Anti-inflammatory, antipyretic and antinociceptive activities of Orally Administered Aqueous Extract of *Carica papaya* Seeds in Animal Models.

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ABSTRACT

It is generally agreed that medicinal plants and their products are safer than their synthetic counterparts, however some plant products may prove efficacious but have low therapeutic index or safety margin. Carica papaya fruits, leaves, seed and latex are used medicinally for various ailments. This study was designed to evaluate the potency of aqueous extract of Carica papaya seeds as an anti-inflammatory, antipyretic and antinociceptive in animal models treated orally with 100mg/kg and 1000mg/kg for groups 3 and 4 respectively. Antipyretic studies was done using 15% suspension of Brewer's yeast in Wistar rats. The antinociceptive activity was done using writhing method in mice and tail immersion method in Wistar rats. The Anti-inflammatory study was done using the xylene-induced ear oedema test and Carrageenan–induced paw oedema. The results showed there was a significant decrease in temperature after 120 mins for both extract groups. For the antinociceptive activity, there was no analgesia with tail immersion test while the writhing test produced 4.5% and 17.4% inhibition for groups 3 and 4 respectively. In the Carrageenan–induced paw oedema, the extract test did not have significant effect while the xylene-induced ear oedema test showed inhibitions of 27.72% and 34.97% for groups 3 and 4 respectively. In Conclusion it can be said that aqueous extract of Carica papaya seed extract has minimal anti-inflammatory and antinociceptive activities with the tested doses in the used animal models, but a better antipyretic activity.

Keywords: Anti-inflammatory, antipyretic, antinociceptive, and *Carica papaya*.

INTRODUCTION

According to Dawson, historians know little about Hippocrates, the physician often referred to as "the father of medicine". However, we do know that he was a strong advocate of the use of medicinal plants to prevent and cure diseases [1]. The history of medicinal plants is intimately connected with the history of civilization. According to the world health organization, about 80% of the population in many third world countries still use traditional medicine (medicinal plants) for their primary health care due to poverty and lack of access to modern medicine [2]. WHO therefore approved the use of herbal products for national policies and drug regulatory measures in order to strengthen research and evaluation of the safety and efficacy of these products [3]. Farnsworth and his co-worker in1985 reported that of the 119 plant derived drugs listed by WHO study, 74% were discovered as a result of chemical studies to isolate the active compounds responsible for the use of original plant in traditional medicine [4]. *Carica papaya* (Pawpaw) is a tree like herbaceous plant in the family *caricaceae*. It is believed to have its origin from the low lands of Eastern Central America, from Mexico to Panama [5]. *Carica papaya* is a known medicinal plant in that it contains substances that can be used for therapeutic purposes. These substances are precursors for

chemopharmaceutical synthesis as such this plant has been used traditionally in cases of kidney failure, low sperm count, dental care and remedy for fibroids in uterus [6]. This plant has been recommended as an anti-ulcerogenic, anti-amoebic, anti-fungal, anti-microbial, anti-tumour, hypolipidaemic and employ in wound-healing activity, free radical scavenging activity, diuretic activity, uterotonic activity and antifertility activity [6,7]. According to Mojica-Henshaw *et al.*, the product improves immunity against common infections and enhances body functioning [8]. Fever is a clinical indicator of a host response, usually to a microbial infection. The conventional view is that infectious agents e.g., Gram-negative bacteria and/or their products that invade the body activate mononuclear phagocytes that then produce and release pyrogenic cytokines. These, in turn, are transported by the bloodstream to the ventromedial preoptic area (VMPO) of the anterior hypothalamus, the ''fever producing center'', where they act and lead to elevation of body temperature [9-12]. Pain is the second leading cause of medically related work absenteeism, resulting in more than 50 million lost workdays each year [13]. Medicinal herbs have been used as a form of therapy for the relief of pain throughout history [14].The treatment of rheumatic disorder is an area in which the practitioners of traditional medicine enjoy patronage and success [15]. Considering that the most important analgesic prototypes (e.g. salicylic acid and morphine) were originally derived from plant sources, the study of plant species traditionally used as pain killers should be seen as a fruitful research strategy in the search of new analgesic and anti-inflammatory drugs [16]. Apart from this, it is believed that some current analgesia-inducing drugs as opioids and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are not useful in all cases because of their side effects and low potency [17]. For instance, morphine causes acute morphine poisoning, hypotension, dependence etc, while the NSAIDs are associated with gastric irritation, bleeding, ulcers and perforation [18]. As a result searches for other alternatives are still essential, necessary and beneficial. Inflammation is a process where white blood cells and plasma leave the blood vessel and go into the surrounding tissues where they release chemicals that protect the body from infection, bacteria and viruses [19]. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. In some diseases, the body's immune system triggers an inflammatory response when there are no foreign substances to fight. The body responds as if normal tissues are infected [20].

