Anti-arthritic and anti-oxidant activities of Antrocaryon micraster seed extract and its fractions

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Research Article

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Anti-arthritic and anti-oxidant activities of *Antrocaryon micraster* seed extract and its fractions

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ABSTRACT

Rheumatoid arthritis (RA) is an incurable debilitating disease which attack the joints and impair quality of life. *Antrocaryon micraster* is used to treat RA in African traditional medicine. However, its anti-arthritic activity has not been pharmacologically studied. This study therefore, reports the anti-arthritic and anti-oxidant activities of *A. micraster* seed extract and its fractions. The seed extract (ASE) was produced by soxhlet extraction and partitioned into petroleum ether (ASEP), ethyl acetate (ASEE) and aqueous (ASEA) fractions. The total polyphenolic content, DPPH anti-oxidant activity and in vitro arthritic activity using the protein denaturation assay were evaluated for ASE and its fractions. The arthritic activity of the crude extract (ASE) and its most effective fraction (ASEA), in the in vitro assay, were then evaluated against CFA-induced arthritis in rats. The polyphenolic constituent of ASE was estimated to be 13.00 ± 0.00 mg/100 mg of GAE. ASEA contained the highest quantity of polyphenolic constituents (10.76 ± 0.00 mg/100 mg of GAE) among the fractions of the extract. ASE and ASEA produced profound anti-oxidant activity (IC_{50} = 20.17 ± 1.291 and 19.35 ± 0.865 respectively) which were similar to that of ascorbic acid (IC_{50} = 17.35 ± 0.500) in the DPPH free radical scavenging assay. Furthermore, in vitro anti-arthritic activity of ASEA was 13.63 and 5.75 times higher than the anti-arthritic activity of the crude extract and diclofenac sodium respectively. In the CFA-induced arthritis assay, both ASE and ASEA significantly ($P < 0.001$) inhibited cachexia, paw edema, infiltration of inflammatory cells,

pannus formation and synovium damage. These results indicates that *A. micraster* seed extract and its fractions possessed significant anti-arthritic activity via inhibition of oxidative stress, inflammation, protein denaturation, infiltration of inflammatory cells and synovium injury due to its constituents such as polyphenols and phytosterols.

Keywords: Polyphenols**,** DPPH assay, Protein denaturation assay, anti-oxidant activity, Phytosterols, *Antrocaryon micraster* seed

1. Introduction

The commonest source of medicine for the treatment of diseases in most developing counties are medicinal plants. Furthermore, finished herbal products and herbal medicines have been incorporated into the orthodox health care delivery systems in countries such as China, India, Ghana and many others. The World Health Organization has reported that about 80% of the world population uses traditional medicine (WHO., 2022). This may be due to the fact that medicinal plants are readily available and accessible at virtually no cost. Additionally, medicinal herbs and their products are perceived to be safer compared to orthodox medicines. Also, most people believe in the healing properties of medicinal herbs used in their localities. Hence, they are unwilling to experiment with unknown orthodox drugs.

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease which manifest in the form of general inflammation of the joints with pain, obliteration of articular tissues, and deformities in the joints with heightened probability of bone injury and cartilage devastation thereby producing extensive deformity (Putterman, 2003). RA has no cure. Hence, the current management strategy focuses on reduction of symptoms at the joints and to slow down it's progression to disability (Bullock et al., 2019).

Apart from the used of pharmaceutical drugs such as disease-modifying antirheumatic drugs (DMARDs) and nonsteroidal anti-inflammatory drugs (NSAIDs) in the management of RA, many medicinal plants and herbal preparations are also employed to treat arthritis. *Antrocaryon micraster* (A. Chev. and Guillaumin) is one of these plants. It is a large tropical rain forest tree, of the Anacardiaceae or cashew family, which shed its leaf annually. The trunk of the plant is upright,

cylindrical and can spread up to 1.5 m in diameter and 50 m tall (Ayarkwa, 2011). The root, stem bark, leaf, fruit and seed of the tree are used as herbal treatments against diseases such as pain and arthritis, cough, chest pain, stomach ache, toothache, chicken pox, headache, inflammation, and boils and malaria (Ayarkwa, 2011; Vigbedor et al., 2008; Ayarkwa, 2012; Addo-Fordjour et al., 2013; Kumatia et al., 2021a). Although, *A. micraster* is used to treat arthritis, the anti-arthritic activity of the plant has not been pharmacologically evaluated. However, the anti-malaria and anti-trypanosomal activities of the plant have been studied (Dofuor et al., 2022; Kumatia et al., 2021). The aim of this study therefore, is to evaluate the anti-arthritic and anti-oxidant activities of *A. micraster* seed extract and its fractions using in vitro and in vivo models.

2. Materials and methods

2.1. Chemicals and reagents

Diclofenac sodium and Complete Freud Adjuvant were acquired from Sigma Chemical Co. (St. Louis, USA). Petroleum ether (40 – 60 ^{o}C) and ethyl acetate were supplied by Fisher Scientific (Loughborough, U.K). Ethanol (99%) was obtained from Midland Ghana Limited (Tema). Normal saline was purchased from Otsuka Pharmaceuticals India Private Limited (Vasana-Chakawadi).

