Journal of Medicinal Plants Research Vol. 2(9), pp. 219-225, September, 2008
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875© 2008 Academic Journals

Full Length Research Paper

Analgesic, anti-inflammatory and antipyretic activities from flavonoid fractions of *Chromolaena odorata*

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Accepted 16 September 2008

The ethanolic extract of *Chromolaena odorata* was fractionated with solvent-solvent extraction technique using the following solvents successively, n-hexane, dichloromethane, ethyl acetate, n-butanol and water. The fractions were evaluated for analgesic, anti-inflammatory and antipyretic activities using standard experimental models which includes; hot plate and formalin paw licking tests for analgesic activities, carrageenan paw oedema and cotton pellet granuloma for anti-inflammatory activities and Brewer's yeast induced pyrexia for antipyretic tests. The dichloromethane (DCF), n-butanol (nBF) and ethyl acetate (EAF) fractions were analyzed using analytical thin-layer chromatography (TLC), and preparative thin-layer chromatography (PTLC). Spectral studies were carried out using ultra-violet (UV) and infra-red (IR) spectroscopy. Phytochemical screening was carried out on the isolated compounds and the R_f values were determined. The result shows that the DCF produced consistent analgesic, anti-inflammatory and antipyretic activities followed by the nBF and EAF. Spectroscopic and phytochemical analyses revealed the presence of flavonoid in DCF. Flavonoids were also detected in nBF and EAF. The biological activities of the extract can therefore be attributed to the presence of flavonoids in the fractions.

Key words: Analgesic, anti-inflammatory, antipyretic, *Chromolaena odorata*, flavonoids, fractions.

INTRODUCTION

Drug discovery involves many steps which must be carefully carried out. One of the most popular and important procedure for drug discovery is bioguided fractionation of extracts (Pieters and Vlietinck, 2005). This procedure involves the fractionation of active extracts and fractions until pure active ingredients are Vlientinck, 2005). In an an attempt to further contribute to the process of Bioassay guided study of *Chromolaena odorata*, the ethanolic extracts of the leaves of the plant which have been previously reported to possess anti- inflammatory, analgesic and antipyretic activities (Owoyele and Soladoye, 2007; Owoyele et al., 2007) was further investigated in this study. The interest in undertaking the study on *C. odorata* is justifiable based on the local uses of the plant for the treatment of headache, and wounds (Gill, 1992; Phan et al., 2001) and the series of scientific investigations into the various activities of the plant. Some of these previous investigations revealed that the ethanolic extract possesses wound healing and anti-oxidant activities (Le et al., 1995; Phan et al., 2001). Furthermore, by using various forms of isolation techniques, about fourteen different flavonoids have been identified from *C. odorata* the latest being 5, 7-dihydroxy-6, 4'-dimethoxyflavanone (Pisutthanan et al., 2006).

The present study was therefore, conducted in order to

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Abbreviations

DCF, dichloromethane fraction; nBF, n-hexane fraction; EAF, ethyl acetate fraction, AQF, aqueous fraction; TLC, analytical thin-layer chromatography; PTLC, Preparative thin-layer chromatography (PTLC); UV, Ultra-violet; IR, infra-red; FRIN, Forestry research institute of Nigeria; FHI, Forestry herbarium Ibadan; Λmax, maximal wavelength.

systematically fractionate and determine the analgesic, anti- inflammatory and antipyretic activities of various active fractions as well as to identify the active groups contained in the most biologically active fraction.

MATERIALS AND METHODS

Plants

The process for the collection and identification of *C. odorata* has been reported earlier (Owoyele et al., 2005). The plant was collected from the outskirt of University of Ilorin and subsequently identified at the Forestry research institute of Nigeria (FRIN) by T. K. Odewo. A voucher specimen (FHI 105769) has since been deposited at herbarium of the Research institute.