MATERIALS AND METHODS

Collection and identification of plant material
The ripe *Carica papaya* fruit (Homestead variety) were purchased from the open market in Zaria, Kaduna State between the months of November and December and were identified and authenticated in the Department of Biological Sciences, Ahmadu Bello University, Zaria. The voucher number 0911 was obtained and same deposited in the herbarium for future reference. The seeds were removed, air dried under shade for a week and then coarsely powdered and used for extraction.

Extraction of plant material
The extraction was done in the Department of Pharmacognosy and Drug Development of Ahmadu Bello University, Zaria. The powdered material was weighed and water was used to soxhlet weighed portion of the powdered seed. The soxhleted material in the distilling pot was then concentrated under reduced pressure using a rotary evaporator and the obtained residue (crude extract) were weighed. The crude extracts were stored at room temperature and used for the study.

Test for anti-inflammatory activity
Carrageenan–induced paw oedema in rats: Pedal inflammation in rats was induced as described by Winter *et al.,* [21]. Carrageenan (Sigma-Aldrich, Poole, UK) was prepared as a 1% W/V solution in 0.9% saline, no more than 24 hours before use (Morris, 2003). Animals were weighed, randomised into groups (n=5), and kept for 1 week to acclimatise to the laboratory conditions. Animals were divided into 4 groups. An injection of 0.1ml of 1% Carrageenan suspension was given into the right hind foot of each rat under the subplantar aponeurosis. The test group rats (groups 3 and 4) were given orally 100 and l000mg/kg of aqueous extracts of plants one hour before the Carrageenan injection while the controls were given the 1ml/100g body weight of 2% Tween80 in normal saline 1h before Carrageenan injection for the control group 1 and the reference group 2 received 150 mg/kg Aspirin. Paw volume measurement was determined by wrapping a piece of cotton thread round the paw of each rat and measuring the circumference with a meter rule [22,23,24] Accurate measurement is dependent on near immobility of the paw and to this end; the rats were placed in a plastic restrainer and the tail and paws left free. This procedure was done prior to (Carrageenan) injection, and at 1, 2, 3, 4 and 5h after injection. The percentages of oedema inhibition in drug treated rats versus control were calculated using the following formula:

% Inhibition= [(V control-V treated)/ V control] x 100

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Where V control= mean oedema volume in rats in controlled group and V treated = oedema volume of each rat in test group.

Xylene Induced Ear Oedema (Weight Parameter).

The animals used in this study method were mice divided into eight groups (n=5), they were fasted overnight and allowed access to water *ad libitum.* The animals were administered with drugs and extracts as follows; group 1 received normal saline (0.2ml), group 2 dexamethasone (15mg/kg), while the test groups (3 and 4) rats were administered 100 and l000mg/kg of aqueous extract of *Carica papaya*. One hour after administration, each animal was administered 0.03ml of xylene using micropipette (Diamond) on anterior and posterior surfaces of the right ear lobe. The left ear was considered as control. One hour after xylene administration, the animals were sacrificed by chloroform anesthesia and both ears were removed. Circular sections were taken, using a cork borer with diameter of 6 mm, and weighed. The percentage of ear oedema was calculated based on the left ear without xylene [25].

$$Inhibition (\%) = 100[1-(Et/Ec)] \text{ where}$$

Et= Average oedema of the treated group
Ec= Average oedema of the control group

Antipyrexic Studies

Four groups of five (5) Wistar rats each were used. A 15% suspension of Brewer's yeast was prepared and used for induction of fever. A digital thermometer was inserted into the rectum, and the initial temperature was recorded. Any animal with a temperature above 37.2°C was excluded. Fever was induced by injection of 10mg/kg body weight of Brewer's yeast suspension subcutaneously in the back below the nape of the neck. Food was withdrawn but they were allowed access to water till after the experiment. They were kept for 18 hours following which temperatures were taken again; only animals with body temperature of at least 38°C and those that had a minimum of 1°C rise in temperature were used for the test. Aqueous extract 100 and 1000mg/kg body weight was administered orally according to body weight to group 3 and 4 while Paracetamol tablet (Emzor brand) was crushed and diluted with distill water and administered orally to group 1 at a dose of 150mg/kg body weight (positive control) and a mixture of normal saline and 2% Tween80 was administered orally to group 2 (negative control) at a dose of 1ml/100g body weight. The rectal temperatures were then recorded at 30 mins time intervals at 0, 30, 60, 90, 120, 150 and 180 minutes after administration of test extract.

