Scientific Review
ISSN(e): 2412-2599, ISSN(p): 2413-8835
Vol. 4, Issue. 5, pp: 34-39, 2018
URL: http://arpgweb.com/?ic=journal&journal=10&info=aims

Academic Research Publishing Group

Original Research | Open Access

Antipyretic Activity of Methanolic Leaf Extract of Acalypha Wilkesiana (Copper Leaf) On Wistar Rats

Wali C. C.*
Department of Anatomy, Faculty of Basic Medical Sciences, Madonna University Elele, Rivers State, Nigeria

Oluwatayo B.
Department of Physiology, Faculty of Basic Medical Sciences, Madonna University Elele, Rivers State, Nigeria

Kolawole T.
Department of Physiology, Faculty of Basic Medical Sciences, Madonna University Elele, Rivers State, Nigeria

Iniodu C.
Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Akwa Ibom State, Nigeria

George U. E.
Department of Anatomy, Faculty of Basic Medical Sciences, Madonna University Elele, Rivers State, Nigeria

Abstract

Acalypha wilkesiana is an ornamental plant that is generally referred to as copper leaf and it is a shrub with mostly glossy green or red leaves. It is used in the management of fever in infants locally. Wistar rats weighing 150-200g were divided into six groups (n=5). Group 1 (control) was administered brewer's yeast (40mg/kg) , group 2 was administered brewer's yeast and aspirin (100mg/kg), while groups 3, 4 and 5 were administered 100, 200 and 300mg/kg of *Acalypha wilkesiana* respectively. Initial rectal temperature was taken before admistration of brewer's yeast. Brewer's yeast was injected subcutaneously to induce fever in the test groups. Temperature was measured after 18hours using rectal thermometer at 0, 1, 2, 3 and 4 hours after the extract and standard drug were administered. Group 5, 4 and 3 (300mg/kg, 200mg/kg and 100mg/kg) significantly reduced brewer's yeast induced pyrexia, (36.46±0.21, 36.90±0.31 and36.84±0.22), when compared with the positive control (brewer's yeast) (37.96±0.22). Group 2 (Aspirin 100mg/kg) also showed significantly reduced brewer's yeast induced pyrexia (36.88 ±0.22) when compared with the positive control (brewer's yeast) (37.96±0.22). This study shows that the antipyretic effect of *Acalypha wilkesiana* is dose dependent, being most effective at the high dose (300mg/kg). This suggests that the methanolic extract of *Acalypha wilkesiana* leaves possesses significant antipyretic activity which is dose dependent and its mechanism could be due to the presence of flavonoids, saponins and alkaloids.

Keywords: *Acalypha wilkesiana;* Brewer's yeast; Aspirin; Antipyretic; Temperature.

CC BY: Creative Commons Attribution License 4.0

1. Introduction

Pyrexia also referred to as fever is a regulated increase of body core temperature characterized by an increased thermoregulatory set point, which results from the interaction of the central nervous and immune system [1]. Normal body temperature is regulated by centers in the hypothalamus that ensures balance between heat loss and heat gain. Fever occurs where there is a disturbance of this hypothalamic "thermostat" which therefore leads to the set point of the body temperature being raised. This will cause readjustment to normal set point by temperature regulating system which operates to reduce the body temperature [2]. Fever is one of the most rampart clinical signs [3]. Infection, tissue damage, malignancy or other diseased conditions can cause fever [4] Fever is described as the body's natural defense mechanism to create an uncondusive environment which infectious agents or damaged tissue cannot withstand [5]. Fever triggers increased muscle tone and chills [6]. Certain endogenous substances which include tumor necrosis factor-alpha (TNFα) and prostaglandins produce fever [7]. Antipyretics are medications that are used to reduce fevers [6]. They are known to act either centrally on the temperature regulation centers of the hypothalamus or peripherally by inducing vasodilatation and heat dissipation [8]. They also act by inhibiting the biosynthesis of prostaglandin E2 [9] possibly by inhibiting COX-2 expression [10, 11]. Long time usage of these antipyretic drugs produces undesirable side effects including gastrointestinal disorders, renal damage and hepatic toxicity [12].