Preparations of the fractions

Crude ethanolic extracts were prepared by cold maceration as described previously (Owoyele and Soladoye, 2007). There after the ethanolic extract was subjected to solvent- solvent extraction. 125 g of the crude extract was extracted using 500 ml of distilled water and four solvents (500 ml each) in successive order; n-hexane, dichloromethane, ethyl acetate and n-butanol. The extract was first dissolved in 500 ml of distilled water and mixed with 500 ml of n-hexane. The mixture was shaken thoroughly for about 4 h after which the n- hexane portion was separated and evaporated to dryness in a pre-weighted beaker.

The main (aqueous portion) was further subjected to successive extraction using the same process and the following solvents successively; dichloromethane, ethyl acetate and n-butanol. Fractions were obtained from each solvent including the residual aqueous portion by evaporation. The fractions obtained were stored in refrigerator prior to the period of biological investigations.

Animals

Male wistar rats (weighing 180 ± 10 g) were used for the study. The rats were bred and housed in the Faculty of Basic Medical Sciences Animal house, University of Ilorin, Ilorin, Nigeria. The animal house was well ventilated and the normal day light cycle was maintained. The animals were fed with mouse cubes (Bendel Feeds, Ilorin) and water was provided *ad libitum*. Animals were divided into groups comprising of five rats each.

Analgesic tests on the fractions

The five fractions obtained (that is, dichloromethane fraction (DCF), n-hexane fraction (nHF), ethyl acetate fraction (EAF), n-butanol fraction (nBF) and aqueous fraction (AQF)) were screened for analgesic activities using the hot plate test and the formalin induced paw licking test as in previous study (Owoyele and Soladoye, 2007). In the Hot plate test animals were orally administered 50 mg/Kg of the fractions or saline (control) or 5 mg/Kg Indomethacin (Reference drug). The animals were each placed on a hot plate (maintained at 50 ± 1^{o}) after 30 min of the administration of the fractions, drug and Saline and the time (Reaction time) it takes each of the animals to jump off the hot plate was noted. Animals were again placed on the hot plate at 60 and 90 min post fractions, Saline and drug administration. The mean of the responses for the animals (5 per group) administered each fraction was compared with the control group.

In the Formalin test the method of Hunskaar and Hole, (1997) was used. The paws of the animals were injected with 100 µL of 3% formalin for 1 h. after the oral administration of fractions (50 mg/Kg), saline or indomethacin. The licking time in the first phase (0 - 5 min post formalin injection and in the second phase (20 – 30 min post formalin injection) was noted and the mean for each group was determined as in the earlier study (Owoyele and Soladoye, 2007).

Anti-inflammatory studies

The carrageenan paw oedema and the cotton pellet –induced granuloma methods were used for this study as described in the report on crude extracts (Owoyele and Soladoye, 2007; Owoyele et al., 2005). A dose of 100 µL of 1% carrageenan was injected into the hind paw of each animal for 1 hr. after the administration of the fractions (50 mg/Kg), saline and indomethacin. The paw size was measured hourly using cotton thread and metre rule from 1 - 5 h post carrageenan injection.

In the cotton pellet granuloma, 30 mg of cotton pellet were surgically inserted into the groin of animals for 7 days with the administration of fractions (50 mg/Kg), Saline and drug once a day for the 7 days period. On the eighth day animals were sacrificed with an overdose of ether. The cotton pellets with the attached granuloma were dissected out, dried and the weights of the dried granuloma were determined. The mean of the granuloma formed in each animal was determined.

Antipyretic study

Pyrexia was induced as in the previous study on the crude extracts (Owoyele et al., 2007). The animals were administered Brewer's yeast in the back and 17 h later rectal temperatures of the hyper-pyrexic rats were measured. After one hour, the fractions (50 mg/Kg) saline and drug were orally administered to the animals. The rectal temperatures of the animals were subsequently measured at 60, 90 and 120 min post fractions, drug and saline administration. The mean of post brewer's yeast rectal temperatures were compared with pre drug temperature (that is, temperature taken at 17 h post brewer's yeast injection).

