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ORIGINAL ARTICLE

Anti-inflammatory and anti-oxidant activities of *Secamone afzelii* (Rhoem) Ascleipiadaceae

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Secamone afzelii **is used traditionally in Ghana mainly as a wound healing agent. This study reports the anti-inflammatory and antioxidant properties of *S. afzelii*. The anti-inflammatory activity was determined by the carrageenan-induced paw oedema method in 7 day old chicks and antioxidant property by the 2,2–diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, total antioxidant and total phenol content determinations. In doses of 30, 100 and 300 mg kg^{-1}, the methanol extract of the leaves reduced carrageenan-induced oedema by 24.70%, 33.41% and 44.26% respectively. The leaf extract exhibited free radical scavenging activity with an IC_{50} of 16.73 µg mL^{-1} compared to ascorbic acid which gave an IC_{50} of 2.01 µg mL^{-1}. In the total phenol content determination, the leaf extract was found to contain 56.86 mg g^{-1} calculated as tannic acid equivalent of the dry weight of extract. The extract also demonstrated remarkable antioxidant effect in the total antioxidant capacity determinations and had an 87% correlation with the amount of phenolic content present.**

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INTRODUCTION

Sub-Saharan Africa is endowed with several medicinal plants with a plethora of historical information for their use in the management of many disease conditions (Lawal *et al*., 2014). Several scientific reports have also justified the biological activities that are traditionally stipulated for these medicinal plants (Agyare *et al*., 2012, Fleischer *et al*., 2013, Kyei *et al*., 2012, Mensah *et al*., 2011, Mensah *et al*., 2001) In recent years, there has been a phenomenal increase in research on medicinal plants used in the in management of inflammatory conditions (Agyare *et al*., 2013, Amponsah *et al*., 2013, Boakye-Gyasi *et al*., 2013, Woode *et al*., 2008) due to severe adverse side effects associated with conventional drugs for the management of inflammation (steroidal and non-steroidal anti-inflammatory drugs). With all efforts therefore geared towards finding suitable herbal remedies for these conditions, studies on *Secamone afzelii* Rhoem (Asclepiadaceae) a creeping woody climber whose aerial parts are appreciated as a medicinal in West Africa (Irvine, 1961) cannot be overlooked. In Ghana, Nigeria and Sierra Leone, the leaves are taken as an infusion, laxative, antispasmodic, analgesic and to treat colic, diarrhoea, urinary tract and sexually transmitted infections. The latex of the leaves is used as a poultice to mature boils and to heal wounds, skin inflammation and breast abscesses. Ground leafy twigs in Shea butter is applied topically to treat nasopharyngeal and respiratory tract infections in children (Kémeuzé, 2010, Burkill, 1985).

Previous studies have shown that *Secamone afzelli* has in vitro antimicrobial activity and the plant extract is able to protect cells against damage by free oxygen radicals (Houghton *et al*., 2005, Mensah *et al*., 2007). The total extract also showed effective free radical scavenging activity in non-enzymatic lipid peroxidation in liposomes which was attributed to α-tocopherol extracted from the leaf (Mensah

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et al., 2004). This study investigates the anti-inflammatory property of the leaf extract of *S. afzelii* using animal models and relates its free radical scavenging and total antioxidant capacity by determining the total phenolic content.

MATERIALS AND METHODS

Plant Collection and Extraction

The leaves of *Secamone afzelii* were collected from the Physique garden of the Faculty of Pharmacy and Pharmaceutical Science, Kwame Nkrumah University of Science & Technology, Kumasi, Ghana in October 2013, authenticated and the voucher specimen, SAS2013 deposited in the herbarium of the Pharmacognosy Department. Two hundred grams (200 g) of the leaves were air dried for 3 days, milled with a mechanical grinder and cold macerated with 500 mL of methanol for 72 hours. The resultant extract was filtered and concentrated to a dark green syrup extract (SA) using a rotary evaporator (R114, Buchi) at temperatures not exceeding 45ºC. The percentage yield was 10.4 % w w^{-1}.