Acalypha wilkesiana is an ornamental plant [13]. The plant is generally referred to as copper leaf and it is a shrub with mostly glossy green or red leaves [14]. *A. wilkesiana* is found all over the world especially in the tropical and subtropical countries [15]. The aqueous extract of the leaf is used in the management of fever in infants as well as abnormal sodium and potassium metabolism that accompanies hypertensions [16]. The leaves of *A. wilkesiana* are popularly used in the north eastern Africa in the treatment of skin infections [17]. The antimicrobial properties of the plant have been reported [18]. In the coastal areas of Nigeria, the plant is used in the treatment of various

*Corresponding Author

gastrointestinal disorders [16]. The analgesic and anti-malarial effects of extracts and fractions of *Acalypha wilkesiana* have been reported [19].The decoction of the leaves is commonly used for the treatment of pain and ulcer by traditional medical practitioners [14]. The presence of saponins, tannins, anthraquinone and glycoside has reportedly been found in the leaves of Acalypha wilkesiana [16]. This dosage for the administration of this leave is not clearly stated by tradomedical practioners. This research is therefore carried out to authenticate the efficacy of the *Acalypha wilkesiana* as an antipyretic at suggested doses.

2. Materials and Methods

2.1. Plant Material and Extraction

Fresh samples of *Acalypha wilkesiana* leaves were collected from a residential farmyard in Abak Akwa Ibom State. The plant material (leaves) was air dried at room temperature and reduced to fine powder by milling.140g of the powdered sample exhaustively extracted with 420ml of methanol (analytical grade) for 12 hours, after which the resultant mixture was filtered and solvent was removed at 55^0 c using a water bath to give a dark green solid extract weighing 40g. Afterwards, the methanolic extract was stored in the refrigerator for subsequent use.

2.2. Animals

The female wistar rats used (150-200g) for this study was procured from the faculty of Veterinary Medicine, University of Nigeria, Nsukka (UNN). They were housed in the institutional animal house in Madonna University Elele. They weighed between 150-200g. The rats were kept in wire mesh cages with a 12hr light /dark cycle, and fed with growers feed and water for one week before the commencement of the experiments.

2.3. Preliminary Phytochemical Screening

Qualitative phytochemical analysis to test for flavonoids, tannins, carbohydrates/ glycosides, saponins, resins, terpenoids and alkaloids were carried out using standard procedures as described by Trease and Evans [20]; Harborne [21].

2.4. Acute Oral Toxicity Studies

Acute toxicity studies of methanolic extract of Acalypha wilkesiana were conducted as per OECD guideline 420 (modified, adopted 17th December 2001) using albino wistar rats.Twenty adult Wistar rats were used for the determination of toxicity study (LD50) of the methanolic leaf extract of *Acalypha Wilkesiana.* These 20 rats were shared into four groups (LD1, LD2, LD3 and LD4) of five rats each. Prior to the toxicity study, all the animals fasted for 24 hours. Each animal was administered methanolic extract by oral route.

2.5. Antipyretic Studies

Rectal temperature was determined by inserting a rectal thermometer 2cm into the rectum. Only wistar rats showing normal rectal temperature were selected for the study. The rats were randomized into five groups. Rats were fasted 24 hours before being induced with pyrexia [22]. The rats were made pyretic by a subcutaneous injection of brewer's yeast 40mg/kg b.w.t. below the nape of the neck [23]. The initial temperature was noted which was the temperature just before brewer's yeast administration [24]. Rats in group 1 were given brewer's yeast (40mg/kg). Group2 were administered brewer's yeast and aspirin (100mg/kg). Group 3, 4 and 5 were administered 100, 200 and 300mg/kg of *Acalypha wilkesiana* respectively. Temperature was measured after 18hours. The temperatures were taken at 0, 1, 2, 3 and 4 hours.

3. Statistical Analysis

Mean ± S.E.M were calculated for each parameter. For the determination of significant intergroup differences, each parameter was analyzed separately and one-way analysis of variance (ANOVA) was carried out. $P<0.05$ was considered significant. +

4. Results

4.1. Phytochemical Analysis

4.1.1. Qualitative Phytochemical Analysis of *Acalypha Wilkesiana*

Qualitative analysis carried out on each extract of *A. wilkesiana* showed the presence of important phytochemical constituents as summarized in Table 1. The result of the phytochemical analysis obtained from the methanolic leave extract of *A. wilkesiania* indicated that tannins were highly present in the extract and saponins in moderate amount. Flavonoids were also present. Carbohydrates, glycosides, alkaloids and steroids and terpenoids were present in low amount. Resins and protein were not present in both the extract.