Analytical TLC and preparative thin layer chromatography

The three most potent fractions (DCF, nBF and EAF) were analyzed using analytically pre-coated TLC plates (MERCK, Kieselgel 60, F_{254} Germany). They were also further analyzed using preparative thin layer chromatography (PTLC) on silica gel GF_{254}, Fluka, Switzerland) 20 x 20cm silica glass plates (designated for PTLC). A mixture of methanol-acetic acid-Water (18: 1: 1) was used as the solvent system for the TLC and PTLC of DCF, while n-hexane-diethyl ether-methanol (3:3:1) solvent system was used for TLC and PTLC of nBF and EAF. The information on the solvent system used for DCF was obtained from the report of Phan et al. (2001) while several trials yielded the best solvent system for nBF and EAF.

The spots developed on the TLC plates were viewed under UV lamp at wavelengths of 254 and 366 nm respectively. The PTLC layers were then scrapped before being eluted with diethyl ether. R_f values for all the spots were recorded.

Phytochemical screening of isolated compounds

The isolated compounds from the PTLC plates were concentrated on rotary evaporator at 60^{o}C to semi solids. The compounds were subjected to phytochemical analysis using Shibata's reaction and Ferric chloride test. Thereafter, the Shibata's test was carried out by adding 2 ml of methanol, small quantity of magnesium turnings and 2 ml of concentrated HCl to 50 mg of the isolated compound. The mixture (in a test tube) was subjected to heat and observed for red

Table 1. Effects of the fractions of ethanolic extract of *Chromolaena odorata* leaves on hot plate test in rats.

Group	Dose (mg/kg) orally	Reaction time (s) (Post drug)[a]		
		30 min	60 min	90 min
A. Control	-	3.2±0.11	2.8±0.34	2.4±0.24
B. n-hexane	50	4.3±0.44*	3.5±0.22	3.4±0.75
C. n-butanol	50	5.8±0.25***	5.0±0.16***	4.0±0.35**
D. Ethylacetate	50	3.8±0.30	2.9±0.45	2.7±0.30
E. Dichloromethane	50	5.0±0.42**	4.8±0.34**	4.8±0.34***
F. Aqueous	50	4.6±0.66	3.9±0.67	3.5±0.52
G. Indomethacin	5	5.0±0.27***	4.7±0.25**	4.9±0.19***

[a]Each value is the mean ± S.E.M. for 5 rats
*P < 0.05; **P < 0.01; *** P < 0.001 compared with control; student's t-test.

Table 2. Effects of the fractions of ethanolic extract of *Chromolaena odorata* leaves on Formalin-induced paw-licking in rats.

Group	Dose (mg/kg) orally	Licking time (s)[a]	
		Early phase	Late phase
A. Control	-	101.0±6.18	88.4±2.48
B. n-hexane	50	80.0±6.28*	72.6±9.82 ns
C. n-butanol	50	67.6±10.85*	25.4±7.06***
D. Ethylacetate	50	85.2±7.46	84.5±4.89
E. Dichloromethane	50	71.1±6.03**	47.4±4.33***
F. Aqueous	50	83.1±8.86	80.4±9.84
G. Indomethacin	5	58.8±7.94**	40.3±4.14***

[a]Each value is the mean ± S.E.M. for 5 rats.
*P < 0.05; **P < 0.01; *** P < 0.001 compared with control; student's t-test.

Table 3. Effects of the fractions of ethanolic extract on *Chromolaena odorata* leaves on carrageenan induced paw oedema in rats.