Anti-Inflammatory Assay

Animals

Day old cockerels (*Gallus gallus*) were obtained from Akate Farms, Kumasi, Ghana and were housed in stainless steel cages (34×57×40 cm) at a population density of 10 to 12 chicks per cage. The chicks were fed on chick mash obtained from GAFCO, Tema, Ghana and water *ad libitum*. Temperature was kept at 29°C and overhead incandescent illumination was maintained on a 12 hour light-dark cycle. Chicks were experimented at 7 day-old and were randomly divided into groups of 5 throughout the study.

Experimental design

The various groups of animals comprised the positive control, negative control and treatment groups. Normal saline which was the vehicle for reconstituting the extracts was administered to the negative control, the positive control received 10, 30 and 100 mg kg^{-1} body weight of diclofenac or 0.3, 1 and 3 mg kg^{-1} body weight of dexamethasone and the experimental groups received the *S. afzelii* extract at 30, 100 and 300 mg kg^{-1} body weight. The vehicle and the extracts were administered orally *(p.o)* whiles the standard drugs (diclofenac and dexamethasone) were administered intraperitoneally *(i.p)*.

Carrageenan-induced foot oedema in the chicks

The carrageenan-induced foot oedema model of inflammation in the chick described by Roach and Sufka (2003) with some modification (Boakye-Gyasi *et al.*, 2013, Roach and Sufka, 2003) was used to investigate the anti-inflammatory property of *Secamone afzelii* leaf extract. The foot volume was measured by water displacement plethysmography as described by Fereidoni *et al* (Fereidoni *et al.*, 2000) and carrageenan (10 µl of a 1% suspension in saline) injected sub-plantar into the right footpads of the chicks. Follow up foot volume measurements were then done. Oedema component of inflammation was quantified by measuring the difference in foot volume before carrageenan injection and at an hourly time interval for 5 hours. All experimental protocols were in compliance with the National Institute of Health guidelines for the care and use of laboratory animals and were approved by the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST Ethics Committee.

Antioxidant Assays

2,2–diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity: This assay was performed by the method described by Blois, (1958). Different concentrations of the extract (100 – 6.25 µg ml^{-1}) and ascorbic acid (25 - 0.78 µg ml^{-1}) were used in the assay. The reaction mixture consisted 1ml of each concentration of extract and 3ml of DPPH (20 mg L^{-1}). The mixtures were allowed to stand for 30 minutes and absorbance was measured at 517 nm. Ascorbic acid and blank methanol were taken through the same procedure to serve as positive and negative controls respectively. The percentage of DPPH scavenged was calculated using the following equation:

$$\%\,DPPH\ scavenged = \frac{(A0 - A1)}{(A0)} \times 100$$

Where A_0 = absorbance of negative control, A_1 = absorbance of different concentrations of extract/ ascorbic acid.

Total antioxidant capacity (TAC)

Total antioxidant capacity of the extract was determined as described by Prieto *et al.,* (1999). Different concentrations were prepared for both ascorbic acid (25 - 0.78 µg mL^{-1}) and extract (100 - 6.25 µg mL^{-1}). The reaction mixture consisted of 1ml of plant extract or standard drug with 3 ml of reagent solution (0.6 M H_2SO_4, 28 mM Na_2HPO_4, and 4 mM ammonium molybdate). The mixtures were incubated at 95ºC for 90 minutes and the absorbance determined at 695 nm. The negative control (methanol only) and positive control (ascorbic acid) were treated in the same manner as extracts. The total antioxidant capacity was expressed in terms of ascorbic acid equivalent of the extract (mg g^{-1} of dry mass).

Total phenol content (TPC) determination:

Total phenol content of the extract was determined using the Folin-Ciocalteu's reagent method (Slinkard and Singleton, 1977). Extract (1 ml of 100 - 6.25 µg mL^{-1}) in methanol was separately mixed with 1 ml Folin-Ciocalteu's reagent (1 ml; diluted 1:10 with distilled water) and 1ml of aqueous Na_2CO_3 (2% w v^{-1}, 1 ml) and incubated at room temperature (28ºC) for 2 h. Absorbance was then read at 760 nm using tannic acid as a reference standard. Methanol was processed in the same way and used as blank. The total phenol content was expressed as mg g^{-1} of tannic acid equivalents (TAEs).

