic since papaverine used as reference antispasmodic product inhibited the activity of both agonists contrary to atropine (Table 7)

In general, in the present study, samples from the seeds of *C. papaya* ripe fruits (RF) exhibited high evaluated activities than those of the seeds from unripe fruits (UF) for the same reasons evoked above. In conclusion, the present study clearly shows that samples from the seeds of *C. papaya* ripe and unripe fruits of *C. papaya* exhibited antibacterial,

antispasmodic end antiamoebic activity at differents extents. Samples from seeds of ripe fruits showed higher activities compared to samples from unripe fruits. These results can partly support and justify the traditional used mainly of the seeds from ripe fruits of this medicinal plant for the treatment of diarrhea and dysentery in traditional medicine in some African countries with success without significant side effects.

**Table 1. Phytochemical screening of ethanol extracts from of the seeds of ripe (ERF-1) and unripe fruits (EUF-4) of *C. papaya.***

| Phytochemical groups | ERF-2 | EUF-5 |
|---|---|---|
| Polyphenols | + | + |
| Catechic tannins | - | - |
| Gallic tannins | - | - |
| Flavonoids | - | - |
| Coumarins | - | - |
| Anthraquinones | - | - |
| Saponins | - | - |
| Cardiotonic heterosides | - | - |
| Steroids | + | + |
| Terpenoids | + | + |
| Alcaloids | + | + |
| Fatty acids | + | + |
| Amined compounds | + | + |

ERF-2 and EUF-5: ethanol extract from ripe and unripe fruits respectively.

The chemical composition was similar to that of both aqueous extracts ARF-2 and AUF-4 respectively.

**Table 2. Antibacterial activity of the aqueous extracts , ethanol extracts and their respective fractions, and total alkaloid extracts of the seeds from ripe (RF) and unripe fruits(UF) of *C. papaya* (MIC, µg/ml)**

| A/B | *B.c* | *E.c* | *K.0* | *K.p* | *P.m* | *P.a* | *S.e* | *S.t* | *S.a* | *S.d* | *S.f* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **ARF-1** | 15.62 | 15.65 | 125 | 250 | 31.25. | 31.25 | 31.25 | 62.5 | 15.62 | 15.62 | 15.62 |
| **ERF-2** | 7.81 | 15.62 | 62.5 | 125 | 15.62 | 15.62 | 15.62 | 31.25 | 7.81 | 7.81 | 7.81 |
| ERF-2.1 | 15.62 | 7.82 | 31.25 | 62.5 | 250 | 250 | 15.62 | 250 | 15.62 | 31.25 | 3.95 |
| ERF-2.2 | 31.25 | 15.62 | 31.25 | 62.5 | >500 | 15.62 | 31.25 | 62.5 | 15.62 | 15.62 | 7..81 |
| ERF-2.3 | 31.25 | 31.25 | 62..5 | 125 | 250 | 125 | 31.5 | 62.5 | 31.75 | 31.25 | 15.62 |
| ERF-2.4 | 62.5 | 125 | 125 | 250 | >500 | >500 | 31.25 | 250 | 31.25 | 62.5 | 31.25 |
| **RF-3** | 3.90 | 15.62 | 31.25 | 31.25 | 62.5 | 62.5 | 7.85 | 31.25 | 3.90 | 7.81 | 3.90 |
| **AUF-4** | 31.25 | 31.25 | 250 | 500 | 62.5 | 62.5 | 62.5 | 125 | 31.25 | 31.25 | 31.25 |
| **EUF-5** | 15.62 | 31.25 | 125 | 250 | 31.25 | 31.25 | 31.25 | 62.5 | 15.62 | 7.81 | 15.62 |
| EUF-5.1 | 31.25 | 15.62 | 62.5 | 125 | 500 | 500 | 31.25 | 500 | 31.25 | 125 | 7.81 |
| EUF-5.2 | 62.5 | 31.25 | 62.5 | > 500 | 125 | 31.25 | 62.5 | 125 | 31.25 | 32.25 | 31.25 |
| EUF-5.3 | 125 | 62.5 | 125 | 500 | 250 | 250 | 62.5 | 125 | 62.5 | 62.5 | 62.5 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| EUF-5.4 | 125 | 250 | 250 | >500 | >500 | >500 | 62.5 | 500 | 62.5 | 125 | 62.5 |
| **UF-6** | 7.81 | 31.25 | 62.50 | 62.5 | 125 | 125 | 15.62 | 62.5 | 7.81 | 15.62 | 15.62 |
| | | | | | | | | | | | |
| **Ampicilline** | 7.81 | 15.62 | 3.90 | 7.81 | 7.81 | 3.90 | 3.90 | 1.95 | 0.97 | 0.97 | 1.95 |
| **Choramphenicol** | 3.90 | 15.62 | 3.90 | 7.81 | 31.25 | 15.62 | 15.62 | 3.90 | 3.90 | 1.95 | 3.90 |

