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## Anti-inflammatory and analgesic activities of *Annickia Polycarpa* stem bark and its constituents

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### ABSTRACT

*Annickia polycarpa* stem bark is used in West Africa to treat ailments related to pain and inflammation in traditional medicine. Yet, these biological activities of the plant have not been investigated. To investigate the anti-inflammatory and analgesic activities of *A. polycarpa* stem bark (ASE) and its constituents. The compounds were isolated using column chromatography and their structures identified by spectroscopic methods. Anti-inflammatory activity was evaluated by carrageenan induced edema test. Analgesic activity was evaluated using the hot plate and the acetic acid-induced writhing tests. ASE at 10 mg/kg p.o demonstrated significant ( $p < 0.01$ ) anti-inflammatory activity of 69.64 %, analgesic activity of 82.54 % and 36.81 % in the hot plate and writhing tests respectively. The results also showed that the chloroform fraction of ASE was the most active. Palmatine and jatrorrhizine were isolated from the chloroform fraction. Palmatine and jatrorrhizine produced ( $p < 0.01$ ) anti-inflammatory and analgesic effects. Their analgesic activity range from 85-94 % at 9 mg/kg p.o. (which was higher than 43.3 % given by tramadol at the same dose) in the hot plate assay. The analgesic activity range from 47.28 - 44.25 % in the writhing assay which was higher than 32.92 % produced by diclofenac at 6 mg/kg p.o. Naloxone abolished the analgesic effects of ASE and palmatine in the hot plate test. This indicates that ASE and its constituents acts as opioids to relieve pain. The findings provide scientific backing for traditional use of *A. polycarpa* stem bark as anti-inflammatory and analgesic agent.

**Keywords:** Alkaloids, Nociceptors, Antagonist, Opioid. Bioassay-guided fractionation

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### INTRODUCTION

*Annickia polycarpa* Sten and Mass is also known as *Enantia polycarpa* Engl. and Diels. It is a tree that belongs to the Annonaceae family of flowering plants and grows up to 18.30m high and 0.91 m wide [1]. The bark is hard and greenish grey revealing a bright yellow color when removed [1]. The wood is dazzling yellow all through but slowly turns brown [1]. The leaf is 20.30-30.50 cm long and 8.90 cm wide, ovate to elliptic-oblong or oblong-lanceolate in

shape without indentation with an obtuse base[1]. The leaf have simple and stellate hairs on their lower surface and 8 to 10 pairs of lateral nerves[1].

The bark of *A. polycarpa* is used in traditional medicine to treat malaria fever, fever, stomach ulcer, eye infections, leprosy wounds, injuries and bacterial infections[1,2,3,4]. Few biological activities such as antibacterial, antitrypanosome and antimalarial activities of the stem bark extracts[3, 5, 6] of this plant were studied.

This study therefore seeks to investigate the anti-inflammatory and analgesic activities and mechanism of antinociceptive effect of the ethanol extracts of the stem bark of *A. polycarpa* with the major goal of isolating the compounds responsible for these activities using bioassay-guided fractionation.

## MATERIALS AND METHODS

### Collection of plant parts and preparation of extracts

*A. polycarpa* stem bark was collected from Bobiri forest Reserved (Kumasi) in the Ashanti region of Ghana in November 2012 and authenticated by Mr. Ntim Gyakari, a botanist. Sample of *A. polycarpa* stem bark was assigned voucher specimen number CPM0512 and deposited in the herbarium of C.P.M.R. The stem bark of *A. polycarpa* was chopped into pieces, air dried for 34 days and pulverized. The pulverized material (4.5 kg) was extracted with 30 L of ethanol at room temperature for 4 days and filtered. The marc was re-extracted with ethanol (26 L x 3) filtered. The extracts were combined and dried with a rotary evaporator to obtain yellowish brown solid which was labeled ASE (312.30 g; 6.94 % w/w). The solid (208.2 g) was suspended in 80 % ethanol-water (2.4 L) and divided into 3 equal portions of 0.8 L each. Each portion was extracted with pet ether (0.8 L x 4). The pet ether fractions obtained were combined and dried in a rotary evaporator at 40 °C. The ethanol was thereafter evaporated from the pet ether insoluble fractions using the rotary evaporator. The mixtures obtained were diluted with extra distilled water and extracted with chloroform (0.8 L x 4), combined and dried in a rotary evaporator at 45 °C to obtain the brownish yellow syrup chloroform fraction. The aqueous fractions left were freeze dried. A total of 18.44 g of pet ether (AP), 87.37 g of chloroform and 102.38 g of aqueous (AER) fractions were obtained.

The results of the pharmacological assays of the various fractions on the stem bark of *A. polycarpa* extract showed that the chloroform fraction was the most active. It was therefore selected for fractionation so as to isolate the active constituents.

The dried chloroform fraction of *A. polycarpa* stem bark, AC (34.0 g), was subjected to column chromatography over normal phase silica gel (500 g) (Merck; 60-120 mesh size). Elution was carried out with stepwise gradient mixture of pet ether/ethyl acetate until 100 % ethyl acetate was achieved. It was followed by gradual introduction of ethanol (at increasing rate of 10 %) to the ethyl acetate until 100 % ethanol was achieved. A total of 150 fractions (about 200 ml per fraction) were collected from the column. The fractions were grouped by their TLC profile to obtain a total of nine groups (A1-A9). Each was concentrated to about 150 ml.

