OPEN ACCESS

African Journal of Biochemistry Research

January-March 2022
ISSN 1996-0778
DOI: 10.5897/AJBR
www.academicjournals.org

ACADEMIC JOURNALS
expand your knowledge

About AJBR

African Journal of Biochemistry Research (AJBR) provides rapid publication (quarterly) of articles in all areas of Biochemistry such as Nutritional biochemistry, Analytical biochemistry, Clinical Biochemistry, Human and Plant Genetics, Molecular and Cell Biology, Enzymology, Toxicology, Plant Biochemistry, Biochemistry Education etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles are peer-reviewed.

Indexing

CAB Abstracts, CABI's Global Health Database, Chemical Abstracts (CAS Source Index), Dimensions Database, Google Scholar, Matrix of Information for The Analysis of Journals (MIAR), Microsoft Academic

Open Access Policy

Open Access is a publication model that enables the dissemination of research articles to the global community without restriction through the internet. All articles published under open access can be accessed by anyone with internet connection.

The African Journal of Biochemistry Research is an Open Access journal. Abstracts and full texts of all articles published in this journal are freely accessible to everyone immediately after publication without any form of restriction.

Article License

All articles published by African Journal of Biochemistry Research are licensed under the Creative Commons Attribution 4.0 International License. This permits anyone to copy, redistribute, remix, transmit and adapt the work provided the original work and source is appropriately cited. Citation should include the article DOI. The article license is displayed on the abstract page the following statement:

This article is published under the terms of the Creative Commons Attribution License 4.0
Please refer to https://creativecommons.org/licenses/by/4.0/legalcode for details about Creative Commons Attribution License 4.0

Article Copyright

When an article is published by in the African Journal of Biochemistry Research, the author(s) of the article retain the copyright of article. Author(s) may republish the article as part of a book or other materials. When reusing a published article, author(s) should; Cite the original source of the publication when reusing the article. i.e. cite that the article was originally published in the African Journal of Biochemistry Research. Include the article DOI Accept that the article remains published by the African Journal of Biochemistry Research (except in occasion of a retraction of the article)
The article is licensed under the Creative Commons Attribution 4.0 International License.

A copyright statement is stated in the abstract page of each article. The following statement is an example of a copyright statement on an abstract page.
Copyright ©2016 Author(s) retains the copyright of this article.

Self-Archiving Policy

The African Journal of Biochemistry Research is a RoMEO green journal. This permits authors to archive any version of their article they find most suitable, including the published version on their institutional repository and any other suitable website.
Please see http://www.sherpa.ac.uk/romeo/search.php?issn=1684-5315

Digital Archiving Policy

The African Journal of Biochemistry Research is committed to the long-term preservation of its content. All articles published by the journal are preserved by Portico. In addition, the journal encourages authors to archive the published version of their articles on their institutional repositories and as well as other appropriate websites.
https://www.portico.org/publishers/ajournals/

Metadata Harvesting

The African Journal of Biochemistry Research encourages metadata harvesting of all its content. The journal fully supports and implement the OAI version 2.0, which comes in a standard XML format. See Harvesting Parameter

Memberships and Standards

Academic Journals strongly supports the Open Access initiative. Abstracts and full texts of all articles published by Academic Journals are freely accessible to everyone immediately after publication.

All articles published by Academic Journals are licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0). This permits anyone to copy, redistribute, remix, transmit and adapt the work provided the original work and source is appropriately cited.

Crossref is an association of scholarly publishers that developed Digital Object Identification (DOI) system for the unique identification published materials. Academic Journals is a member of Crossref and uses the DOI system. All articles published by Academic Journals are issued DOI.

Similarity Check powered by iThenticate is an initiative started by CrossRef to help its members actively engage in efforts to prevent scholarly and professional plagiarism. Academic Journals is a member of Similarity Check.

CrossRef Cited-by Linking (formerly Forward Linking) is a service that allows you to discover how your publications are being cited and to incorporate that information into your online publication platform. Academic Journals is a member of CrossRef Cited-by.

Academic Journals is a member of the International Digital Publishing Forum (IDPF). The IDPF is the global trade and standards organization dedicated to the development and promotion of electronic publishing and content consumption.

Contact

Editorial Office: ajbr@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJBR

Submit manuscript online http://ms.academicjournals.org

Academic Journals
73023 Victoria Island, Lagos, Nigeria
ICEA Building, 17th Floor,
Kenyatta Avenue, Nairobi, Kenya.

Editor

Prof. Johnson Lin
School of Biochemistry, Genetics, Microbiology and Plant Pathology
University of KwaZulu-Natal (Westville)
Private Bag X 54001, Durban
Republic of South Africa

Dr. Madhu M. Ouseph
Department of Pathology and Laboratory Medicine,
Brown University and Rhode Island Hospital,
USA

Editorial Board Members

Dr. Ahmed Malki
Biochemistry Department
Faculty of Science
Alexandria University
Alexandria,
Egypt.

Dr. Rouabhi Rachid
Biology Department
Tebessa University
Algeria.

Dr. Ercan Bursal
Department Of Chemistry,
Mus Alparslan University,
Turkey.

Ass. Prof. Alfonso Baldi
Dept. Biochemistry, Sect. Pathology
Second University of Naples,
Italy.

Dr. Oluwole Ariyo
Allen University
USA.

Prof. Belkhodja Moulay
University of Senia Oran
Algeria.

Prof. Emmanuel Anosike
Department of Biochemistry
University of Port Harcourt
Nigeria.

Ahmed Ragab Gaber
Division of Anatomy and Embryology, Zoology department,
Faculty of Science, Beni-Suef University,
Egypt.

Table of Content

The anti-inflammatory activities of twelve Nigerian medicinal plants: Inhibition of NfκB, activation of Nrf2, and antioxidant content 1
Dorothy VanderJagt, Lucy Hunsaker, Sunday O. Otimenyin, Mary O. Uguru

Medicinal effects of Saffron on obese animals by reducing Castelli's risk index to protect from cardio-pathology and psychological distress 11
Saara Ahmad, Hamna Rafiq, Asra Khan, Prashant Tikmani, Saima Khaliq, Saad Bilal Ahmed, Muhammad Misbah ur Rehman

Amino acids and carbohydrates composition in breast milk of lactating mothers of different age group from Aleto Health Center, Rivers State 19
Ndidi NWACHOKO, Udiomine B. AKURU, Fatima M. ODEJAYI, Jack Gbenenee TETAM

Changes in lipid class and fatty acid composition during the development of African pear (Dacryodes edulis) fruit pulp 24
Uhunmwangho E. S., Omoregie E. S.

Vol. 16(1), pp. 1-10, January-March 2022
DOI: 10.5897/AJBR2021.1134
Article Number: F50226A68493
ISSN 1996-0778
Copyright © 2022
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJBR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biochemistry Research

Full Length Research Paper

The anti-inflammatory activities of twelve Nigerian medicinal plants: Inhibition of NfκB, activation of Nrf2, and antioxidant content

Dorothy VanderJagt[1*], Lucy Hunsaker[1], Sunday O. Otimenyin[2] and Mary O. Uguru[2]

[1]Department of Biochemistry and Molecular Biology, University of New Mexico, USA.
[2]Department of Pharmacologgy, Faculty of Pharmaceutical Science, University of Jos, Nigeria.

Received 10 September, 2021; Accepted 7 January, 2022

Chronic non-communicable diseases are responsible for a large majority of deaths worldwide. These include cardiovascular and respiratory diseases, diabetes and cancer. Inflammation has been reported to be involved in the initiation or progression of these conditions. Populations in developing or low to middle- income countries often rely on traditional medicine using locally available herbs and plants for their medical care. This study examined the anti-inflammatory potential of aqueous extracts of twelve medicinal plants used in Nigeria. The antioxidant activity was estimated using the total radical-trapping antioxidant parameter (TRAP) and ferric reducing ability (FRAP) assays. The abilities to inhibit nuclear factor kappa light chain enhancer of activated B cells (NfkB), a key regulator of the inflammatory response, and to activate nuclear factorE2 related factor 2 (Nrf2), a transcription factor that regulates cellular antioxidant defense systems, were determined using *in vitro* cell based assays. Extracts of *Erythrina senegalensis* (leaves), *Sclerocarya birrea* (bark), *Boswellia dalzielli* (leaves and bark), *Pseudrocedrila kotschyi* (bark), *Sterculia setigera* (stem bark), and *Sarcocephalus esculentus* (bark) contained the highest levels of antioxidant activity. Extracts that showed the greatest inhibition of NfkB were *S. esculentus* (bark), 91.8%; *E. senegalensis* (leaves), 81.4%; *S. birrea* (stem bark), 77.5%; and *S. setigera* (stem bark), 75.5%. *B. dalzielli* (leaves) and *Xylopia aethiopica* (leaves) gave 7.4 and 7.7 fold activation of Nrf2, respectively. These were comparable to activation by sulphorophane.

Key words: NfkB inhibition, Nrf2 activation, antioxidant activity, inflammation, medicinal plants, Nigeria.

