

In vivo Comparative Anti-Inflammatory and Analgesic Activities of Root Bark, Stem and Leaf Extracts of *Capparis erythrocarpus* (Capparaceae)

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ABSTRACT

Introduction: The root bark powder of the medicinal plant *Capparis erythrocarpus* is used at the Centre for Plant Medicine Research (In Ghana) against inflammatory pain disorders. The whole plant is destroyed to obtain the root bark which constitutes only 11.1 % of the plant. Hence, the plant is going extinct. We therefore sought to evaluate anti-inflammatory and analgesic activities of the root bark, stem and leaf of *C. erythrocarpus* in order to explore them as substitute(s) anti-inflammatory and analgesic agent to the root bark. **Methods:** Carrageenan induced paw oedema assay was used to evaluate anti-inflammatory activity. Hot plate and acetic acid induced writhing assays were employed to ascertain analgesic activity. **Results:** The extracts of the root bark (CRB) and leaf (CL) produced significant ($p < 0.05$) anti-inflammatory activity of 48.93 and 37.42 % at 50 and 200 mg/kg p.o. respectively whereas the stem extract (CS) was inactive. Moreover, the extracts produced significant ($p < 0.05$) analgesic activity. The analgesic activity (178.20 – 248.70 %) of the extracts were higher than that of morphine (136.70%) at 5 mg/kg i.m. in the hot plate assay. CS (200 mg/kg p.o.) produced the highest analgesic activity (54.61%) among the extracts and diclofenac sodium (41.15%) at 5 mg/kg p.o. in the writhing assay. **Conclusion:** These results indicates that the leaf of *C. erythrocarpus* can be substituted for the root as analgesic and anti-inflammatory agent. While, the stem can also be used as analgesic agent. This will save the plant from extinction.

Key words: Pain, Phytochemical constituents, Hot plate, Writhing assay, Oedema.

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INTRODUCTION

Capparis erythrocarpus is a shrub that belongs to the Capparaceae family of flowering plants. The plant is mostly scandent with 3-14 cm long and 1.5-5.0 cm broad leaves of different shapes that ranges from abovate through elliptic to ovate and lanceolate. Furthermore, the leaf has cuneate to rounded base and sharply acuminate to obtuse apex.¹ The plant produces 5.0 cm long scarlet fruits from flowers which sprouts as cluster of stout pedicels at the end of young branches.¹ The stem is full of sharp long thorns. The leaf is also full of clusters of tiny thorns.

The root of *C. erythrocarpus* is used in traditional medicine to treat rheumatoid arthritis, blurred vision, partial male impotence, urinogenital pain, otitis, ear pain and gas in the stomach.^{1,2} The powdered root bark of *C. erythrocarpus* (Known as Serapac) is an oral herbal medication dispense at the Centre for Plant Medicine research at Mampong - Akwapim, Ghana for treatment of arthritis, pain and inflammatory disorders. Chronic toxicity studies, anti-arthritis and anti-nociceptive activities of the root bark of

C. erythrocarpus have been evaluated.³⁻⁵ However, there has not been any scientific investigation of the medicinal properties of the stem and leaf of the plant. Furthermore, the whole plant is destroyed to obtain the root bark. This is causing extinction of the plant. This research therefore, seeks to evaluate the anti-inflammatory and analgesic activities of the root bark, stem and leaf of *C. erythrocarpus* in order to explore the stem and leaf as an alternate anti-inflammatory and/or analgesic agent to the root and hence, save the plant from extinction.

MATERIALS AND METHODS

Identification, Collection and Processing of Plant Materials

Five plants were uprooted at Dodowa in the Greater Accra Region of Ghana in January, 2017. The plant was identified by Mr. H. R. Blagoggee, a botanist at Centre for Plant Medicine Research (CPMR), Ghana. The root, stem and leaf were given voucher specimen number 4880, 4881 and 4883 and stored

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in the herbarium of CPMR. The root was cut off and washed with portable water. The root bark was removed and chopped into pieces. The leaves were also removed from the branches. The stem was also chopped into pieces. The plant materials were then sun dried for 8 days and their weights determined separately.