**A8C** (15 mg) was obtained as bright orange amorphous solid from fraction A6 eluted with ethyl acetate/ethanol 30:70. The solid obtained was thoroughly washed with a mixture of acetone with few drops of methanol.

**A6C** (100 mg) was obtained from fraction A8 eluted with ethyl acetate/ethanol 10:90 as yellow crystals.

### Drugs and other chemicals

Carrageenan, Naloxone hydrochloride dehydrate, Atropine sulphate and Acetic acid were procured from Sigma Chemical Co. (St. Louis USA). Indomethacin was procured from Cayman Chemical Company. Diclofenac Sodium Chloride was purchased from Bliss GVS, (India), Tramadol Hydrochloride was purchased from Bristol Laboratories Ltd and Tween 80 was from VWR International, PROLABO (CE).

### Animals

Animals were acquired from the Animal House Unit of C.P.M. R. They were accommodated in aluminum cages under standard temperature and pressure and given free access to sterile water and feed *ad libitum*. Mice used in the acetic acid-induced assays were fasted overnight just before the experiment. Care and handling of the animals were done according to Foundation for Biomedical Research rules and methods on the use of animals in research [7].

**Dose regime**

Right doses of ASE and all the standard drugs used were prepared and administered at a rate of 2 ml/rat or 0.2 ml/mouse. The vehicle controls were also administered at the corresponding rates.

**Acute toxicity studies**

The safety or toxicity associated with the short term use of the ASE was assessed in 2 groups (n=6) each of male Sprague-Dawley rats, Swiss albino mice and C57/BL6 mice. ASE at 2 500 and 5 000 mg/kg p.o in 2 % Tween 80 aqueous solutions were administered at 10 ml/kg body weight per each animal. The rats were observed for signs of toxicity such as pilo-erection, motor impairment, sedation, salivation, hyper excitability and death within 24 h and for extra 16 days.

**ANTI-INFLAMMATORY ACTIVITY STUDIES****Carrageenan-induced paw edema assay**

The anti-inflammatory activity of the extracts was studied in rats using the carrageenan induced paw edema model [8]. Sprague – Dawley rats (35) of either sex were divided into 7 groups (n=5). ASE were administered at 10, 100 and 1 000 mg/kg p.o (Group 1-3); Indomethacin at 9, 15, 30 mg/kg p.o (Group 4-6) as the reference drug, and 2% Tween 80 aqueous solution p.o (Group 7) as vehicle control. Inflammation was induced by injection of 1 % w/v carrageenan in 0.9 % normal saline (at 0.1 ml each) into the sub plantar area of right hind paw of each rats 1 h post each treatment. Paw volumes were measured by volume displacement using plythesmometre (UGO Basile 7140) before (Vo) and at an hour interval (Vt) from 1-5 h after carrageenan injection.  $V_t - V_o$  equals edema [9]. The percentage inhibition of the inflammation was calculated as:

$$\text{anti-inflammatory activity} = ((V_t - V_o)_{\text{tec}} - (V_t - V_o)_{\text{ted}}) / (V_t - V_o)_{\text{tec}} \times 100 \%$$

Where  $(V_t - V_o)_{\text{tec}}$  = Total edema response of vehicle treated control group.  $(V_t - V_o)_{\text{ted}}$  = Total edema response of drug treated group.

**ANALGESIC ACTIVITY STUDIES****Hot plate assay**

The mouse hot plate test used to evaluate the antinociceptive action of ASE was performed as previously reported [10]. C57/BL6 mice (28) of either sex were divided into 10 groups (n=4). The mice were separately placed on an electric hot plate (UGO Basile hot/cold plate 35 100) maintained at  $55 \pm 0.5$  °C and the time taken to lick, lift, shake or stamp any of the hind limbs or jump constitute latency time, recorded as antinociceptive response. Baseline latencies ( $T_o$ ) were obtained as mean of two determinations prior to any treatment. Only mice with baseline latency of 3.5–9 s were used. Extracts were administered at 10, 100, and 1000 mg/kg p.o. (Group 1-3); tramadol at 3, 9 and 15 mg/kg p.o. (Group 4-6); 2% Tween 80 aqueous solution was used as vehicle control (group 10). Latency of each mouse was measured at an hour ( $T_t$ ) interval for 5 h post treatment. The antinociceptive response were expressed as a percentage of pain threshold inhibition (%PTI) [11] calculated as:

$$\%PTI = (T_t - T_o) / T_o \times 100$$

Overall analgesic response (%OPTI) for each group was obtained as the sum of mean %PTI of all animals per group over the experimental period.

**Acetic acid induced-writhing test**

Analgesic effect of ASE was also evaluated in the acetic acid induced-writhing model as described [12]. Swiss albino mice (35) were divided into 10 groups (n = 5). ASE was administered at 10, 100, 1000 mg/kg p.o (Group 1–3); diclofenac sodium at 2, 10, 50 mg/kg p.o (group 7-9); 2 % Tween 80 aqueous solution served as vehicle control (group 10). Aqueous acetic acid (1 % v/v) was injected at 1 ml/100g (i.p) per mouse 45 min post treatment. The number of writhing movements and stomach contortions in each mouse was counted for 20 min after induction. The inhibition of writhing movements in the treated group(s) compared with the control group was taken as the percentage analgesic effect (%AE) [11] which was calculated using the formula:

$$\%AE = (MR_c - MR_t) / MR_c \times 100$$

Where:  $MR_c$  = mean writhing count of the control;  $MR_t$  = mean writhing count of CRE or drug treated group.