INTRODUCTION

According to the World Health Organization, more than 70% of deaths worldwide can be attributed to chronic non-communicable diseases. These include cardiovascular diseases, cancer, respiratory diseases, and diabetes (WHO, 2018; Unwin and Albert, 2006). The majority of these deaths occur in developing and low- to middle-income countries where people rely on traditional medicine for their everyday health care (WHO, 2019). Inflammation is reported to play a role in the initiation and/or progression of many of these conditions (Kosmas et al., 2019; Christodoulidis et al., 2014; Kundu and Surh, 2008; Fernandes et al., 2015; Wang et al., 2018; Shaikh

*Corresponding author. E-mail: DVanderjagt@salud.unm.edu.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

et al., 2019; Tsalamandris et al., 2019;Baker et al., 2011).

Inflammation is initiated by the innate immune system in response to infection or tissue injury. Activated macrophages and neutrophils release reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, nitric oxide, and pro-inflammatory cytokines that include TNFα (Abbas et al., 2012). Both ROS and TNFα activate NFκB, a proinflammatory transcription factor that is a critical regulator of the inflammatory response (Liu et al., 2017; Kunnumakkara et al., 2020).NFκB regulates a large number of genes involved in processes of the immune and inflammatory responses as well as the expression of enzymes involved in the generation of ROS including NADPH oxidase (Anrather et al., 2006), NO synthase (Li et al., 2007), cyclooxygenase (Deng et al., 2003), xanthine oxidase (Xu et al., 1996), and phospholipase A2 (Schutze et al., 1992; Szymczak-Pajor et al., 2020).

Although mounting an immune response to infection or injury is essential to life, unregulated production of ROS may result in chronic inflammation. The generation of ROS beyond cellular antioxidant capacity can damage DNA, proteins, lipids and cell membranes (Farber, 1994) and result in the initiation of various chronic diseases. Balancing the production of ROS with their removal is attained by activating cellular antioxidant defense systems. Nrf2 is the primary transcription factor that regulates the synthesis of various detoxification enzymes and proteins such as glutathione-S transferases, NADPH: quinone oxidoreductaase, gamma-glutamylcysteine synthase, ferritin, and heme oxygenase (Chen and Kunsch, 2004). Inhibiting NFκB and increasing Nrf2 activity in the occurrence of excessive ROS production may provide a therapeutic approach to treating chronic inflammation (Chen and Kunsch, 2004; Gupta et al., 2010; Zhang et al., 2017; Sivandzade et al., 2019).

Although steroids and other non-steroidal drugs are commonly used for treating inflammatory disorders, populations in many low- income and developing countries rely on traditional medicine for their health care because conventional pharmaceuticals are costly or may not be accessible (WHO, 2019; Oyebode et al., 2016; James et al., 2018). Traditional medicine practitioners commonly use infusions or decoctions made from plants available in their area (Gurib-Fakim, 2006). Plant extracts contain antioxidants and other phytochemicals that have been demonstrated to affect various transcription factors and cytokines involved in the inflammatory process (Spelman et al., 2006; Talhouk et al, 2007; Orlando et al., 2010; Ghosh et al., 2016; Qin and Hou, 2016).

It is estimated that more than 80% of Nigerians rely on traditional healers and herbal remedies made from whole plants or plant parts such as leaves, bark, and roots (Odugbemi, 2006). The present study aimed to determine the antioxidant capacity of aqueous extracts of 12 plants commonly used by traditional medical practitioners in Nigeria. The ability of these preparations to inhibit NFκB and activate Nrf2 activities in cell-based assays was also determined.

MATERIALS AND METHODS

Specimen collection

Plants used for medicinal purposes were collected in the Babale Ward in the Jos North Local Government area of Plateau State, Nigeria. Plants were identified and authenticated by Dr. M. Adul-Kareem from the Department of Horticulture at the Federal College of Forestry, Plateau State, Nigeria. Voucher specimens were deposited at the Herbarium of the Federal College of Forestry at the same location.

After removing dirt and debris by rinsing with water, the plants were in the shade. The plants were separated into parts (leaves, stem bark, bark). Some specimens were analyzed in the whole plant form. Samples were transported to Albuquerque, NM, USA for analyses. Prior to analyses, specimens were ground to a fine powder in a stainless steel grinder and dried in a vacuum desiccator to constant weight. A list of the plant materials collected for analyses and some of their common uses are presented in Table 1.

Preparation of aqueous extracts

Aqueous plant extracts were prepared by adding 10 ml of distilled water to 0.5 g of sample in a 16 × 150 mm Pyrex glass tube. After vortexing, samples were heated at 80°C for 30 min. The samples were then centrifuged (Fisherbrand 614 series centrifuge, Fisher Scientific, Waltham, MA, USA)at 1200 g for 10 min to clarify the extract. The clarified samples were filter-sterilized and stored at 4°C in the dark until analyzed or at -70° for longer term storage.

Antioxidant capacity determination

The term antioxidant includes substances with different mechanisms of action. Since no single assay can recognize all the different types of antioxidants, the antioxidant capacity of the aqueous extracts was determined by two different methods (Schleiser et al., 2002; Huang et al., 2005): the total radical-trapping antioxidant parameter assay (TRAP) (Re et al., 1999) and the ferric reducing ability assay (FRAP) (Benzie and Strain, 1999). The TRAP assay measures the ability of antioxidants in the sample to interfere with the reaction between peroxyl radicals and a target probe. It is useful for determining the content of non-enzymatic antioxidants, such as glutathione and ascorbic acid. The FRAP assay reflects the electron reducing power of antioxidants (Moon and Shibamoto, 2009).

TRAP assay

For the TRAP assay, 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (Sigma Aldrich, St. Louis. MO, USA) was reacted with potassium persulfate in the dark, overnight, to generate the colored $ABTS^{\cdot +}$ radical cation, which has an absorption maximum at 734 nm. The activity of the plant preparations was determined by their ability to quench the color of the radical cation. Duplicate 10 µl aliquots of the extracts were used for analysis. Dilutions of the sample extracts were madcorrespond to the standard curve generated using Trolox (6-hydroxy-2, 5, 7, 8 tetramethylchroman-2-carboxylic acid (Sigma Aldrich, St. Louis, MO, USA) as the reference material. Duplicate measurements

Table 1. Nigerian medicinal plants investigated for their antioxidant and anti-inflammatory properties.

Local name (Hausa)		Plant parts analyzed	Some common uses (Borokini, 2011; Odugbemi, 2006)
Annona senegalensis	Gwandar daaji	Bark, leaves	Dysentery, venereal disease, toothache, cough, wound healing, gastrointestinal disorders
Boswellia dalzielli	Ararrabi, haro, harrabi	Bark, leaves	Trypanosomiasis, malaria
Erythrina senegalensis	Mingirya	Whole plant, Leaves	Jaundice, amenorrhea, dysentery, pneumonia, infections, Schistosomiasis
Ficus thonningii	Che'diya	Bark, leaves	Fever, dysentery, wound healing
Khaya senegalensis	Madaacii	Bark	Malaria
Kigelia Africana	Rawaya	Whole plant	Dysentery, rheumatism, cough, malaria, gonorrhea
Momordica balsamina	Garahuni	Whole plant	Malaria, fever, jaundice, stomach ache
Pseudocedrela kotschyi	Tonas	Bark	Fever, dysentery, hemorrhoids
Sarcocephalus esculentes	Tabashiiye	Whole plant, bark	Fever
Sclerocarya bierra	Dania	Stem bark, leaves	Dysentery, rheumatism, gonorrhea, hypertension, Malaria prophylaxis
Sterculia setigera		Stem bark, leaves	Diarrhea, dysentery, ulcers, boils, gastrointestinal disorders
Xylopia aethiopica	Kimba dutse	Leaves	Neuralgia, stomach ache, bronchitis, gonorrhea, antimicrobial activity
Annona senegalensis	Gwandar daaji	Bark, leaves	Dysentery, venereal disease, toothache, cough, wound healing, gastrointestinal disorders
Boswellia dalzielli	Ararrabi, haro, harrabi	Bark, leaves	Trypanosomiasis, malaria
Erythrina senegalensis	Mingirya	Whole plant, Leaves	Jaundice, amenorrhea, dysentery, pneumonia, infections, Schistosomiasis
Ficus thonningii	Che'diya	Bark, leaves	Fever, dysentery, wound healing
Khaya senegalensis	Madaacii	Bark	Malaria
Kigelia Africana	Rawaya	Whole plant	Dysentery, rheumatism, cough, malaria, gonorrhea
Momordica balsamina	Garahuni	Whole plant	Malaria, fever, jaundice, stomach ache
Pseudocedrela kotschyi	Tonas	Bark	Fever, dysentery, hemorrhoids
Sarcocephalus esculentes	Tabashiiye	Whole plant, bark	Fever
Sclerocarya bierra	Dania	Stem bark, leaves	Dysentery, rheumatism, gonorrhea, hypertension, Malaria prophylaxis
Sterculia setigera		Stem bark, leaves	Diarrhea, dysentery, ulcers, boils, gastrointestinal disorders
Xylopia aethiopica	Kimba dutse	Leaves	Neuralgia, stomach ache, bronchitis, gonorrhea, antimicrobial activity

were made and given as the average. Results are expressed as the mmol Trolox equivalents/ml aqueous extract.