Test for antinociceptive activity

Acetic Acid – Induced Writhing In Mice: Swiss mice weighing between 25-30gm were divided into 4 groups of 5 mice each, with two treatment and two control groups. Writhing was induced by the method of Koster *et al.,* [26]. The test groups 3 and 4 were administered 100 and 1000mg/kg body weight of aqueous extracts of *Carica papaya* orally, while the control group 1 received 0.2ml normal saline orally. The reference group 2 received 150 mg/kg body weight of aspirin orally. The animals were fasted for 16 h prior to the treatments. One hour after treatment, the mice were injected intraperitoneally with 0.2ml of 3% acetic acid solution (Merck Chemicals Ltd. Germany) to induce the writhing. The number of abdominal constrictions (writhing) and stretching with a jerk of the hind limb were counted between 5 and 15 minutes after acetic acid injection (A writhe is indicated by abdominal constriction and full extension of hind limb). The response of the extract and aspirin treated groups were compared with those of the animals in the control group (0.2ml saline and 2% Tween80). Percentage protections against writhing movement (% inhibition of writhing) were taken as an index of analgesia and it was calculated as follows:

$$\% \ Inhibition = Wr \ (Control) – Wr \ (test \ group) \ / \ Wr \ (Control) \ X \ 100$$

Where Wr = Mean number of writhing.

Tail Immersion Method

Analgesia was assessed according to the method of Luiz *et al*., [27]. Mice were divided into four groups of five each. They were held in position in a suitable restrainer with the tail extending out. Three to four centimetres (3-4 cm) area of the tails were marked and immersed in the water bath thermo-statistically maintained at 51°C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency. The maximum cut-off time for immersion was set at 15 seconds to avoid the injury of the tissues of tail. 0.2 ml of 0.9% NaCI and 2%Tween 80 solution was administered to control animals; and intraperitoneal (IP) pentazocine 10mg/kg body weight as reference drug. Plant extracts in doses of 100 and 1000mg/kg were given orally by intubation to groups 3 and 4 respectively. The initial reading was taken immediately before administration of test and standard drugs and then 60, 90, 120,150 and 180 minutes after the administration. A criterion for analgesia was withdrawal

time of more than 6 seconds. (The criterion for analgesia was post drug latency greater than two times the pre-drug average latency) [28].

RESULTS AND DISCUSSION

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant [29]. Several compounds including alkaloids, flavonoids, lignans, phenols and terpenes have been isolated from *Carica papaya* Linn seed [30,31,32]. According to Adeneye *et al.,* in traditional medicine, *Carica papaya* Linn seed is used for its hepatoprotective, anti-diabetic, antihypertensive, analgesic, anti-inflammatory and antimicrobial properties [33].

Table 1: Anti-inflammatory effects of orally administered *C. papaya* extracts on xylene-induced ear oedema

Parameters	Group 1 Mean±SEM N/S	Group 2 Mean±SEM Dexamethasone 15mg/kg	Group 3 Mean±SEM Aq100mg/kg	Group 4 Mean±SEM Aq 1000mg/kg
Rt ear (mg)	17.62±0.413	13.078±0.171	16.040±0.121	14.940±0.121
Lt ear (mg)	9.90±0.342	10.32±0.312	10.46±0.174	9.92±0.22
Difference (mg)	7.72±0.080	3.046±0.234	5.58±0. .218	5.02±0.198
% Inhibition	_	60.54	27.72	34.97

N=5   Data expressed as Mean±SEM

Table 2: Anti-inflammatory effects of orally administered *C. papaya* seed extracts on Carrageenan induced paw oedema in rats

