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Studies on *In-vitro* Anti-inflammatory and Antioxidant Potentials of *Annona muricata* Leaf Extracts

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ABSTRACT

Inflammation has stimulated significant worldwide scientific interest because of its implication in many human diseases. Most inflammations are caused by reactive oxygen species or free radicals. *Annona muricata* leaf extracts were investigated for their *in-vitro* antioxidant and anti-inflammatory potentials. *Annona muricata* leaves were dried at room temperature, blended using a mill, and extracted with solvents of varying degree of polarities. The solvents used were hexane, ethyl acetate, and ethanol. After sequential extraction, the crude extracts were examined for their *in-vitro* anti-inflammatory activities on lipoxygenase inhibition, proteinase inhibition, albumin denaturation inhibition, and red blood cell membrane stabilization assays, while the antioxidant activities were examined using DPPH, ABTS and hydrogen peroxide assays. The results showed that the ethanol extract had significantly higher albumin denaturation inhibition activity at 500 µg/mL ($p < 0.01$). The activity of all the extracts on proteinase inhibition decreased with the increase in concentration of the extracts. Indomethacin (standard), ethanol extract, and ethyl acetate extract exhibited a dose dependent increase in lipoxygenase activity. The ethanol extract showed high red blood cell membrane stabilization activity at 500 µg/mL and the activity was comparable with that of the standard (diclofenac). Hydrogen peroxide scavenging activity of the extracts and standard (Vitamin C) were comparable at 20 – 100 µg/mL. The ethanol extract showed significantly higher ($p < 0.01$) DPPH radical scavenging activity compared with other extracts. A similar trend was also observed for ABTS radical scavenging activity. Generally, the ethanol extract exhibited higher anti-inflammatory and antioxidant activities in most of the assays, this could be attributed to the polar compounds present in the extract.

Introduction

Inflammation is a complex biological response of vascular tissues to harmful stimuli (Adegbola et al., 2017). Wounds and damage to tissues would not be able to heal without an inflammatory response, but chronic inflammation can cause severe health conditions including cancers. It is a major cause of mortality in the world (Krishnamoorthy et al., 2016). Inflammations are also caused by reactive oxygen

species such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxy nitrite. The reactive oxygen species produced by the action of free radicals on molecular oxygen causes an imbalance between the oxidizing molecules and the antioxidant system of the body, which in turn results in serious inflammation conditions (Amri et al., 2018). Non-steroidal anti-inflammatory drugs (NSAIDs) which are commonly prescribed for the treatment of inflammatory conditions are associated with side effects, such as gastrointestinal bleeding and

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suppressed function of the immune system (Adebayo et al., 2015). Due to the complications associated with synthetic anti-inflammatory drugs, there is need for safe and natural anti-inflammatory and antioxidant agents which could have better activities than the harmful synthetic ones.

Annona muricata is of the family Annonaceae and genus *Annona*. It is cultivated in the tropical regions of Central and South America and Tropical Africa. Annonaceae is a family of about 130 genera and 2000 species. The plant is also called sour sop because the fruit has a characteristic sour taste and flavour (Mejia-Jimenez et al., 2002). All parts of the tree are used in traditional medicine in the tropics, including the bark, leaves, root, fruits and seeds. The plant has been found to be useful as an antispasmodic, a sedative, hypoglycaemic, hypotensive, and smooth muscle relaxant (Holdsworth, 1990). *Annona muricata* has edible fruits which are normally eaten by mothers after childbirth to boost breast milk (Abdul Wahab et al., 2018; Moghadamtousi et al., 2015). There is no report on the use of *Annona muricata* leaf as a food ingredient or as functional food, this may be due to the hard nature of the leaf. Considering the wide traditional applications of this plant, it is necessary to carry out investigations of the anti-inflammatory and antioxidant potentials of the leaf extracts.