FRAP assay

For the FRAP assay, the ferric complex of 2,4,6–tripyridyl-*s*-triazine was prepared at acidic pH, and the anti-oxidant activities of the plant preparations were determined by their abilities to reduce the ferric complex to the ferrous complex, monitored by formation of the ferrous complex at 593 nm. Results are expressed as the mmol Trolox equivalents/ml aqueous extract.

Inhibition of NFκB activation

An NFkB reporter stable cell line derived from human 293T embryonic kidney cells (293T/NFκB-luc) (Panomics, Inc. Redwood

City, CA) was grown in a humidified atmosphere at 37°C in 5% CO_2/95% air. The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM-high glucose containing 4 mM glutamine) supplemented with 10% fetal bovine serum (FBS), 1 mM sodium pyruvate, 100 units/ml penicillin, 100 µg/ml streptomycin and 100 µg/ml hygromycin (Gibco/Invitrogen, Carlsbad, CA) to maintain cell selection. One day before treatment with the plant extracts, the 293T/NFkB-luc cells were plated into 24-well cell culture plates (Costar, Cambridge, MA) at approximately 70% confluency in the above media without hygromycin. The following day cells were fed fresh media 1 h prior to treatment. Media, with or without recombinant tumor necrosis factor (TNFα, 2ng/ml) (R&D Biosciences/clontech, Palo Alto, CA), was then applied to the cells followed by immediate addition of 50µL plant extracts. The cells were then placed in a humidified atmosphere in 5% CO_2/95% air for 7 h. Plate wells were gently washed with 1x passive lysis buffer (Promega, Madison, WI). The subsequent lysates were analyzed using the Luciferase Assay System (Promega) utilizing a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA). The firefly luciferase relative light units were normalized to protein (mg/ml) with BCA Protein Assay Kit (Pierce, Rockford, Il) and standardized to percent of control (TNFα control). Results (percent of control) are given as the average of duplicate analyses.

Activation of Nrf2

As previously described (Deck et al., 2017), a Nrf2-ARE reporter-Hep G2 stable cell line (BPS Bioscience, San Diego, CA, USA) was grown in a humidified atmosphere at 37°C in CO_2 (5%)/air (95%). The cells were maintained in MEM medium with Earles balanced salts and L-glutamine (Gibco, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS), 1 mM sodium pyruvate, 1% non-essential amino acids, penicillin (100 units/ml), streptomycin (100 µg/ml), and Geneticin (400 µg/ml) to maintain cell selection.

One day prior to treatment, the Nrf2-ARE cells were plated into 24-well cell culture plates (costar, Cambridge, MA, USA) at approximately 30% confluency in the above media without Geneticin. The following day, fresh media with or without sulforaphane (15 µM) was added to appropriate wells as controls. Plant extracts (50 µl) were then applied to the sample wells. The cells were again placed in a humidified atmosphere at 37°, CO_2 (5%), air (95%) for 24 h. Cells were washed with phosphate buffered saline (PBS), pH 7.4, and lysed with 1x passive lysis buffer (Biotium, Hayward, CA, USA). The lysates were analyzed using the Luciferase Assay System (Biotium, Hayward, CA, USA) and a E-5311 GloMax 20/20 luminometer (Promega, Sunnyvale CA, USA). The firefly luciferase relative light units were normalized to protein (mg/ml) determined using the BCA Protein Assay Kit (Pierce, Rockford, IL, USA) and then standardized to percent of control activation.

Plant extract toxicity

Plants commonly used in traditional medicine are assumed to be safe of acute toxicity based on their long-term usage. However, some recent studies have shown that many plants may contain toxic, mutagenic, or carcinogenic compounds (Letsyo et al., 2017; Mounanga et al., 2015; Valdivia-Correa et al., 2016). For determination of cell viability in the presence of the plant extracts, 293T/NFκB-luc cells were prepared as described above and then plated at approximately 5,000 cells/well in a 96-well plate. Triplicate plant extracts, 10 µl in 100 µl media, were then added to the wells. Appropriate dilutions of the plant extracts were made to equal the amount used in the NFκB inhibition analyses. After incubating for 6 h, 10 µl WST-1 Cell Proliferation Reagent (Roche Applied Science, Indianapolis, IN, USA) was added and the cells were incubated as described above for 45 min. The WST-1 dye is reduced by metabolically active cells and was quantified 450 nm using a Spectromax plate reader (Molecular Devices Co., Sunnyvale CA, USA). Results are expressed as the percent absorption of control (untreated) cells

RESULTS

Antioxidant activity

In the TRAP assay, *Erythrina senegalensis* (leaves) and *Sclerocarya birrea* (bark) exhibited antioxidant activities approximately five times higher than the following two highest plants: *Boswellia dalzielli* (both leaves and bark) and *Pseudocedrela kotschyi* (bark) (Figure 1). *Sterculia setigera* (stem bark) and *Sarcocephalus esculentus* (bark) had the next highest activities.

In the FRAP assay, *E. senegalensis* (leaves), *P. kotschyi* (bark), and *S. birrea* (bark) had the highest activity, followed by *B. dalzielli* (bark and leaves) (Figure 2).

NFkB inhibition

The inhibition of NFκB activation by the plant extracts in the presence of TNFα is shown in Figure 3. Plant extracts that inhibited the activation by more than 50% are: *S. esculentus* (bark) 91.8%; *E. senegalensis* (leavesl) 81.4%; *S. bierra* (stem bark) 77.5%; *S. setigera* (stem bark) 75.5%; *Khaya senegalensis* (bark) 62.4%; and *Annona senegalensis* (bark) 53.1%. The remainder of the plant extracts gave less than 50% inhibition.

Activation of Nrf2

Two plant preparations gave activation of Nrf2 comparable to sulphorophane, a known promoter of Nrf2 activity (Keum, 2011) (Figure 4). Extracts of *B. dalzielli* (leaves) and *Xylopia aethiopica* (leaves) demonstrated a 7.4 and 7.7 fold increase in Nrf2 over baseline (no sulphorophane), respectively. These activities were equal to 83 and 81% of the sulphorophane activation.

Correlation of antioxidant capacity and NFκB inhibition

The antioxidant capacity as determined by the TRAP assay was significantly correlated with NFkB inhibition ($p=0.002$, $r=-0.64$). A significant relation was also observed between antioxidant capacity determined by the FRAP assay and NFκB inhibition ($p=0.008$, $r=-0.569$). Correlation between the FRAP and TRAP antioxidant activities was also found ($p=0.001$, $r=0.89$).

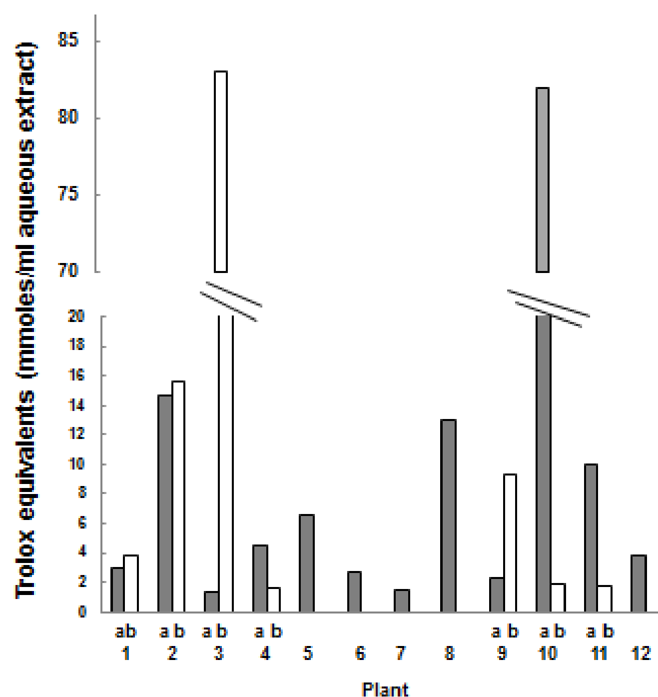


Figure 1. TRAP assay: mmoles Trolox equivalents/ml aqueous extract. Plant: 1. *Annona senegalensis* (a) bark, (b) leaves; 2. *Boswellia dalzielli (*a) bark, (b) leaves*;* 3. *Erythrina senegalensis (*a) whole plant, (b) leaves; 4. *Ficus thonningi* (a) bark, (b) leaves; 5. *Khaya senegalensis*, bark; 6. *Kigelia Africana*, whole plant; 7. *Momordica balsamina*, whole plant; 8. *Pseudocedrela kotschyi,* bark; 9. *Sarcocephalus esculentus* (a) whole plant, (b) bark; 10. *Sclerocarya birrea* (a) stem bark, (b) leaves; 11*. Sterculia setigera* (a) stem bark, (b) leaves; 12. *Xylopia aethiopica*, leaves.