2.2. Sourcing of A. micraster seed

The dried seed of *A. micraster* (AS) was purchased from a vendor at the GPRTU bus station in Koforidua (the capital town of the Eastern Region of Ghana) in February, 2021. The identity of the seed was authenticated by Mr. Jonathan Dabo, of the Forestry Research Institute of Ghana (FORIG) Kumasi. The seed was assigned a voucher specimen (FORIG 0015) and kept at the FORIG herbarium.

2.3. Processing and extraction

Sample of the seed was crushed into pieces and then milled into course powder. A total of 400 g of the AS course powder was extracted using the soxhlet apparatus. Briefly, 200 g of AS was sequentially extracted with 1.5 L of 70% ethanol three times after every 4 hours. The second batch of 200 g was also treated with the same procedure. The extracts from the six extractions were combined and concentrated in vacuum (Eyeler N1110, Tokyo-Japan) at 46 ^{o}C to 650 mL. Sample of the extract (400 mL) was transferred into a separating funnel and diluted with 100 mL of

distilled water. It was then successively partitioned three times each with 500 mL petroleum ether, followed by 500 mL each of ethyl acetate three times. Each fraction was separately combined and dried in vacuum at 40 ºC. The aqueous fraction was also freeze-dried. The dried solids were labelled ASE, ASEP, ASEE and ASEA which corresponds to the crude extract, petroleum ether, ethyl acetate and aqueous fractions respectively.

2.4. Animals and ethical considerations

The rats were kept in aluminum cages containing saw dust at 22°C ± 2°C and were fed on powdered commercial feed and sterilized water. The study was performed in compliance with the UK. Animals (Scientific Procedures) Act, 1986. The permission to perform the study was given by the Centre for Plant Medicine Research institutional review committee under 2010/63/EU animal experimental guidelines. All precautions were taken in order not to cause the animal undue pain or distress.

2.5. Phytochemical screening of ASE

The phytochemical constituents present or absent in the extract were analyzed using methods described by Fong et al., (1977).

2.6. Quantification of polyphenols

Folin–Ciocalteau method (Singleton et al. 1999) some with modification was employed to determine the total polyphenol content of the extract and the fractions. In summary, 2 mL of Folin–Ciocalteau reagent (10%) was mixed with 0.4mL of 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625 and 0.0078125 mg/mL each of the sample prior to addition of 1.6 mL and kept in the dark for 8 min. Thereafter, the mixture was added to 1.6 mL of 7.5% sodium carbonate solution in 15 mL test tubes, mixed and incubated again in the dark for 90 min. The absorbance was then measured at 765 nm using double beam UV/VIS spectrophotometer (BK-D590, Shandong, China). A standard calibration curve was also generated using Gallic acid under similar experimental conditions from which the polyphenol concentrations in the extract and fractions were derived as Gallic acid equivalent (GAE) per milligram of dry sample. The tests were done in triplicate.

2.7. Evaluation of antioxidant activity

The antioxidant activity of the extract and the fractions was evaluated by using DPPH free radical scavenging activity assay described by Kostyuk et al., (1990). Approximately, 4 mL of 0.2 mM, 2, 2-diphenyl-1-picrylhydrazyl (DPPH)-methanol solution was mixed with 2 mL each of 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625 or 0.0078125 mg/mL each of the test sample concentrations and incubated at room temperature (25 ºC) for 30 min in the dark. The absorbance of the mixture was measured at 517 nm with double beam UV/VIS spectrophotometer (BK-D590, Shandong, China). Ascorbic acid (Ac-A) and methanol were used as the standard drug. A blank sample was prepared by mixing 2 mL of methanol in 0.2 mM of 2 mL DPPH solution. The tests were performed in triplicate. The % inhibition was estimated using the equation below.

$$\%\text{Inhibition} = \frac{\text{Absorbance of the blank} - \text{absorbance of test sample}}{\text{Absorbance of the blank}} \text{ x } 100\%$$

The % inhibition of the sample was then used to plot the log normalized response curve using GraphPad Prism 9.5.1 from which the antioxidant activity was estimated as the IC_{50} values.

2.8. In vitro protein denaturation assay

Protein denaturation assay was performed as described by (Padmanabhan and Jangle, 2012) with slight modifications. Briefly, sodium diclofenac salt/extract concentrations of 3.90-2000 µg/mL (2 mL) were each added to 0.2 mL of egg albumin from fresh egg and 2.8 mL of phosphate buffer saline (PBS, pH 6.4) and mixed thoroughly. The mixture was incubated at room temperature for 10 min and then heated at 70 ºC for 20 min and finally left to cool down. The turbidity of the mixture was read at an absorbance of 660 nm. The negative control consists of 10% DMSO in distilled water (v/v). The percentage inhibition of protein denaturation was calculated as:

$$\%\text{Inhibition} = \frac{\text{A(NC)} - \text{A(S)}}{\text{A(NC)}} \text{x } 100\%$$

Where: A (NC) is the absorbance of the negative control and A(S) is the absorbance of the sample. Graph pad prism was used to computed the 50% inhibitory concentration (IC_{50}).

2.9. Acute toxicity test

Acute toxicity effect of ASE was tested in female SDR rats (N = 6) by adopting the method of OECD, (2001) with some amendments. A single dose of 5 g/kg of the extract was suspended in distilled water` and administered to the rats using oral gavage at a rate of 10 mL/100 g body weight. The rats were then monitored at regular time intervals for indications of toxicity like paralysis, salivation, change in fur alignment, change in food and water intakes, lacrimation, unconsciousness or death for 14 days.

