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## Stability study and haematological profile of aqueous leaves extract of *Carica Papaya*

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### ABSTRACT

The present investigation was aimed to prepare aqueous based leaves extract of *C. papaya* and determine the stability of extracts by adding preservatives on long term storage. The stable extract was considered for haematological changes and clotting time in wister rat. The constituent of papaya leaves was extracted using distilled water. The phytochemical investigation was carried out using standard literature procedure. The stability of extract was determined based on the turbidity of extract. The ability of extract to resistance in microbial growth was determined by agar disc diffusion technique (zone of inhibition) in different strains of bacteria. The haematological parameter like platelet count, RBC and clotting time was determined on wister rat for 21 days. The percentage yield of the extract was 19.5%. The phytochemical investigation proof the presence of tannins, saponins, cardiac glycosides and steroids as a major constituent in the extract. The stability result showed the extract contained propyl paraben and methyl paraben (0.3% v/v) as a preservative are more stable. The efficacy of the extract was determined on wister rat. The body weight of both test and control groups was stable during the trial. This study clearly showed increasing platelet and RBC count in healthy rats after feeding with a short course of papaya leaf extract. The clotting time also increased in the case of the test. without causing any acute toxicity.

**Key Word:** Dengue, *Carica Papaya*, Methylparaben, Propylparaben, Platelet, RBC, Clotting time

### INTRODUCTION

Dengue viruses carried by the mosquito of the *Flaviviridae* family, are the causative agents of dengue fever [1]. Dengue is one of the most important emerging viral disease of humans being in recent decades. Dengue is found in tropical and sub-tropical regions around the world, predominantly in urban and semi-urban areas [2].

It is estimated that there are between 50 and 100 million cases of dengue fever (DF) and about 500 000 cases of dengue haemorrhagic fever (DHF) each year which require hospitalization [3]. Dengue fever is spread through the bite of an infected *Aedes aegypti* mosquito. The mosquito gets the virus by biting an infected person [4]. The first symptom of the disease appears in about 5-7 days after the infected mosquito bites a healthy person. It is possible to become infected by dengue multiple times because the virus has four different serotypes. Although each infection confers lifelong immunity to that particular serotype, a subsequent infection with a different serotype increases the risk of contracting the much deadlier form known as dengue haemorrhagic fever (DHF) [5].

The symptoms of dengue fever include high fever, rash, and a severe headache (dengue triad). Additional symptoms include severe joint and muscular pain (breakbone fever), nausea, vomiting, and eye pain. Although dengue fever itself is rarely fatal, it can be an extraordinarily painful and disabling illness and may become the epidemic in a population following the introduction of a new serotype. One sinister feature in dengue fever is the rapid fall in the platelet counts (PLT) which may lead to deadly complications such as Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).

With the recent dengue outbreak, the use of papaya leaves as the natural cure for dengue has received much interest among the public, and in the lay press [6]. Being easily available and affordable, the use of papaya leaves occurs indiscriminately. The physician, however, remains unclear of his or her stand on the issue. The therapeutic effects of aqueous extract of papaya (*Carica papaya*) leaves are presumed to be due to several active components such as papain, chymopapain, cystatin, L-tocopherol, ascorbic acid, flavonoids, cyanogenic glycosides, and glucosinolates. These are antioxidants that reduce lipid peroxidation, exhibit anti-tumour activity and immune modulatory effects [7]

Animal studies suggest that papaya leaf extracts have the potential therapeutic effect on disease processes causing destabilization of biological membranes [8] as they inhibit hemolysis *in vitro* and may cause increased platelet and red blood cell counts [9]. A recent open-labelled trial from Malaysia demonstrated significantly higher platelet count after 40-48 hours of the first dose of papaya leaves juice [10]. Others have also reported encouraging findings. In spite of these small scale studies, the fact remains that dengue is a mostly a self-limiting disease with a spontaneous increase in platelets during recovery. The role of papaya leaves cannot be scientifically substantiated based on a few positive preliminary reports. The need of the day is to commission high-quality trials in humans to provide scientific evidence for or against papaya leaves. Herbal products are assumed to be safe because they are natural, but this assumption cannot be valued more than mere conventional wisdom. As reported, anticoagulant effect of warfarin was found to be potentiated after consuming an extract of *Carica papaya* [11].

## MATERIALS AND METHODS

Distilled water was prepared in the lab by the double distillation process. Nutrient agar methylparaben and propylparaben were obtained from CDH Chemicals Ltd. Microbial cell of *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 10418) and *Bacillus subtilis* (NCTC 8853) were from Pharmaceutical Microbiology Laboratory of Faculty of Pharmacy of the University of Uyo in Nigeria.