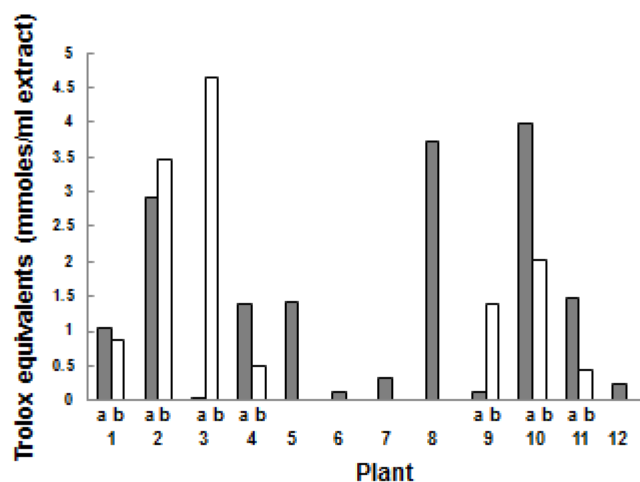


Figure 2. FRAP assay: mmoles Trolox equivalents/ml aqueous extract. Plant: 1. *Annona senegalensis* (a) bark, (b) leaves; 2. *Boswellia dalzielli (*a) bark, (b) leaves*;* 3. *Erythrina senegalensis (*a) whole plant, (b) leaves; 4. *Ficus thonningi* (a) bark, (b) leaves; 5. *Khaya senegalensis*, bark; 6. *Kigelia Africana*, whole plant; 7. *Momordica balsamina*, whole plant; 8. *Pseudocedrela kotschyi*, bark; 9. *Sarcocephalus esculentus* (a) whole plant, (b) bark; 10. *Sclerocarya birrea* (a) stem bark, (b) leaves; 11*. Sterculia setigera* (a) stem bark, (b) leaves; 12. *Xylopia aethiopica*, leaves.

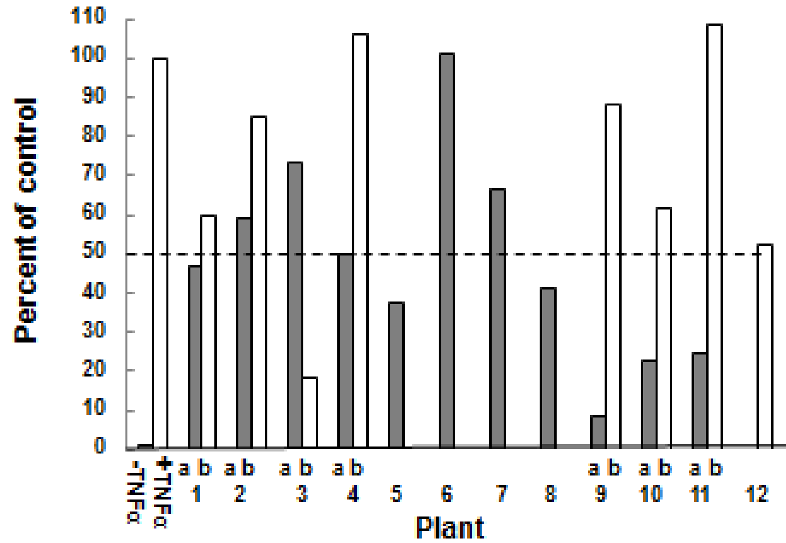


Figure 3. Percent NfkB inhibition per 10µl aqueous extract: Plant: 1. *Annona senegalensis* (a) bark, (b) leaves;2. *Boswellia dalzielli (*a) bark, (b) leaves*;* 3. *Erythrina senegalensis (*a) whole plant, (b) leaves; 4. *Ficus thonningi* (a) bark, (b) leaves; 5. *Khaya senegalensis*, bark; 6.*Kigelia Africana*, whole plant; 7. *Momordica balsamina*, whole plant; 8. *Pseudocedrela kotschyi*, bark; 9.*Sarcocephalus esculentus* bark (a) whole plant, (b) bark; 10. *Sclerocarya birrea* (a) stem bark, (b) leaves; 11*. Sterculia setigera* (a) stem bark, (b) leaves; 12. *Xylopia aethiopica*, leaves.

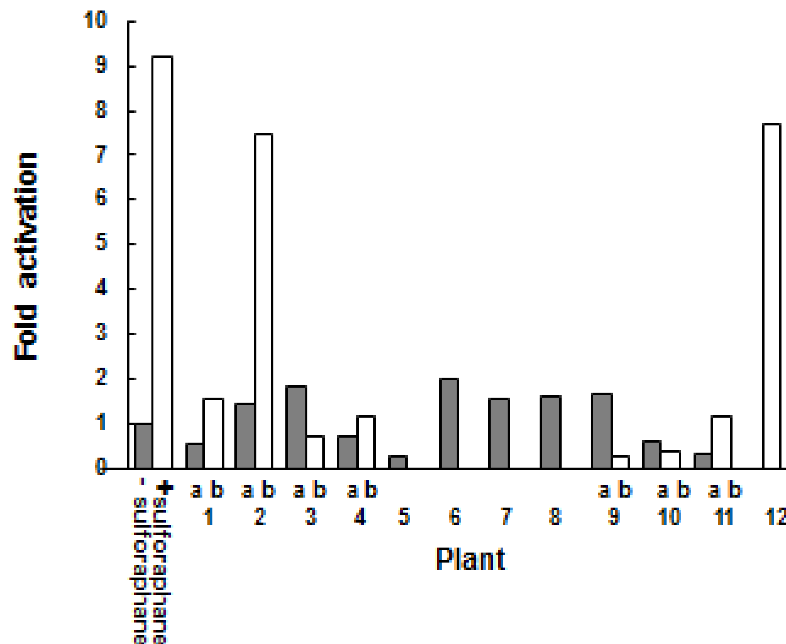


Figure 4. Nrf2 fold activation per 10µl extract. Plant: 1. *Annona senegalensis* (a) bark, (b) leaves: 2. *Boswellia dalzielli (*a) bark, (b) leaves*;* 3. *Erythrina senegalensis (*a) whole plant, (b) leaves; 4. *Ficus thonningi* (a) bark, (b) leaves; 5. *Khaya senegalensis*, bark; 6. *Kigelia Africana*, whole plant; 7. *Momordica balsamina*, whole plant; 8. *Pseudocedrela kotschyi*, bark; 9. *Sarcocephalus esculentus* (a) whole plant, (b) bark; 10. *Sclerocarya birrea* (a) stem bark, (b) leaves; 11*. Sterculia setigera* (a) stem bark, (b) leaves; 12. *Xylopia aethiopica*, leaves.

Plant extract toxicity

293TN/NFκB cells treated with plant extracts had 58 to 100% viability compared to control (untreated cells): *S. birrea* (stem bark) 58%; *S. esculentus* (bark) 65%; *E. senegalensis* (leaves) 68%; *Annona senegalensis* (bark) 70%; *Pseudocedrela kotschyi* (bark) 71%; *Annona senegalensis* (leaves) 76%; *Khaya senegalensis* (bark) 80%; *S. setigera (stem bark) 76*%; *Khaya senegalensis* (bark) 80%; *Ficus thonningii* (bark) 89%; B. *dalzielli* (bark) 84%; *S.birrea* (leaves) 90%; *X. aethiopica* (leaves) 92%*;* *B.dalzielli* (leaves) 98%; *S. setigera* (leaves) 100%; *Momordica balsamina* (whole plant) 100%; *Kigelia africana* (whole plant) 100%; *Ficus thonningii* (leaves) 100%; *E. senegalensis (*whole plant) 100%; *S. esculentus* (whole plant) 100%.

DISCUSSION

Aqueous extracts of several of the Nigerian medicinal plants examined in this study, namely *E. senegalensis (*leaves), *S. birrea* (stem bark), *S. esculentus* (bark), *S. setigera* (stem bark), *B. dalzielli* (bark), and *X. aethiopica* (leaves) inhibited the pro-inflammatory NFκB signaling pathway or activated the antioxidant NrF2 signaling pathway.

E. senegalensis (leaves) had the highest antioxidant activity of the plants tested and was also a promising inhibitor of pro-inflammatory NFkB activation (81.4%). A spiny tree found in west tropical Africa, it is commonly used as an ornamental shrub (Tropical Plants Database, 2020). Decoctions of *E. senegalensis* leaves are traditionally used for treating wounds, various fevers, gastrointestinal disorders, and infertility in women (Togola et al., 2005). An aqueous leaf extract was reported to provide a protective effect against oxidative liver damage in rats exposed to CCl_4 (Wakawa and Hauwa, 2013). In an *in vivo* study using the egg- albumin induced acute edema model, an aqueous extract of the stem bark demonstrated significant analgesic and anti-inflammatory activity in rats (Saidu et al., 2000). An ethanol extract of *Erythrina senegalensis* leaves was also reported to have wound healing activity in albino rats (Ilodigwe et al., 2014). Various phytochemicals have been isolated from the plant and may provide some of the anti-inflammatory and antioxidant activities observed (Kone et al., 2011).