Statistical analysis

The raw scores for foot volume increase at each hour (T_1, T_2, T_3, T_4 and T_5) for each animal was normalized as the percentage difference from the initial foot volume at time zero (T_0) and was determined as follows:

$$\%\ Increase\ of\ foot\ volume = \frac{(Foot\ Volume\ at\ T1 - Foot\ Volume\ at\ T0)}{(Foot\ Volume\ T1)} \times 100$$

These values were then averaged for each treatment group and the total foot volume for each treatment group calculated in arbitrary unit as the area under the curve (AUC). Percentage inhibition of oedema for each treatment group was determined as follows:

$$\%\ Inhibition\ of\ oedema = \frac{(AUC\ control - AUC\ treatment)}{(AUC\ control)} \times 100$$

Differences in AUCs were analyzed by one way analysis of variance (ANOVA) followed by Student - Newman Keuls' post test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Secamone afzelii Rhoem (Asclepiadaceae) is used widely in the sub-Saharan region for the treatment of pain, abscess, boils and wounds among others. One major factor underlying most of these diseases is inflammation and associated oxidative stress. In this work, the anti-inflammatory property of the

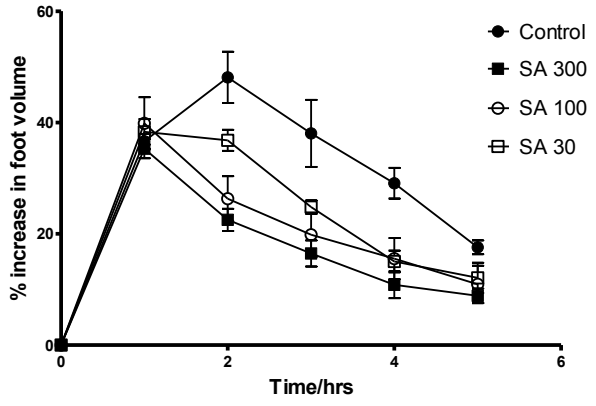

Figure 1a: Time course curve for progression of inflammation for SA (300, 100, 30 mg kg^{-1})

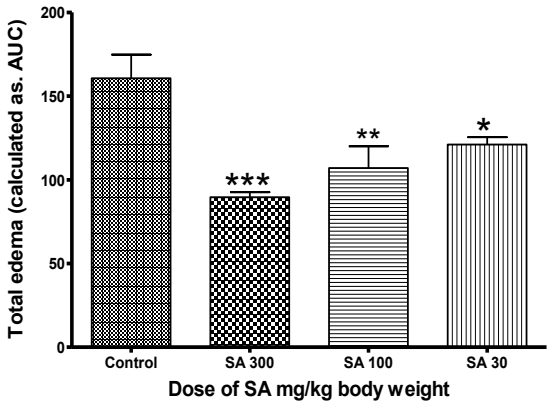

Figure 1b: Total oedema response for SA (300, 100, 30 mg kg^{-1}) calculated as AUCs

leaves of *S. afzelii* was investigated in order to give scientific justification to the reported traditional uses. The total phenol content, total antioxidant capacity as well as and free radical scavenging ability were also investigated. Phytochemical screening of the powdered bark of *S. afzelii,* revealed the presence of bioactive components specifically flavonoids, triterpenes and coumarins which confirms previous works done (Mensah *et al.*, 2004, Mensah *et al.*, 2007, Zabri *et al.*, 2008).