Group	Dose (mg/kg) orally	Paw size (mm) a		Inhibition (%)	
		3h	5h	3h	5h
A. Control	-	8.6±0.93	10.0±1.58	-	-
B. n-hexane	50	4.2±0.66*	8.6± 0.98	51.2	14.0
C. Dichloromethane	50	6.2± 0.49*	2.8±0.97*	27.9	72.0
D. Ethylacetate	50	4.4±0.98**	2.0±1.10*	48.8	80.0
E. n-butanol	50	8.0± 0.89	10.4±0.98	7.0	0
F. Aqueous	50	4.8± 0.37*	1.6± 0.75**	44.2	84
G. Indomethacin	5	5.8± 0.07*	1.0± 0.63**	32.6	90

a Each value is the mean ± S.E.M. for 5 rats.
*P < 0.05; **P < 0.01; *** P < 0.001 compared with control; student's t-test

colouration of flavonoids (Markham, 1982). In the Ferric chloride test, 2 ml of water and three drops of freshly prepared $FeCl_3$ were added to 50 mg of the isolated compound (in a test tube). The mixture was warmed and observed for the bluish green coloration of flavonoids (Trease and Evans, 1993).

Physical measurement using ultraviolet and infrared spectroscopy

The isolated compounds absorption bands in the ultraviolet (UV) spectroscopy were carried out on Aguamate spectrophotometer v4.60, Germany at the Department of Chemistry, University of Ilorin. Nigeria. (Table 6) The functional groups of the active compound were carried out using model M500 IR- spectrophotometer at the Department of Chemistry, Ladoke Akintola University, Ogbomoso, Nigeria. The wave numbers (cm^{-1}) were printed out and tabulated (Table 7a, b and c)

Statistical analysis: Results were expressed as mean ± SEM and statistical significance was obtained using the Student's t – test.

Table 4. Effects of the fractions of ethanolic extract on *Chromolaena odorata* leaves on Cotton pellet-induced granuloma in rats.

Group	Dose (mg/kg) orally	Increase in weight of pellet (mg)[a]	Inhibition (%)
A. Control	-	0.69±0.01	-
B. n-hexane	50	0.27±0.05**	60.9
C. Dichloromethane	50	0.25±0.04***	63.8
D. Ethylacetate	50	0.57±0.03*	17.3
E. n-butanol	50	0.51±0.03**	18.0
F. Aqueous	50	0.24±0.02***	65.2
G. Indomethacin	5	0.10±0.02***	85.5

[a]Each value is the mean ± S.E.M. for 5 rats.
*P < 0.05; **P < 0.01; *** P < 0.001 compared with control; Student's t-test.

Table 5. Results of TLC (analytical and preparatory) performed on dichloromethane, n-butanol and ethyl acetate fractions of ethanolic extract of *Chromolaena odorata*.

Fractions	Spots	Colour of spots (UV lamp)	R_f
Dichloromethane	1	Orange	0.82
n-butanol	1	Orange	0.82
	2	Dark brown	0.72
	3	Dark brown	0.48
Ethyl acetate	1	Orange	0.82
	2	Yellow	0.73
	3	Brown	0.62
	4	Dirty brown	0.47
	5	Light brown	0.29

Results were regarded as significantly different from the control when p< 0.05.

RESULTS

Analgesic studies

The results of the hot plate test show that only the nBF and DCF fractions produced consistent and significant increase in reaction time to the thermal stimuli (p< 0.05) (Table 1). Likewise only the nBF and DCF produced significant decrease in paw licking time in the formalin-induced paw licking test (p< 0.05) (Table 2).

Anti-inflammatory studies

In the carrageenan-induced paw oedema, only the EAF, DCF and Aqueous fractions produced consistent reduction in paw size after 3 and 5 h of carrageenan injection (Table 3). In the cotton pellet granuloma test, all the fractions produced significant reduction in granuloma weight but the highest inhibition of granuloma formation was produced by the aqueous (65.2%) followed by the DCF (63.8%) and nHF (60.9%)(Table 4) (p< 0.05).