Time	Group 1 N/S+2%Tween80 1ml/100g	Group 2 ASA 150mg/Kg	Group 3 Aqueous 100mg/kg	Group 4 Aqueous 1000mg/kg
0 mins	2.77±0.039	2.71±0.083	2.76±0.055	2.74±0.024
1Hour	4.12±0.066	3.74 ±0.278	4.21±0.043	4.15±0.058
2 Hours	5.70±0.125	4.52±0.086**	5.75±0.111	5.45±0.181
3 Hours	6.47±0.059	3.77±0.248**	6.34±0.047	5.98±0.039
4 Hours	6.25±0.040	3.45±0.158**	6.11±0.097	5.88±0.039
5 Hours	6.17±0.022	3.06±0.091**	5.98±0.068	5.79±0.118

N =5   Data expressed as Mean±SEM   p<0.05, p<0.001***

Table 3: Anti-inflammatory effects of orally administered *C. papaya* extracts on carrageenan induced paw oedema in rats.

Time	Group 1 N/S+2%Tween80 1ml/100g	Group 2 ASA 150mg/Kg	Group 3 Aqueous 100mg/kg	Group 4 Aqueous 1000mg/kg
0 mins	2.77±0.039	2.0	0	0
1 Hour	4.12±0.066	9.4	0	0
2 Hours	5.70±0.125	20.8	0	4.3
3Hours	6.47±0.059	41.9	2.3	7.8
4 Hours	6.25±0.040	44.8	2.2	5.8
5 Hours	6.17±0.022	50.4	3.0	6.1

Percentage (%) Inhibition of Paw Diameter with Corresponding Time Intervals

Table 4: Antipyretic effects of orally administered *C. papaya* seed extracts on Wistar rats

Time	Group 1 N/S+2%Tween80 1ml/100g	Group 2 PCM 150mg/kg	Group 3 Aqueous 100mg/kg	Group 4 Aqueous 1000mg/kg
0 mins	38.92±0.09	39.0±0.08	38.72±0.12	38.88±0.05
30 mins	38.68 ±0.73	38.56 ±0.51	38.68 ±0.07	38.62 ±0.07
60 mins	38.88 ±0.07	38 ±0.03**	38.72 ±0.13	38.6 ±0.21
90 mins	38.82 ±0.09	37.68 ±0.02**	38.56 ±0.10	38.1 ±0.20*
120 mins	38.76 ±0.08	37.34 ±0.13**	38.36 ±0.14	37.86 ±0.27
150 mins	38.58 ±0.10	37.04 ±0.12**	38.22 ±0.07*	37.68 ±0.14*
180 mins	38.7 ±0.13	36.88 ±0.04**	37.98 ±0.05**	37.64 ±0.05**

Mean   Temperature (°C) Values with Corresponding Time Intervals   p<0.05, p<0.001***

Table 5 Antinociceptive activity of *C. papaya* seed extracts; acetic acid writhe test Showing Mean Writhing response

Parameters	Group 1 N/S+ 2% Tween80	Group 2 Aspirin 150mg/Kg	Group 3 Aqueous 100mg/kg	Group 4 Aqueous 1000mg/kg
Mean writhing	35.60±1.249	8.80±0.374**	34.0±0.71	29.4±0.51**
% Inhibition	_	75.2	4.5	17.4

N =5   Data expressed as Mean±SEM   P<0.05, P<0.001***

Table 6: Antinociceptive activity of *C. papaya* seed extracts; tail immersion test Showing Tail Withdrawal Time (Secs) With Corresponding Time Intervals

Time	Group 1 N/S+2%Tween80 1ml/100g	Group 2 IP pentazocine 10mg/kg	Group 3 Aqueous 100mg/kg	Group 4 Aqueous 1000mg/kg
0 mins	2.50±0.224	3.0±0.447	2.6±0.367	2.8±0.200
60 mins	2.74±0.20	4.2 ±0.374*	3.0±0.316	2.8±0.374
90 mins	2.80±0.20	6.0±0.316**	3.2±0.20	2.8±0.20
120 mins	2.7±0.20	6.4±0.510**	3.4±0.245	3.0±0.316
150 mins	2.7±0.20	8.2±0.663**	2.8±0.374	3.0±0.548
180 mins	2.8±0.374	8.8±0.490**	3.4±0.40	3.8±0.374