The anti-inflammatory activity was evaluated using the carrageenan-induced paw oedema method in chicks. Carrageenan-induced paw oedema in laboratory animals was first introduced by Winter (Winter *et al.*, 1962) and has been used widely to screen new and potential anti-inflammatory drugs. In this experiment, subcutaneous injection of 1% carrageenan caused a time dependent increase in foot volume which began approximately after one hour of administration and peaked at the third hour. It began to reduce slowly throughout the 5 hour period of the experiment (Figure 1a-3a) indicating the body's owns ability to fight inflammation. However, there was an observable decrease in foot volume which occurred at a faster rate compared to the negative control after ingestion of SA (30-300 mg kg^{-1}) whose effect was dose-dependent. The highest dose of SA gave a 44.26% inhibition of carrageenan induced oedema (Figure 1b) followed by SA 100 mg kg^{-1} (33.41%) and SA 30 mg kg^{-1} (24.70%). The highest effect given by the extract was however lower than that of standard drugs used. The standard drugs used, diclofenac (Figure 2b) and dexamethasone (Figure 3b), showed significant inhibition ($p<0.001$) of oedema at all doses. The highest dose of diclofenac (100 mg kg^{-1}), however gave a better anti-inflammatory effect (71.5±9.3%).

According to Vinegar *et al*, the development of the carrageenan induced inflammation is due to the release of cytoplasmic enzymes and serotonin from mast cells and the increase of prostaglandin release to the inflamed area (Vinegar *et al.*, 1987). While the anti-inflammatory activity of diclofenac is mediated chiefly through inhibition of the cyclooxygenase pathway (COX 1 and COX 2), particularly prostaglandins and that of dexamethasone is mediated through their suppressive effects on the inflammatory cytokines and on other lipid mediators of inflammation (Enomoto, 2007), the exact mechanism by which *Secamone afzelii* reduces inflammation is not known. It is however possible that the extract may inhibit the release of inflammatory mediators released during carrageenan-induced inflammation.

The antioxidant activity of the methanolic extract of *S. afzelii* leaves was also examined by the DPPH assay, total antioxidant capacity (TAC) and total phenol content (TPC) determination. DPPH is a stable nitrogen-centred free radical with a deep violet colour and absorption maxima at 517 nm. It is

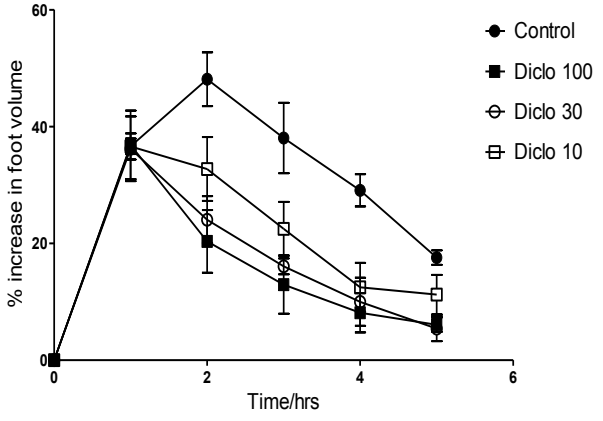

Figure 2a: Time course curve for progression of inflammation for diclofenac (100, 30, 10 mg kg^{-1})

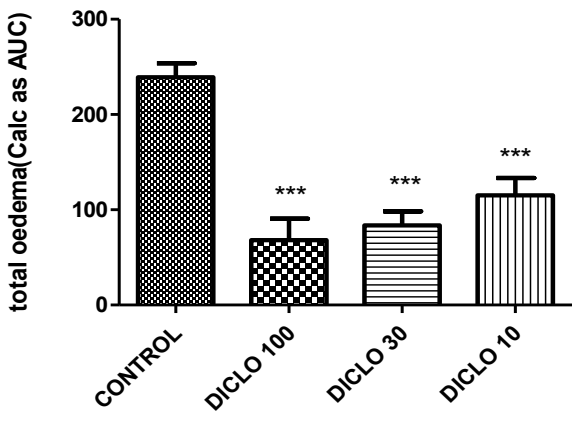

Figure 2b: Total oedema response for diclofenac (100, 30, 10 mg kg^{-1}) calculated as AUCs

