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ANALGESIC ACTIVITY OF SEQUENTIAL FRACTIONS OF *GREWIA CRENATA* LEAVES

***A. N. Ukwuani¹, M. G. Abubakar², S. W. Hassan² and B. M. Agaie³**

¹*Department of Biochemistry, Faculty of science, Kebbi State University of Science and Technology, Aliero, Kebbi state, Nigeria*

²*Department of Biochemistry, Faculty of science, Usmanu Danfodiyo University, Sokoto, Sokoto state, Nigeria*

³*Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Sokoto state, Nigeria.*

**Author for Correspondence*

ABSTRACT

In Nigeria, many plant products are used for their analgesic and anti-inflammatory effects and their efficacy is traditionally acclaimed. *Grewia crenata* is an example of such plant with analgesic and anti-inflammatory claim amongst the traditional bone setters in northern-west Nigeria. Powdered *Grewia crenata* leaves (GCL) was sequentially extracted with solvents of increasing polarity: hexane, chloroform, ethyl acetate and butanol. Sequential fractionation of *Grewia crenata* leaves yielded 22.84g (2.28%) hexane fraction, 3.88g (0.38%) chloroform fraction, 26.95g (2.69%) ethyl acetate fraction, 20.15g (2.01%) butanol fraction and last remaining aqueous fraction of 28.80g (2.88%). Analgesic screening of sequential fractions showed all fractions to evoke significant ($P < 0.05$) dose dependent reduction in the number of writhes with hexane (54.48%) and ethyl acetate (54.48%) fractions respectively showing the highest percentage inhibition but incomparable to the standard drug Diclofenac-sodium with 85.07%. The effect of the extract on hot plate reaction time showed significant ($p < 0.05$) and a dose dependent elevation of the post-treatment reaction time to thermal pain was evident in all the fraction treated groups as well as the standard drug. Phytochemical screening of these fractions revealed the presence of cardiac and saponogenic glycosides, saponins, tannins, flavonoids, pseudotannins, balsam, anthraquinone, steroids. The results above support the traditional use of this plant in some painful and inflammatory conditions and suggest the presence of biologically active components.

Keywords: *Grewia crenata*, Leaves, Sequential, Fractions

INTRODUCTION

Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain (Okigbo *et al.*, 2008). Besides food, shelter and clothing, they are important sources of fine chemicals which find their application in pharmaceutical industries. Herbal-derived substances remain the bases for a large proportion of commercial medications used for the treatment of pain, inflammation, asthma and other illnesses. Indeed, natural products have proved to be a rich source of therapeutic agents. Due to the side effects caused mostly by synthetic drugs, interest in natural products is growing rapidly and research into natural products has advanced tremendously in academia and pharmaceutical companies. Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well being and the bioprospecting of new plant-derived drugs (Lucy and Edgar, 1999). The ongoing growing recognition of medicinal plants is due to several reasons, including escalating faith in herbal medicine (Kala, 2005).

Pain is a subjective, complex, multidimensional and unpleasant experience. It is one of the most prevalent conditions that require medical attention (Enkelejdja *et al.*, 2013). Despite the tremendous progress in the medical science during the last years, the treatment of some inflammatory painful diseases is yet problematic and complex. Nowadays the number of patients that are using herbal remedies and complementary and alternative medicine for treatment of pain is growing rapidly.

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Grewia crenata (Kamomowa) is a 4 - 5 meter tall shrub distributed in subtropical and tropical regions of world. In folk medicine, the leaves are applied to the skin for wounds and cuts and to relieve irritation and painful rashes. In northwest Nigeria, it is used in the treatment of fractured bones, wound healing and inflammatory conditions. The present study was designed to evaluate the analgesic activity of sequential fractions of *Grewia crenata* leaves in albino rats.

MATERIALS AND METHODS

Plant material collection and identification

Preliminary collection and identification of *Grewia crenata* leaves (GCL) was done in October, 2010 from the wilds of various local government areas of Sokoto state, Nigeria. Herbarium specimens were prepared and photographs were taken to aid in the confirmation of the identity of the plants. Voucher specimens were deposited in the Herbarium, Botany unit, Biological Science Department, Usmanu Danfodiyo University, Sokoto-Nigeria.