2.10. Evaluation of anti-arthritic activity against CFA-induced arthritis

Anti-arthritic activity of the ASE and its fraction was evaluated by employing previously described methods (Pearson 1956; Kumatia et al., 2023). Briefly, arthritis was induced in the left hind paw of female Sprague-Dawley rats (135-147 g) by intradermal injection of the left footpad with 0.2 mL Complete Freund's Adjuvant (CFA). The rats were grouped into 6 (N = 5). The crude seed extract (ASE), its aqueous fraction (ASEA) and the standard drug (D-Na) were dissolved in water to form a uniform solution and orally administered with gavage at 2.0 mL per rat daily for 14 consecutive days.

Group 1 – ASE 25 mg/kg p.o.

Group 2 – ASE 100 mg/kg p.o.

Group 3 – ASEA 25 mg/kg p.o.

Group 4 – ASEA 100 mg/kg p.o.

Group 5 – D-Na 5 mg/kg p.o.

Group 6 - 2 mL of distilled water (CFA/Arthritic control).

The baseline paw volume of the left hind paw was measured on day 0 (Vo) as the average of two measurements by Plethysmometer, (UGO Basile, Italy) 3 h prior to the induction of the arthritis. Measurements of the paw volumes of the left hind limbs of each rat was repeated at 2 days intervals (Vt) from day 0 – day 28. The administration of the extracts and the standard drug was started day 14th day after the arthritis induction. The change in paw volume which was taken as edema was calculated as (Vt-Vo).

2.11. Body weight measurement

The rats were weighed (Wo) before the commencement of the experiment. They were again weighed after every two (Wt) until the 28th day. The mean change in the body weight of the rats was then calculated as shown below.

$$\text{Mean change in body weight} = \frac{\sum(\text{Weight of animal at Wt} - \text{Weight of animal at Wo})}{\text{Total number of mice in a group}}$$

2.12. Histopathological examination of the ankle joints

The process was executed as described by Patil et al., (2012). On 29th day, the rats were euthanized and their arthritic ankle joints removed preserved in buffered formalin (10%) for 14 days. They were decalcified in 5% formic acid, fixed in paraffin and split into 5 µ sections. The sections were stained with hematoxylin-eosin solution and analyzed under light microscope (Carl Zeiss, Primos Star, Germany) at x10 magnification for indicators of arthritis such as inflammatory cells infiltration, congested interstitial space, development of pannus.

2.13. Statistical Analysis

Graph Pad Prism version 6 statistical soft was employed for the data analysis and the results written as mean ± SEM. Variations were ascertained by comparative analysis using one-way or two-ways analysis of variance (ANOVA), followed by Dunnett's or Tukey's multiple comparison test where applicable. Statistically significant was determined using $P < 0.05$.

3. Results

3.1. Phytochemical analysis of the extract

The extract (ASE) contained phytoconstituents such as phytosterols, polyphenols and free reducing sugars. Flavonoids, alkaloids, polyuronoids, cyanogenic glycosides, triterpenes, saponins and antracenosides were absent.

3.2. Yield and polyphenols contents of the extract and its fractions

The yields of the extract and its fractions and their polyphenol contents is shown below (Table 1). The yield and polyphenol content of the seed extract and its fractions were moderately low.

TABLE 1: Weight, yields and polyphenol contents of the ASE and its fractions

Sample	**Weight (g)**	**Yield (%w/w)**	**Polyphenol content (mg/100 mg of GAE)**
ASE	18.15	4.54	13.00 ± 0.00
ASEP	3.86	1.45	1.70 ± 0.01
ASEE	5.04	1.89	0.40 ± 0.02
ASEA	3.20	1.20	10.76 ± 0.00

3.3. In vitro anti-oxidant activity

The results of the IC_{50}s obtained for ASE and its fractions in the DPPH assay is presented below in Table 2.

TABLE 2: DPPH free radical scavenging activity of ASE and its fractions

Sample	**IC_{50} ± SEM (µg/mL)**
Ac-A	17.35 ± 0.500
ASE	20.17 ± 1.291
ASEP	69.35± 1.21
ASEE	93.22 ± 1.005
ASEA	19.35 ± 0.865

ASE, ASEA and the standard compound, Ascorbic acid (Ac-A) gave similar IC_{50} values against the inhibition of free radical formation from DPPH. The IC_{50}s of ASEP and ASEE were 4 and 5.4 times higher than that of Ac-A.

3.4. Protein denaturation

The result of the in vitro anti-arthritic activity measurement in the protein denaturation test is shown below in Table 3.

TABLE 3: Summary of the IC_{50} values of sodium diclofenac, ASE and its fractions against protein denaturation.

Sample	**IC_{50} ± SEM (mg/mL)**
D-Na	0.092 ± 0.00
ASE	0.218 ± 0.00
ASEP	0.180 ± 0.01
ASEE	0.362 ± 0.00
ASEA	0.016 ± 0.00

ASE and all of its fractions demonstrated very good anti-arthritic activity with very low IC_{50} values. However, ASEA produced the lowest IC_{50} (Table 3).