Antipyretic studies

In this study, the nBF and DCF were the only fractions that produced significant reductions in the temperature at the entire interval (60, 90 and 120 min) at which rectal temperatures were measured (p< 0.05) (Figure 1).

Result of UV irradiation and spectroscopy

UV irradiation of the different spots on the TLC plates of DCF, nBF and EAF shows that each of the three fractions has at least one spot with orange coloration when viewed under UV lamp at 366 nm (Table 5). The results for the UV spectroscopy are tabulated in Table 6. The result shows that the only isolated compound (R_f value = 0.82) from the most active fraction (DCF) has absorption bands corresponding to those of flavonoids [Λmax: 254, 278.5 and 285.5 nm].This supports the result of Pisutthanan et al. (2006). The UV- spectra for the nBF and EAF are also reflected on the Table 6.

Results of IR spectroscopy

The result of the IR spectroscopic analysis of the isolated

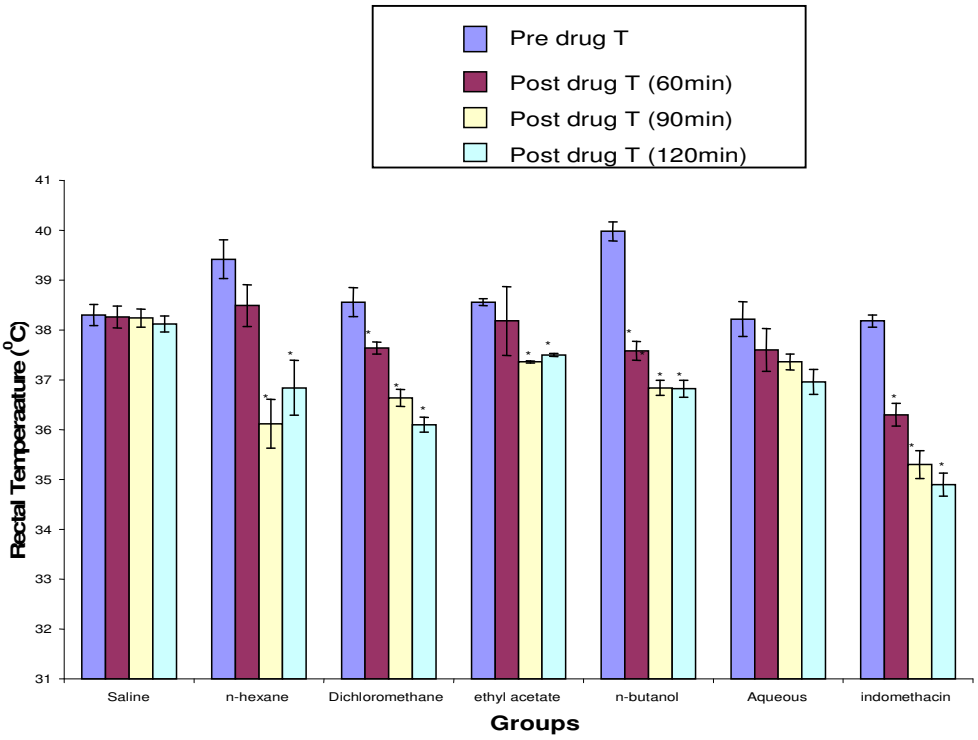

Figure 1. Effects of the fractions from ethanolic extract of *Chromolaena odorata* leaves on brewers yeast induced pyrexia in rats.

Table 6. Summary of the UV spectra obtained from fractions (Dichloromethane, n- butanol and Ethyl acetate) of ethanolic extract of *Chromolaena odorata*

Fractions	UV Spectrum(nm)
1. Dichloromethane	
Compound 1	254, 278.5, 285.5
2. n- butanol	
Compound 1	209, 220, 274
Compound 2	254, 281.5, 323.5
Compound 3	269
3. Ethyl acetate	
Compound 1	274, 385, 395
Compound 2	384, 387, 654
Compound 3	388.5, 392.5, 399.5
Compound 4	388.5, 393.5, 656.5
Compound 5	245.5, 285.5, 368.5

constituent from DCF reveal the presence of many functional groups which include –OH, -CH, -C=C-, C-O stretch of aromatic hydrocarbons. The results of the IR spectroscopic analysis of DCF, nBF and EAF are shown in Table 7a, b, c respectively.