N =5   *Data expressed as Mean±SEM*   *P<0.05* P<0.001***

Its use as an anti-inflammatory remedy is not strongly justified by the result of this extract study. On xylene-induced ear oedema (Table 1), the extract at the different doses showed some degree of inhibition which was dose dependent. Aqueous extract 100mg and 1000 mg/kg body weight showed some percentage inhibitions of 27.72% and 34.97%, respectively. They were however much less than the inhibitory effect shown by 15mg/kg body weight of dexamethasone which was 60.54%. Table 2 and 3 shows the results of the anti-inflammatory effects of orally administered *C. papaya* extracts on Carrageenan paw oedema in rats. The figures in Table 2 shows that aspirin had a significant effect on the paw circumference when compared to groups 1, 3 and 4 as the oedema and was significantly different from these groups from the 3rd hour reaching a peak by the 5th hour. After the first one hour of administration, aspirin had 9.4% inhibition only with the extract showing none (Table 3). By the fifth hour, the there was a significant inhibition by aspirin 50.4% while aqueous extract 100mg/kg body weight had 3.0% and 1000mg/kg body weight had 6.1%. In this model, the standard drug, aspirin (150mg/kg body weight, orally), produced a greater anti-inflammatory effect at all times, inhibiting the development of oedema from the first to the fifth hour in percentages of 9.4%, 20.8 %, 41.9%, 44.8 and 50.4% respectively (Table 3). In a similar study using methanol extract of *Carica papaya* seed at 300mg/kg body weight resulted in 61% reduction in paw oedema which was nearly equivalent to that of 10 mg/kg body weight of the standard drug aspirin. These maybe species difference as the variety used here was the homestead variety, it may also be as a result of the solvent used which is alcohol as against water used in this study. The development of oedema in the rat paw after the injection of carrageenan has been described as having two phases. The first phase starts immediately after injection and last an hour. The second phase of swelling begins after 1 hour and remains through 3 hours; this is due to the release of prostaglandin-like substances [34,35]. It has been reported that the second phase of oedema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents. Generally NSAIDs strongly inhibit the second phase of Carrageenan-induced oedema while some others inhibit both phases [34,35]. The pyrexia induced results from the activity of Brewer's yeast because of its ability to cause infections, tissue damage and inflammation. The resultant infections serves as a pyrogenic stimulus and the pyrogens are phagocytised by the Kupffer cells, monocytes, macrophages etc. leading to the release of cytokines. Cytokines have the capacity to raise the set point of normal body temperature. Temperature was taken at 0, 30, 60, 90, 120, 150, and 180 minutes. After 60 mins there was a significant difference in paracetamol treated group when compared to negative control group, but aqueous 100mg/kg and 1000mg/kg body weight were still not significantly different from control. The 1000mg/kg body weight extract group produced a significant decrease in temperature after 90mins. However, aqueous 100mg/kg still did not show any significant difference in temperature until after 120 mins and this remained so for the remaining 60 mins of the experiment as shown on Table 4. The 100mg/kg body weight group produced the least effect on temperature while paracetamol group produced the most significant effect. The results obtained with acetic acid induced writhing are shown in Table 5. The reference drug (aspirin) and the aqueous extract at 1000mg/kg had significant difference with the control group while group 3 (100mg/kg body weight) showed no significant effect on the number of abdominal contractions when compared to the negative control group 1. However the percentage inhibitions were very small when compared to that of group 1 showing 75.2%, 4.5% and 17.4% respectively for groups 2, 3 and 4. This means that the extract has very little effect on peripheral pains as the writhe test is used to test for peripheral analgesia. However the control drug aspirin produced 7.60±0.510 writhes and it was significantly different compared to the other treated groups, producing a 75.2% inhibition when compared with the control group treated with Normal Saline+Tween80. The Negative control group produced 32.60±0.678 writhes. Acetic acid induced writhe is a sensitive procedure in detecting analgesic effect of medicinal agents [36]. This pain mechanism is believed to involve local peritoneal receptors [37]. Intraperitoneal administration of acetic acid releases prostaglandins and sympathomimetic system mediators like prostaglandin E2 (PGE2) and prostaglandin GF2α (PGF2α) and their levels are increased in the peritoneal fluid [38]. The abdominal constriction observed during the test is related to the sensitization of nociceptive receptors by these prostaglandins. It is therefore possible that aqueous extract of *Carica papaya* have significant effect by inhibition of the synthesis or action of prostaglandins, thereby having inhibitory effects on peripheral pains. This did not preclude the fact that it might have done this centrally hence the need for the tail immersion tests that are most sensitive to centrally acting analgesics. The results obtained with Tail immersion test are shown in Table 6. At 60 mins, pre-treatment pentazocine produced significant difference