3.5. Acute toxicity

None of the rats given the extract died or shows any sign of toxicity within the 14 days observational period. Hence, the LD_{50} of the extract is above 5000mg/kg body weight by oral administration.

3.6. Effect of treatment of ASE and ASEA on body weight of arthritic rats

The effect of the treatment of ASE, its aqueous fraction and D-Na on the body weight alteration during arthritis is shown below (Table 4).

Table 4

Mean change in body weight during the treatment period

Sample	**Mean change in body weight (g)**
CFA-Control	- 8.63 ± 3.76
D-Na 10 mg/kg p.o.	10.93 ± 3.90**
ASE 25 mg/kg p.o.	12.54 ± 3.12***
ASE 100 mg/kg p.o.	19.29 ± 74.84***
ASEA 25 mg/kg p.o.	10.07 ± 1.06*
ASEA 100 mg/kg p.o.	16.14 ± 2.14***

*P < 0.05; **P < 0.01; ***P < 0.001 compared to CFA-control where rats were treated with the substances in the last 14 days (day 14-28) out of the 28 days period of the experiment**.**

The arthritic rats experienced weight lost (- 8.63 ± 3.76 g) in the last 14 days. However, treatment ASE and ASEA, dose-dependently and significantly ($P < 0.05$ - 0.001) increased their body weights. In the same manner, D-Na 5 mg/kg p.o., also significantly ($P < 0.01$) increased the body weight of the rats (Table 4).

3.7. Effect of ASE and ASEA on edema inhibition during CFA-induced arthritis

The results of edema inhibition of ASE and ASEA in CFA-induced arthritis is shown below in Fig. 1.

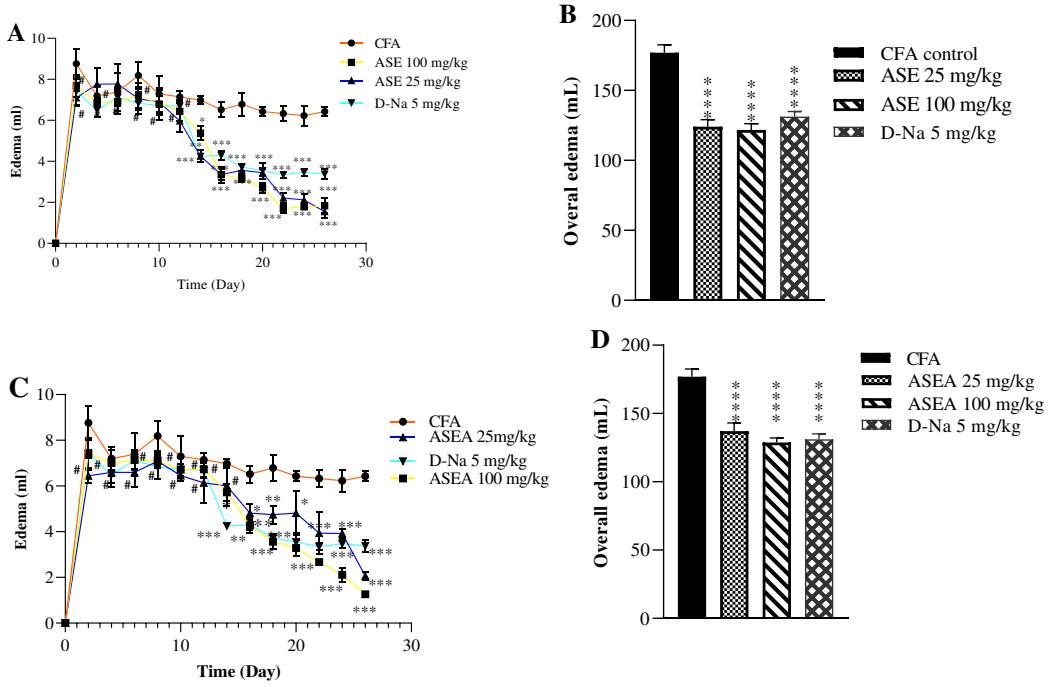

FIGURE 1: Effect of ASE and ASEA on edema formation on the time course curve (A and C) and overall edema (B and D) calculated as the area under the curve, during CFA-induced arthritis in rats' paw. Each point represents Mean ± S.E.M. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ or $^{\#}P > 0.05$ compared to CFA-control rats.

ASE and ASEA reduced edema formation during CFA-induced arthritis in rats. The activity became statistically significant on day 14-28 on the time coursed curve after the commencement of treatment (Fig. 1. A and C). The overall edema inhibition was also statistically significant ($P < 0.0001$) and dose-dependently for both ASE and ASEA (Fig. 1. B and D).

3.8. Histopathology of the arthritic ankle joints

The results of the histopathological assessment of the arthritic joints after termination is shown below in Fig 2 A-E.