**Mechanism of antinociceptive activity**

The effect of the opioid antagonist, naloxone on ASE induced-analgesia was evaluated in the hot plate assay using 3 groups (n=4) of C57BL/6 mice. Administration was done as follows: ASE 10 mg/kg p.o. followed by naloxone 2 mg/kg (i.p) (in distilled water) at 10 ml/100 g was administered 45 min after ASE was given (Group 1). ASE at 10mg/kg p.o administered alone constitutes group 2 and 2% Tween 80 aqueous solution formed the control set (Group 3). The experimental procedures were carried out as described in the hot plate test above. The effect of, atropine on analgesic activity of ASE was also evaluated in the acetic acid-induced writhing assay. Three (3) groups of Swiss albino mice (n=4) were used. Administration was done as follows: ASE at 10 mg/kg p.o. preceding atropine 5 mg/kg (i.p) (in water) 30 min (Group 1), ASE at 10 mg/kg p.o. (Group 2) and 2 % tween 80 aqueous solution as vehicle control (Group 3).

**Statistical analysis**

All statistical analysis was performed using Graph Pad Prism Version 5.03 with the level of significant set at 95 % confidence interval of difference.

**RESULTS****Acute toxicity studies**

ASE administered at 2 500 and 5 000 mg/kg p.o showed no sign of toxicity or death of the SDR rats, ICR or C57/BL6 mice during the observation period.

**Identification of the isolated compounds**

A6C: Bright yellow crystals.  $R_f = (\text{CH}_3\text{OH}: \text{C}_2\text{H}_5\text{COOH}): 30: 1 = 0.723; 40:1 = 0.3784$ . UV (recorded in methanol)  $\lambda_{\text{max}} = 279\text{-}436 \text{ nm}$ . IR (KBr)  $\nu_{\text{max}} = 3496, 3072, 2979, 1634, 1384 \text{ and } 1242 \text{ cm}^{-1}$ . GC-MS (recorded in methanol): m/z (relative abundance) 389 (2.5 %), 279 (5 %), 167.01 (32.5 %), 149.14 (100%), 150.14 (10 %), 104.22 (5 %), 71.28 (21.25) and 55.24 (8.75 %). The molecular ion peak at m/z 389 represents  $M^+ + 2$ , suggestive of the formula  $\text{C}_{22}\text{H}_{21}\text{NO}_4$  calculated for Palmatine chloride.

$^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz),  $\delta$  7.65 (1H, s, H-1), 7.04 (1H, s, H-4), 3.30 (2H, t, J = 8.80 Hz, H-5), 4.93 (2H, t, J = 6.36 Hz, H-6), 9.75 (1H, brs, H-8), 8.09 (1H, d, J = 9.28 Hz, H-11), 8.01 (1H, d, J = 8.76 Hz, H-12), 8.80 (1H, s, H-13), 3.96 (3H, s, 2-OCH<sub>3</sub>), 4.02 (3H, s, 3-OCH<sub>3</sub>), 4.23 (3H, s, 9-OCH<sub>3</sub>), 4.17 (3H, s, 10-OCH<sub>3</sub>).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz),  $\delta$  109.9 (C-1), 151.3 (C-2), 154.2 (C-3), 112.7 (C-4), 130.4 (C-4a), 28.2 (C-5), 56.4 (C-6), 146.7 (C-8), 123.6 (C-8a), 146.1 (C-9), 152.3 (C-10), 128.4 (C-11), 124.9 (C-12), 135.6 (C-12a), 121.7 (C-13), 140.1 (C-13a), 120.8 (C-13b), 57.5 (2-OCH<sub>3</sub>), 57.2 (3-OCH<sub>3</sub>), 63.0 (9-OCH<sub>3</sub>), 57.8 (10-OCH<sub>3</sub>).

A8C was obtained as orange crystal:  $R_f = (\text{CH}_3\text{OH}: \text{C}_2\text{H}_5\text{COOH}): 30:1 = 0.761; 50:1 = 0.653$  UV (methanol)  $\nu_{\text{max}} = 270\text{-}442 \text{ nm}$ : IR (KBr)  $\lambda_{\text{max}} = 3428, 3353, 3067, 2941, 1364 \text{ and } 1276 \text{ cm}^{-1}$  indicating the presence of OH group, C=N+H group, benzene ring(s) and methylene ( $\text{C}_2\text{H}_2$ ) groups in the compound. GC-MS (recorded in methanol): m/z (relative abundance) 316.82245 (100%), 419.011 (47.50%), 396.6316, (12.5%) The molecular ion peak at m/z = 396.6316 represents  $M + \text{Na}$  which is consistent with the  $\text{C}_{20}\text{H}_{20}\text{NO}_4^+\text{Cl}^-$  (Molecular formula 273 g/mol).