Materials and methods

Preparation and extraction of plant material

The plant materials were collected from a local garden in Ilorin, Kwara State, Nigeria. The sample was identified and documented at the Herbarium of Plant Biology Department, University of Ilorin, with voucher number UIH001/1106. The leaves of *Annona muricata* were dried at room temperature and ground to powder using a mill. The cold extraction method was used for the extraction of secondary metabolites from the plant. About 2.5 kg of leaf powder was extracted with hexane for three days. The crude extract solution was decanted, filtered with Whatman No. 1 filter paper, and concentrated in vacuo. The hexane crude extract which weighed 52 g was coded as AMH and stored in the refrigerator until further analyses. Ethyl acetate was used to extract the remaining plant material for three days. The crude extract was decanted, filtered, and concentrated. The ethyl acetate extract was coded as AMEA and the weight was 110 g. It was stored in the refrigerator until further analyses. The residual plant material was extracted with ethanol for three days. The resulting ethanol crude extract which weighed 66 g was coded as AME and stored in the refrigerator until further analyses.

The crude extracts; AMH, AMEA, and AME were dark green oils.

Anti-inflammatory assays

Anti-inflammatory assays were carried out on AMH, AMEA, and AME. The anti-inflammatory assays include the inhibition of lipoxygenase, proteinase inhibitory activity, albumin denaturation inhibition, and red blood cell (RBC) membrane stabilization. The assays were carried out using the procedures described by Leelaprakash and Das (2011). In the inhibition of lipoxygenase assay, indomethacin was used as a standard. Indomethacin is a nonsteroidal anti-inflammatory drug and numerous comparative studies have affirmed the clinical utility of the drug (Nalamachu and Wortmann, 2014). Diclofenac, a potent anti-inflammatory drug, was used as a standard for the RBC membrane stabilization assay (Altman et al., 2015).

Anti-oxidant assays

AMH, AMEA, and AME were analysed for their anti-oxidant activities. The parameters that were considered include; 1,1-diphenyl-2-picryl hydroxyl (DPPH) radical scavenging, 2,2-azinobis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS) radical scavenging, and hydrogen peroxide scavenging activities. The experiments were carried out as described by Nishaa et al. (2012). Vitamin C was used as standard for ABTS and hydrogen peroxide scavenging assay due to its potent antioxidant activity. It has also been used in several antioxidant studies (Padayatty et al., 2003; Pisoschi and Negulescu 2011). Quercetin has been used in several studies for the DPPH radical scavenging assay due to its high antioxidant activity (Priyanga and Vijayalakshmi, 2017).

Results and discussions

Results of anti-inflammatory studies

Results of albumin denaturation inhibition

The results showed that AME was more active than other extracts at 200 and 500 µg/mL (Fig. 1). At 500 µg/mL, AME exhibited significantly higher albumin denaturation inhibition activity ($p < 0.01$). The activity of AME and AMH were dose dependent. Albumin denaturation is the partial or complete alteration of the structures of proteins or nucleic acids resulting in a loss of bioactivity and cell death.

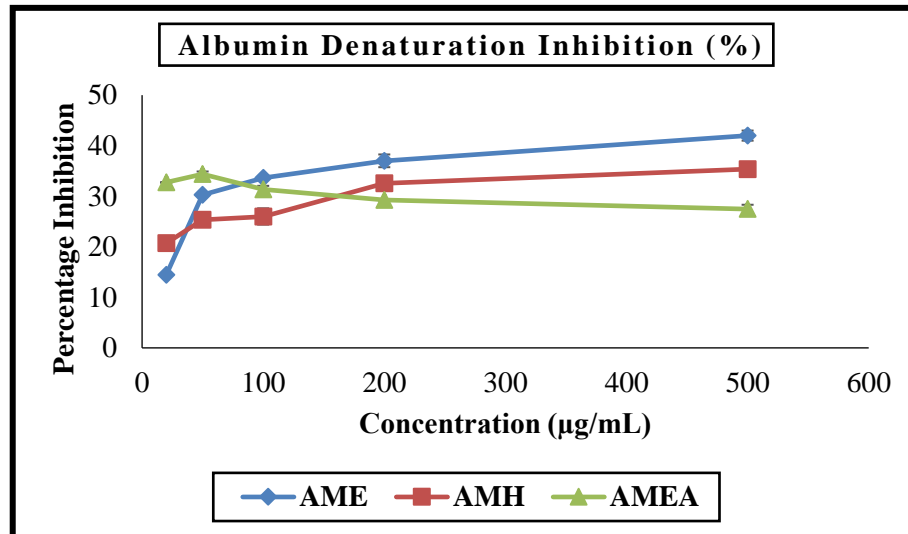


Fig. 1. *In-vitro* Albumin denaturation inhibition activity of *Annona muricata* leaf extracts. Each point on the line graph represents the mean of triplicate readings (n=3) while the error bar represents standard error (SE) of the means. AME=*Annona muricata* ethanol extract; AMH=*Annona muricata* hexane extract; AMEA=*Annona muricata* ethyl acetate extract