Table-1. Result of the Preliminary Qualitative phytochemical analysis of *Acalypha wilkesiana*

Active Ingredients	Methanolic Extract
Tannins	+++
Saponins	++
Resins	-
Alkaloids	+
Glycosides	+
Flavonoids	+
Steroids and Terpenoids	+
Protein	-
Carbohydrates	+

+ = slightly Present, ++ = moderately Present, +++ =highly present, - = Not detected.

4.1.2. Quantitative Phytochemical Analysis of *Acalypha Wilkesiana*

The result of the preliminary quantitative phytochemical screening of *Acalypha wilkesiana* is shown in Table 2. The results showed that methanolic extract of *A. wilkesiana* had a high tannin and phenol content.

Table-2. Result of the Preliminary Quantitative Phytochemical Screening of *Acalypha wilkesiana*

Active ingredients/ metabolites	Methanol extract
Flavonoids $^{++}$	0.0506±0.035
Flavonol $^{++}$	1.575±0.044
Phenol *	0.331±0.024
Tannins *	0.066±0.024

Data represented as Mean+- SEM of triplicate analyses
*Expressed as mg gallic acid equivalents (GAE)/ mg dry weight plant extract
$^{++}$Expressed as mg quercetin equivalents (QE)/ g dry weight plant extract

4.2. Acute Toxicity Study

Table 3 shows the observations during the toxicity study of methanolic leaf extract of *Acalypha Wilkesiana* in Wistar rats. While control presented no physical and behavioural changes, the group that received 375mg/kg of the leaf extract of *Acalypha Wilkesiana*, presented moderate changes in physical and behavioural observation and no change was recorded during the 24th and 72 hours post ingestion. On the other hand, the groups that received 750mg/kg and 1500mg/kg leaf extract of *Acalypha Wilkesiana* presented severe physical and behavioural changes that were observed from the first 3 hours post ingestion to 48th hours. No death was recorded in any group for the time span of the toxicity study.

Table-3. Toxicity study of different doses of leaf extract of *Acalypha Wilkesiana.*

Toxicity study	Time (hrs)	LD1 (control)	LD2(375mg/kg)	LD3(750mg/kg)	LD4(1500mg/kg)
Physical changes (weakness, abnormal movements, diarrhea)	3	- - -	+ - -	+ - -	+ + -
	6	- - -	+ - -	+ - -	+ + -
	12	- - -	- - -	+ - -	- - -
	24	- - -	- - -	- - -	+ - -
	48	- - -	- - -	- - -	- - -
	72	- - -	- - -	- - -	- - -
Behavioural changes (aggressiveness, rotational movement, biting, hyperactivity, tremors)	3	- - - - -	- - - - -	+ + - + -	- - - - -
	6	- - - - -	- - - - +	+ + - + -	- + + - +
	12	- - - - -	- - - - +	- - - + -	+ + + + +
	24	- - - - -	- - - - -	- - - - -	+ + + + -
	48	- - - - -	- - - - -	- - - - -	- - + - -
	72	- - - - -	- - - - -	- - - - -	- - - - -
Death	3	-	-	-	-
	6	-	-	-	-
	12	-	-	-	-
	24	-	-	-	-
	48	-	-	-	-
	72	-	-	-	-

Table-4. Effect of methanolic extract of *acalypha wilkesiana* on brewer's yeast induced pyrexia in rats.

Groups	Initial rectal temp. °c	Temp at 0hr °c	Temp at 1hr °c	Temp at 2hr °c	Temp at 3hr °c	Temp at 4hr °c
GR1Positive Control (brewer's yeast).	36.34±0.12	38.16±0.32	37.96±0.22	38.98±0.10	38.26±0.20	39.18±0.20
GR2 Aspirin (100mg/kg)	36.22±0.12	38.56±0.12	37.10±0.52	37.72±0.39	36.88 ±0.22*	36.60±0.19*
GR3 A.wilkesiana (100mg/kg)	36.32±0.10	38.40±0.29	37.82±0.37	37.16±0.23	36.84±0.22*	36.94±0.11*
GR4 A. wilkesiana (200mg/kg)	36.30±0.07	38.02±0.13	37.00±0.09	36.90±0.31*	36.76±0.28*	36.94±0.27*
GR5 A. wilkesiana (300mg/kg)	36.20±0.04	38.24±0.14	36.46±0.21*	36.58±0.33*	37.04±0.32	36.76±0.17*

Values are mean± S.E.M. (n=5)

Table 4 shows that 18hours after brewer's yeast injection, hyperthermia was recorded (0 hour). Group 5 (300mg/kg) showed significant reduction in the temperature. This was seen as from the first hour, when compared with the positive control (brewer's yeast). Group 4 200mg/kg) showed significant reduction in the temperature as from the second hour (2hr). Group 3 (100mg/kg) showed significant reduction in the temperature as from the third hour (3hr). Group 2 (Asprin 100mg/kg) showed significant reduction in temperature as from the third hour.