### Harvesting and identification of plant material

The Fresh leaves of the plant were harvested in the Botanical garden of IIMT College of Medical Sciences. The leaves were thoroughly washed with clean water and properly rinsed. They were identified at the Herbarium Unit of the Department of Pharmacognosy, Faculty of Pharmacy, IIMT College of medical Sciences. A voucher specimen was submitted in the herbarium.

### Extraction procedure of papaya leaves:

The Fresh leaves were chopped to small pieces and washed with water and weight. Approx 300 g of the papaya leaves was completely soaked in 500 ml of distilled water at room temperature for 48h. The mixture was executed with a muslin cloth. The extract was collected in the beaker. The process was continued at least three times with fresh water. The filtrate was concentrated on the rotary evaporator and stored in an airtight container for further studies.

### Yield of extract

The percentage yield of the extract was obtained after extraction using Eq. (1)

$$\text{Percentage Yield (\%)} = \frac{W_a}{W_b} \times 100$$

### Phytochemical screening

The extract was tested for the presence of different phytochemicals constituent. Which was determined by using the standard methods prescribed in Trease and Evans [12].

### Stability testing of the extract:

The ability of the extract to resist microbial growth and degradation on storage was assayed. This was carried out on the extract alone and after the incorporation of some additives. The different extracts were designated as follows. The total study period was 30 days.

Extract alone (E1)

Extract + methylparaben (0.3% v/v): (E2)

Extract + propylparaben (0.3% v/v): (E3)

Extract + propylparaben + methylparaben (0.3% v/v): (E4)

**Antibacterial sensitivity tests:**

The Agar diffusion method was used to evaluate the antibacterial activity. 4 mg of the extract was freshly diluted using distilled water to obtain a concentration of 200 mg/mL and used immediately for the test. Sterile Petri dishes (diameter = 10 cm) were prepared with a base layer of nutrient agar and allowed to solidify. Bacteria cells at the density of 10<sup>6</sup>-10<sup>8</sup> cfu were inoculated on the solid agar. Wells of 5 mm were made in the agar with a sterile cork borer and filled with 0.1 mL of the 200 mg/mL concentration of the extract. Also 0.1 mL of 1 mg/mL concentration of Ampiclox (standard). Petri dishes were incubated at 37°C for 24 h after standing for 2 h at room temperature to allow for diffusion. The diameters of clear zones, which are indicative of inhibition of the organisms by the extract were measured in millimeters, as demonstrated by Abayomi [13]. The experiments were carried out in triplicates. The procedure was repeated with *B. subtilis* and *E. coli* as test organisms.

**Effect of storage time of the extract on the antibacterial activity**

The Effect of storage time of the extract on the antibacterial activity was determined by 30 days protocol by using the above-described agar-well diffusion method. A fresh dilution of the extract was prepared using distilled water to obtain a concentration of 200 mg/mL of the extract which was stored at room temperature for 1 h, 24 h, 48 h and 72 h respectively before determination of activity.

**Experimental animals**

The Wistar Rats of either sex was considered for the study. The animals were taken from the animal house of IIMT College of Medical sciences. The animals were kept in the fully aerated room at room temperature (RT) 23-24°C and maintained with ad libitum access to water and food. All experiments were approved by the institutional animal Ethical committee OF IIMT College of Medical Sciences.

**Experimental procedure [14]**

Body weights of all mice were recorded before feeding started and weighing was repeated every second day during the study period. Behavioural activities were recorded once a day. Blood smears were prepared to evaluate the platelet and red blood cell (RBC) counts from both test and control groups on alternate days and on days of sacrifice (on Days 1, 7, 14 and 21). The rats were divided into four groups of six animals each:

Group I was treated with saline (10ml/kg p.o) for a period of 21 days.

Group II was considered as control and treated with cyclophosphamide (50mg/kg s.c) for a period of first three days.

Group III was considered as Test-1 treated with *Carica papaya* leaf aqueous extract (400 mg/kg p.o) for a period of fifteen days along with cyclophosphamide (50mg/kg s.c) for first three days.

Group IV was considered as Test-2 and treated with *Carica papaya* leaf aqueous extract (800 mg/kg p.o) for a period of fifteen days along with cyclophosphamide (50mg/kg s.c) for first three days.

Blood was withdrawn from retro-orbital plexus on the 1st, 7th, 14th and 21st day of study. after subjecting the animals to light anesthesia using ether and platelet count was determined by using automated cell counter Coulter Act-Diff [15].