$^1\text{H}$  NMR ( $\text{CD}_3\text{OH}$ , 400 MHz),  $\delta$ : 7.63 (1H, s, H-1), 6.84 (1H, s, H-4), 4.09 (2H, t, J = 4.88 Hz, H-5), 4.85 (2H, t, J = 10.24 Hz, H-6), 9.72 (1H, brs, H-8), 8.08 (1H, d, J = 7.36 Hz, H-11) 8.00 (1H, d, J = 7.88 Hz, H-12), 8.75 (1H, s, H-13), 4.01 (3H, s, 2-OCH<sub>3</sub>), 4.08 (3H, s, 9-OCH<sub>3</sub>), 4.20 (3H, s, 10-OCH<sub>3</sub>).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OH}$ , 400 MHz),  $\delta$ : 110.02 (C-1), 149.64 (C-2), 146.15 (C-3), 115.89 (C-4), 130.25 (C-4a), 27.65 (C-5), 56.96 (C-6), 146.15 (C-8), 119.41 (C-8a), 151.76 (C-9), 145.64 (C-10), 123.16 (C-11), 124.39 (C-12), 128.02 (C-12a), 119.41 (C-13), 135.40 (C-13a), 120.93 (C-13b), 57.39 (2-CH<sub>3</sub>), 62.52 (9-CH<sub>3</sub>), 57.63 (10-CH<sub>3</sub>).

The spectra data obtained for A6C and A8C compared favorably with those obtained for palmatine and jatrorrhizine from literature [13, 14]. Hence **A6C** was identified as **palmatine** and **A8C** as **jatrorrhizine**.

The structures of the isolated compounds are shown below in Figure 1.