5. Discussion

In the present study, the antipyretic activity of the methanolic extract of *Acalypha wilkesiana* was studied. Yeast induced hyperpyrexia in a rat model was employed in this study [25]. A significant elevation of body temperature occurred after inducement of pyrexia. This was in consonance with work by Irfan, *et al.* [26].

The qualitative phytochemical constituents of the extract were also studied. Phytochemicals are plant chemicals which are found in plants. Some of them serve as antioxidants while some of them contain toxins which have potential side effects on the body [27]. In the present study the presence of tannins, saponins, alkaloids, glycosides, flavonoids, steroids, terpenoids and carbohydrates was revealed by the qualitative analysis. The qualitative phytochemical analysis of the aqueous extract of Acalypha wilkesiana in another study revealed the presence also of tannins and flavonoids but other constituents were absent. Instead triterpeniods, gallic acid, corilagin and geranin were seen [28]. Phytochemicals are generally used to refer to chemicals that may affect health [29].

Elevation in body temperature occurs when the concentration of prostaglandin E2 (PGE2) increases within parts of the brain. Such an elevation contributes to a considerable alteration in the firing rate of neurons that control the thermoregulation process in the hypothalamus. Evidence points to the fact that antipyretic drugs exert their action by inhibiting the enzymatic activity of cyclooxygenase and consequently reducing the levels of PGE2 within the hypothalamic region [30]. Flanvonids and tannings were reportedly found in the phytochemical analysis of the extract. Flavonoids have been shown to exert an antipyretic effect by suppressing TNF-α [31]. Flavonoids and tannins are known to inhibit prostaglandin synthesis as reported by earlier research [6]. It is therefore suggested that the flavonoids and tannins present in the plant's extract played a role in the observed antipyretic effect. Alkaloids have also been reported to inhibit the synthesis of prostaglandin E2 [32], which could eventually reduce elevations of body temperature. This phytochemical is also suggested to have played a part in the observed antipyretic effect.

The extract exhibited antipyretic activity in rats made hyperthemic by brewer's yeast injection. This study corresponds with the study of Zakaria, *et al.* [33] in which compounds like flavonoids and saponins were suggested to act synergistically to exert observed pharmacological activity.

Antipyretic activity is commonly mentioned as the characteristics of drugs or compounds which have an inhibitory effect on prostaglandin biosynthesis. NSAIDS produce their antipyretic action through inhibition of prostaglandin synthetase within the hypothalamus [34, 35]. The extract is likely to reduce pyrexia by reducing brain concentration of prostaglandin E2 especially in the hypothalamus through its action on COX-3 or by enhancement of the production of the body's own antipyretic substances like vasopressin and arginine [36]. The result of the present study indicates that the methanolic extract of *Acalypha wilkesiana* possesses significant antipyretic activity on brewer's yeast induced hyperthermia in rats. This may be due to the presence of flavonoids, saponins and alkaloids in the extract.

6. Conclusion

In conclusion this study suggests that the methanolic extract of *Acalypha wilkesiana* leaves possesses significant antipyretic activity which is dose dependent and its mechanism could be due to the presence of flavonoids, saponins and alkaloids.

References

[1] Kluge, M. J., 1991. "Fever: role of pyrogens and cryogens." *Physiol Rev.*, vol. 71, pp. 93-127.

[2] Kelly, G., 2006. "Body temperature variability (Part 1): a review of the history of body temperature and its variability due to site selection, biological rhythms, fitness and aging." *Alternat. Med. Rev.*, vol. 11, pp. 278-293.

[3] Karakitsos, D. and Karabinis, A., 2008. "Hypothermia therapy after traumatic brain injury in children." *N. Engl. J. Med.*, vol. 359, pp. 1179-1180.