Phytochemical analysis

Phytochemical analysis on the isolated compounds from DCF, nBF and EAF confirms the presence of flavonoid in the isolate from DCF as well as in those isolated from nBF and EAF that produced orange colour when viewed under UV lamp (366 nm).

DISCUSSION

The present study was an attempt to investigate the nature of the active constituents that were responsible for the previously described analgesic, anti-inflammatory and analgesic activities of *C. odorata* (Owoyele and Soladoye, 2007; Owoyele et al., 2007). The result obtained from this study shows that the active constituent can be selectively isolated using different solvents.

In order to identify the fractions with high activity in the three biological tests (analgesic, anti-inflammatory and antipyretic) all the fractions were subjected to the same tests that the ethanolic extract was previously subjected to (Owoyele and Soladoye, 2007; Owoyele et al., 2007). In the analgesic test the DCF and nBF showed consistent activities. This indicates that the fraction contain high amount of active constituents that can inhibit both the centrally and tonic pain induced by Hot plate and Formalin tests (Prado et al., 1990; Parkhouse and Pleuvry, 1999; Tjølsen et al., 1992; Santos et al., 1997; Hunskaar and Hole, 1997).

In the anti-inflammatory test only the DCF, EAF and Aqueous fraction produced consistent and high anti-inflammatory activities in the acute and chronic models of

Table 7a. Summary of the IR spectra obtained from Dichloromethane fraction of ethanolic extract of *Chromolaena odorata*

Compound 1	IR Spectrum(cm^{-1})
	3400.22 (s) 2976.44-2899.88(m) 1650.58(st) 1409.79(m) 1087.74-1047.11(st) 879.75-668.07(st)

m and st = medium and strong peaks

inflammation represented by the Carrageenan and Cotton pellet granuloma models respectively (Di Rosa et al., 1972; Olajide et al., 2003). These indicate that the three fractions significantly retained the active constituents of the ethanolic extract. In the antipyretic test the DCF and nBF demonstrate significant antipyretic activities. Thus, indicating that the two fractions significantly retained the antipyretic agents contained in the ethanolic extract.

The result of the biological screening on the fractions from ethanolic extract of *C. odorata* confirms that the DCF showed consistent activities in the analgesic, anti-inflammatory and antipyretic tests. It is the DCF that actually combined these three activities effectively as observed with the previous study on the ethanolic extracts (Owoyele and Soladoye, 2007; Owoyele et al., 2007) while the activity of nBF and EAF followed that of DCF. It is common to screen a plant for analgesic, anti-inflammatory and antipyretic tests since most substances or drugs (e.g. Non steroidal anti-inflammatory drugs) have various degrees of the three (analgesic, anti-inflammatory and antipyretic) biological activities (Steinmeyer, 2000; Neal, 1991).

The result of the PTLC indicates that nine substances were isolated from the three fractions (DCF, nBF and EAF) analyzed. The only isolate from the DCF was confirmed to be a flavonoid using UV Λmax and IR spectroscopy as well as the phytochemical analysis. Likewise the same procedure was used to identify the active compound from the isolate of nBF and EAF which was screened to be flavonoid compound. Many studies have shown that flavonoids are mainly responsible for the activities *of C. odorata*. These activities include, wound healing (Le et al., 2007; Phan et al., 2001), anti-oxidant activities (Phan et al., 2001), antimicrobial activities (Akah, 1990). Therefore this present study further unveils the additional activities of the flavonoids of *C. odorata* and thus showing that the flavonoid possesses anti-inflammatory, analgesic and antipyretic activities.