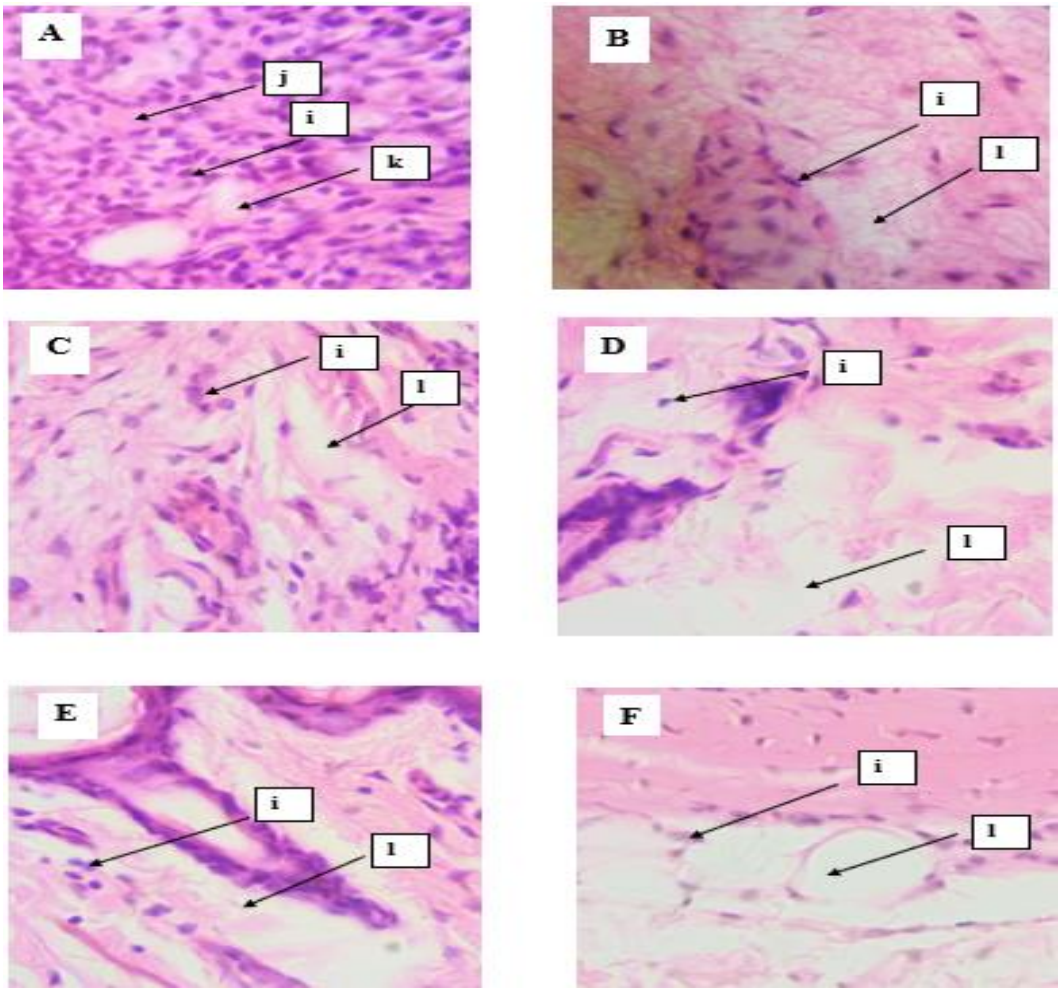

FIGURE 2: A (Arthritic control); B (DF); C (ASE 25 mg/kg p.o.); D (ASE 100 mg/kg p.o.); E (ASEA 25 mg/kg p.o.); F (ASEA 100 mg/kg p.o.); i (inflammatory cells); j (congested interstitial space); k (pannus) and l (clear interstitial space).

It can be seen from the micrographs that there is synovial hyperplasia, disintegration of cartilage, wearing away of bone and development of pannus which are very much pronounced in the nontreated arthritic control rats (Fig 2. A) as compared to the ASE, ASEA and D-Na treated rats (Fig 2. A).

4. Discussion

The yield and polyphenol content of 4.54 %w/w and 13.00 ± 0.00 mg/100 mg of GAE obtained respectively for the soxhleted extract of *A. micraster* seed is quite very low, especially when compared to the yield and polyphenol content of 20.65 %w/w and 234.960 ± 0.026 mg/g of GAE respectively reported for the stem bark of the same plant extracted by cold maceration (Kumatia et al., 2021a), a method which gives a poorer yield than soxhlet extraction. The poor yield of the seed is due to its extreme hard nature. Surprisingly, also, the polyphenol content of the fractions of the extract (ASE) is inversely proportional to their yields (Table 1). Thus, when the fractions of ASE are in the order of increasing yield as ASEA < ASEP < ASEE, the reverse order of ASEE < ASEP < ASEA holds for the polyphenol content.

It had been reported that generation of auto-antigens in some inflammatory or arthritic conditions could be as a result of denaturation of proteins in the affected tissue (Opie, 1962; Umapathy et al., 2010). Furthermore, it was shown that medicinal plant extracts which inhibits protein denaturation in vitro mostly demonstrates in vivo anti-inflammatory activity (Chandra et al., 2012). Moreover, the egg albumen protein denaturation test is very cheap, easy to performed and required less resources as compared to an in vivo animal study. The egg albumen protein denaturation test was therefore, employed in this study to screen ASE and its fractions (ASEP, ASEE and ASEA) in other to assess their anti-inflammatory activity which was an indication of their anti-arthritic activity. The results show that the extract and its fractions produced very low $1C_{50}$ values which indicates their effective anti-inflammatory action. The in vitro anti-inflammatory activity of the test agents can be arranged as follows: ASEE < ASP < ASE < D-Na < ASEA. In real terms, D-Na the stand drug is only 2.4, 1.96 or 3.93 times more potent than ASE, ASP or ASEE. However, the aqueous fraction of the extract, ASEA was 5.75 more potent than D-Na and the most potent fraction among the three. The result also shows that although all the three fractions of ASE were active, ASEA and ASEP were more active than the parent extract, ASE. This shows that the most active anti-inflammatory constituents of ASE is/are both very polar compounds which resides in the aqueous fraction followed by very nonpolar ones in the petroleum ether fraction. It also shows that anti-inflammatory action of the active constituents of the extract was blocked by the other constituents when the fractions were together as one whole extract (ASE).