Extraction

The plant materials were separately pulverized and stored in air tight plastic bags. The pulverized root bark (1.0 kg) was extracted with 70 % ethanol (5 L x 2) for 5 days. The extracts were combined filtered and concentrated with rotary evaporator at 45°C. The aqueous extract obtained was lyophilized to obtain a brownish gummy solid which was coded CRB. The stem and leaf (1.0 kg each) were also taken through similar procedure to obtain brownish gummy solid and dark green solid which were coded CS and CL respectively and stored in air tight glass containers. The yields of the extracts were calculated.

Phytochemical Screening of the Extracts

The extracts (30 ml each) were taken and screened for the presence or absence of ten (10) groups of phytochemical compounds according to Trease and Evans.⁶ They were: alkaloids, flavonoids, triterpenes, saponins, free reducing sugars, phenolic compounds, polyuronoids, cyanogenic glycosides, atheracenosides and phytosterols.

Animals

Rats and mice were obtained from the Animal House Unit of CPMR and housed in aluminum cages under standard temperature and pressure with free access to sterile water and feed *ad libitum*. Mice used in the acetic acid-induced assays were fasted overnight just before the experiment. Guidelines and procedures provided by Foundation for Biomedical Research on the use of animals in research was applied in caring for the animals and handling them.⁷

Drugs and chemicals

Carrageenan and Acetic acid were procured from Sigma Chemical Co. (St. Louis, USA). Diclofenac sodium salt powder was purchased from Sigma-Aldrich (St. Louis, USA). Morphine sulphate injection (i.v.) was purchased from Laboratories STEROP (Brussels). Normal saline was purchased from Otsuka Pharmaceuticals India Private Limited (Vasana-Chakawadi, India).

Anti-Inflammatory Activity Assay

Anti-inflammatory activity of the extracts was evaluated using carrageenan induced paw oedema model in rats.^{8,9} Male Sprague-Dawley rats (185 - 200 g) were divided into 11 groups ($n=5$). Group 1- 3 were given CRB 5 - 200 mg/kg p.o. Group 4 - 6 were also given CL 5 - 200 mg/kg p.o. Furthermore, Group 7 - 9 were also given CS 5 - 200 mg/kg p.o. Group 10-11 were given 2 mg/kg p.o. of diclofenac sodium as standard drug or 1 ml each of distilled water as vehicle control respectively. Prior to drug/extract administration, oedema was induced by the sub-plantar injection of 0.1 ml Carrageenan in normal saline (1% w/v) in the right hind paw of each animal. The paw volume of the right hind limb of each animal was measured prior to induction of oedema (V_o) and hourly (V_t) until the 4th hr post drug/extract administration using plethysmometer. ($V_t - V_o$) which represent change in paw volume is taken as oedema. Anti-inflammatory activity (AI) was calculated using the formula:

$$AI = \frac{((\text{Mean } (V_t - V_o)_c) - (\text{Mean } (V_t - V_o)_t))}{(\text{Mean } (V_t - V_o)_c)} \times 100 \%$$

Where: Mean ($V_t - V_o$)_c and Mean ($V_t - V_o$)_t represents mean oedema of the control and treated groups respectively over the duration of the experiment.

Analgesic Activity Assays

Hot Plate Assay

The mouse hot plate test used to evaluate the analgesic activity of the extracts was performed according to Eddy and Leimbach.^{9,10} Briefly, C57/BL6 mice (15-25 g) of either sex were divided into 8 Groups ($n=4$). Each mouse was placed on an electric hot plate (UGO Basile hot/cold plate 35 100) maintained at $55 \pm 0.5^\circ\text{C}$. The time taken for a mouse to lick, lift, shake or stamp any of the hind limbs or jump constitutes latency time, recorded as analgesic response. Baseline latencies (T_o) were calculated as averages of two measurements before any treatment of mice. Average baseline latency of mice used for this test ranged from 3.3 - 8.0 s. Extracts were administered as follows. Group 1-3 were given CR 5 - 200 mg/kg p.o. Group 4 - 6 also received CL 5-200 mg/kg p.o., whereas Group 7 - 8 received Morphine sulphate 5 mg/kg i.m. as standard drug or 0.2 ml each of distilled water as vehicle control respectively. Latency of each mouse was then obtained hourly (T_t) for 5 h after each treatment. Analgesic activity was calculated as the sum of percentage Maximal probable effect (% MPE) over the experimental period. % MPE was calculated as:¹¹

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

The analgesic activity in the hot plate assay was calculated as overall analgesic activity for each group in the hot plate test which was obtained as mean % MPE $\pm$ S.E.M.