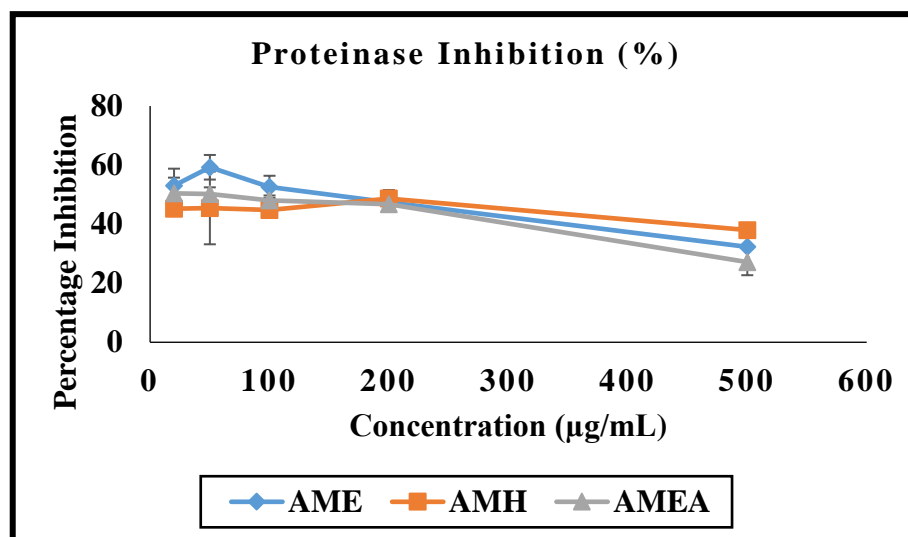


Fig. 2. *In-vitro* Proteinase inhibition activity of *Annona muricata* leaf extracts. Each point on the line graph represents the mean of triplicate readings (n=3) while the error bar represents standard error (SE) of the means. AME=*Annona muricata* ethanol extract; AMH=*Annona muricata* hexane extract; AMEA=*Annona muricata* ethyl acetate extract.

Results of proteinase inhibition

The activities of the samples decreased with the increase in concentration of the extracts. The activity of AME at 50 µg/mL was significantly higher ($p < 0.01$) than other treatments (Fig. 2). Proteinases are involved in regulating physiological functions such as cell growth. They also mediate various pathological conditions such as hypertension, cancer, and malaria. Strict regulation of the activities of proteinases is necessary to prevent unrestrained cleavage of proteins. Proteinase inhibitors play a vital role in the regulation of proteinase activities and also show therapeutic effects against diseases in humans (Perera et al., 2016).

Results of lipoxygenase inhibition

All the tested samples showed high lipoxygenase inhibition activity (Fig. 3). AMH was most active at 20–100 µg/mL compared with other extracts and the standard. Ondua et al. (2019) reported that the hexane extract of the medicinal plant *Typha capensis* had higher lipoxygenase activity than quercetin which was used as a standard. The activity of AME was dose dependent. The products of lipoxygenase mediated pathways play a major role in the manifestation of chronic inflammatory diseases. Inhibitors of lipoxygenase are promising therapeutic targets.