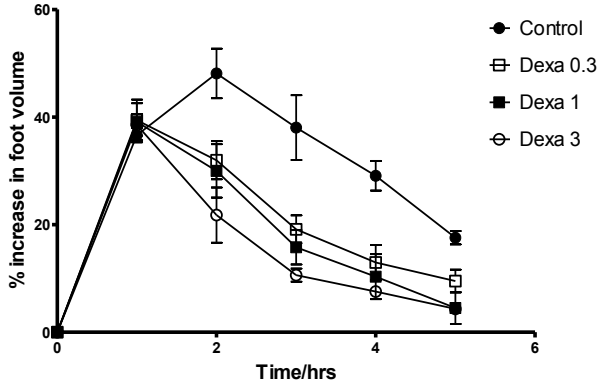

Figure 3a: Time course curve for progression of inflammation for dexamethasone (3, 1, 0.3 mg kg^{-1})

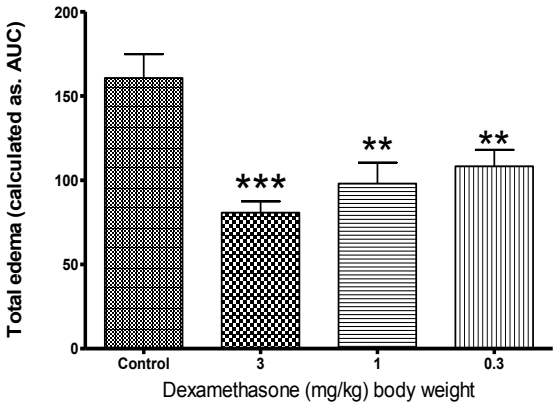

Figure 3b: Total oedema response for dexamethasone (3, 1, 0.3 mg kg^{-1}) calculated as AUCs

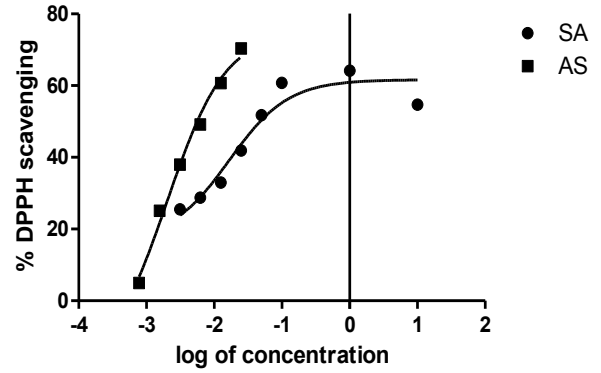

Figure 4: %DPPH absorbance vrs. log concentration of SA extract and ascorbic acid (AS)

Table 1: EC_{50} of SA and ascorbic acid in the DPPH free radical scavenging assay

Drug	EC_{50} (µg mL^{-1})
Secamone afzelii	16.73
Ascorbic acid	2.018

decolorized when it accepts an electron from the antioxidant compound to form DPPH-H. The amount of residual DPPH is quantitatively measured from changes in UV absorbance at 517 nm (Blois, 1958). The methanolic extract (SA) caused a dose dependent decrease in DPPH absorbance, with an EC_{50} of 16.73 µg mL^{-1}. This effect was lower than that of the standard drug, ascorbic acid which had an EC_{50} of 2.01 µg mL^{-1} (Figure 4; Table 1).

The phosphomolybdate method was used to evaluate the total antioxidant capacity (TAC) of the extract. The TAC was expressed as the number of equivalents of ascorbic acid in mg g^{-1} of the dry mass of extract. It is based on the reduction of Mo (VI) to Mo (V) by the antioxidant agent (i.e. *Secamone afzelii* or ascorbic acid) and the formation of a green phosphate complex (Athukorala *et al.*, 2006). An increased amount of complex compound formed causes an increase in absorbance and indicates better antioxidant capacity. The results of the experiment indicated that *Secamone afzelii* has antioxidant activity which increases with increasing concentration. The highest TAC recorded for *S. afzelii* leaves was 112.86 mg g^{-1} (ascorbic acid equivalent). The total anti-oxidant capacity increase with increasing concentration of the extract (Figure 5).