A/B: Sample codes/microorganisms, ARF-1: aqueous extract, ERF-2: ethanol extract of the seeds from ripe fruits, ERF-2.1, ERF-2.2, ERF-2.3 and ERF-2.4 : chloroform, ethylacetate, *n*-butanol and aqueous phase from the partition of ERF-2 and RF-3: total alkaloids extract of the seeds from ripe fruits, AUF-4: aqueous extract, EUF-5: ethanol extract, EUF-5.1, EUF-5.2, EUF-5.3 and EUF-5.4: chloroform, ethyl acetate, *n*-butanol and aqueous phase from the repartition of EUF-5 and UF-6: total alkaloids extract of the seeds from the unripe fruits. *B.s.*: *Bacillus subtilis, E.c.: Escherichia colis, K.o: Klebsiella oxytocica, Klebsiella pneumonia, P.m: Proteus mirabilis, P.a: Pseumonas aeruginosa, S.e: Salmonella enteritidis, S.t: Salmonella thyhimurium, S.a: Staphylococcus aureus, S.d: Shigella dysenteria, S.f: Shigella flexneri.*

**Table 3. Bactericidal activity of the aqueous extracts , ethanol extracts and their respective fractions, and respective total alkaloid extracts of the seeds from ripe (RF) and unripe fruits(UF) of *C. papaya* (MBC, µg/ml)**

| A/B | B.c | E.c | K.0 | K.p | P.m | P.a | S.e | S.t | S.a | S.d | S.f |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **ARF-1** | 31.25 | 31.25 | 250 | >500 | 62.50. | 62.5 | 62.5 | 125 | 15.62 | 15.62 | 31.25 |
| **ERF-2** | 15.62 | 31.25 | 125 | 250 | 15.62 | 31.25 | 31.25 | 31.25 | 15..62 | 15.62 | 15.62 |
| ERF-2.1 | 31.25 | 15.62 | 62.5 | 125 | 250 | 250 | 15.25 | 250 | 15.62 | 31.25 | 7.81 |
| ERF-2.2 | 62.5 | 31.25 | 62.5 | 125 | >500 | 31.25 | 31.25 | 62.5 | 15.62 | 15.62 | 15.62 |
| ERF-2.3 | 62.5 | 62.5 | 31.25 | 62.5 | 250 | 125 | 62.5 | 125 | 31.25 | 62.5 | 31.25 |
| ERF-2.4 | 125 | 62.5 | 125 | 250 | >500 | >500 | 62.5 | 250 | 62.5 | 62.5 | 31.25 |
| **RF-3** | 3.90 | 7.80 | 62.5 | 31.25 | 62.5 | 62.5 | 15.62 | 15.62 | 3.90 | 15.81 | 3.90 |
| | | | | | | | | | | | |
| **AUF-4** | 31.25 | 125 | 250 | >500 | 125 | 250 | 250 | 125 | 62.5 | 62.25 | 62.5 |
| **EUF-5** | 15.62 | 62.5 | 125 | 500 | 31.25 | 62.5 | 62.5 | 62.5 | 62.5 | 31.25 | 31.25 |
| EUF-5.1 | 31.25 | 31.25 | 62.5 | 125 | 250 | 250 | 250 | 250 | 31.25 | 62.5 | 15.62 |
| EUF-5.2 | 62.5 | 125 | 62.5 | 250 | 500 | 500 | 500 | >500 | 62.5 | 62.5 | 31.25 |
| EUF-5.3 | 125 | 125 | 125 | 125 | 250 | 250 | >500 | 125 | 125 | 125 | 62.5 |
| EUF-5.4 | 250 | 250 | 125 | 250 | 500 | >500 | 125 | 250 | 250 | 62.5 | 125 |
| **UF-6** | 15.62 | 31.25 | 62.5 | 62.5 | 125 | 125 | 125 | 31.25 | 31.25 | 15.81 | 15.62 |
| | | | | | | | | | | | |
| **Ampicilline** | 7.81 | 15.62 | 3.90 | 7.81 | 7.81 | 3.90 | 3.90 | 1.95 | 0.97 | 1.94 | 1.95 |
| **Choramphenicol** | 3.90 | 15.62 | 3.90 | 7.81 | 31.25 | 15.62 | 15.62 | 3.90 | 3.90 | 3.90 | 3.90 |