Since, the crude extract is what is used to treat diseases in traditional medicine, it was therefore selected with ASEA for the in vivo anti-arthritic activity test.

IC_{50} is defined as the minimum concentration of the substance is required to produce 50% inhibition of the oxidation of DPPH molecule. Hence, the higher the IC_{50} value of the substance, the lager the amount required to prevent the oxidation and less effective is the substance. On the other hand, if the IC_{50} value is small, then little concentration of the substance is required to inhibit the oxidation of the DPPH molecule. And hence, more effective is the substance. In the DPPH free radical scavenging activity assay (Table 2), similar IC_{50} values were obtained for ASE, ASEA and the standard compound, Ac-A. Whereas, the IC_{50}s of ASEP and ASEE were 4 and 5.4 times higher than that of Ac-A. This indicates that ASE and ASEA possessed strong anti-oxidant activity similar to that of ascorbic acid (Ac-A). But, ASEP and ASEE exhibited moderate anti-oxidant activity. It also indicates that the antioxidant constituents of *A. micraster* seed is more concentrated in its aqueous fraction than the petroleum ether and ethyl acetate fractions. Radical scavenging properties of substances are very vital in the prevention of the damaging effects of excess free radicals especially in chronic disease conditions such as diabetes, hypertension, cancers and arthritis. Anti-oxidants such as Polyphenols and tocopherols prevent these damages by donation of their hydrogen atoms (Huang et al., 2005; Baumann et al., 1979). It has also, been reported that free radical scavenging antioxidant activity of plant extracts is vastly associated with their total phenolic contents (Huang et al., 2005). Therefore, the fraction of the extract (ASEA) which contains the higher quantity of polyphenols (Table 2) also demonstrated the most effective anti-oxidant activity in the DPPH assay. Thus, the antioxidant and anti-arthritic activity of *A. micraster* seed extract and its fraction is be due to its polyphenol and phytosterol constituents as shown by the qualitative and quantitative phytochemical tests.

Studies have shown that an effective in vitro DPPH free radical scavenging capacity of a plant extract indicates a corresponding in vivo anti-oxidant potency (Kumatia et al, 2021b; Kumatia et al, 2021c). Therefore, ASE and ASEA may also be very effective anti-oxidant agents in vivo which contributes to its anti-arthritic activity.

Paw swelling is one of the classical signs of CFA-induced arthritis. And it can be measured with easy thereby providing a quick technique for the assessment of the efficacy of anti-arthritic agents

(Rajendran and Krishnakumar, 2021). The paw swelling is as a result of cellular infiltration of the inflamed region, vascular permeability, and increase in fluid exudation (Bose et al., 2014). An effective anti-arthritic agent is therefore expected to significantly inhibit the causes of paw edema with corresponding decrease in paw swelling. Our results show that ASE and ASEA are very effective anti-arthritic agent since they significantly ($P < 0.0001$) inhibited paw edema formation of their treatment groups (Fig. 1. A-D).

Rheumatoid arthritis assaults the synovial membrane and cause the inflammation of the joint which are visible by histopathological examination (Zheng et al., 2014). It has also been reported that the release of NF-kB and proinflammatory cytokines during rheumatoid arthritis leads to the classical symptoms such as synovial hyperplasia, pannus formation, joint and bone disintegration in addition to the infiltration of the synovium by inflammatory cells which then destroys the synovial membrane (Shabbir et al., 2014; Alvarez, 2009). Hence, the histopathological examination of the joints was performed to assess the treatment outcomes on these symptoms. The results from our study indicated synovial hyperplasia, disintegration of cartilage, wearing away of bone, congested interstitial space and development of pannus in the arthritic joints of the nontreated arthritic control rats (Fig. 2. A). On the other hand, no pannus development, very few inflammatory cells, clear interstitial spaces and intact synovial membranes were observed in the ASE and ASEA and D-Na treatment groups (Fig. 2. B-F). These results confirmed that *A. micraster* seed extract possessed antiarthritic activity by its ability to inhibit the damaging effects of proinflammatory cytokines on the joints.

Another classical symptom of rheumatoid arthritis is wasting away, a condition known as cachexia. Rheumatoid cachexia has been reported to be due to inflammation as a result of inability of the gut to absorb nutrients from food which is reserved by the intake of anti-inflammatory drugs (Jalalpure et al., 2011; Roubenoff et al., 1994). The results shows that administration of *A. micraster* seed extract and its aqueous fraction significantly ($P < 0.05$) enhanced weight gain dose-dependently compared to the arthritic control rats, confirmation that the extract is an effective anti-inflammatory agent and hence restores nutrients absorption from food.