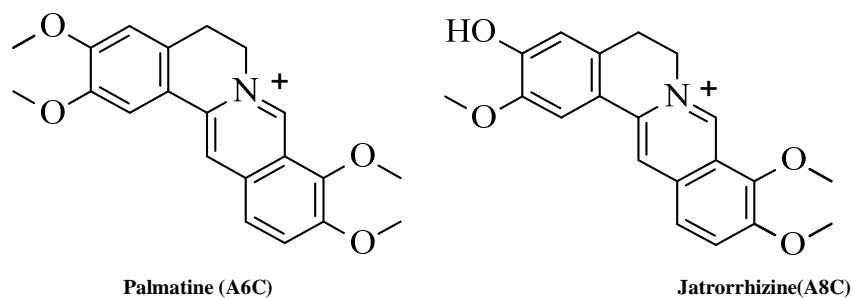
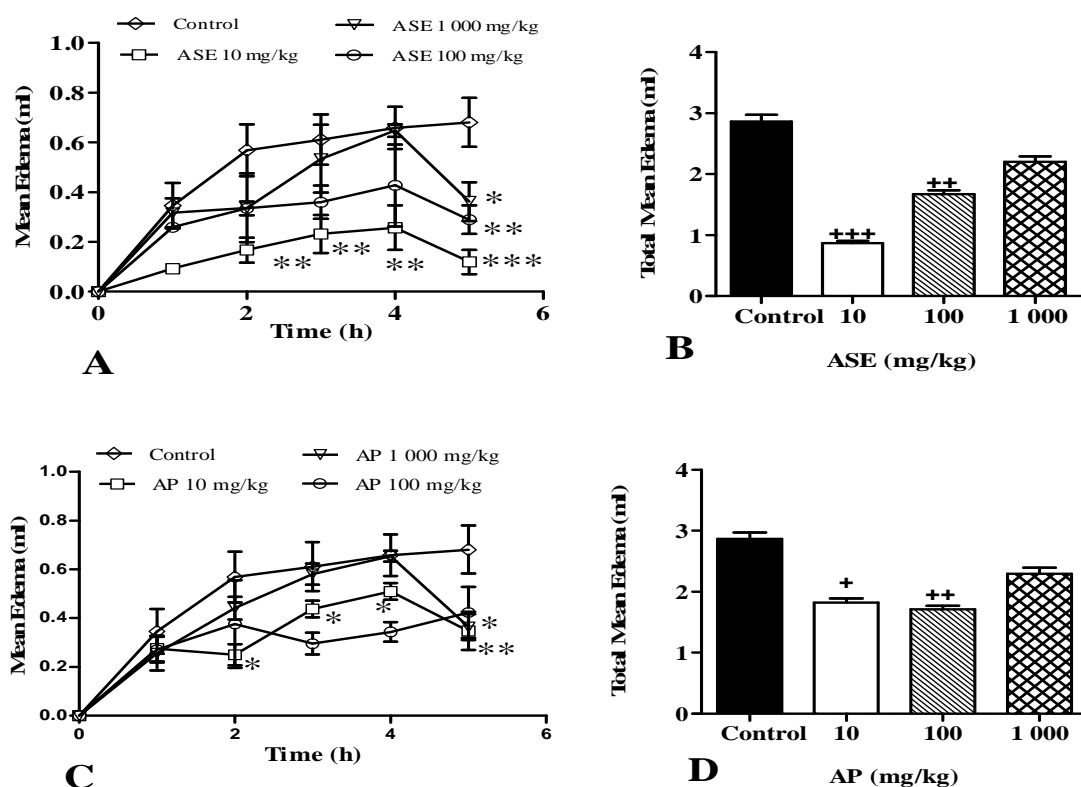
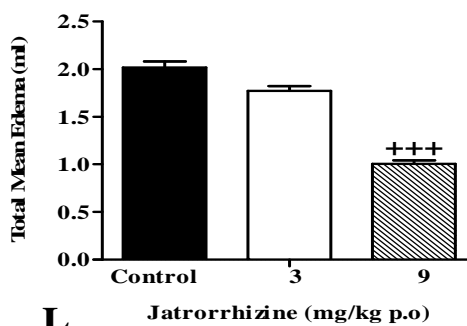
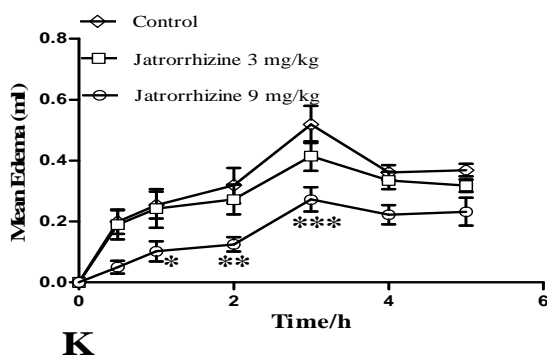
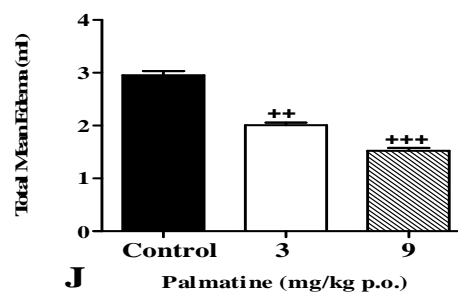
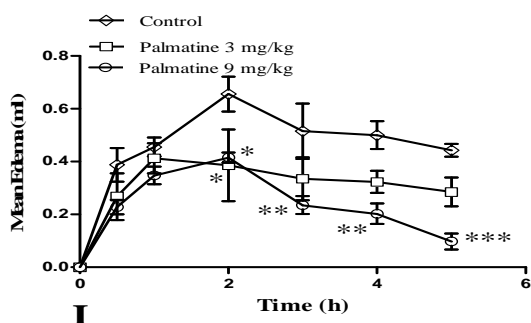
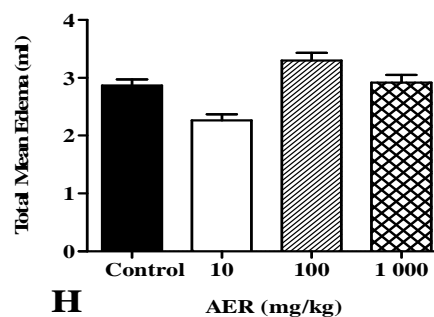
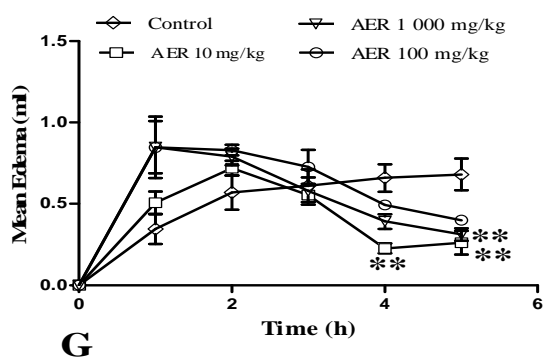
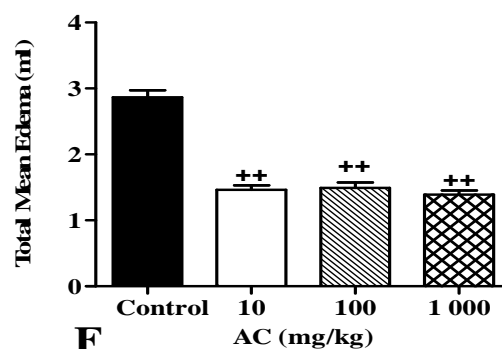
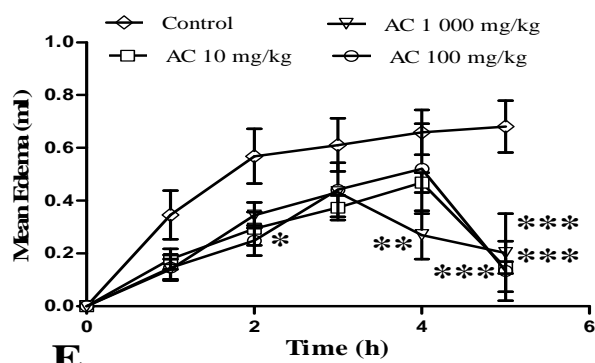


Figure1: Structures of isolated compound

**Anti-inflammatory effect**

The results of the anti-inflammatory activity of ASE, its fractions, isolated constituents and indomethacin are shown below in Figure 1 and Table 1. ASE showed significant ( $p < 0.01$ ) anti-inflammatory effect from 2-5 h.