S. birrea is a medium –size deciduous tree found in the semi-arid and savannah regions of sub-Saharan Africa (National Research Council, 2008) and its fruit is a traditional African food. Preparations from various parts of the tree are widely used throughout Africa for treating dysentery, diarrhea, indigestion, fungal infections, snake bite, and diabetes (Ojewole et al., 2010). In the present study, the aqueous bark extract had antioxidant levels comparable to *Erythrina senegalensis* and was also a good inhibitor of NFκB activation (77.5%). In an *in vivo* test of anti-inflammatory activity, both aqueous and methanol extracts of the stem bark were reported to reduce rat paw edema induced by egg albumin in Wistar rats (Ojewole, 2003; Fotio et al., 2009). Analyses of the stem bark and leaves of *S. birrea* demonstrated the presence of various phytochemicals such as phenols, flavonoids and flavonols that could contribute to the antioxidant and anti- inflammatory properties (Tanih and Ndip, 2012; Braca et al., 2003).

The bark of the *S. esculentus,* widel*y* found throughout West Africa (Dalziel, 1937, a) showed the most promising inhibition of NFκB activation of the extracts investigated in this study (91.8%). Bark preparations are used for fevers, indigestion related halitosis, vomiting, and toothache. Otimenyin and co-workers (Otimenyin et al., 2008) reported that aqueous extracts of the roots of *S. esculentus* inhibited egg-albumin induced rat paw edema and acetic acid-induced writhing in mice in a dose-dependent manner.

The two plants in this study that exhibited activation of Nrf2 comparable to sulforophane were *B. dalzielli* (bark) and X. *aethiopica* (leaves). *B. dalzielli*, found in northern Nigeria and the West African savannah (Dalziel, 1937b), is a species of the frankincense tree. Extracts of the leaf are used for diarrhea and extracts of the stem bark are commonly used for fever, rheumatism, and gastrointestinal disorders. (Mbiantcha et al., 2018) reported that a methanol extract of the stem bark had an anti-inflammatory effect in several models of rat paw edema including the carrageenan, arachidonic acid, histamine and bradykinin models (Mbiancha, 20018). The extract also significantly reduced the production and release of ROS from isolated human polymorphonuclear cells and mouse peritoneal macrophages and decreased the production of the inflammatory cytokines TNFα and IL-1b. In another study of the anti-inflammatory properties of the *B. dalzielli* stem bark, Kafuti et al. (2019) reported that the methanol and aqueous fractions of the extract had significant antioxidant activity measured by both a radical scavenging assay and the FRAP assay. These results are in agreement with those obtained in our study using an aqueous preparation.

X. aethiopica, an aromatic evergreen plant that is widespread in West Central and Southern Africa, is used for the treatment of fever, cough, dyspepsia, skin infections and muscular and rheumatic pain (Dalziel, 1937). An ethanol/water extract of the leaves was reported to decrease TNFα and IL-6 levels in LPS activated THP-1 derived macrophages, suggesting its usefulness as an anti-inflammatory agent (Macedo et al., 2020). Moukette et al., (2015) tested ethanol and ethanol/water extracts of the *X. aethiopica* bark in liver homogenates and found that both extracts exhibited antioxidant activity in both free radical scavenging and the FRAP assays. The present study used the unfractionated aqueous extract of the plants and did not identify individual components. A variety of compounds

may be present in plant extracts depending on the part of the plant used and the solvent used for preparing the extract. Constituents may include various vitamins (e.g., vitamin C, vitamin E, carotenoids) and other phytochemicals such as polyphenols. This may allow synergistic effects of multiple components to contribute to the antioxidant and anti-inflammatory effects observed (Gilbert and Alves, 2003).

When using plant preparations for treatment, several factors need to be considered: the bioavailability of the active components, the co-extraction of toxic compounds, and interference in the efficacy of conventional pharmaceuticals when used simultaneously with the herbal preparations. Polyphenols occur in multiple forms including glycosides and polymers. Depending on the particular polyphenol, the compounds must be acted on by intestinal enzymes prior to absorption by enterocytes and then modified by the liver prior to export into the circulation. The rate of absorption has been shown to be dependent on the structure of the polyphenol (Kawabata et al., 2019; Silberberg et al., 2006). Herbal preparations may also interfere with the effectiveness of conventional pharmaceuticals by causing alterations in gastrointestinal function affecting absorption, inhibiting or inducing enzymes involved in metabolism, or affecting the renal excretion of drugs or their metabolites (Fasinu et al., 2012).

In addition to beneficial components, plants may also contain toxic compounds. For example, Letsyo and coworkers analyzed 70 herbal medicine products commonly used in Ghana and other West African countries and reported that 60% of them contained pyrrolizidine alkaloids (Letsyo et al., 2017).These are present as a defense against herbivores and occur predominantly as N-oxides. They show little or no toxicity in that form but are metabolized by the liver to toxic tertiary pyrrolizidine alkaloids. All of the plant extracts examined in this study gave viability of 65% or greater in the cell toxicity tests.

In summary, aqueous extracts of the plants examined in this study showed anti-inflammatory activity by various mechanisms such as the inhibition of the pro-inflammatory NfkB signaling pathway or the activation of the Nrf2 antioxidant signaling pathway in *in vitro* assays. Further studies should include the identification of active components present in these extracts and the determination of their bioavailability in *vivo*

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abbas AK, Lichtman AH, Pillai S (2012). Cellular and Molecular Immunology 7th ed. Elsevier Saunders, Philadelphia.

Anrather J, Racchumi G, Iadecola C (2006). NF-kappaB regulates phagocytic NADPH oxidase by inducing the expression of gp91phox. Journal of Biological Chemistry 281(9):5657-5667.

Baker RG, Hayden MS, Ghosh S (2011). NF-kB, inflammation, and metabolic disease. Cell Metabolism 13(1):11-22.

Benzie IF, Strain JJ (1999). Ferric reducing/antioxidant power assay: direct measurement of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology 299:15-27.

Borokini T (2011). Conservation and Utilization of Medicinal Plants in Nigeria. Lambert Academic Publishing GMBH&Co.KG; Berlin, 2011.

Braca A, Politi M, Sanogo R, Sanou H, Morelli I, Pizza C, de Tommasi N (2003). Chemical composition and antioxidant activity of phenolic compounds from wild and cultivated *Sclerocarya birrea* (Anacardiaceae) leaves. Journal of Agriculture and Food Chemistry 51(23):6689-6695.

Chen XL, Kunsch C (2004). Induction of cytoprotective genes through Nrf2/antioxidant response element pathway: a new therapeutic approach for the treatment of inflammatory diseases. Current Pharmaceutical Design 10(8):879-91.

Christodoulidis G, Vittoria TJ, Fudim M, Lerakis S, Kosmas CE (2014). Inflammation in coronary artery disease. Cardiology in Review 22(6):279288.

Dalziel JM (1937). The useful plants of West Tropical Africa. The Crown Agents for the Colonies, London pp. 52-560.

Deck LM, Whalen LJ, Hunsaker LA, Royer RE, Vander Jagt D (2017). Activation of anti-oxidant Nrf2 signaling by substituted trans stilbenes. Bioorganic and Medicinal Chemistry 25(4):1423-1430.

Deng WG, Zhu Y, Wu KK (2003). Up-regulation of p300 binding and p50 acetylation in in tumor necrosis factor-alpha-induced cyclooxygenase-2 promoter activation. Journal of Biological Chemistry 278(7):4770-4777.

Farber J (1994). Mechanism of cell injury by activated oxygen. Environmental Health Perspectives 102(suppl 10):17-24.

Fasinu PS, Bouic PJ, Rosenkranz B (2012). An overview of the evidence and mechanisms of herb-drug interactions. Frontiers in Pharmacology 3:69.

Fernandes JV, Cobucci RN, Jatoba CA, Fernandes TA, de Azevedo JW, de Araujo JM (2015). The role of the mediators of inflammation in cancer development. Pathology &Oncology Research 21(3):527-534.

Fotio AL, Dimo T, Nguelefack TB, Dzeufiet PD, Ngo Lemba E, Temdie RJ, Ngueguim F, Olleros ML, Vesin D, Dongo E, Kamtchouing P, Garcia I (2009). Acute and chronic anti-inflammatory properties of the stem bark aqueous and methanol extracts of *Sclerocarya birrea*. Inflammopharmacology 17(4):229-237.

Ghosh N, Ali A, Ghosh R, Das S, Mandal SC, Pal M (2016). Chronic inflammatory diseases: progress and prospect with herbal medicine. Current Pharmaceutical Design 22(2):247-264.

Gilbert B, Alves LF (2003).Synergy in plant medicines. Current medicinal chemistry 10(1):13-20. doi: 10.2174/0929867033368583.

Gupta SC, Sundaram C, Reuter S, Aggarwal BB (2010). Inhibiting NF-kappaB activation by small molecules as a therapeutic strategy. Biochimica Biophysica Acta 1799(10-12):775-787.

Gurib-Fakim A (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine 27(1):1-93.

Huang D, Ou B, Prior RL (2005). The chemistry behind antioxidant capacity assays. Journal of Agricultural Food Chemistry 53(6):1841-1856.