Flavonoids have been linked with analgesic, anti-inflammatory and antipyretic activities (Cody et al., 1986; Mutalik et al., 2003; Venkatesh et al., 2003). Such flavonoids include quercetin, solon etc (Phan et al., 2001; Zayachkivska et al., 2005, Owoyele et al., 2006). It is not

Table 7b. Summary of the IR spectra obtained from n-butanol fraction of ethanolic extract of *Chromolaena odorata*

Compound 1	IR Spectrum(cm^{-1})
	3381.00(m) 2975.48-2897.6(m) 1923.64(m) 1650.72(st) 1453.49(st) 1410.73(m) 1382.69(m) 1328.09(m) 1274.32(m) 1088.68-1048.26(m) 880.19-653.13(st)
Compound 2	
	3850.76(st) 3383.94(st) 2975.56-2897.6(m) 1651.08(st) 1454.35(m) 1382.86(m) 1328.34(m) 1274.28(m) 1088.71-1048.27(st) 88019-665.12(st)
Compound 3	
	3380.94(m) 2975.18-2896.68(st) 1924.15(m) 1651.40(st) 1454.0(m) 1416.67-1382.10(m) 1328.71-1274.28(m) 1089.13-657.40(m) 880.50-657.40(st)

m and st = medium and strong peaks

Table 7c. Summary of the IR spectra obtained from ethylacetate fraction of ethanolic extract of *Chromolaena odorata*

Compound 1	IR Spectrum(cm^{-1})
	3396.04 (st) 2975.59-2926.78(m) 1463.25(st) 1412.05(m) 1273.18(m) 1088.11-1047.65(st) 879.7(m) 656.8(m)

m and st = medium and strong peaks.

not surprising therefore to observe the three activities in the DCF since it contained only flavonoids. The activities observed in the nBF and EAF might also be due to the flavonoid constituents. The relative reduction in the activities observed with the two fractions might be due to interaction between the flavonoid and other non-flavonoid constituent of the fractions or it may be due to actual reduction of the activity of the flavonoids by the other components. This explanation agrees with the observation of Likhitwitayawuid et al. (2002) on *Dracaena loureiri* where activities of a fraction were based on the interaction between the components of the fraction. However recent research has shown that up to 14 flavonoids have been identified in *C. odorata* including 5, 7-dihydroxy-6, 4'-dimethoxyflavanone (Pisutthanan et al., 2006). The thrust of further studies will involve specifically the identification, characterization as well as the evaluation of the toxicity of the isolated flavonoids.

In conclusion this study has shown that the active constituent that is responsible for analgesic, anti-inflammatory and antipyretic activities of *C. odorata* are mainly flavonoid compounds. Furthermore, the flavonoids can easily be isolated using ethanol/dichloromethane as solvents.

ACKNOWLEDGEMENTS

The authors thank Mr Ajala of Chemistry Department, University of Ilorin. Ilorin. Nigeria for excellent technical assistance.

REFERENCES

Akah PA (1990). Anti microbial effects of crude *Chromolaena odorata* leaf extract. Intern J. Crude Drug Res. 28: 233–235.

Cody V, Middleton E, Harborne JB (1986) Plant Flavonoid in Biology and Medicine, vol. I and II, Alan, R. Liss New York.

DiRosa M (1972). Biological properties of Carrageenan. J. Pharm. Pharmacol. 24:89-102.

Gill LS (1992). Ethnomedical Uses of Plants in Nigeria. Uniben Press Benin,Nigeria.

Hoareau L, Da Silva EJ (1999). Medicinal plants: a re-emerging health aid. Eur. J. Biotechnol. 2:56-70.

Hunskaar S, Hole K (1997). The formalin test in mice-dissociation between inflammatory and non inflammatory pain. Pain 30:103–114.