Acetic Acid Induced Writhing Assay

Analgesic activity of the extracts was also evaluated in the acetic acid induced-writhing model.¹² Female Swiss albino mice (21-29 g) were divided into 8 groups ($n=4$). Group 1-3 were given CR 5 - 200 mg/kg p.o. Group 4 - 6 were also given CR 5 - 200 mg/kg p.o. Finally, Group 7 and 8 received diclofenac sodium, 10 mg/kg p.o. as standard drug and 0.2 ml of distilled water as vehicle control respectively. Thereafter, each mouse was administered 1 ml/100g (i.p.) of 1 % v/v aqueous acetic acid 45 min after treatment and isolated into transparent Perspex cage. Writhing movements and stomach contortions produced by each mouse after induction was counted for 20 min. Percentage analgesic activity (%AE) of the extracts was calculated by comparing the average writhing movements of the vehicle control group and drug treated group which is given by the formula:

$$\%AE = ((MRC - MRC_t)/MRC) \times 100$$

Where: MRC = Average writhing count of the vehicle treated control group; MRC_t = Average writhing count of treated group.

Statistical Analysis

Graphpad Prism 5 was used for all statistical analysis. Each point on the time course curve or column in the bar chart represents mean $\pm$ S.E.M. Data obtained for the time course curves were analysed using Two Way ANOVA followed by Bonferroni's Post hoc test to determine statistical significant. Whereas, One-way ANOVA was employed to analyse data for the bar charts followed by Dunnett's Post hoc test for statistical significant. Results were considered to be statistically significant when $p < 0.05$ by comparing the test subjects to the control group.

RESULTS AND DISCUSSION

Percentage Composition of Morphological Parts of *C. erythrocarpus* and Yield on Extraction

Table 1 shows the composition of the various part of the plant and their percentage yield upon extraction. The stem formed the highest percentage of the plant (55.6 %). The root bark constitutes only 11.1 % of the plant. Hence discarding the stem and leaf of the plant means 66.7 % of the plant is being wasted.

Table 1: Percentage Composition of the Various Part of *C. erythrocarpus* and Yield on Extraction.

Plant part	Weight per plant (kg)	Composition per plant (%)	Yield on extraction	
			g	(% w/w)
Root bark	0.4	11.1	144.50	14.4
Wood in the root	0.6	16.7	-	-
Stem	2.0	55.6	80.81	8.0
Leaf	0.6	16.7	46.64	4.6

Not extracted = -

Anti-Inflammatory Activity

Figure 1 A, C and E shows the effects of ethanol extracts of the root bark (CRB), stem (CS) and leaf (CL) of *C. erythrocarpus* on inflammatory response in Sprague-Dawley rats on time course curve. Whereas, B, D and F represents the mean oedema of extracts, Diclofenac sodium (DS) and control treated groups respectively. From the results DS (2 mg/kg p.o.) and CRB 5–200 mg/kg p.o. suppressed the development of inflammation caused by carrageenan injection. The effect of the extract was dose-independent (Figure 1 A and B).

Moreover, the results also show that CS has no inhibitory effect on the development of inflammation caused by carrageenan injection relative to the negative control (Figure 1 C and D). This indicates that CS was inactive as an anti-inflammatory agent.

Furthermore, CL dose – dependently suppressed the development of inflammation caused by carrageenan injection. The observed effect was however not comparable to the positive control (Diclofenac). Also, the inhibitory effect of the root bark extract (Figure 1 A and B) appears more pronounced than that of the leaves extract (Figure 1 E and F). The anti-inflammatory activity of CRB 5–200 mg/kg p.o. was calculated to be 44.81, 46.35, 48.93 % respectively. And that of CL 5–200 mg/kg p.o. were 1.06, 16.32 and 37.42 % for the respective doses.