Ilodigwe E, Okonkwo BE, Agbata CA, Ajaghaku DL, Eze PM. (2014). Wound healing activity of ethanol leaf extract of *Erythrina senegalensis*. British Journal of Pharmaceutical Research 4:531-540.

James PB, Wardle J, Steel A, Adams J (2018). Traditional, complementary and alternative medicine use in Sub-Saharan Africa: a systematic review. British Medical Journal Global Health 3(5):e000895.

Kafuti YS, Alemika TE, Ojerinde OS, Balogun O, Alamika TE, Taba KM, Mpiana PT, Kindombe NM (2019). Antioxidant and antiproliferative activities of the stem bark extract and fractions of *Boswellia dalzielii* Hutch. International Journal of Pharmacognosy and Phytochemical Research 11(3):177-182.

Kawabata K, Yoshioka Y, Terao J (2019). Role of intestinal microbiota

in the bioavailability and physiological functions of dietary polyphenols. Molecules 24(2):370-395.

Keum YS (2011). Regulation of the Keap1/Nrf2 system by chemopreventive sulforaphane: implications of posttranslational modifications. Annals of the New York Academies of Science 1229(1):184-189.

Kone WM, Solange KN, Dosso M (2011). Assessing Sub-Saharan *Erythrina* for efficacy: Traditional uses, biological activities and phytochemistry. Pakistan Journal of Biological Science 14(10):560-571.

Kosmas CE, Silverio D, Sourlas A, Montan PD, Guzman E, Garcia M (2019). Anti-inflammatory therapy for cardiovascular disease. Annals of Translational Medicine 7(7):147-153.

Kundu JK, Surh YJ. (2008). Inflammation: gearing the journey to cancer. Mutation Research Review 659(1-2):15-30.

Kunnumakkara AB, Shabnam B, Girisa S, Harsha C, Banik K, Devi TB, Choudhury R, Sahu H, ParamaD, Sailo B, Thakur KK, GuptaSC, Aggarwal BB. (2020). Inflammation, NF-kB, and chronic diseases: how are they linked? Critical Review of Immunology 40(1):1-39.

Letsyo E, Jerz G, Winterhalter P, Beuerle T (2017). Toxic pyrrolizidine alkaloids in medicines commonly used in Ghana. Journal of Ethnopharmacology 202:154-161.

Li Y, Zhao Y, Li G, Wang J, Li W, Lu J (2007). Regulation of neuronal nitric oxide synthase exon 1f gene expression by nuclear factor-kappaB acetylation in human neuroblastoma cells. Journal of Neurochemistry 101(5):1194-1204.

Liu T, Zhang L, Joo D, Sun S-C (2017). NF-kB signaling in inflammation. Signal Transduction and Targeted Therapy 2:17023.

Macedo T, Ribeiro V, Oliveira AP, Pereira DM, Fernandes F, Gomes NGM, Araujo L, Valentao P, Andrade PB (2020). Anti-inflammatory properties of *Xylopia aethiopica* leaves: Interference with pro-inflammatory cytokines in THP-1-derived macrophages and flavonoid profiling. Journal of Ethnopharrmacolgy 248:112312.

Mbiantcha M, Almas J, Atsamo AD, Ateufack G, Shabana SU, Tatsinkou DF, Yousseu Nana W, Nida D (2018). Anti-inflammatory and anti-arthritic effects of methanol extract of the stem bark of *Boswellia dalzielli* Hutch (Burseraceae). Inflammopharmacology 26(6):13383-1398.

Moon J-K, Shibamoto T (2009). Antioxidant assays for plant and food components. Agriculture and Food Chemistry 57(5):1655-1666.

Moukette BM, Pieme CA, Nya Biapa PC, Ngogang JY (2015). In vitro antioxidant and anti-lipoperoxidative activities of bark extracts of *Xylopia aethiopica* against ion-mediated toxicity on liver homogenates. Journal of Complementary and Integrative Medicine 12(3):195-204.

Mounanga MB, Mewono L, Angone SA (2015). Toxicity studies of medicinal plants used in sub-Saharan Africa. Journal of Ethnopharmacology 174:618-627.

National Research Council (2008). “Marula” in Lost Crops of Africa: Volume III: Fruits. Lost Crops of Africa 3 Washington, DC. National Academies Press P 117.

Odugbemi T (2006). Outlines and Pictures of Medicinal Plants from Nigeria., University of Lagos Press.

Ojewole JA, Mawoza T, Chiwororo WD, Owira PM (2010). *Sclerocarya birrea* (A. Rich) Hochst. [‘Marula’] (Anacardiaceae): A review of its phytochemistry, pharmacology and toxicology and its ethnomedicinal uses. Phytotherapy Research 24(5):633-639.

Ojewole JAO (2003). Evaluation of the anti-inflammatory properties of *Sclerocarya birrea* (A.Rich.) Hochst. (family: Anacardiaceae) stem-bark extracts in rats. Journal of Ethnopharmacology 85(2-3):217-220.

Orlando RA, Gonzales AM, Hunsaker LA, Franco CR, Royer RE, Vander Jagt DL, VanderJagt DJ (2010). Inhibition of nuclear factor kB activation and cyclooxygenase-2 expression by aqueous extracts of Hispanic medicinal herbs. Journal of Medicinal Food 13(4):888-895.

Otimenyin SO, Uguru MO, Auta A (2008). Anti-inflammatory and analgesic activities of *Cassia goratensis* and *Sarcocephalus esculentus* extracts. Journal of Herbs, Spices and Medicinal Plants 13(2):59-67.

Oyebode O, Kandala NB, Chilton PJ, Lilford RJ (2016). Use of traditional medicine in middle-income countries: a WHO-SAGE study. Health Policy Plan 31(8):984-991.

Qin S, Hou D-X (2016). Multiple regulations of Keap1/Nrf2 system by dietary phytochemicals. Molecular Nutrition Food Research 60(8):1731-1755.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolonization assay. Free Radical Biology and Medicine 26(9-10):1231-1237.

Saidu K, Onah J, Orisadipe A, Olusola A, Wambebe C, Gamaniel K (2000). Antiplasmodial, analgesic, and anti-inflammatory activities of the aqueous extract of the stem bark *Erythrina senegalensis*. Journal of Ethnopharmacology 71(1-2):275-280.

Schleiser K, Harwat M, Bohm V, Bitsch R (2002) Assessment of antioxidant activity by using different *in vitro* methods. Free Radical Research 36(2):177-187.

Schutze S, Machleidt T, Kronke M (1992). Mechanisms of tumor necrosis factor action. Seminars in Oncology 19(2 suppl 4):16-24.

Shaikh SB, Prabhu A, Bhandary YP (2019). Interleukin-17A a potential therapeutic target in chronic lung diseases. Endocrine, Metabolic &Immune Disorders- Drug Targets 19(7):921-928.

Silberberg M, Morand C, Mathevon T, Besson C, Manach C, Scalbert A, Remesy C (2006). The bioavailability of polyphenols is highly governed by the capacity of the intestine and of the liver to secrete conjugated metabolites. European Journal of Nutrition 45(2):88-96.

Sivandzade F, Prasad S, Bhalerao A, Cucullo L (2019). Nrf2 and NF-kB interplay in cerebrovascular and neurodegenerative disorders: Molecular mechanisms and possible therapeutic approaches. Redox Biology 21:101059.

Spelman K, Burns J, Nichols D, Winters N, Ottersberg S, Tenborg M (2006). Modulation of cytokine expression by traditional medicines: a review of herbal immunomodulators. Alternative Medicine Review 11(2):128-150.

Szymczak-Pajor I, Kleniewska P, Wieczfinska J, Pawliczak R (2020) Wide-range effects of 1,25(OH)2D3 on group 4A phospholipases is related to nuclear factor k-B and phospholipase-A2 activating protein activity in mast cells. International Archives of Allergy and Immunology 181(1):56-70.

Talhouk RS, Karam C, Fostok S, El-Jouni W, Barbour EK (2007). Anti-inflammatory bioactivities in plant extracts. Journal of Medicinal Food 10(1):1-10.

Tanih NF, Ndip RN (2012). Evaluation of the acetone and aqueous extracts of mature stem bark of *Sclerocarya birrea* for antioxidant and antimicrobial properties. Evidence-Based Complementary and Alternative Medicine 834156.

Togola A, Diallo D, Dembele S, Barsett H, Paulsen BS (2005). Ethnopharmacological survey of different uses of seven medicinal plants from Mali, (West Africa) in the regions Doila, Kolokani and Silby. Journal of Ethnobiology and Ethnomedicine 1:7.

Tropical Plants Database (2020). Ken Fern.tropical.the ferns.info.2020-02-03. (http://tropical.theferns.info/viewtropical.php?id=Erythrina+senegalensis)

Tsalamandris S, Antonopoulos AS, OIkonomou E, Papamikroulis GA, Vogiatzi G, Papaioannoou S, Deftereos S, Tousoulis D (2019). The role of inflammation in diabetes: Current concepts and future perspectives. European Cardiology Review 14(1):50-59.

Unwin N, Albert KG. (2006). Chronic non-communicable diseases. Annals of Tropical Medicine and Parasitology 100(5-6):455-64.