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(p<0.05) from control group 1 with a withdrawal time of 4.2 ±0.374. This was the trend up to 180 minutes as the withdrawal time kept increasing. The withdrawal time for the extract groups remained low throughout the 180 minutes and was not significantly different from the control group 1. At 180 minutes the times where 2.8±0.374, 8.8±0.490, 3.4±0.40 and 3.8±0.374 for groups 1 to 4 respectively with only group 2 showing significant different when compared to control group 1. The result indicates that the effect of the extract on central pain inhibition is practically nonexistent as the tail test is for central analgesia. The little observed anti-inflammatory and antinociceptive activities of aqueous extract of *Carica papaya* may be due to a combination of different biological constituents rather than any single compound, being that it contains alkaloids, flavonoids and the triterpenoids and polyphenolic compounds [30]. Recent reports have also indicated that many flavonoids possess anti-inflammatory activity [32]. In addition to this, studies have also shown that flavonoids and saponins isolated from about 50 plants possess analgesic and anti-inflammatory activities against several experimental models in mice and rats [39,40,41].

CONCLUSION

In conclusion it can be said that aqueous extract of *Carica papaya* seed extract has minimal anti-inflammatory and antinociceptive in the tested models, but a greater antipyretic activity.

However, the exact mechanism by which *Carica papaya* seed extract produce these effects is subject to further studies.

REFERENCES

[1] Dawson Emma; The Medicinal Properties of the Papaya, Carica papaya L Southern Illinois University Carbondale / Ethnobotanical Leaflets . http://www.ethnoleaflets.com/leaflets/papaya.htm 15-May-98.
[2] Silva, T. de (**1997**). Industrial utilization of medicinal plants in developing countries: 34-44. In: Bodeker, G., Bhat, K.K.S., Burley, J. & Vantomme, P. (eds.) Medicinal Plants for Forest Conservation and Health Care. FAO (Non-wood Forest Products 11), Rome.
[3] Saxena M J (**2001**). *Vet. Med. Assoc*. pp. 14-16.
[4] Farnsworth, N.R., O. Akelere, A.S. Bingel, D.D. Soejarto and Z. Guo, (**1985**). *Bull World Health Org*., 63: 965-981.
[5] Nakasone, H.Y., Paul, R.E., (**1998**). *Tropical Fruits*, CAB International Walling Ford.
[6] Aravind. G, Debjit B, Duraivel. S, Harish. G. (**2013**). *Journal of Medicinal Plants Studies*. 1(1): 7-15.
[7] Krishna, K L, Paridhavi, M, Patel, J. A,: *Indian Journal of Natural Products and Resources* (**2008**). Vol 7 (4) 364-373 ISSN: 0975-1092 (Online); 0972-592X (Print)
[8] Mojica-Henshaw MP, Francisco AD, De Guzman F, Tingo XT (**2003**). *Clin. Hemorheol. Micro*. 29:219-229.
[9] Saper, C.B. (**1998**). *Ann. N.Y. Acad. Sci.,* 856: 90–94.
[10] Roth, J. and De Souza, G.E. (**2001**) *Braz. J. Med. Biol. Res*., 34: 301–314.
[11] Dunn, A.J. (**2002**). *Int. Rev. Neurobiol*., 52: 43–65.
[12] Dinarello, C.A. (**2004**). *J. Endotoxin Res*., 10: 201–222.
[13] American Pain Society (**2000**). "Pain Assessment and Treatment in the Managed Care Environment." http://www.ampainsoc.org/advocacy/assess_treat_mce.htm.
[14] Almeida, R. N., Navarro, D. S and Barbosa-Filho. J. M. (**2001**). *Phytomedicne* 8: 310-322.
[15] Akah. P. A. and Nwambie, A. I. (**1994**). *J. Ethnopharmacol*., 42: 179-182.
[16] Shanmugasundaram P. and Venkataraman S, (**2005**). *J. Traditional, Complementary and Alternative Medicines* 2 (1): 62- 69
[17] Ahmadiani A, Fereidoni M, Semnanian S, Kamalinejad M, Sureni S (**1998**). *J. Ethnopharmacol*. 61: 229-235.
[18] Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG (**1996**). The *Pharmacological Basis of Therapeutics.* 9th ed. McGraw-Hill, San Francisco, USA, pp 374 – 655.
[19] Woolf, Wotherspoon, and Young (**2002**). Basic Pathological Processes. Acute inflammation, healing, and repair in *Essentials of Pathology http://www.fleshandbones.com/readingroom/pdf/221.pdf*
[20] Tamara Mitchell (**2011**), Feeling Swell: *Inflammation* Edited by Sally Longyear http://www.working-well.org/articles/pdf/Inflammation_1.pdf
[21] Winter, C. A., Risley, E. A ., and Nuss, G. W. (**1962**). *Proc. Soc. Exp. Biol.* 111, 544–547
[22] Morris, Christopher J. (**2003**). Inflammation Protocols Methods in *Molecular Biology*, Volume 225, II, 115-121, DOI: 10.1385/1-59259-374-7:115 ED Paul G. Winyard and Derek A. Willoughby.
[23] Hess SM, Milonig RC (**1972**). Assay for inflammatory drugs: In Lepow IH, Ward PA (eds) *Inflammation, Mechanisms and control*, Academic Press, New York. pp. 1–12.
[24] Bamgbose S.O.A, Noamesi B.K., (**1981**). *Planta medica* 42, 392 – 396.
[25] Junping K, Yun N, Wang N, Liang L, Zhi-Hong H, (**2005**). *Biological Pharmaceutical Bulletin* , 28, 176-180.
[26] Koster, R., M. Anderson and E.J. de Beer, (**1959**). Acetic-acid for analgesic screening. *Fed. Proc.,* 18: 412-418.