Carrageenan-induced oedema in rat paws is an established assay which involves triphasic progressive release of pro-inflammatory mediators.^{8,12} The first Phase which occurs from 0-1.5 h after carrageenan injection comprises the production of histamine and serotonin. The second phase which occurs from 1.5 - 2.5 h is mediated by release of bradykinin.¹³ The last phase is controlled by production of prostaglandins from 2.5-6 h after induction.¹⁴ CRB and CL produced significant ($p < 0.05$) inhibition of inflammation from 3-4h period which corresponds to the third Phase on the time course curve in this assay (Figure 1 A and E). This indicates that CRB and CL produce anti-inflammatory activity by suppressing the synthesis or action of prostaglandins. Although, CRB, CL and DS produced pronounced anti-inflammatory activity in this assay, their overall anti-inflammatory activities calculated as inhibition of mean oedema formation. insignificant ($p > 0.05$) (Figure 1 B and F). This may be due to the low doses of the extracts and drug used or the short duration in which the experiment was performed (1-4 h) instead of (1-6 h).

Analgesic Activity

Hot Plate Assay

The results of the analgesic activity of CRB, CS and CL are shown in Figure 2 G, I and K on time course curves. H, J and L represents the mean analgesic activities of the extracts over the duration of the experiment. All the three extracts produced very significant ($p < 0.05$) analgesic activity in the hot plate assay. The analgesic activity of CRB appears to be dose-dependent between 5 –50 mg/kg p.o. and levels off at a certain dose between 50 – 200 mg where increasing the dose of the extract does not result in the corresponding increase in activity (Figure 2 H). The

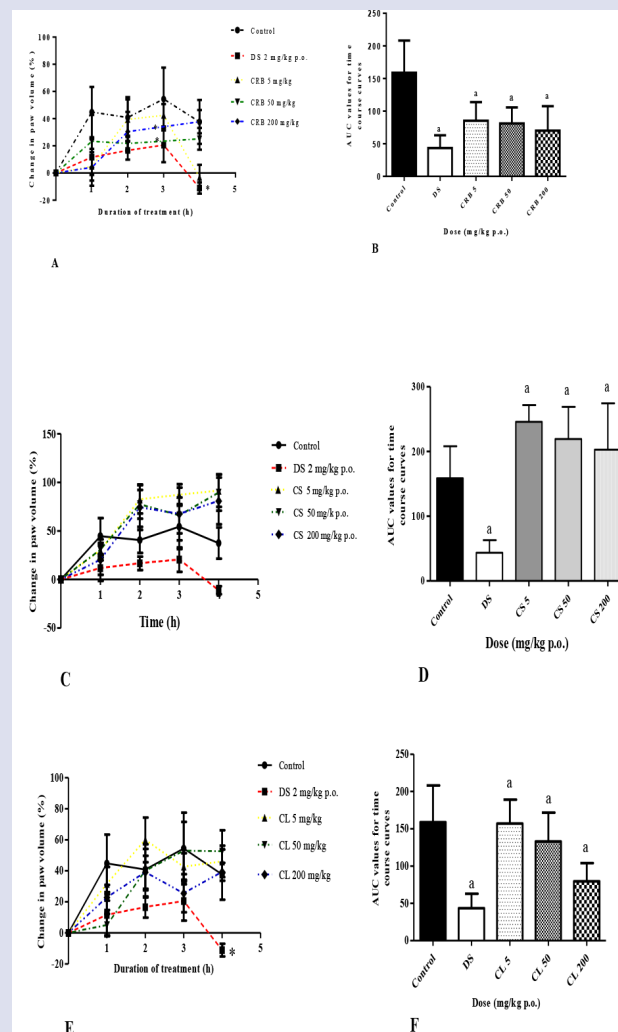


Figure 1: Effect of CR (5 – 200 mg/kg p.o.), CL (5 – 200 mg/kg p.o.) and diclofenac sodium (2 mg/kg p.o.) on inhibition of carrageenan-induced paw oedema in rat's paw on time course curve (A and C) and total area under the curve (B and D). Each point or column represents mean $\pm$ SEM ($n=5$). * $p < 0.05$ compared to vehicle treated control group (Two-way ANOVA followed by Bonferroni's post hoc test). ^ $p > 0.05$ compared to vehicle treated control group (One-way ANOVA followed by Dunnett's post hoc test).