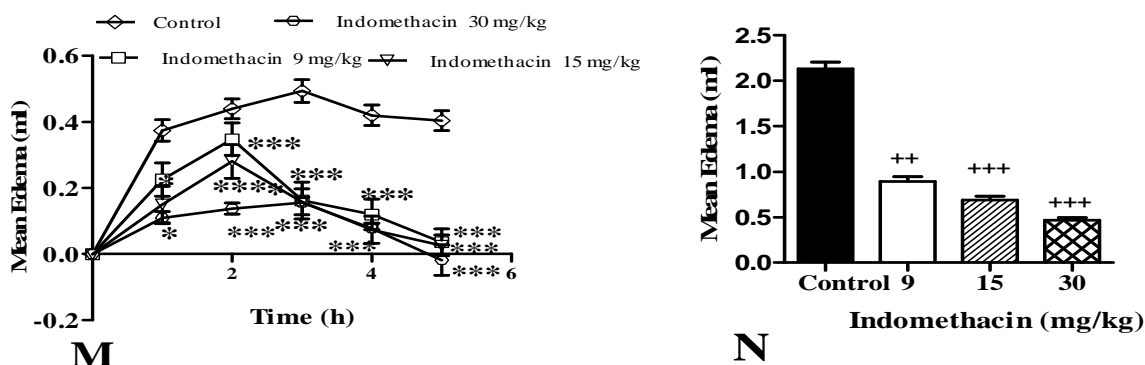


Figure 2: Anti-inflammatory effect of ASE, its pet ether (AP), chloroform (AC) or aqueous fractions (AER) (at 10-1000 mg/kg p.o) respectively; palmitine and jatrorrhizine (at 3-9 mg/kg p.o) on time course curves (A, C, E, G, I, K, M) and total mean edema response (B, D, F, H, J, L, N) respectively on carrageenan induced inflammation in rats. Values are expressed as means  $\pm$  SEM ( $n=5$ ). \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  compared to vehicle treated control group (Two-way ANOVA followed by Bonferroni's post hoc test). + $p < 0.05$ ; ++ $p < 0.01$ ; +++ $p < 0.001$  compared to vehicle treated control group (One-way repeated measures ANOVA followed by Tukey's post hoc test).

Table 1: Anti-inflammatory activity of ASE, its fractions and isolated constituents

	Anti-inflammatory activity(%)		
Dose (mg/kg p.o)	10	100	1000
ASE	69.64	41.63	23.24
AP	36.50	40.30	19.81
AC	49.03	59.50	47.98
AER	20.94	inactive	inactive
Dose (mg/kg p.o)	3	9	15 30
Palmitine	32.05	48.39	-
Jatrorrhizine	12.23	50.25	-
Indomethacin (dose mg/kg p.o)	58.15 (9)	67.71 (15)	78.23 (30)

## ANALGESIC EFFECTS

### Hot plate test

ASE demonstrated remarkable analgesic activity in the hot plate test. The highest overall analgesic response of 82.54 % at 10 mg/kg p.o was very remarkable ( $p < 0.01$ ). The analgesic activity of ASE, ITS fractions and isolates are shown below in Table 2.

Table 2: Analgesic activity(%) of ASE, its fractions and isolates in the hot plate test

	Analgesic activity, AE, (%)		
Dose (mg/kg p.o)	10	100	1000
ASE	82.54**	77.07**	53.07*
AP	-4.34	33.06	73.04*
AC	57.92	95.93**	89.87*
AER	-3.94	18.51	21.96
Dose (mg/kg p.o)	3	9	15
Palmitine	45.22	85.56**	-
Jatrorrhizine	51.35	93.87**	-
Tramadol	30.10	43.30*	45.50*

Values are expressed as means  $\pm$  SEM ( $n=5$ ). \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  compared to vehicle treated control group (One-way repeated measures ANOVA followed by Tukey's post hoc test).

### Acetic acid-induced writhing test

ASE showed significant ( $p < 0.05$ ) analgesic activity by protecting mice against writhing responses induced by acetic acid. The results of analgesic activities (%AE) calculated for ASE, and its fractions in addition to palmitine and jatrorrhizine are shown in Table 3 below.



Table 3: Analgesic activity (%) of ASE, its fractions and isolates in acetic acid-induced writhing assay

	Analgesic activity, AE, (%)		
Dose (mg/kg p.o)	10	100	1000
ASE	36.81*	32.59*	44.03**
AP	inactive	inactive	inactive
AC	47.33*	1.23	inactive
AER	1.23	40.33*	12.20
Dose (mg/kg p.o)	2	6	10
Palmatine	Inactive	47.28*	-
Jatrorrhizine	40.59**	44.25**	-
Diclofenac	23.51	32.92*	47.65*

Values are expressed as means  $\pm$  SEM (n = 4). \* $p$  < 0.05; \*\* $p$  < 0.01; \*\*\* $p$  < 0.001 compared to vehicle treated control group (One-way ANOVA followed by Tukey's post hoc test).

### Mechanism tests

ASE at 10 mg/kg p.o alone group of mice produced overall analgesic response of 82.54 %. But that of ASE at 10 mg/kg p.o followed by naloxone at 2 mg/kg i.p was greatly abolished ( $p$  < 0.01) to -44.72 % (Fig.3: P and Q). The protoberberine alkaloid, palmatine also demonstrated analgesic activity of 84.91%. This was significantly ( $p$  < 0.05) reduced to -19.56 % when it was co-administered with naloxone. In the writhing test, there were no significant ( $p$  > 0.05) difference in analgesic activity of ASE alone when compared with the group that was given the ASE/palmatine in addition to atropine at 5 mg/kg i.p (Fig.3T and U). The effect of naloxone on analgesic effect of jatrorrhizine could not be evaluated in this experiment. However, atropine had no significant effect on the analgesic activity of jatrorrhizine in the writhing test.