Also, the qualitative phytochemical screening indicates that the extract contains polyphenols and phytosterols. Further quantitative assay was then employed to calculate the amount of polyphenols in the extract. It was reported that polyphenols act on the inflammatory, oxidative, and apoptotic pathways to reduce the progression of RA. They also interfere with the inflammatory system through the MAPK track and NFATC1 quality in osteoblasts by modulation of MAPK, ILs 1 and 6, TNF-α, NF-κB, JNK, extracellular signal-directed kinase (ERK1/2), activator protein-1 (AP-1), and COX-2 (McInnes and Schett, 2011). Moreover, a recent study also shows that phytosterols such as beta-sitosterol 3-palmatate and beta-sitosterol 3-myristate possessed significant anti-arthritic activity at 3 µmol/kg (p.o.) via inhibition of inflammation by 31.02 and 39.14% respectively against CFA-induced arthritis in rats which were equivalent to 30.79% anti-inflammatory activity of the standard, diclofenac sodium at the same dose. Therefore, the presence of polyphenols and phytosterols in the extract and its fractions are responsible for its anti-arthritis activity by acting on the inflammatory, oxidative, and apoptotic pathways of the CFA-induced arthritis.

Finally, since neither of the rats shows any symptom of toxicity nor died during the acute toxicity test and the LD_{50} of the extract was above 5000 mg/kg p.o. per body weight, indicates that the 70% soxhleted ethanol extract of *A. micraster* seed (ASE) is safe for human consumption.

5. Conclusion

This is the first study to provide scientific validation of the use of *A. micraster* seed as a medicinal agent. The study has also, shown that the 70% soxhleted ethanol extract of *A. micraster* seed (ASE) and its petroleum ether (ASEP), ethyl acetate (ASEE) and aqueous (ASEA) fractions possessed considerable anti-arthritic and anti-oxidant activities due to their polyphenolic and phytosterol contents. ASEA was the most active fraction among the three. The crude extract (ASE) and ASEA attenuates arthritis in rats through the inhibition of protein denaturation, inflammation, cachexia and protection of the joints against adverse pathological alterations.

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Conflict of interest

The authors declare that they have no conflict of interest in this study.

Data availability

The data upon which the conclusion in this article is based can be obtained from the corresponding author upon reasonable request.

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References

World Health Organization (WHO). 2022. https://www.who.int/news/item/25-03-2022-who-establishes-the-global-centre-for-traditional-medicine-in-india. Accessed on 11-01-2023.

Putterman, C., 2006. Introduction: New treatment paradigms in rheumatoid arthritis. Am. J. Orthopaed. 36, S2–S3.

Bullock, J., Rizvi, S.A.A., Saleh, A.M., Ahmed, S.S., Do, D.P., Ansari, R.A., Ahmed, J., 2019. Rheumatoid arthritis: a brief overview of the treatment. Med. Princ. Pract. 27(6), 501-507.

Ayarkwa, J. 2011. *Antrocaryon micraster*. In: Louppe, D., Otteng-Amoako, A. A., Brink, M., Editors. Plant Resources of Tropical Africa Databank for Commodity. PROTA Foundation, Wageningen, Nertherlands: Backhuys Publishers. http://www.prota4u.org/search.asp. Accessed 16 May 2019.

Vigbedor, B.Y., Osafo, A.S., Gyan, A.Y., 2008. Ethnobotanical survey of plants used to treat malaria in the Sekyere Central District of the Ashanti Region of Ghana. Int. J. Novel Res. Life Sci. 2 (6), 17-25.

Ayarkwa, J., 2012. *Antrocaryon micraster*. in: Louppe, D., Otteng-Amoako, A.A., Brink, M., editors. Plant Resources of Tropical Africa Databank for Commodity Group 2. Timbers 2. PROTA Foundation, Wageningen, Nertherlands. Backhuys Publishers. http://www.prota4u.org/search.asp. Accessed 16 May 2019.

Addo-Fordjour, P., Belford, E. J. D., Akonnor, D., 2013. Diversity and conservation of medicinal plants in the Bomaa community of the Brong Ahafo Region of Ghana. J. Med. Plant Res. 2 (9), 226-233.

Kumatia, E.K., Ayertey, F., Appiah-Opong, R., Bolah, P., Ehun, E., Dabo, J., 2021a. *Antrocaryon micraster* (A. Chev. And Guillaumin) stem bark extract demonstrated anti-malaria action and normalized hematological indices in plasmodium berghei infested mice in the Rane's test, J. Ethnopharmacol. 266 (2021), 113427.

Dofuor, A.K., Kumatia, E.K., Chirawurah, J.D., Ayertey, F., 2022. Antiplasmodial, Antitrypanosomal, and Cytotoxic Effects of *Anthonotha macrophylla, Annickia polycarpa, Tieghemella heckelii, and Antrocaryon micraster* Extracts. Adv. Pharmacol. Pharmaceut. Sci. 2022 (9195753), 1-7.

Fong, H.S., Tin-Wa, M., Farnsworth, N.R., Dobberstein, R.H., 1977. Phytochemical Screening Methods, College of Pharmacy, University of Illinois at the Medical Centre, Chicago, IL, USA, 1977.

Organization for Economic Co-operation and Development (OECD) guidelines for testing of chemicals. Acute oral toxicity - Acute toxic class. Geneva, WHO, 2001.