The presence of phenolic compounds and flavonoids in plants has been associated with their antioxidant action in biological systems (Chanda and Dave, 2009). Due to their structural chemistry, plant phenols act as hydrogen or electron donors to stabilize the unpaired radical (Rice-Evans *et al.*, 1997). During the inflammatory process, the excessive production of reactive oxygen metabolites by

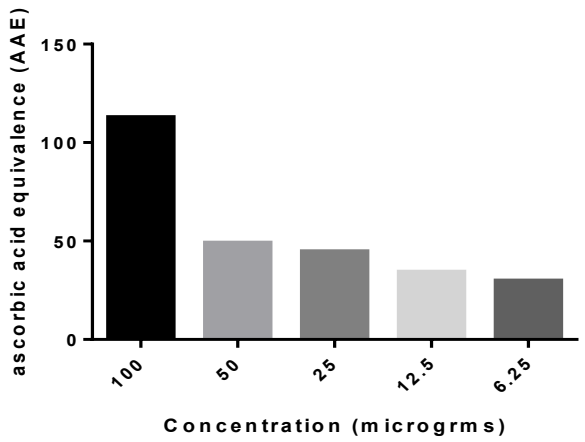

Figure 5: Total antioxidant capacity of *S. afzelii* measured as ascorbic acid equivalent in mg/g at different concentrations of extract.

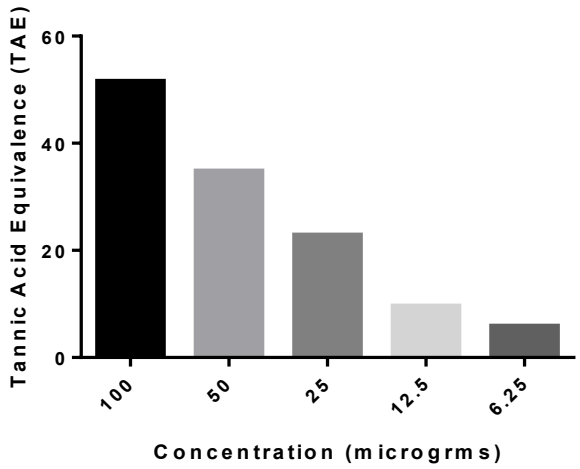

Figure 6: Total phenolic content of *S. afzelii* measured as tannic acid equivalent in mg g^{-1} at different concentrations of extract.

phagocytic leucocytes causes tissue injury which in turn augments the state of inflammation and lead to chronic inflammatory states. Antioxidants, which scavenge these reactive oxygen metabolites, have been found to complement the anti-inflammatory process, enhance tissue repair and promote wound healing (Wu *et al.*, 2006, Vinegar *et al.*, 1987). From the results, *Secamone afzelii* contains phenols. The TPC was 56.86 mg g^{-1} of dry mass of extract ex-

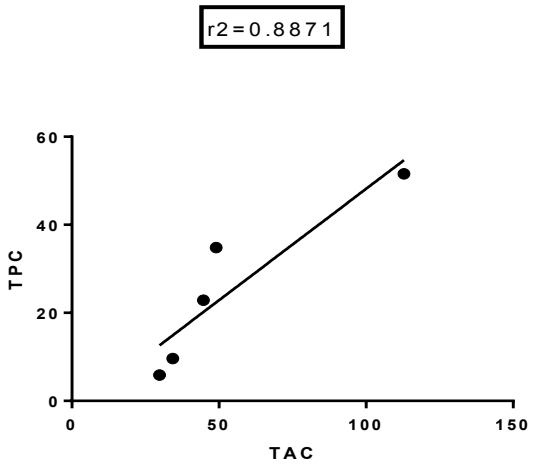

Figure 7: Correlation between TPC and TAC

pressed as the number of equivalent tannic acid. As concentration of the extract increase, there was an observable increase in absorbance corresponding to an increase in phenolic content (Figure 6). A linearity graph of TPC against TAC was also obtained to show the relationship between the phenol content of the extract and its antioxidant capacity (Figure 7). From the linearity graph, 88.7% of its antioxidant activity is due to the presence of phenols and the remaining 12% from other components of the plant. The antioxidant activity of the extracts may therefore partly contribute to its anti-inflammatory activity because inflammatory tissue injuries are mediated by reactive oxygen metabolites from phagocytic leucocytes that invade the tissue (Zaikov, 2000). The antioxidant activity of the plant may support its traditional use in Ghana as a wound healing agent.