Experimental animals

Swiss albino mice of both sexes (18-25g) were used for this study. The animals were obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria. They were kept in well-ventilated environment and have free access to rodent pellets (Vital Feeds Ltd, Nigeria) and water *ad libitum*. The animals were allowed to acclimatize for 3 weeks and were fasted over night prior to experiments.

Fractionation of the GCL extract

The method described by Yesilada and Kupeli (2007) was employed with mild modification. One kilogram (1kg = 1000g) of coarse powder of GCL was extracted thrice with methanol: water (7:3). The combined hydromethanolic extracts were filtered and evaporated using rotary evaporator to yield 108.3g (10.83%) hydromethanolic extract. The hydromethanolic GCL extract was redissolved in 500ml of distilled H₂O and partitioned twice with hexane (500ml each). The combined hexane fraction (HF) was separated and evaporated using rotary evaporator to yield 22.84g (2.28%). The remaining aqueous extract obtained from separation of the hexane extract was then partitioned twice times with chloroform and the combined chloroform fraction (CF) were separated and evaporated to yield 3.88g (0.38%). This procedure was repeated using ethyl acetate and butanol respectively yielding 26.95 g (2.69%) ethyl acetate fraction (EAF) and 20.15g (2.01%) butanol fraction (BF). The last remaining aqueous fraction (LWF) was evaporated to yield 28.80g (2.88%).

Preliminary Phytochemical Screening

Phytochemical analysis was performed using standard procedures to identify chemical constituents as described by Trease and Evans (1989), Harbone (1973) and Sofowora (1993).

Analgesic activity

Mouse writhing assay: The acetic acid-induced abdominal writhing test was performed according to the procedure described previously by Koster *et al.* (1959). Twenty five (25) Swiss albino mice were divided into 5 groups of 5 mice each. The first group was given 10 ml/kg of Normal saline i.p. and served as control, group 2 receive Diclofenac 10mg/kg as a positive control, 3 and 4 received 250 and 500 mg of plant extract per kg of body weight i.p. respectively. Thirty minutes later, mice in all the groups were treated with Acetic acid (0.7% v/v, 1ml per 100g body weight i.p.). Five minutes after Acetic acid injection, mice were placed in individual cages and the number of abdominal contractions was counted for each mouse for a period of 10 minutes. Percentage inhibition of writhing was calculated using the formula:

$$\text{Percentage Inhibition(\%)} = \frac{\text{mean no. of writhings(control)} - \text{mean no. of writhings(test)}}{\text{mean no. of writhings(control)}}$$

Hot-plate test: Mice (20-25g) of both sexes were fasted overnight before the study. Experimental animals of either sex were randomly selected and divided into five groups designated as group I - V consisting of five mice per group. Each group received a particular treatment i.e. control (Normal saline, 10ml/kg, p.o.), positive controls (Diclofenac sodium 10 mg/kg, p.o. and morphine 5mg/kg, p.o.) and the extract treated groups (methanolic extract of 250mg/kg, p.o. & 500 mg/kg, p.o. respectively). The animals were

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positioned on Ugo Basile's hot plate kept at a temperature of 55 ± 0.5 °C in order to obtain the animal's response to electric heat-induced nociceptive pain stimulus (licking of the forepaws and eventually jumping out of the glass beaker). Jumping out of the beaker was taken as an indicator of the animal's response to heat-induced nociceptive pain stimulus. The time taken for each mouse to jump out of the beaker (i.e. reaction time) was recorded in seconds. Readings were taken at intervals of 30, 60 and 90 minutes after extract administration. A cut off period of 15 s (Franzotti *et al.*, 2000) was observed to avoid damage to the paw.

RESULTS

The phytochemical analysis of the extract/fraction obtained is shown in Table 1. All fractions of GCL evoked significant ($p < 0.05$) and dose dependent reduction in the number of writhes in rats (Table 2). Hexane (54.48%) and ethyl acetate (54.48%) fractions respectively showed the highest percentage inhibition but incomparable to the standard drug Diclofenac-sodium with 85.07% inhibition. The effect of the extract on hot plate reaction time is shown in Table 3. A significant ($p < 0.05$) and a dose dependent elevation of the after-treatment reaction time to thermal pain was evident in the GCL fraction treated groups as well as the standard drug. The effect of the Ethyl acetate fraction (200mg/kg) was comparable to that produced by 10mg/kg of Diclofenac-sodium.