[4] Chattopadhyay, D., Arunachalam, G., Ghosh, L., Rajendran, K., Mandal, A. B., and Bhattacharya, S. K., 2015. "Antipyretic activity of Alstonia macrophylla Wall ex A. DC: An ethnomedicine of Andaman." *Islands. J Pharm. and Pharma. Sci.*, vol. 8, pp. 558-564.

[5] Hajare, S. W., Tandan, S. K., Sarma, J., Lal, J., and Telang, A. G., 2000. "Analgesic and antipyretic activities of dalbergia sissoo leaves." *Indian J. pharmacol.*, vol. 32, pp. 357-360.

[6] Tosan, C. A., Adeoti, O. K., Ojewuyi, O. B., and Olayemi, J. O., 2014. "Antipyretic activity of abutilon mauritianum (Jacq.) roots in wistar rats." *International Journal of Pharma Sciences and Research*, vol. 5, pp. 42-46.

[7] Kluger, M. J., 1991. " Fever: role of pyrogens and cryogens." *Physiol. Rev.*, vol. 71, pp. 93-127.

[8] Adesokan, A. A., Yakubu, M. T., Owoyele, B. V., Akanji, M. A., Soladoye, A. O., and Lawal, O. K., 2008. "Effect of administration of aqueous and ethanolic extracts of Enantia chlorantha stem bark on brewer's yeast induced pyresis in rats." *Afr. J. Biochem. Res.*, vol. 2, pp. 165-169.

[9] Kurokawa, M., Imakita, M., Kumeda, C. A., Yukawa, T. A., and Shiraki, K., 1996. "Kakkon-to suppressed interleukin-1a production responsive to interferon and alleviated influenza infection in mice." *J. Tradit. Med.*, vol. 13, pp. 201-209.

[10] Luo, C., He, M. L., and Bohlin, L., 2005. "Is COX-2 a perpetrator oraprotector? Selective COX -2 inhibitors remain controversial." *Acta Pharmacol. Sin.*, vol. 26, pp. 926-933.

[11] Gege-Adebayo, G. I., Bassi, A. S., Igbokwe, V. U., and Shafe, M. O., 2013. "Antipyretic effect of ocimum gratissium on brewer's yeast induced fever in wistar rats." *J. Med. Med. Sci.*, vol. 4, pp. 247-251.

[12] Chaudhary, S. K., 2010. *Quitessence of medical pharmacology*. 3rd ed. New Dehli: New Central Book Agency (P) Ltd. p. 707.

[13] Jekayinfa, A. O., George, A. O., and Jaiyeoba, K. T., 1997. "Acalypha wilkesiana: preliminary in vitro microbiological and clinical trial on dermatitis." *Afr J Health Sci.*, vol. 4, pp. 39-42.

[14] Owoyele, B. V., Okoye, O. C., Dolor, R. O., Oloruntola, O. P., and Soladoye, A. O., 2011. "Analgesic, anti-inflammatory and antipyretic effects of the ethanol extract of Acalypha wilkesiana leaves in rats." *Nig. J. Physiol. Sci.*, vol. 26, pp. 077-082.

[15] Madziga, H. A., S., S., and Sandabe, U. K., 2010. "Elemental analysis of Acalypha wilkesiana leaf." *Journal of American Science*, vol. 6, pp. 510-514.

[16] Oladunmoye, M. K., 2006. "Comparative evaluation of antimicrobial activities and phytochemical screening of two varieties of acalypha wilkesiana." *Int. J. Trop. Med.*, vol. 1, pp. 134-136.

[17] Madziga, H. A., Sanni, S., and Sandabe, U. K., 2010. "Phytochemical and elemental analysis of acalypha wilkesiana leaf." *Journal of American Science*, vol. 6, pp. 15-32.

[18] Gotep, J. G., Agada, G. O. A., Gbise, D. S., and Chollom, S., 2010. "Antibacterial activity of ethanolic extract of Acalypha wilkesiana leaves growing in Jos, Plateau State Nigeria." *Malaysian J Microbiol*, vol. 6, pp. 69-74.

[19] Udobang, J. A., Nwafor, P. A., and Okokon, J. E., 2010. "Analgesic and Antimalarial activities of crude extract and fractions of Acalypha wilkensiana." *J. Ethnopharmacol*, vol. 127, pp. 373-378.

[20] Trease, G. E. and Evans, W. C., 1989. *Pharmacology*. 11th ed. London: Bailliere Tindall Ltd. pp. 60-75. .