Le TT (1995). The use of Eupolin prepared from *Eupatorium odoratum* to treat soft tissue wounds. The 5th European Tissue repair society Annual Meeting, Padora Italy. (Abstract 30).

Likhitwitayawuid K, Sawasdee K, Kirtikara K (2002) Flavonoids and stilbenoids with COX-1 and COX-2 inhibitory activity from *Dracaena Loureiri*. Planta Medica 68: 841-843.

Markham KR (1982) Techniques of flavonoids identification Academic press Publication. New York, pp111-113

Mutalik S, Paridhavi K, Mallikarjuna Rao C, Udupa N (2003) Antipyretic and analgesic effect of leaves of *Solanum melongena* Linn in rodents. Indian J Pharmacol 35: 312-315

Neal MJ (1991) Medical Pharmacology at a Glance. Blackwell series, pp 62-67.

Olajide OA, Makinde JM, Okpako DT (2003). Evaluation of the anti-inflammatory property of the extract of *Combretum micranthum*. G .Don (Combretaceae). Inflammopharmacol 11:293-298

Owoyele BV, Oladimeji A, David A J, Soladoye AO (2007). Antipyretic activities of the leaf extract of *Chromolaena odorata*. Trop. J. Health Sci. 14:1-3.

Owoyele B V, Jegede T O, Soladoye AO (2006) Analgesic and antipyretic activities of ethanolic extract of *Psidium guajava* in rats. Recent Progr Med Plants 13:485-492.

Owoyele BV, Adediji JO, Soladoye AO (2005) Anti-inflammatory activity of aqueous leaf extract of *Chromolaena odorata*. Inflammopharmacol 13:479-484.

Owoyele BV, Soladoye AO (2007) Analgesic and anti-inflammatory activity of ethanolic extract of *Chromolaena odorata* leaves. Recent Progr. Med. Plants 18:397-406.

Parkhouse J, Pleuvry B J (1979) Analgesic Drug. Oxford, Black Well pp 1-5.

Phan TT, Wang L, See P, Grayer RJ, Chan SY, See ST (2001) Phenolic compounds of *Chromolaena odorata* protect cultured skin cells from oxidative damage: implication for cutaneous wound healing. Biol. Pharm. Bull. 24: 1373-1379

Pieters L, Vlietinck A J (2005). Bioguided isolation of pharmacologically active Plant components, still a valuable strategy for the finding of new lead compounds? J. Ethnopharmacol 100: 57–60.

Pisutthanan N, Liawruangrath B, Liawruangrath S, Bremner JB (2006). A new flavonoid from *Chromolaena odorata*. Nat. Prod. Res. 20:1192-1198.

Prado WA, Tonussi CR, Rego EM, Corrado AP (1990). Antinociception induced by intraperitoneal injection of gentamicin in rats and mice. Pain 41:365-371.

Santos FA, Rao VSN, Silveira ER (1997). Anti-inflammatory and analgesic activities of the essential oil of *Psidium guianense* Fitoter 68:65–68.

Steinmeyer J (2000). Pharmacological basis for the therapy of pain and inflammation with nonsteroidal anti-inflammatory drugs. Arthritis Res. 25:379-385.

Tjølsen A, Berge O, Hunskaar S, Rosland JH, Hole K (1992). The formalin test; an evaluation of the method. Pain 51:5-17.

Trease GE, Evans WC (1989). A Textbook of Pharmacognosy 13th edn. Bailliere-Tindall Ltd, London.

Venkatesh S, Madhava Reddy B, Dayanand Reddy R, Ramesh M (2003). Antipyretic activity of *Rumex nepalensis* roots. J. Nat. Prod. Med. 07:53-55.

Zayachkivska O S, Konturek S J, Drozdowicz D, Konturek PC, Brzozowski T, Ghegotsky MR (2005). Gastroprotective effects of flavonoids in plant extracts. J. Physiol. Pharmacol. 56 (suppl1):219-231.