Table 1: Phytochemical screening of activity guided fractions of GCL extract

Phytochemical	Crude extract	GCL Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Butanol fraction	Last remaining aqueous fraction
Alkaloid	ND	ND	ND	ND	ND	ND
Glycosides	++	ND	ND	+++	+++	+++
Cardiac glycoside	+	ND	ND	+	+	ND
Saponin glycoside	++	+	ND	+++	+++	+
Flavonoid	+	+	ND	+++	+++	ND
Saponins	+++	++	ND	+++	+++	++
Tannins	+++	ND	ND	+++	+++	+
Pseudotannins	+++	+	+	+++	++	++
Balsam	+	ND	+	+++	+++	++
Anthraquinone	++	ND	ND	++	++	ND
Steroids	+++	ND	ND	++	ND	ND
Volatile oil	ND	ND	ND	ND	ND	+

+ = trace amount, ++ = mildly present, +++ = highly present, ND = not detected

Table 2: Effects of GCL fractions on Acetic acid induced writhing in rat

Treatment group	Dose (mg/kg)	Mean no. of writhing	%inhibition
Control	5ml/kg	33.50 ± 5.98	-
	50	15.25 ± 2.39*	54.48
Hexane fraction	100	16.75 ± 1.89*	50
	200	17.50 ± 2.40*	47.76
	50	26.50 ± 4.29	20.9
Chloroform fraction	100	19.50 ± 1.32*	41.79
	200	18.25 ± 2.10*	46.21
	50	18.00 ± 1.35*	46.27
Ethyl acetate fraction	100	15.25 ± 1.32*	54.48
	200	15.50 ± 0.65*	53.73
	50	22.00 ± 2.12*	34.85
Butanol fraction	100	20.25 ± 2.87*	40.15
	200	18.25 ± 1.11*	46.21
Last remaining aqueous fraction	50	18.25 ± 2.96*	46.21
	100	20.25 ± 4.39*	40.15
	200	20.50 ± 1.19*	38.8
Diclofenac sodium	10	5.00 ± 2.04**	85.07

* $P < 0.05$ compared to control ** $p < 0.01$ compared to control using Graph pad InStat Software (San Diego, USA)

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Table 3: Effects of activity guided GCL fractions on hot plate test

Treatment group	Dose (mg/kg)	Response time (sec)			
		0 minutes	30 minutes	60 minutes	90 minutes
Control	5ml/kg	2.60 ± 0.15	2.63 ± 0.27	2.00 ± 1.44	2.10 ± 0.15
	50	3.08 ± 0.23	3.58 ± 0.70	4.98 ± 0.25*	5.38 ± 1.42*
Hexane fraction	100	2.60 ± 0.14	4.08 ± 0.67*	4.98 ± 0.40*	6.20 ± 0.54*
	200	2.95 ± 0.31	5.28 ± 0.26*	5.30 ± 0.47*	6.85 ± 0.39*
Chloroform fraction	50	3.08 ± 0.62	4.87 ± 0.61*	6.30 ± 0.37*	5.95 ± 0.83*
	100	3.70 ± 0.38	4.35 ± 0.58*	5.18 ± 0.92*	5.45 ± 0.61*
Ethyl acetate fraction	200	3.53 ± 0.91	4.50 ± 0.61*	5.03 ± 0.63*	5.50 ± 0.31*
	50	3.45 ± 0.24	3.58 ± 0.13	3.70 ± 0.79	4.58 ± 0.45*
Butanol fraction	100	3.38 ± 0.20	3.13 ± 0.51	6.60 ± 0.51*	6.95 ± 0.29**
	200	2.85 ± 0.26	4.00 ± 0.22*	6.10 ± 0.65*	7.07 ± 0.30**
Last remaining aqueous fraction	50	3.48 ± 0.36	5.08 ± 0.31*	6.38 ± 0.13*	6.33 ± 0.40*
	100	3.10 ± 0.32	5.05 ± 0.44*	6.30 ± 0.36*	6.83 ± 0.64*
Diclofenac sodium	200	3.55 ± 0.28	5.08 ± 0.43*	6.13 ± 1.25*	6.85 ± 0.79 *
	50	2.30 ± 0.18	3.80 ± 0.53	4.15 ± 0.88*	4.10 ± 0.64*
Diclofenac sodium	100	3.28 ± 0.13	3.55 ± 0.38	5.22 ± 0.46*	3.65 ± 0.48
	200	3.55 ± 0.25	4.23 ± 0.23*	4.85 ± 0.66*	5.83 ± 0.39*
Diclofenac sodium	10	3.63 ± 0.14	5.70 ± 0.95*	6.55 ± 0.71*	8.60 ± 0.66**