Valdivia-Correa B, Gomez-Guttierrez C, Uribe M, Mendez-Sanchez N. (2016). Herbal Medicine in Mexico: a cause of hepatotoxicity, A critical review. International Journal of Molecular Sciences 17(2):235-244.

Wakawa HY, Hauwa M (2013). Protective effect of *Erythrina senegalensis (DC)* leaf extract on carbon tetrachloride-induced liver injury in rats. Asian Journal of Biological Science 6(4):234-238 (20130501).

Wang Y, Xu J, Meng Y, Adcock IM, Yao X (2018). Role of inflammatory cells in airway remodeling in COPD. International Journal of Chronic Obstructive Pulmonary Disease 13:3341-3348.

World Health Organization (WHO) (2018). https://www.who.int/health-topics/noncommunicable-diseases

World Health Organization (WHO) (2019) WHO global Report on traditional and complementary medicine.

https://www.who.int/traditional-complementary-integrative-medicine/WhoGlobalReportOnTraditionalAndComplementaryMedicine2019.pdf

Xu P, Hueckstead TP, Hoidal JR (1996). Molecular cloning and characterization of the human xanthine dehydrogenase gene (XDH). Genomics 34:173-180.

Zhang R, Xu M, Wang Y, Xie F, Zhang G, Qin X (2017). Nrf2-a promising therapeutic target for defensing against oxidative stress in stroke. Molecular Neurobiology 54(8):6006-6017.

Vol. 16(1), pp. 11-18, January-March 2022
DOI: 10.5897/AJBR2021.1137
Article Number: DC8738B68570
ISSN 1996-0778
Copyright © 2022
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJBR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biochemistry Research

Full Length Research Paper

Medicinal effects of Saffron on obese animals by reducing Castelli's risk index to protect from cardio-pathology and psychological distress

Saara Ahmad[1*], Hamna Rafiq[1], Asra Khan[1], Prashant Tikmani[1], Saima Khaliq[2], Saad Bilal Ahmed[3] and Muhammad Misbah ur Rehman[4]

[1]Department of Biological and Biomedical Sciences, the Aga Khan University, Karachi, Pakistan.
[2]Department of Biochemistry, Federal Urdu University of Science, Arts and Technology, Karachi, Pakistan.
[3]Department of Geriatrics, Saint John of God Health Care, Australia.
[4]Department of Surgery, Hussain Lakhani Hospital, Karachi, Pakistan.

Received 29 September, 2021; Accepted 17 November, 2021

A high-fat diet is a major factor to the global obesity and diabetes epidemic. The mechanism underlying chronic fat-rich food exposure may raise cholesterol and inflammatory mediator levels. One of the key pathological hallmarks of cardiovascular disorders is the development of inflammatory markers (CVDs). The pathological action may also inhibit the insulin secretion, which triggers diabetes-associated psychological distress. Saffron is used for the beneficial effects on different diseases. Therefore, the presented study was conducted to assess the therapeutic profile of saffron on cardio-pathology and neuropsychology in rats, subjected on high-fat diet. The study used Sprague-dawley divided in normal diet (healthy control), high-fat diet treated control animals (HFDCs) and high-fat diet + Saffron (10 mg/kg). After two weeks of treatment, Castelli's risk index, glucose, and insulin levels were analysed, followed by cervical dislocation. Saffron supplements resulted in a lower Castelli's risk index and an increase in insulin level with normoglycemic effects, implying cardioprotective and antidiabetic benefits.

Key words: Cardiopathology, Castelli's risk index, depression, cholesterol, insulin, saffron.

INTRODUCTION

Cardiovascular diseases (CVDs) and depression are the leading causes of mortality and morbidity worldwide. It is known to evolve from multiple factors such as dyslipidemia, inflammation and endothelial dysfunction, the hallmark of CVD. Depression entails a cluster of transient emotions including distressed feeling and flat mood that may become severe enough to expose clinical symptoms (Mäkinen et al., 2014). It has been reported in many clinical trials and aetiological studies that depression is more frequently seen in Patients with CVD.

*Corresponding author. E-mail: saara_ahmad@hotmail.com, saara.muddasir@aku.edu. Tel: +92-21-36642030, +92-334-3448258.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

The emerging trend of fat rich diets nowadays has escalated the occurrence of CVD. Fat rich diets contain trans fatty acids that alter lipid regulation crucial to maintain homeostasis of physiological systems. Augmented concentration of *trans*-fats in high fatty diets (HFD) may lead to interruption of normal mechanism and initiates risks of chronic diseases due to dyslipidemia and generation of inflammatory cytokines (Mayberry et al., 2013). Dyslipidemia is mainly characterized as the elevated levels of total cholesterol, low density lipoprotein cholesterol (LDL-C), triglycerides and low levels of high-density lipoprotein cholesterol (HDL-C). Furthermore, the HFD are also responsible for induction of stress induced activation of hypothalamic-pituitary-adrenal axis (HPA axis) which stimulate the production of glucocorticoids and long term exposure of HPA axis may contribute to vicious cycle of increased inflammatory mediators with augmented risk of depression and severity in CVDs (Liu et al., 2013).

The Castelli risk index-I (CRI-I) has a significant diagnostic value comparable to total cholesterol assessment. It reflects production of coronary plaques and has a good correlation with insulin resistance (IR). IR is characterized as the impaired activation of insulin in liver muscles and adipocytes and appeared as metabolic disturbances (Vargas et al., 2014). A fat rich diet may elevate the level of lipolysis a key contributor of IR in adipocytes. As a result, free fatty acid turnover is enhanced, resulting in raised circulating levels, which trigger the pathogenic processes of CVDs and depression (Li et al., 2015).

The management of CVDs involves therapeutics known as beta blockers, calcium channel blocker and anticoagulants; however, for depression antidepressant like selective serotonin reuptake inhibitors (SSRIs) are the most often. It is also important to mention here that some of the CVDs medications are reported to cause depressive symptoms as a side effect and may exaggerate the clinical symptoms of depression. Likewise, there are many controversial studies providing evidence of compromised cardiac health by the use of antidepressants (De Souza et al., 2015). As a result, the use of natural herbs as medicine, which has been practiced for millennia, has recently advanced in current CVD and depression treatments. Researchers are interested in studying the possible impacts of numerous natural substances and their bioactive compounds, which operate on several targets to alleviate chronic illnesses and their comorbidities.

Crocus sativus L. is a useful spicy herb for the treatment of various ailments. Many clinical studies demonstrated its efficacy against depression and found it quite comparable with SSRI (Fluoxetine) as the administration of saffron increases the level of monoamine neurotransmitters in the synapse (Faridi et al., 2019). Emerging research also indicates that saffron has a significant potential to prevent CVDs by inhibiting platelet aggregation and lowering LDL-C, since herbs with medicinal characteristics are now widely regarded as an important treatment option with fewer side effects. The main chemical constituents of saffron, causing modulation of the monoamine levels in brain and reduction of LDL-c in the body, are crocetin, crocin, saffranal, carotenoids, lycopene, anthocyanins and saponins (Lopresti and Drummond, 2014).

Likewise, saffron, is known for its natural antioxidant properties. The existence of anthocyanins has gotten a lot of attention in this respect, and they, together with other ingredients, allow for a reduction in prostaglandin production by inhibiting the cyclooxygenase 1 and 2 enzymes. Anthocyanins have also been shown to inhibit the endoplasmic reticulum's ability to create inflammatory cytokines (Gwarzo et al., 2014). Therefore, the present study intended to evaluate the therapeutic effects of saffron in rats fed with high fat diet in related with neuropsychology and cardio-protection.

METHODOLOGY

Collection of plant

Dried stamens of saffron were bought from local market of Karachi. Specimen was preserved in herbarium with voucher number CS-ST-08-18-05 in Natural Products Research Division, Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi.

Methanolic extract preparation

After cleaned of adulterants, weighed approximately 40 g saffron were soaked in 2 L of 70% methanol for 3 days with intermittent shaking followed by filtration with muslin cloth and then "whatman qualitative grade-1 filter paper." All filtrate were then combined and subjected to rotary evaporator in order to concentrate the filtrate with maintained temperature 35-40°C and the subnormal pressure. The ultimate product was crude (Wt.Cr) with 13% yield (wt/wt) was obtained which was soluble in saline as well as in distilled water. Fresh dilutions were prepared every time prior the administration.

Experimental animals

The experiment was carried out using eighty 200 g Sprague-dawley rats from the Aga Khan University's animal house in Karachi, according to a protocol authorized by the Ethical Committee of Animal Care and Use under number 68 ECACU-BBS-17. The guidelines made by National Institute of Health (NIH) Care and Use of laboratory animals were also strictly followed (Publication No. 85-23, revised 1985. All animals were kept in clean plastic cages in a strictly regulated environment with a 12 h light/dark cycle and a temperature of 22°C, with free access to a standard rodent feed and clean tap water. Animals were individually treated respective to their group name as Normal diet (Healthy control), High fat diet treated control animals (HFDC) and High fat diet+ Saffron (Saff). Saffron were given to animals individually at dose 10 mg/kg (orally).