analgesic activity of CS also followed the same trend as that of CRB in the hot plate assay (Figure 2 J). On the other hand, CL appears to produce inverse dose depended analgesia. The 50 mg/kg p.o. dose of CRB and CS produced the highest analgesic activities of 248.7 and 178.2% respectively. Whereas, CL 5 mg/kg p.o. produced the highest analgesic activity of 184.5%. CRB demonstrated the highest analgesic activity among the extracts in the hot plate assay. The analgesic activity of the standard drug, Morphine sulphate (MS 5 mg/kg i.m.), was calculated to be 136.70%. The extracts at 50 – 200 mg/kg p.o. showed higher analgesic activity than MS but at 5 mg/kg p.o. only the leaf showed higher activity than MS (Figure 2 H, J and L).

The hot plate assay is used to evaluate the analgesic activity of drugs which elicit analgesia through the central nervous system.¹⁵ Since CRB, CS and CL showed tremendous analgesia in the hot plate assay, it indi-

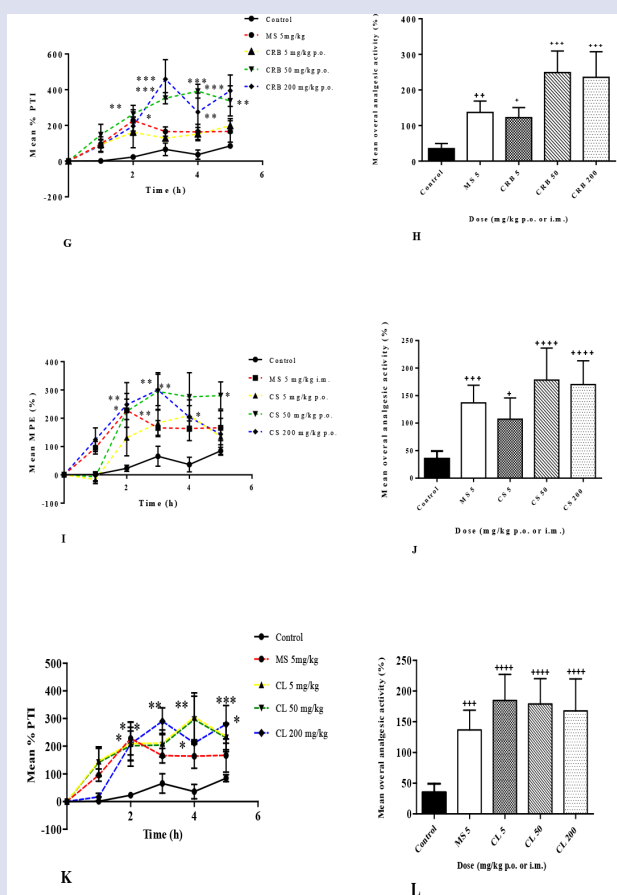


Figure 2: Effect of CRB, CS or CL (5–200 mg/kg p.o.) and MS, (5 mg/kg i.m.) on prolongation of latency of mice to react to pain on time course curve (G, I and K) and mean overall analgesic activity (H, J and L) in hot plate assay. Each point or column represents mean $\pm$ SEM ($n=4$). * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ compared to the control group (Two-way ANOVA followed by Bonferroni's post hoc test). + $p<0.05$, ++ $p<0.01$, +++ $p<0.001$ and ++++ $p<0.0001$ compared to the control group. (One-way ANOVA followed by Dunnett's post hoc test).

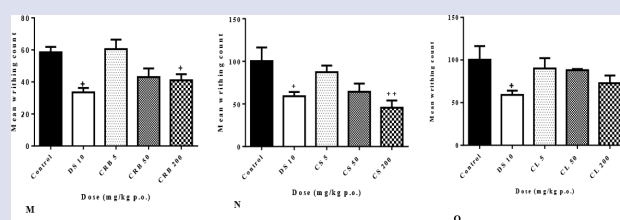


Figure 3: Effect of CRB, CS or CL (5–200 mg/kg p.o.) and diclofenac sodium (10 mg/kg p.o.) on inhibition of acetic acid-induced writhing movements in mice (M, N and O). Each column represents mean $\pm$ SEM ($n=5$). + $p<0.05$, ++ $p<0.01$ compared to vehicle treated control group (One-way ANOVA followed by Dunnett's post hoc test).