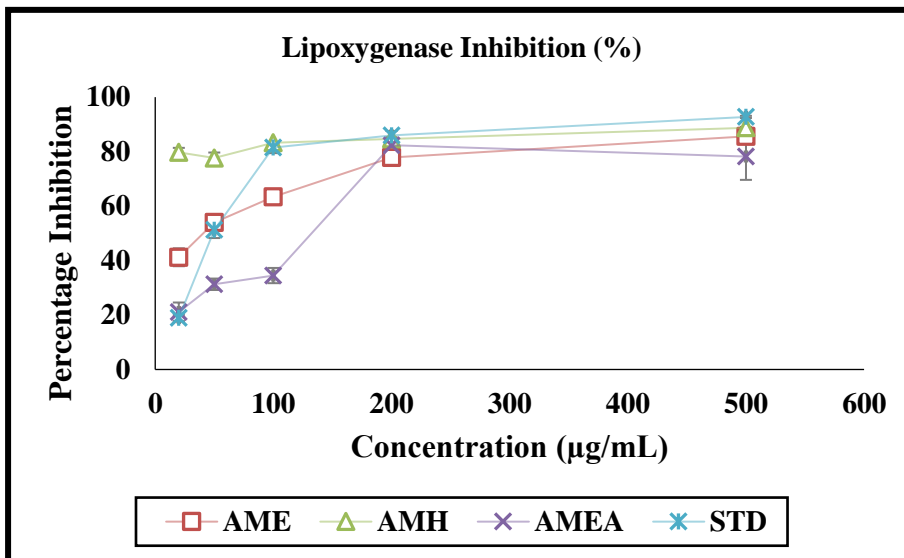


Fig. 3. In-vitro lipoxygenase inhibition activity of *Annona muricata* leaf extracts. Each point on the line graph represents the mean of triplicate readings (n=3) while the error bar represents standard error (SE) of the means. AME=*Annona muricata* ethanol extract; AMH=*Annona muricata* hexane extract; AMEA=*Annona muricata* ethyl acetate extract; STD=Standard (Indomethacin).

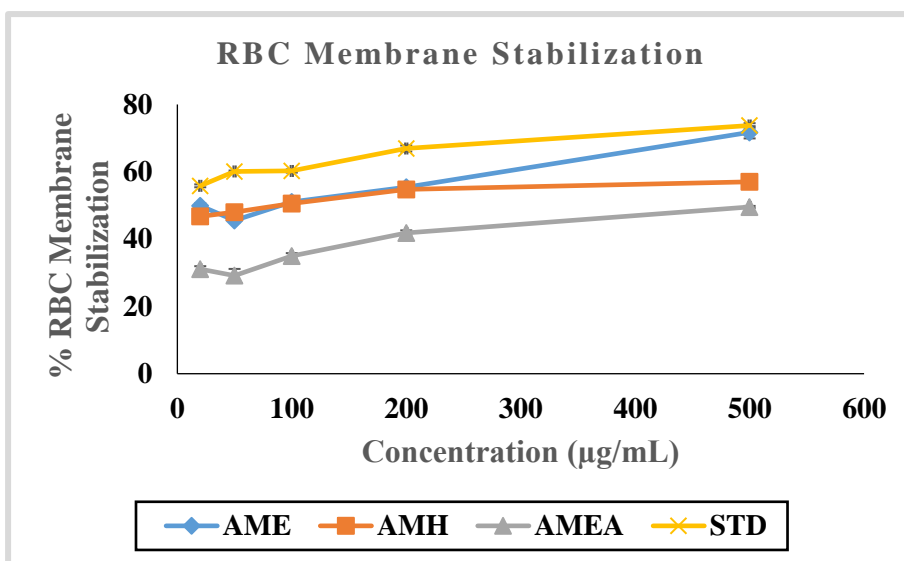


Fig. 4. In-vitro RBC membrane stabilization activity of *Annona muricata* leaf extracts. Each point on the line graph represents the mean of triplicate readings (n=3) while the error bar represents standard error (SE) of the means. AME=*Annona muricata* ethanol extract; AMH=*Annona muricata* hexane extract; AMEA=*Annona muricata* ethyl acetate extract; STD=Standard (Diclofenac).