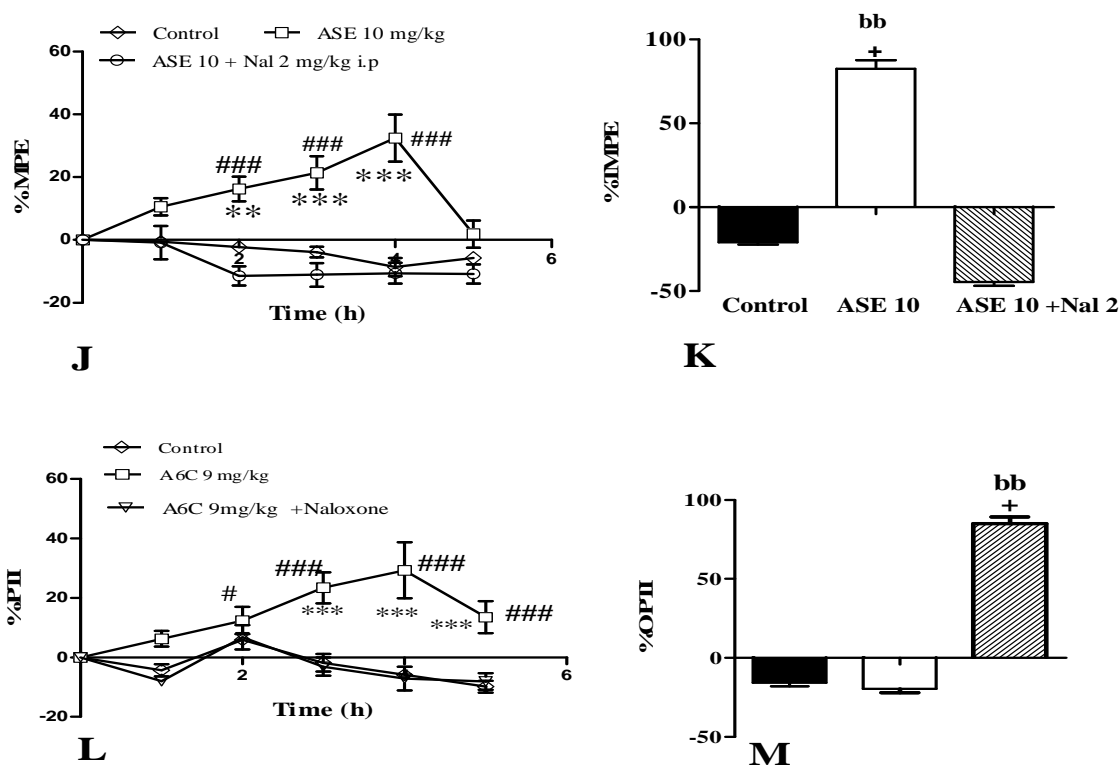


Figure3: Effect of Naloxone (2 mg/kg i.p.) on analgesic effect of ASE and CRE in the hot plate test on time course curves (P, R) and total pain response (Q, S); and atropine (5 mg/kg i.p) in the acetic acid-induced writhing test (T, U).

Values are expressed as means  $\pm$  SEM. (n = 4). \* $p$  < 0.05; \*\* $p$  < 0.01 compared with vehicle treated control group; <sup>a</sup> $p$  < 0.05; <sup>##</sup> $p$  < 0.001 mg/kg p.o. Agonist 9 mg/kg + Naloxone 2 mg/kg i.p (Two-way ANOVA followed by Bonferroni's post hoc test). <sup>++</sup> $p$  < 0.01, <sup>a</sup> $p$  > 0.05 compared with vehicle treated control or ASE + atropine (5 mg/kg i.p.) (One-way repeated measures ANOVA followed by Tukey's post hoc test). <sup>b</sup> $p$  < 0.05, <sup>bb</sup> $p$  < 0.01 compared with agonist 9 mg/kg p.o + naloxone (2 mg/kg i.p) (One-way repeated measures ANOVA followed by Tukey's post hoc test).



## DISCUSSION

The results obtained from the acute toxicity assay indicates that the mean lethal dose (LD<sub>50</sub>) of ASE was above 5 000 mg/kg p.o. and that ASE is safe for the short term use when taken orally even up to 5 000 mg/kg at once.

In all the three tests performed on ASE and its fractions, the results obtained indicate that; ASE possessed potent anti-inflammatory and analgesic activities. The pet ether fraction (AP) was inactive in the writhing test but showed activity in the other 2 assays. The aqueous fraction (ARE) was also inactive or showed insignificant ( $p > 0.05$ ) anti-inflammatory and analgesic activities in the carrageenan induced edema test and the hot plate test respectively. Hence AP and AER were not considered for chromatography. It was only AC that showed significant anti-inflammatory and analgesic activity in all the 3 assays. This shows that the anti-inflammatory and analgesic principles of *A. polycarpa* stem bark resides in its chloroform fraction (AC). AC was therefore selected and chromatographed.

Carrageenan-induced edema in rat paws is a notable test used to screen anti-inflammatory substances [8] and had been established to be a three phase process mediated by sequential release of several mediators [15]. The 0-1.5 h after carrageenan injection involves the release of histamine and serotonin and constitute Phase 1 followed by Phase 2 mediated by liberation of bradykinin from the 1.5 to 2.5 hours [16] and the final phase mediated by prostaglandins (PGI<sub>2</sub>) and slow reaction agents release from 2.5 to 6 hours [17]. ASE demonstrated significant ( $p < 0.05$ ) anti-inflammatory action starting from the 2 h to the 5 h. These periods correspond to the Phase 2 and Phase 3 of the acute inflammatory process mediated by bradykinins and prostaglandins. Therefore, the mechanism of anti-inflammatory action of ASE may be due to its inhibition of bradykinins and prostaglandins synthesis or action.