See Table 2.

**Table 4. Effects of aqueous extracts of the seeds from the ripe (ARF-1) and unripe (AUF-4) fruits of *C. papaya* on *Entamoeba hystolitihca* growth.**

| | | | | ARF-1 | | | | | | AUF-4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Conc., µg/ml** | **Number of amoeba** | **Day 1** | **Day 2** | **Day 3** | **Day 4** | **Day 5** | **Day 6** | **Day 1** | **Day 2** | **Day 3** | **Day 4** | **Day 5** | **Day 6** |
| 50 | 27 | 9 | 4 | - | - | - | - | 14 | 11 | 9 | 7 | - | - |
| 25 | 27 | 11 | 8 | 2 | - | - | - | 20 | 18 | 15 | 14 | 5 | - |
| 12.5 | 26 | 17 | 8 | 10 | 5 | - | - | 23 | 20 | 17 | 16 | 20 | 22 |
| 6.25 | 26 | 20 | 17 | 13 | 3 | - | - | 25 | 23 | 20 | 18 | 21 | 25 |
| 3.12 | 26 | 23 | 19 | 10 | 1 | - | - | 22 | 22 | 20 | 23 | 25 | 28 |
| 1.56 | 26 | 27 | 12 | 12 | 5 | 3 | 1 | | | | | | |
| 0.78 | 25 | 23 | 27 | 13 | 6 | 3. | 2 | | | | | | |
| N.C. | 26 | 28 | 32 | 34 | 36 | 34 | 33 | | | | | | |
| | | | | | | | | | | | | | |
| P.C-1 | 26 | 13 | - | - | - | - | - | | | | | | |
| P.C-2 | 25 | 10 | - | - | - | - | - | | | | | | |

See Table 3. ARF-1 ad ARF-4: aqueous extract from the seeds of ripe and unripe fruits respectively. N. of amoeba: number of amoeba x $10^6$/ml in test tube, ERF-2 and EUF-5: ethanol extract of the seeds from the seeds of ripe and unripe fruits respectively, N.C: negative control, P.C-1: positive controle metronidazole and P.C-2: positive control dehydroemetine tested at concentration of 10 µg/ml.

**Table 5. Effects of ethanol extracts from the seeds of the ripe (ERF-2) and unripe (EUF-5) fruits of *C. papaya* on *Entamoeba histolytica* growth**