The numbers of platelets and RBCs were counted in 10 fields or more under oil immersion (×100). The procedure was repeated in thin or thick areas of the film if the distribution was uneven. Then, average numbers of platelets and RBCs were determined by dividing the total number by the number of fields viewed. Finally, the average number of platelets and RBCs was multiplied by the established field factor to determine the estimated count. On the 21st day, the clotting time of blood was determined by the capillary method.

**Statistical analysis**

The results were shown as Mean ±

SD and comparison between standard and test compounds

were made by one way ANOVA followed by Dunnetts test.

Values of  $p \leq 0.001$  were considered as significant.

**RESULTS AND DISCUSSION****Yield of extract:**

The percentage yield of the extract which was obtained after aqueous extraction of the plant and Calculated by Eq. (1) was 19.5%. This is appreciable considering that water used for the extraction is a polar solvent and will only extract polar constituents of the crushed plant leaves.

**Phytochemical screening:**

The phytochemical screening which carried out on pulverized leaves the result, as shown in Table 1. Alkaloids, anthraquinones, and flavonoids were absent, while tannins, saponins, cardiac glycosides and steroids were present; saponins, cardiac glycosides and steroids were present in trace amounts while tannins were the major phytochemical present in the leaves extract.

**Table 1: Phytochemical screening of papaya leaf extract**

Phytochemical	Abundance
Alkaloids	-
Anthraquinones	-
Flavonoids	+
Tannins	++
Saponins	+
Cardiac glycosides	+
Steroids	+

**Stability testing of the extract:**

This study was carried out to determine the ability of the extract to resist microbial growth and degradation on storage alone and when some additives were incorporated. It was also done to determine whether the extract could be used for further studies after storage for about 1 month. On visual observation, a clear preparation was considered stable while a cloudy preparation was labelled unstable. The results were presented in Table 4. The results showed that the Extract + propylparaben + methylparaben (0.3%v/v): (E4) showed more stability compared to others and chosen for further study.

**Table 2: Result of stability testing of the Extract alone and with preservatives**

Day	Preparation			
	E1	E2	E3	E4
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-
7	-	-	-	-
8	-	-	-	-
9	-	-	-	-
10	+	-	-	-
11	+	-	-	-
12	+	-	-	-
13	++	-	-	-
14	++	+	-	-
15	+++	+	-	-
16	+++	+	-	-
17	+++	+	-	-
18	+++	+	-	-
19	+++	+	-	-
20	+++	+	-	-
21	+++	+	+	-
22	+++	+	+	-
23	+++	+	+	-
24	+++	+	+	-
25	+++	++	+	-
26	+++	++	+	-
27	+++	++	+	-
28	+++	++	+	-
29	+++	++	+	-
30	+++	++	+	-

'+' Indicates microbial growth in the extract . '-' Indicates clean extract without microbial growth. The result indicates, The extract E4 (-) are more stable compared to other extracts

**Antibacterial tests:**

The antibacterial screening of the extract was done by the agar-well diffusion method. The mean inhibition zone diameter (IZD) which was measured was recorded in millimetre (mm). The obtained results are presented in Table 3. It showed that Ampiclox a broad spectrum antibiotic exerted strong inhibitions against the tested strains of *S. aureus*, *E. coli*, and *B. subtilis*. The result showed that the extract inhibited the growth of tested organisms at the test concentration of 200 mg/mL. It exhibited the highest and equal growth inhibition on *S. aureus* and *E. coli* with

zones of inhibition values of 33.7 mm, but it produced the least growth inhibition on *B. subtilis* with zone inhibition value of 5.76 mm.

**Table 3. Result of antibacterial screening of extract**

Test organisms	Mean IZD± SD (mm)	
	Extract (20mg/ml)	Ampiclox (1mg/ml)
<i>Staphylococcus aureus</i>	33.70 ± 2.3	61.0 ± 5.3
<i>Escherichia coli</i>	33.70 ± 1.2	51.7 ± 5.8
<i>Bacillus subtilis</i>	5.76 ± 1.2	27.0 ± 3.5

Data analyzed by one-way ANOVA followed by Dunnett's test (n = 3). \*\*\*P ≤ 0.001 were considered as significant. \*\*P ≤ 0.05 were considered as moderate in activity

#### Effect of storage time of the extract on antibacterial activity:

This test was carried out to determine if there would be significant (p < 0.05) change in antibacterial activity of the extract after storage for a predetermined time intervals of 1, 7, 14 and 30 day, respectively. The obtained results are presented in Table 4. From the result of the effect about storage time of the extract on the antibacterial activity of the plant on the test microorganisms showed an increase in antibacterial activity of the extract with increased duration of storage up to 30 day for *S. aureus* and Data analyzed by one-way ANOVA followed by Dunnett's test (n = 3). \*\*\*P ≤ 0.001 were considered as significant. \*\*P ≤ 0.05 were considered as moderate in activity *B. subtilis*, after which there was a declined inhibition during which most of these microbes have been cleared off. On the other hand, the antibacterial activity of the extract against *E. coli* increased steadily throughout the duration of the study.