[21] Harborne, J. B., 1973. *Photochemical methods: A guide to modern techniques of plant analysis* vol. 279. London: Chapman A. & Hall.

[22] Bensita, M. B., Pakianathan, N., and Divakar, M. C., 1998. "On the antipyretic, anti-inflammatory, analgesic and molluscicidal properties of polyscias fruticosa (L.) harms." *Ancient Science of Life*, vol. 17, pp. 313-319.

[23] Somezeet, P., Choudhury, N. S. K., Jagannath, P. V., Dipti, K. P., and Goutam, K. J., 2009. "Analgesic, antipyretic and anti-inflammatory effect of the whole plant extract of desmostachya bipinnata stapf (poaceae) in albino rats." *DIT.*, vol. 1, pp. 150-153.

[24] Roszkowski, A. P., Rooks, W. H., Tomolonis, A. J., and Miller, L. M., 1971. "Anti-infl amatory and analgetic properties of d-2-(6'-methoxy-2'-naphthyl)-propionic acid (naproxen)." *J Pharmacol Exp Ther.*, vol. 179, pp. 114-123.

[25] Vane, J., 1987. "The evolution of non steroidal anti inflammatory drugs and their mechanisms of action." *Drugs*, vol. 33, pp. 18-27.

[26] Irfan, U., Jamshaid, A. K., Muhammad, S., Ajmal, K., Achyut, A., Peer, A. H., J., I., Faisal, S., and Umar, F., 2016. "Pharmacological screening of monotheca buxifolia (Falc.) A. DC. for antinociceptive, anti-inflammatory and antipyretic activities " *BMC Complementary and Alternative Medicine.*, vol. 16, p. 273.

[27] Onuah, C. L., Monago, C. C., and Omeodu, S. I., 2016. "Ethanol extract of acalypha wilkesiana muel arg leaves ameliorates paracetamol-induced hepatotoxicity in rats." *International Journal of Biochemistry Research & Review*, vol. 12, pp. 1-7.

[28] Sule, O. J., Elekwa, I., and Ayalogu, E. O., 2012. "Effect of acalypha wilkesiana muell arg. On haematological parameters in wistar albino rats." *International Journal of Biological & Medical Research*, vol. 3, pp. 1234-1237.

[29] Rishikesh, M., R. Md., Rasheful, G. M., Muhammad, R. A. M., Parboti, R. D., and Abdullah, A. M. M., 2013. "Phytochemical and pharmacological investigation of achyranthes aspera linn." *Scholars Academic Journal of Pharmacy*, vol. 2, pp. 74-80.

[30] Rajani, G. P., Deepak, G., Sowjanya, K., and Sahithi, B., 2011. "Screening of antipyretic activity of aerial parts of Nelumbo nucifera gaertn in yeast induced pyrexia." *Pharmacologyonline*, vol. 1, pp. 1120-1124.

[31] Chang, C. P., Huang, W. T., Cheng, B. C., Hsu, C. C., and Lin, M. T., 2007. "The flavonoid baicalin protects against cerebrovascular dysfunction and brain inflammation in experimental heatstroke." *Neuropharmacology*, vol. 52, pp. 1024-1033.

[32] Backhouse, N., Delporte, C., Givernau, M., Cassels, B. K., Valenzuela, A., and Speisky, H., 1994. "Anti-inflammatory and antipyretic effects of boldine." *Agents Actions*, vol. 42, pp. 114-117.

[33] Zakaria, Z., Loo, Y. W., Nurul, I. A. R., A., A. H. A., Mohd, R. S., and Hanan, K. G., 2007. "Antinociceptive, anti-inflammatory and antipyretic properties of the aqueous extract of bauhinia purpurea leaves in experimental animals." *Medical Principles and Practice*, vol. 16, pp. 443-449.

[34] Spacer, C. B. and Breder, C. D., 1994. "The neurologic basis of fever." *New England Journal of Medicine*, vol. 330, pp. 1880-1886.

[35] Zeil, R. and Krupp, P., 1975. *Schorbaum E, Lomax P, Jacob J editors. Temp regulation and drug action*. New York: AG Publisher pp. 233-241.

[36] Okokon, J. E. and Nwafor, P., 2010. "Antiinflammatory, analgesic and antipyretic activities of ethanol root extract of Croton zambesicus." *Pak. J. Pharm. Sci.*, vol. 23, pp. 385-392.