| | | | | ERF-2 | | | | | | EUF-5 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Conc. µg/ml** | **N. of amoeba** | **Day 1** | **Day 2** | **Day 3** | **Day 4** | **Day 5** | **Day 6** | **Day 1** | **Day 2** | **Day 3** | **Day 4** | **Day 5** | **Day 6** |
| 50 | 27 | 4 | - | - | - | - | - | 18 | 16 | 7 | - | - | - |
| 25 | 28 | 6 | - | - | - | - | - | 20 | 17 | 9 | - | - | - |
| 12.5 | 26 | 7 | - | - | - | - | - | 23 | 21 | 15 | 9 | - | - |
| 6.25 | 26 | 12 | - | - | - | - | - | 26 | 20 | 18 | 7 | 3 | - |
| 3.12 | 25 | 15 | 2 | - | - | - | - | 25 | 23 | 20 | 13 | 5 | - |
| 1.56 | 25 | 18 | 7 | 4 | 1 | - | - | 26 | 25 | 22 | 11 | 8 | 3 |
| 0.78 | 25 | 18 | 9 | 5 | 3 | 1 | 3 | 27 | 29 | 31 | 33 | 37 | 39 |
| N. C | 26 | 28 | 32 | 34 | 36 | 34 | 33 | | | | | | |
| | | | | | | | | | | | | | |
| P.C-1 | 26 | 13 | - | - | - | - | - | | | | | | |
| P.C-2 | 25 | 10 | - | - | - | - | - | | | | | | |

See Table 6, ERF-2 and EUF-5: ethanol extract of the seeds from ripe and unripe fruits respectively.

**Table 6. Antiamoebic activity of the aqueous extracts, ethanol extracts and their respective fractions, and respective total alkaloid extracts from the seeds of ripe (RF) and unripe (UF) *C. papaya* fruits (MAC, µg/ml)**

| Sample codes of seeds from ripe fruits (RF) | MAC (µg/ml) | Sample codes of seeds from unripe fruits (UF) | MAC (µg/ml) |
|---|---|---|---|
| **ARF-1** | 3.12 | **AUF-4** | 25 |
| **ERF-2** | 1.56 | **EUF-5** | 3.12 |
| ERF-2.1 | 6.25 | EUF-5.1 | 12.50 |
| ERF-2.2 | 3.12 | EUF-5.2 | 6.25 |
| ERF-2.3 | 12.50 | EUF-5.3 | 12.50 |
| ERF-2.4 | 12.50 | EUF-5.4 | 25 |
| **RF-3** | 0.39 | **UF-6** | 0.78 |
| | | | |
| **Metronidazole** | 0.52 | **Dehydroemetine** | 0.31 |
| **Dehydroemetine** | 0.31 | **Metronidazole** | 0.52 |

See Table 1. MAC: minimal amoebicidal concentration

**Table 7. % Inhibition of ACh and the depolarized solution rich in KCl induced contractions on guinea-pig isolated ileum by samples from the seeds of ripe (RF) and unripe (UF) fruits of *C. papaya.***

| Sample codes of seeds from ripe fruits (RF) | ACh | Depolarized solution rich in KCl | Sample codes of seeds from unripe fruits (UF) | ACh | Depolarized solution rich in KCl |
|---|---|---|---|---|---|
| **ARF-1** | 75.41 ± 1.21 | 73.34 ± 0.50 | **AUF-4** | 72.33 ± 0.75 | 71.80 ± 0.24 |
| **ERF-2** | 85.64 ± 0.42 | 81.37 ± 0.80 | **EUF-5** | 80.47 ± 0.72 | 78.72 ± 0.31 |
| ERF-2.1 | 72.30 ± 0.27 | 70.80 ± 0.44 | EUF-5.1 | 68.22 ± 0.50 | 66.12 ± 0.17 |
| ERF-2.2 | 72.34 ± 0.71 | 70.84 ± 0.41 | EUF-5.2 | 69.27 ± 1.24 | 67.55 ± 0.94 |
| ERF-2.3 | 66.30 ± 0.11 | 65.77 ±0.30 | EUF-5.3 | 64.30 ± 0.81 | 61.8 2± 0.65 |
| ERF-2.4 | 67.82 ± 0.72 | 65.87± 0.20 | EUF-5.4 | 65.25 ± 0.42 | 62.82 ± 0.72 |
| **RF-3** | 79.67 ± 1.20 | 76.12 ± 1.53 | **UF-6** | 76.32 ± 0.23 | 74.84 ± 0.52 |
| **Atropine** | 100.0 ±0.0 | 0.0 ± 0.0 | | | |
| **Papaverine** | 98.6 ± 0.2 | 97.3 ± 0.4 | | | |

See Table 1, ACh: acethylcholine.

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