**Table 4. Result of effect of storage time of extract on antibacterial activity**

Test organism	Mean IZD±SD (mm)				
	Extract (20mg/ml)				
	1Day	7 Day	14 Day	21 Day	30day
<i>S. aureus</i>	28.3 ± 5.8	38.3 ± 5.8	41.4 ± 2.8	34.3 ± 3.1	51.7 ± 5.8
<i>E. coli</i>	30.3 ± 1.4	34.3 ± 0.0	39.4 ± 0.0	40.8 ± 1.7	38.0 ± 3.5
<i>B. subtilis</i>	6.5 ± 1.0	7.2 ± 0.0	7.5 ± 1.2	7.0 ± 0.0	25.0 ± 2.0

Data analyzed by one-way ANOVA followed by Dunnett's test (n = 3). \*\*\*P ≤ 0.001 were considered as significant. \*\*P ≤ 0.05 were considered as moderate in activity

#### Biological evaluation:

##### Changes in Bodyweight

The average body weight of rat at the start of the 1st-day trial was checked in the control and test groups. However, it was observed that the average body weight was unchanged in the control and test respectively for 21 days after the start of treatment showed in Table-5

**Table-5: Change in body weight, platelet count, RBC count and clotting time**

Parameter	1 day		7day		14day		21day					
	Control	Test		Control	Test		Control	Test				
		400 mg/kg	800 mg/kg		400 mg/kg	800 mg/kg		400 mg/kg	800 mg/kg			
Body Weight (gram)	125±0.32	120±0.31	121±0.41	125±0.35	126±0.33	128±0.30	124±0.37	129±0.14	130±0.11	124±0.32	132±0.22	136±0.21
Platelet count (x10 <sup>5</sup> /μL)	3.67±0.12	3.15±0.13	4.52±0.15	3.15±0.19	5.31±0.12	7.15±0.13	2.15±0.18	8.15±0.11	9.16±0.12	2.89±0.12	8.18±0.12	9.30±0.12
RBC (x10 <sup>6</sup> /μL)	5.63±0.15	5.67±0.12	5.95±0.18	4.54±0.12	6.67±0.12	7.18±0.10	3.13±0.19	7.80±0.13	7.85±0.12	4.31±0.12	8.63±0.12	8.61±0.12
Clotting time (Sec)										123 ± 0.12	215.13±0.12	250±0.21

Data analyzed by oneway ANOVA followed by Dunnett's test (n = 6). \*\*\*P ≤ 0.001 were considered as significant. \*\*P ≤ 0.05 were considered as moderate in activity

#### Hematological investigation

Average platelet counts of the test and control groups were of the normal value of average 4.31×10<sup>5</sup>/μL and 4.21×10<sup>5</sup>/μL respectively before the experiment. There was no significant difference in the platelet counts during the first 3 days in either group. However, platelet counts within the test group started to rise steadily after Day 7 and reached a peak level at Day 14. Subsequently, the platelet count remained relatively constant at a range of 8.15×10<sup>5</sup> to 9.30×10<sup>5</sup>/μL. The platelet count of the test groups was increased at the end of the experiment compared to that of the control group. Control and test groups had nearly similar RBC counts of 5.63×10<sup>6</sup>/μL and 5.87×10<sup>6</sup>/μL in control and test groups respectively on Day 1. The average RBC counts in the control group remained at a constant

level of  $(6.00 \pm 0.31) \times 10^6/\mu\text{L}$  with minor fluctuations. In contrast, the average RBC count in the test group increased steadily during 7-day trial. Thereafter, it remained at a constant level around 14 days (Table-5). Our results clearly indicated that there was a significant increase in average platelet counts in the test group, as against a slight increase in the control group. RBC count was also significantly increased in the test group compared to controls. The clotting time was checked on 21 days of the treatment. The test groups were found to have considerably higher value than that of toxicant.

### CONCLUSION

The stability result showed the extract contained propyl paraben and methyl paraben (0.3% v/v) as a preservative are more stable. It is also clear that an oral feeding of pure extract of *C. papaya* leaves causes considerable increases in platelet and RBC counts in the murine model without causing any acute/subacute toxicity. Therefore, we suggest that aqueous juice of *C. papaya* with 0.3% v/v exhibit more stability. The extract also can be used to boost haemopoiesis and thrombopoiesis when these have been suppressed by disease.

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