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[27] Luiz, CDS., Mirtes, C, Sigrid, LJ., Mizuikirizawa, M., Cecelia, G. and Jrolin. G. (**1988**). *J. of Ethnopharmacology,* 24: 205-211.

[28] Janssen PAJ, Niemegeers CJE, Dony JGH (**1963**). *Arzneim Forsch* 13:502–507.

[29] Joseph, B. and S.J. Raj, (**2010**). *Int. J. Pharma. Bio Sci.*, 1: 246-253.

[30] Tona LK, Kambu N, Ngimbi K, Cimanga K, Vlietinck AJ (**1998**). *J. Ethnopharmacol. 61: 57-65.*

[31] Jaime A, Teixeira da S, Zinia R, Duong TN, Dharini S, Abed G, Manoel TS, Paula FT (**2007**). *Tree Forest. Sci. Biotechnol.* 1(1): 47-73.

[32] Anaga AO, Onehi EV (**2010**). *Afr. J. Pharm. Pharmacol.* 4(4): 140-144.

[33] Adeneye, A.A.; Ajagbonna, O.P.; Adeleke, T.I.; Bello, S.O. (**2006**). *J. Ethnopharmacol.* 2006, 105, 374-379.

[34] Di Rosa M, Giroud JP, Willoughby DA. (**1971**). *J. Pathol.* 104: 15-29. http://dx.doi.org/10.1002/path.1711040103 PMid:4398139

[35] Holsapple MP, Schunner M, Yim GKW (**1980**). *Agents Actions* 10: 368-373.

[36] Collier HOJ, Dinneen LC, Johnson CA, Schineider C (**1968**). *Br. J. Pharmacol.* 32: 293-310

[37] Bentley GA, Newton SH, Starr JB (**1983**). *Br. J. Pharmacol.* 79: 125-134.

[38] Deraedt, R., Joughney, S., Delevakee. F. and Falhour, M. (**1980**). *Eur. J. Pharmacol.* 51: 17-24.

[39] Lacaille-Dubois, M.A. and H. Wagner, (**1996**). *Phytomedicine*, 2: 363-386

[40] Kerber VA (**1999**). Análise dos alcalóides de Psychotria brachiceras Mull. Arg. E Psychotria umbellate Vell, e o estabelecimento ecaracterização de cultura de céllulas de P. umbellata Vell. Tese de doutorado, Curso de Pós – Graduação em Ciências Farmacêuticas. Universidade Federal do Rio Grande do Sul.

[41] Fernanda LB, Victor AK, Amelia TH, Elisabetsky E (**2002**). *Pharm. Biol.* 44: 54-56

[42] Luiz, CDS., Mirtes, C, Sigrid, LJ., Mizuikirizawa, M., Cecelia, G. and Jrolin. G. (**1988**). *J. of Ethnopharmacology,* 24: 205-211.