CONCLUSION

The anti-inflammatory activity observed for *S. afzelii* gives some scientific justification for its use in the treatment of inflammatory conditions and wounds. The presence of phenolic matter and antioxidant activity is further confirmed and may be involved in the wound healing process of the plant.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Agyare, C., Dwobeng, A. S., Agyepong, N., Boakye, Y. D., Mensah, K. B., Ayande, P. G. & Adarkwa-Yiadom, M. 2013. Antimicrobial, Antioxidant, and Wound Healing Properties of *Kigelia africana* (Lam.) Beneth. and *Strophanthus hispidus* DC. *Advances in pharmacological sciences*, 1-10.

Agyare, C., Koffuor, G. A., Boamah, V. E., Adu, F., Mensah, K. B. & Adu-Amoah, L. 2012. Antimicrobial and anti-inflammatory activities of *Pterygota macrocarpa* and *Cola gigantea* (Sterculiaceae). *Evidence-Based Complementary and Alternative Medicine*, 1-10.

Amponsah, I. K., Fleischer, T. C., Dickson, R. A., Annan, K. & Thoss, V. 2013. Evaluation of anti-inflammatory and antioxidant activity of Furanocoumarins and Sterolin from the stem bark of *Ficus exasperata* Vahl (Moraceae). *Journal of Scientific and Innovative Research,* 2**,** 880-887.

Athukorala, Y., Kim, K. N. & Jeon, Y. J. 2006. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. *Food and Chemical Toxicology,* 44**,** 1065-1074.

Blois, M. S. 1958. Antioxidant determinations by the use of a stable free radical.

Boakye-Gyasi, E., Abotsi, W. K. & Woode, E. 2013. Anti-inflammatory and analgesic effects of ethanolic leaf extract of *Newbouldia laevis* (P. Beauv.) Seemann Ex Bureau (Bignoniaceae). *Der Pharmacia Sinica,* 4.

Burkill, H. 1985. *The useful plants of west tropical Africa. Edition 2. Vol. 1: families AD*.

Chanda, S. & Dave, R. 2009. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research,* 3**,** 981-996.

Enomoto, R., Suzuki, C., Koshiba, C., Nishino, T., Nakayama, M., Hirano, H., Yokoi, T. & Lee, E. 2007. Wogonin prevents immunosuppressive action but not anti-inflammatory effect induced by glucocorticoid. *Annals of the New York Academy of Sciences,* 1095**,** 412-417.

Fereidoni, M., Ahmadiani, A. & Samnanian, S. 2000. An accurate and simple method for measurement of paw oedema. *Journal of Pharmacology and Toxicological Methods* 43**,** 11 -14.

Fleischer, T. C., Annan, K., Dickson, R. A., Mensah, A. Y. & Sarpong, F. M. 2013. Anti-inflammatory, antioxidant and antimicrobial activity of the stem bark extract and fractions of *Ficus exasperata* Vahl. (Moraceae). *Journal of Pharmacognosy and Phytochemistry,* 2**,** 38-44.

Houghton, P. J., Hylands, P. J., Mensah, A. Y., Hensel, A. & Deters, A. M. 2005. In vitro tests and ethnopharmacological investigations: wound healing as an example. *Journal of Ethnopharmacology,* 100**,** 100-107.

Irvine, F. R. 1961. Woody plants of Ghana. *Woody plants of Ghana*.