The results obtained from this experiment also indicates that Palmatine inhibits inflammation ( $p < 0.05-0.001$ ) from 2-5 h. This period corresponds to the 2<sup>nd</sup> and 3<sup>rd</sup> phase of the acute inflammatory reaction. Therefore, palmatine exerts anti-inflammatory activity by inhibition of inflammatory mediators such as histamine, serotonin and prostaglandins.

The anti-inflammatory action of palmatine was well document as demonstrated in its ability to significantly inhibit xylene-induced ear inflammation [18], acetic acid-induced increase in vascular permeability and serotonin-induced hind paw edema [19]. This compound was also reported to exhibit antioxidant properties [20]. Xylene-induced ear edema and increased vascular permeability assays involve released of inflammation mediators subsequent to induction. This promotes dilation of arterioles and venules in addition to increased vascular permeability [21]. Thus the anti-inflammatory effects of palmatine is not only due to its inhibitory effects on mediators such as bradykinins and prostaglandins as described here in the carrageenan induced test or serotonins [19] but may also be due to its membrane-stabilizing property that reduces capillary permeability and/or antioxidant actions.

The result from the anti-inflammatory studies indicates that jatrorrhizine is a potent anti-inflammatory agent. The anti-inflammatory action of jatrorrhizine became statistically significant on the time-course curve between 1-3 h. This period corresponds to the first and the second phase of inflammatory response. Hence the anti-inflammatory action of jatrorrhizine may be mainly due to inhibition of histamine, serotonin and bradykinin production by the injured cells. The anti-inflammatory activity of jatrorrhizine was already known [22]. In addition, jatrorrhizine also exhibited anti-oxidant activity in various test models [23]. Therefore, the presence of Jatrorrhizine in the stem bark of *A. polycarpa* may also contribute to its anti-inflammatory action

The central analgesic effect of substances are evaluated using the hot plate test which is thermal-induced nociception model [11]. The ethanol extract of *A. polycarpa* stem bark (ASE) exhibited the most potent analgesic effect of 82.54 % at 10 mg/ kg p.o in the hot plate test among the crude ethanol extracts which was higher than that calculated for the highest response given by tramadol (45.50 %) at 15 mg/kg p.o. This suggests that ASE possessed immense central analgesic activity and could be a good source of analgesic agents that act by exerting their effect on the central nervous system.

Palmatine also demonstrated enormous analgesia in the mouse hot plate test with overall analgesic effect of 85.56 %. Thus palmatine also possessed substantial central antinociceptive action. Jatrorrhizine also demonstrated immense analgesic activity in the hot plate test indicating that this compound also has central antinociceptive effect. Therefore, the presence of palmatine and jatrorrhizine in the stem bark of this plant lend credence to the plant's use

in the treatment of various ailments in traditional medicine. To the best of our knowledge, this is the first study to report the central analgesic actions of palmatine and jatrorrhizine.

The acetic acid-induced writhing test is a visceral pain model [24] used in evaluating both central and the peripheral analgesic activities of substances [11]. Intraperitoneal injection of acetic acid into rats or mice abdominal cavity results in elevated levels of prostaglandins in the peritoneal exudates after about 30 min [25]. The stretching and abdominal contortions obtained in the writhing test therefore correlates with sensitization of nociceptors to the production of prostaglandins [11]. ASE and its constituents, palmatine and jatrorrhizine demonstrated significant inhibition of mean writhing counts in the mouse writhing assay in this experiment. Thus they possessed substantial analgesic effect. Hence, ASE, palmatine and jatrorrhizine are analgesic agent which act by inhibiting the cyclooxygenase enzyme and reduce the biosynthesis of prostaglandins in the mass cells of mice abdominal cavity.

The results from the mechanism assays indicate that naloxone antagonized the analgesic action of ASE and palmatine in the hot plate test but atropine did not have any significant effect on the analgesic actions of these substances in the writhing assay. It can therefore be concluded that ASE and palmatine produce analgesia by exerting their effects on the central endogenous opioid peptide nociceptors. ASE and palmatine therefore acts as opioid analgesic agents. The effect of naloxone on analgesic effect of jatrorrhizine could not be evaluated. But since atropine did not antagonize the analgesia of jatrorrhizine in the writhing assay we can assume that jatrorrhizine may also be acting through the central endogenous opioid nociceptors

This is the first study to report the anti-inflammatory and analgesic activities of *A. polycarpa* addition to its mechanism of antinociception. Furthermore, this study is the first to employ bioassay-guided fractionation to identify the anti-inflammatory and analgesic constituents of *A. polycarpa* stem bark as palmatine and jatrorrhizine and to establish the analgesic activity of jatrorrhizine and the mechanism of antinociception of palmatine.

## CONCLUSION

The present studies demonstrated that the ethanol extract of the stem bark of *A. polycarpa* (ASE) possessed immense anti-inflammatory and analgesic properties. It has also been shown that ASE and its isolated constituents, namely palmatine and jatrorrhizine acts as both non-steroidal anti-inflammatory and opioid analgesic agents. Toxicity studies also revealed that ASE safe for use in acute conditions. This study has therefore, provided some scientific basis for the traditional uses of *A. polycarpa* as therapeutic agent.

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