Kostyuk VA, Potapovich AI, Kovaleva JI., 1990. Simple and sensitive method of defnition of superoxide dismutase, based on reaction of oxidation of kvercetine. Quest Med Chem 2:88–91.

Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substances and antioxidants by means of folin-ciocalteu reagent. Meth Enzymol 299:152–178.

Padmanabhan, P., Jangle, S.N., 2012. Evaluation of in-vitro anti-inflammatory activity of herbal preparation, a combination of four medicinal plants. Inter. J. Basic Appl. Med. Sci. 2 (1), 109-116,

Pearson, C.M. 1956. Development of arthritis, periarthritis and periostitis in rats given adjuvants Proc. Soc. Exp. Bio. Med. 91; 95-110.

Kumatia, E.K., Ocloo, A., Tung, N.H. 2023. Triterpenoid esters from *Capparis erythrocarpos* (Isert), Capparaceae, root bark ameliorates CFA-induced arthritis. Saudi Pharmaceutical Journal 31 (2023), 698–705.

Patil M, Kandhare A, Bhise S (2012) Anti-arthritic and anti-inflammatory activity of *Xanthium srtumarium* L. ethanolic extract in Freund's complete adjuvant induced arthritis. Biomed Aging Pathol 2: 6–15.

Umapathy, E., Ndebia, E., Meeme, A., Adam, B., Menziwa, P., Chungag, B.N., 2010. An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. J. Med. Plants Res. s2010 (4), 789-795.

Opie, E., 1962. On the relation of necrosis and inflammation to denaturation of proteins. J. Exp. Med. 115, 597–600.

Chandra, S., Chatterjee, P., Dey, P., Sanjib, S., 2012. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. Asian Pac. J. Trop. Biomed. 178-180.

Huang, D., Ou, B., Prior, R.I., 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53:1841–1856.

Baumann, J., Wurn, G., Bruchlausen, F.V., 1979. Prostaglandin synthetase inhibiting O2 radical scavenging properties of some flavonoids and related phenolic compounds. Deutsche Pharmakologische Gesellschaft abstracts of the 20th spring meeting, Naunyn-Schmiedebergs abstract no: R27. Arc. Pharmacol. 307: R1–77.

Kumatia, E.K., Ayertey, F., Appiah-Opong, R., Ofori-Attah, E., Bolah, P., Antwi, S., Appiah, A.A., Ocloo, A., 2021b. Effect of Anthonotha macrophyla (P. Beauv) leaf extract on carrageenan-induced paw oedema, oxidative stress makers and hyperalgesia in murine models. Adv. Trad. Med. https://doi.org/10.1007/s13596-020-00541-8.

Kumatia, E.K., Appiah-Opong, R., 2021c. The hydroethanolic stem bark extract of *Tieghemella heckelii* (A.Chev.) Pierre ex Dubard (Sapotaceae) produced N-Methyl-D-Aspartate (NMDA) receptor-dependent analgesia and attenuates acute inflammatory pain via disruption of oxidative stress. Evid. Based Complement. Alternat. Med. 3466757. https://doi.org/10.1155/2021/3466757

Bose, M., Chakraborty, M., Bhattacharya, S., Bhattacharjee, P., Mandal, S., Kar, M., Mishra, R., 2014. Suppression of NF-κ B p65 nuclear translocation and TNF-α *by Pongamia pinnata* seed extract in adjuvant-induced arthritis. J. Immunotoxicol. 11, 222 - 230.

Rajendran, R., Krishnakumar, E., 2010. Anti-arthritic activity of *Premna serratifolia* Linn., wood against adjuvant induced arthritis. Avicenna J. Med. Biotechnol. 2, 101.

Zheng, C.J., Zhao, X.X., Ai, H.-W., Lin, B., Han, T., Jiang, Y.-P., Xing, X., Qin, L.-P. 2014. Therapeutic effects of standardized Vitex negundo seeds extract on complete Freund's adjuvant induced arthritis in rats. Phytomedicine. 21, 838-846.

Shabbir, A., Shahzad, M., Ali, A., Ziaur-Rehman, M., 2014. Anti-arthritic activity of N′-[(2, 4-dihydroxyphenyl) methylidene]-2-(3, 4- dimethyl-5, 5-dioxidopyrazolo [4, 3-c][1, 2] benzothiazin-1 (4H)-yl) acetohydrazide. Eur. J. Pharmacol. 738, 263 - 272.

Alvarez, J.L.P., 2009. Interleukin 6 in the physiopathology of rheumatoid arthritis. Reumatol. Clin. 5, 34−39.

Jalalpure, S.S., Mandavkar, Y.D., Khalure, P.R., Shinde, G.S., Shelar, P.A., Shah, A.S., 2011. Antiarthritic activity of various extracts of Mesua ferrea Linn. seed. J. Ethnopharmacol. 138, 700 -704.

Roubenoff, R., Roubenoff, R.A., Cannon, J.G., Kehayias, J. J., Zhuang, H., Dawson-Hughes, B., Dinarello, C.A., Rosenberg, I.H., 1994. Rheumatoid cachexia: cytokine-driven hypermetabolism accompanying reduced body cell mass in chronic inflammation. J. Clin. Invest. 93, 2379 - 2386.

McInnes, I.B., Schett, G. 2011. The Pathogenesis of Rheumatoid Arthritis. N. Engl. J. Med. 365, 2205- 2219.