Results of RBC membrane stabilization

The results showed that the RBC membrane stabilization activity of AME at 500 µg/mL was comparable with that of the standard (Diclofenac) and the two treatments were significantly higher than that of other treatments (Fig. 4). Oyekachukwu et al. (2017) reported that the chloroform extract of the *Annona muricata* leaf significantly caused red blood

cell membrane stabilization in a manner comparable with indomethacin which was used as a standard. It was reported in the study of Abd Hamid et al. (2010) that ethanol extract of *Annona muricata* leaf significantly inhibits inflammation in a dose dependent manner. The RBC membrane stabilization is a result of anti-inflammatory agents inhibiting the rupture of erythrocytes by stabilizing the cell membrane.

Results of antioxidant studies

Results of hydrogen peroxide scavenging activity

The activities of the samples were comparable with that of the standard (Vitamin C) at 20 – 200 µg/mL. The activity of AME, AMEA, and AMH fall within the range of 30 – 44% (Fig. 5). The activity of AME at 500 µg/mL was higher than that of other extracts. Nishaa et al. (2012) reported that at 500 µg/mL, ethanol extract of *Maranta arundinacea* rhizomes showed hydrogen peroxide scavenging of 69.49%, which is higher than the activity of *Annona muricata* extract reported in this study.

Results of DPPH radical scavenging activity

From the results of DPPH radical scavenging, AME showed significantly higher ($p < 0.01$) antioxidant activity than AMH and AMEA at all concentrations tested, but the activity of AME was not comparable with the standard (Quercetin) (Fig. 6). Correa et al. (2018), in their studies with *Pfaffia townsendii*, revealed that the ethanol extract of the leaf has higher DPPH antioxidant activity than the hexane extract.

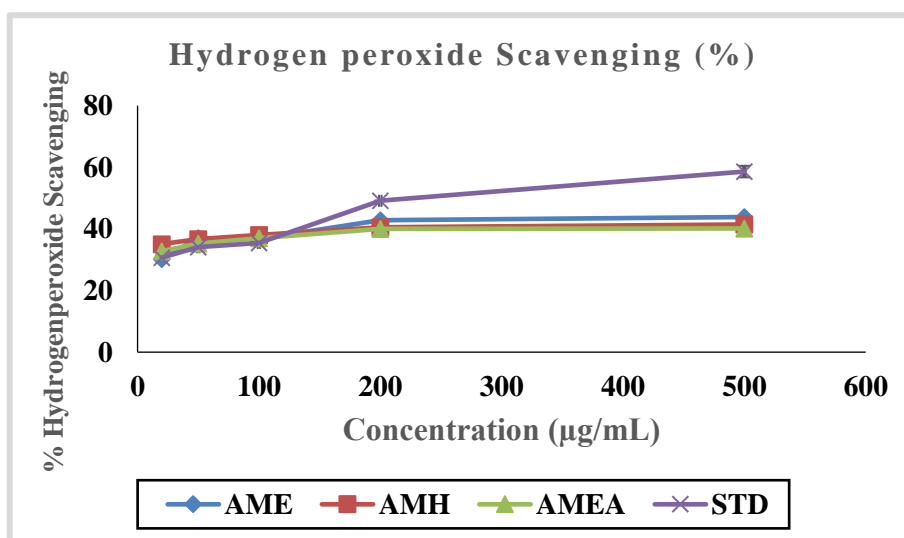


Fig. 5. In-vitro hydrogen peroxide scavenging activity of *Annona muricata* leaf extracts. Each point on the line graph represents the mean of triplicate readings (n=3) while the error bar represents standard error (SE) of the means. AME=*Annona muricata* ethanol extract; AMH=*Annona muricata* hexane extract; AMEA=*Annona muricata* ethyl acetate extract; STD=Standard (Vitamin C).