numbers in the mouse writhing assay. Hence, the analgesic activity of CRB and CL extracts may be due to their ability to suppress the cyclooxygenase enzyme and decrease the biosynthesis of prostaglandins. The result obtained for CRB and CL correlated with the results obtained for them in the carrageenan-induced oedema assay. Thus, CRB and CL may also possess peripheral analgesic property in addition to the central analgesic property. These results also indicate that the anti-inflammatory constituents of CRB and CL are also responsible for their analgesic activity in the acetic acid-induced writhing assay. CS on the other hand, demonstrated strong analgesic activity in both hot plate and acetic acid induced writhing assays but did not show any anti-inflammatory activity in the carrageenan induced oedema assay. This indicates that CS may possess only central analgesic property without peripheral analgesic property.

Previous reports indicated that *C. erythrocarpus* root possessed central analgesic property probably mediated by adenosinergic receptors in the formalin assay.⁵ However, this is first report on the analgesic and anti-inflammatory activities of stem and leaf extracts of *C. erythrocarpus*.

Phytochemical screening of *C. erythrocarpus*

The root bark of the plant contains the highest number (Five) of phytochemical constituents while the stem and the leaf contains equal numbers (Four each). Reducing sugar, triterpenes and phytosterols were present in all the three extracts. In addition, saponins were present in the root bark and leaf but absent in the stem. Alkaloids were also present in the root bark and stem extracts but not in the leaf.

Finally, some classes of phytochemical constituents such as saponins, triterpenes, phytosterols and alkaloids are known to possess anti-inflammatory and analgesic properties. The presence of these constituents in the root bark, stem and leaf of *C. erythrocarpus* contributes to the anti-inflammatory and / or analgesic effects of the plant extracts.

CONCLUSION

This study showed that, the 70 % ethanol leaf extract of *C. erythrocarpus* (CL) possessed anti-inflammatory and analgesic properties similar to that of the 70% ethanol extract of the root bark (CRB). CL may therefore, be substituted in place of CRB to treat inflammatory pain in order to prevent the plant from going extinct. However, the 70% ethanol extract of the stem (CS) of the plant did not show anti-inflammatory activity. CS also appears to possess only central analgesic properties. Hence, CS can be used to treat other forms of pain, but not inflammatory pain.

This is the first work that reports on the anti-inflammatory and analgesic activity of *C. erythrocarpus* stem and leaf. The anti-nociceptive activity of the root of this plant had already been established.

cates that the 70 % ethanol extract *C. erythrocarpus* root bark, stem and leaf may possess central analgesic effect.

Writhing assay

Figure 3 M, N and O. All the extracts (CRB, CS and CL) showed dose dependent analgesic activity in the acetic acid-induced writhing assay. The highest dose of CRB (200 mg/kg p.o.) produced ($p<0.05$) analgesic activity of 17.45 %. CS at (200 mg/kg p.o.) also produced significant ($p<0.01$) analgesic activity of 54.61 % (Figure 3). The highest dose of CL (200 mg/kg p.o.) also produced the highest analgesic response of 27.43 %. The analgesic activity of the standard drug, diclofenac sodium, was calculated to be 41.15 %.

The acetic acid-induced writhing assay is used to evaluate both central and peripheral analgesic activities of substances.¹⁵ 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.¹⁶ The stretching and abdominal contortions obtained in the writhing test therefore correlates with sensitization of nociceptors to the production of prostaglandins.¹⁵ CRB and CL significantly suppressed writhing