Kémeuzé, V. A. 2010. *Secamone afzelii* (Schult.) K.Schum; In: Schmelzer, G.H. & Gurib-Fakim, A.(Editors). *Medicinal plants/Plantes médicinales 2*. Wageningen, Netherlands: PROTA, .

Kyei, S., Koffuor, G. A. & Boampong, J. N. 2012. Antiarthritic effect of aqueous and ethanolic leaf extracts of *Pistia stratiotes* in adjuvant-induced arthritis in Sprague-Dawley rats. *Journal of Experimental Pharmacology,* 4**,** 41-51.

Lawal, I., Grierson, D. & Afolayan, A. 2014. Phytotherapeutic Information on Plants Used for the Treatment of Tuberculosis in Eastern Cape Province, South Africa. *Evidence-Based Complementary and Alternative Medicine*.

Mensah, A. Y., Donkor, P. O. & Fleischer, T. C. 2011. Anti-Inflammatory And Antioxidant Activities Of The Leaves Of *Wissadula Amplissima* Var Rostrata. *African Journal of Traditional, Complementary and Alternative Medicines,* 8.

Mensah, A. Y., Houghton, P. J., Agyare, C., Komlaga, G., Mensah, M. L. K., Fleisher, T. C. & Sarpong, K. 2007. Investigation of Activities Related to Wound Healing of *Secamone Afzelii*. *Journal of Science and Technology (Ghana),* 26**,** 83-89.

Mensah, A. Y., Houghton, P. J., Akyirem, G. N. A., Fleischer, T. C., Mensah, M. L. K., Sarpong, K. & Adosraku, R. 2004. Evaluation of the antioxidant and free radical scavenging properties of *Secamone afzelii* Rhoem. *Phytotherapy Research,* 18**,** 1031-1032.

Mensah, A. Y., Sampson, J., Houghton, P. J., Hylands, P. J., Westbrook, J., Dunn, M., Hughes, M. A. & Cherry, G. W. 2001. Effects of *Buddleja globosa* leaf and its constituents relevant to wound healing. *Journal of Ethnopharmacology,* 77**,** 219-226.

Prieto, P., Pineda, M. & Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry,* 269**,** 337-341.

Rice-Evans, C., Miller, N. & Paganga, G. 1997. Antioxidant properties of phenolic compounds. *Trends in plant science,* 2**,** 152-159.

Roach, J. T. & Sufka, K. J. 2003. Characterization of the chick carrageenan response. *Brain research,* 994**,** 216-225.

Slinkard, K. & Singleton, V. L. 1977. Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture,* 28**,** 49-55.

Vinegar, R., Truax, J. F., Selph, J. L., Johnston, P. R., Venable, A. L. L. & Mckenzie, K. K. 1987. Pathway to carrageenan-induced inflammation in the hind limb of the rat. *Federation proceedings.*

Winter, C. A., Risley, E. A. & Nuss, G. W. 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. *Proc Soc Exp Biol Med,* 111**,** 544-7.

Woode, E., Ansah, C., Ainooson, G. K., Abotsi, W. M., Mensah, A. Y. & Duweijua, M. 2008. Anti-inflammatory and antioxidant properties of the root extract of *Carissa edulis* (Forsk.) Vahl (Apocynaceae). *Journal of Science and Technology (Ghana),* 27**,** 5-15.

Wu, S. J., Tsai, J. Y., Chang, S. P., Lin, D. L., Wang, S. S., Huang, S. N. & Ng, L. T. 2006. Supercritical carbon dioxide extract exhibits enhanced antioxidant and anti-inflammatory activities of *Physalis peruviana. Journal of ethnopharmacology,* 108**,** 407-413.

Zabri, H., Kodjo, C., Benie, A., Bekro, J. M. & Bekro, Y. A. 2008. Phytochemical screening and determination of flavonoids in *Secamone afzelii* (Asclepiadaceae) extracts. *African Journal of Pure and Applied Chemistry,* 2**,** 080-082.

Zaikov, G. E. 2000. *Biotic type antioxidants: The prospective search area for novel chemical drugs*, VSP.

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