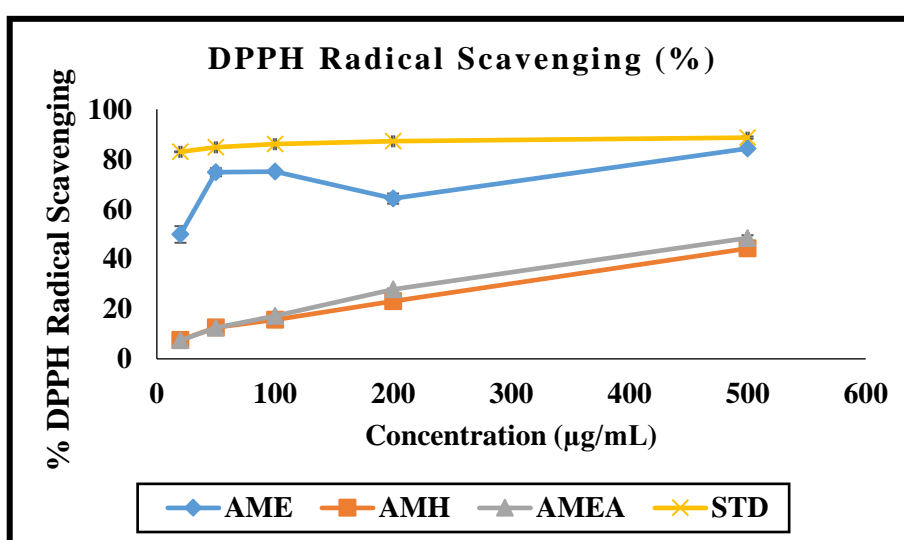


Fig. 6. In-vitro DPPH radical scavenging activity of *Annona muricata* leaf extracts. Each point on the line graph represents the mean of triplicate readings (n=3) while the error bar represents standard error (SE) of the means. AME=*Annona muricata* ethanol extract; AMH=*Annona muricata* hexane extract; AMEA=*Annona muricata* ethyl acetate extract; STD=Standard (Quercetin).

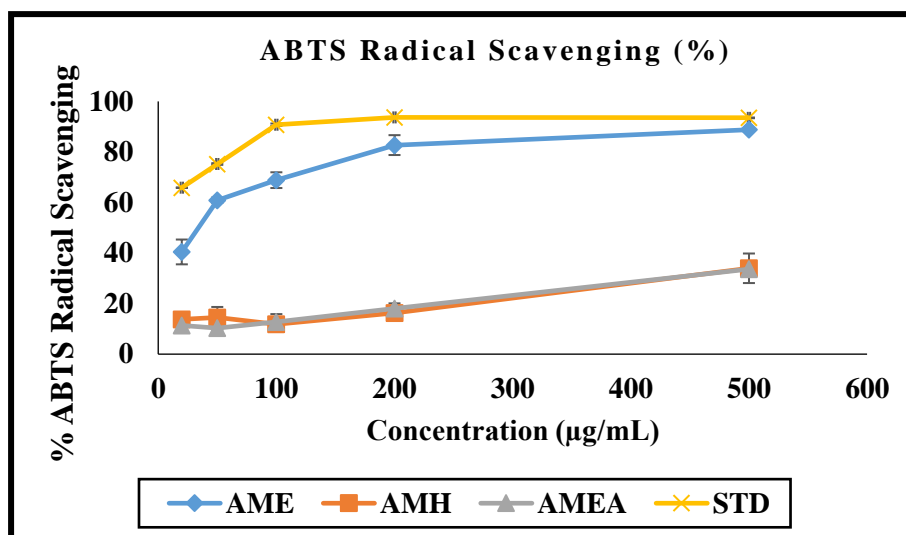


Fig. 7. *In-vitro* ABTS radical scavenging activity of *Annona muricata* leaf extracts. Each point on the line graph represents the mean of triplicate readings (n=3) while the error bar represents standard error (SE) of the means. AME=*Annona muricata* ethanol extract; AMH=*Annona muricata* hexane extract; AMEA=*Annona muricata* ethyl acetate extract; STD=Standard (Vitamin C).

Results of ABTS radical scavenging activity

The results of ABTS radical scavenging activity showed that AME was dose dependent and had significant higher antioxidant activity than AMH and AMEA (Fig. 7). The activities of AMH and AMEA were low and comparable at all concentrations.

Conclusion

The ethanol extract showed highest anti-inflammatory and antioxidant activity compared with ethyl acetate and hexane extracts. The high activity of this extract could be attributed to the presence of highly polar compounds. The ethanol extract of *Annona muricata* could be utilized for the treatment of oxidative and inflammatory disorders. Further studies are needed to isolate and characterize the bioactive compounds that are responsible for the activities and determine their mechanism of action.

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