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

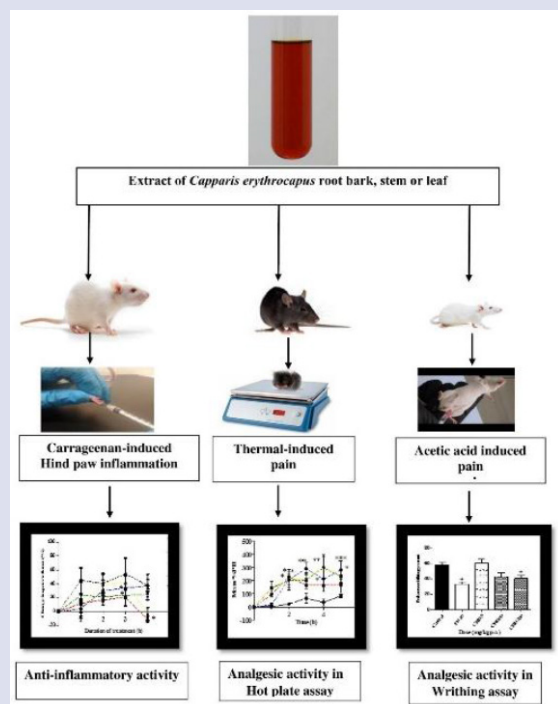
ABBREVIATIONS

ANOVA: Analysis of variance; **CRB:** *C. erythrocarpus* root bark extract; **CS:** *C. erythrocarpus*; **CL:** *C. erythrocarpus* leaf; **CPMR:** Centre for Plant Medicine Research; **DS:** Diclofenac sodium; **i.m.:** Intramuscular; **i.v.:** Intravenous; **AUC:** Area under the curve; **MPE:** Maximum probable effect; **T:** Latency; **t:** Time; **MRC:** Mean Writhing count of control group; **MRT:** Mean writhing count of treated group; **SEM:** Standard error of the mean.

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GRAPHICAL ABSTRACT



SUMMARY

- Problem Statement and Hypothesis:** *C. erythrocarpus*, an important medicinal plant whose root is used to prepare an herbal medicine that is employed to treat arthritis and other inflammatory pain conditions, is now difficult to obtain in Ghana, because of the indiscriminate destruction of the plant to obtain the root. The present study was conducted to ascertain and compare the anti-inflammatory and analgesic activity of the leaf, stem and root bark of *C. erythrocarpus* so as to substitute the leaf and / or stem for the root bark in order to save the plant from extinction.
- Implication of the Results:** This study demonstrated that the leaf of *C. erythrocarpus* can be substituted for the root bark as an anti-inflammatory and analgesic agent while the stem can only be used as analgesic agent. This will save the plant from extinction.
- Way Forward:** Work is underway to isolate the constituents of the plant, characterize and assay them for their analgesic and anti-inflammatory activities.

ABOUT AUTHORS



Dr. Emmanuel Kofi Kumatia holds a Ph.D. in Pharmacognosy. He is the Head of the Phytochemistry Department of the Centre for Plant Medicine Research. He has authored and co-authored research articles which were published in peer reviewed scientific journals in the fields of phytochemistry and pharmacognosy. His research areas of interest include isolation and structure elucidation of active principles from plants, inflammation, pain, diabetes, malaria, hypertension and microbiology.



Mr. Stephen Antwi holds an MPhil. in Pharmacology. He is the Head of the Animal Experimentation Unit of the Centre for Plant Medicine Research. He has authored and co-authored research articles which were published in peer reviewed scientific journals in the fields of efficacy, toxicology and mechanism of action of herbal extracts/medicines.



Mr. Henry Brew-Daniels holds an MPhil. in Chemistry. He was the Acting Head of the Phytochemistry Department of the Centre for Plant Medicine Research. He has co-authored research articles which were published in peer reviewed scientific journals. His research areas include heavy metals analysis, pesticide residue analysis and phytochemistry.



Dr. Alfred Ampomah Appiah is a Pharmacist and holds a Ph.D. in Chemistry. He is a Principal Research Officer and the Deputy Executive Director of the Centre for Plant Medicine Research. He has authored and co-authored many research articles which were published in peer reviewed scientific journals in the fields phytochemistry, pharmacognosy and others. His research areas of interest include isolation and structure elucidation of active principles from plants, prostate enlargement, Parkinson disease and noncommunicable diseases.



Prof. Augustine Ocloo holds a Ph.D. in Cell and Molecular Biology. He is the Executive Director of the Centre for Plant Medicine Research and a former Head of the Department of Biochemistry at University of Ghana. He has authored and co-authored many research articles which were published in peer reviewed scientific journals in the fields of cell and molecular biology, biochemistry of medicinal plant extracts and others. His research areas of interest include mitochondria as a target for drug discovery, biochemistry and pharmacology of medicinal plant extracts.

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