*P<0.05 compared to control **p<0.01 compared to control using Graph pad Instat Software (San Diego, USA)

DISCUSSION

Exhaustive extraction is usually carried out with different solvents of increasing polarity in order to extract as much as possible the most active components with highest biological activity. In the present study, powdered GCL was sequentially extracted with solvents of increasing polarity: hexane, chloroform, ethyl acetate and butanol.

Pain and inflammation are associated with pathology of various clinical conditions like arthritis, cancer, and vascular diseases (Weitzmann and Gordan, 1990). The GCL fractions exhibited a significant analgesic activity in different animal models of pain. The abdominal constriction method is very sensitive and can detect anti-nociceptive effect of substances at a dose that cannot be detected by other methods, such as the tail-flick test (Collier *et al.*, 1968) while hot plate method has been found to be suitable for evaluation of centrally acting analgesics (Ibironke and Ajiboye, 2007).

In acetic acid induced method, pain is generated indirectly via endogenous mediators like prostaglandin, which stimulates peripheral nociceptive neurons. These neuronal fibers are sensitive to both narcotics and non steroidal anti-inflammatory drugs (Collier *et al.*, 1968). Acetic acid induced writhing in mice which is attributed to visceral pain has been widely used in screening analgesic drugs (Hasan *et al.*, 2010). Abdominal constriction responses have been reported to partly involve local peritoneal receptors (Bentley *et al.*, 1981). In this study GCL fractions significantly inhibited the acetic acid induced pain but not comparable to the standard drug Diclofenac. This strongly suggests that the mechanism of action of these fractions may be linked partly to lipoygenases and/or cyclo-oxygenases (Ukwuani *et al.*, 2013).

Hot plate method is based on the mice paw sensitivity to heat at temperatures not damaging the skin which is observed as jumping, paw licking and paw withdrawal (Rao *et al.*, 2008). The time until these responses occur is prolonged after administration of centrally acting analgesics. In the present study, GCL fractions was found to affect jumping, withdrawal or paw licking response which makes it evident that it is centrally acting. This suggests implication of μ receptors in the analgesic effect. The significant analgesic activity exhibited by GCL fractions suggests an advantage of its use as an anti-inflammatory agent. GCL fraction has inhibited both types of pain which suggests that the fractions may act as a opioid analgesic. Previous studies on the hydromethanolic extract of GCL have also been reported to act centrally and peripherally (Ukwuani *et al.*, 2013).

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Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases (Cragg *et al.*,` 1999). Many of these natural products have been shown to present interesting biological and pharmacological activities and are used as chemotherapeutic agents or serve as the starting point in the development of modern medicines (Verpoorte, 1998; Verpoorte, 2000). In this study, phytochemical screening revealed the presence of flavonoids, steroids, tannins, anthraquinones, cardiac glycosides and saponins mainly. Flavonoids and tannins are known to target prostaglandins which are involved in late phase of acute inflammation and pain perception (Rajnarayana *et al.*,` 2001; Mule *et al.*,` 2008). Hence, the presence of flavonoids and tannins may be contributory to the analgesic activity of GCL fractions. Alkaloids, glycosides and tannins have been reported for analgesic activity (Deb *et al.*,` 2010; Starec *et al.*,` 1988).

Conclusion

On the bases of the outcome of the present study, it is concluded that GCL fractions is endowed with potential analgesic activity and this further scientifically justifies the use in folklore remedies as an analgesic and anti-inflammatory agent. Although the mechanism of action was not determined, the clinical implication of these findings must await further studies.

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