Composition of normal and high fat diet

Normal diet (ND) was developed at the animal house of Aga Khan

University. ND consists of (gram of dietary constituents per kilogram body weight of rats): fiber 380, flour 380, molasses 12, powdered milk 150, sodium chloride 5.8, vegetable oil 38, potassium metabisulphate 1.2, nutrivet-L 2.5, and fish meal 170.

In addition to typical diet components, a high fat diet (HFD) were constructed using a combination of cholesterol, cholic acid, and butter fat (2, 0.5, and 5% w/w, respectively).

Experimental protocol

Animals were randomly divided into 3 group (n=10) as healthy control (HC), HFDC and High fat diet test group of methanolic extract of saffron (Saff). All animals treated according to their groups for two weeks. At the end of experiment blood and brain samples of animals were collected through cervical dislocation. Blood samples were collected in heparin-containing test tubes and stored at room temperature for 30 min before being centrifuged at 15,000 g for 10 min. Brain samples were promptly washed with saline and kept at -20°C until analysis.

Behavioral assessment

Forced swim test (FST)

Forced swim test is the assessment of animal depressive behavior. The apparatus for this test was consist of a container with 56 cm height and 30 cm width. The container was filled with water at the height of 22 cm and temperature of 25°C. The depth of water was adjusted to avoid the contact of tail to the bottom as well as to escape from the apparatus. Animals were subjected to swim and struggling time (Latency to immobilize) recorded for six minutes.

Biochemical estimations

Estimation of blood TG/LDL/HDL-cholesterol

The determinants of lipid content of TG/LDL/HDL-cholesterol were done by using Roche kits Cobas c 111 (Roche, Pakistan).

Determination of Castelli's risk index

The indices were calculated using the following formulae:

Castelli's risk index-I (CRI-I) = TC/HDL-c
Castelli's Risk Index (CRI-II) = LDL-c/HDL-c.

Estimation of insulin

Insulin level (U/l) determination was done by Ultra-Sensitive Rat Insulin ELISA Kit (Crsytal Chem, USA).

Estimation of fasting blood glucose levels

The animals were fasted overnight, and blood was collected from their tails to calculate fasting blood glucose levels (mmol/lit) using an Accu-Check Performa glucometer.

Estimation of brain derived neurotropic factor (BDNF) level

Serum BDNF was quantified using an ELISA method (Cloud Clone Corp. BDNF Sandwich ELISA kit, SEA011Mi, USA) following the instructions of the manufacturer.

Statistical analysis

Results are represented as mean ± SD. Data of behavioral and biochemical analysis evaluated by one-way ANOVA of SPSS version 20. Tukey's test was used for post hoc analysis. Non-significant was defined as p >0.05.

RESULTS

Effects on Castelli's risk index

Figure 1 presents the effects of saffron for two weeks on CRI-I in high fat diet treated rats. Statistical analysis by one-way ANOVA revealed a significant effect of saffron ($F_{2.27} = 33.5$, $p<0.05$). The *post hoc* Tukey's test revealed that the ratio of TC/HDL-cholesterol was significantly higher in high fat diet treated rats (HFDC) than normal diet treated rats (HC) ($p<0.05$). However, the saffron group showed significantly ($p<0.05$) lower CRI-I than HFDC group.

Figure 2 presents the effects of saffron for two weeks on CRI-II in high fat diet treated rats. Statistical analysis by one-way ANOVA revealed the significant effect of saffron on CRI-II ($F_{2.27} = 59.795$; $p<0.05$). The Tukey's test showed that the ratio of LDL/HDL-cholesterol was significantly higher in high fat diet treated rats (HFDC) than normal diet treated rats (HC) ($p<0.05$). The effects of saffron reduced the CRI-II in Saff group, the effects were significant in HFDC ($p<0.05$) and non-significant relation was found between HC and Saff group.

Effects on insulin level

Figure 3 presents the effects of saffron for two weeks on insulin in high fat diet treated rats. The effect of therapy on insulin ($F_{2.27} = 41.99$; p0.05) was significant, according to statistical analysis using ANOVA (one-way). The level of insulin in HFDC was substantially greater than in HC ($p<0.05$) after post hoc analysis using Tukey's test. However, saffron was significantly ($p<0.05$) reduced the insulin level than HFDC rats.

Effects on glucose level

Figure 4 presents the effects of saffron for two weeks on glucose in high fat diet treated rats. Statistical analysis by ANOVA (one-way) revealed that the effect of treatment on the glucose ($F_{2.27} = 10.740$; $p<0.05$) was significant. Results of *post hoc* analysis by Tukey's test revealed that long term consumption of high fat diet in HFDC group increased the level of glucose ($p<0.05$) than HC group. However, saffron reduced the glucose level than HFDC rats.

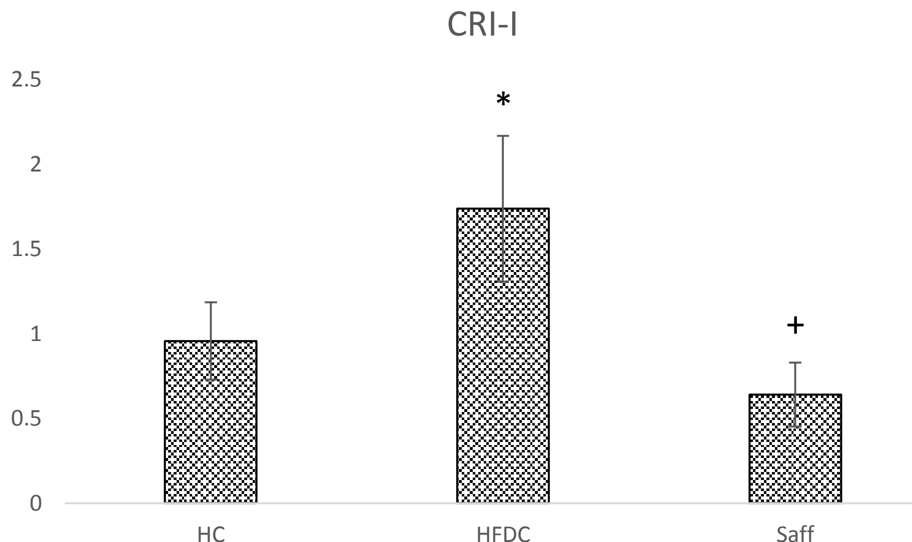


Figure 1. Effects of saffron on CRI-I in High fat diet treated rats. Values are presented as means ± SD (n=10). Tukey's test Significant values:* $p<0.05$ from control HC group, + $p<0.05$ from HFDC group

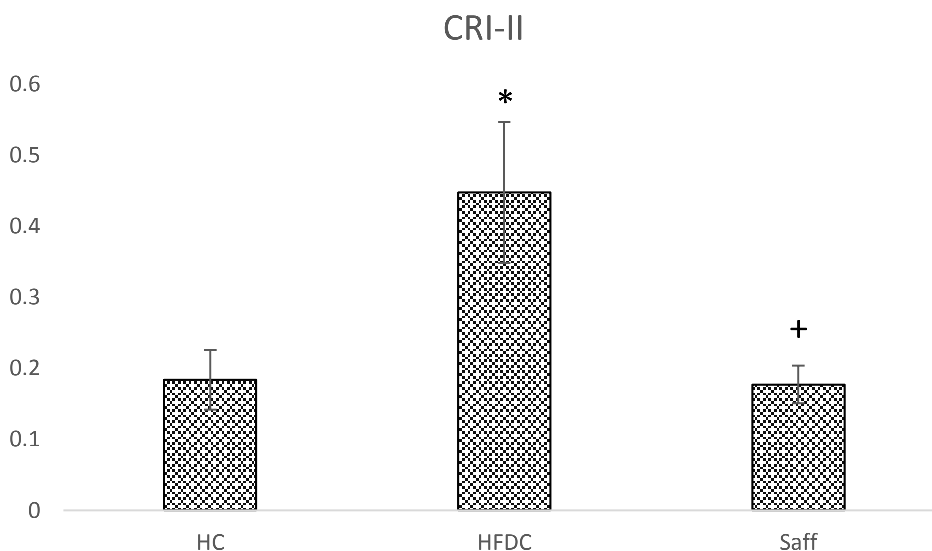


Figure 2. Effects of saffron on CRI-II in High fat diet treated rats. Values are presented as means ± SD (n=10). Tukey's test Significant values:* $p<0.05$ from control HC group, + $p<0.05$ from HFDC group.

Effects on FST

Figure 5 presents the effects of saffron for two weeks on FST in high fat diet treated rats. Statistical analysis by one-way ANOVA revealed the effect of saffron ($F_{2.27}$ = 40.9, $p<0.05$) was significant. The *post hoc* Tukey's test revealed that the latency time to immobilize was significantly reduced after the consumption of high fat diet in HFDC group as compared to normal diet treated rats (HC) ($p<0.05$). However, the saffron group showed significantly ($p<0.05$) increased latency time to immobilization time in HFDC group.

Effects on BDNF level

Figure 6 presents the effects of saffron for two weeks on BDNF in high fat diet treated rats. Statistical analysis by

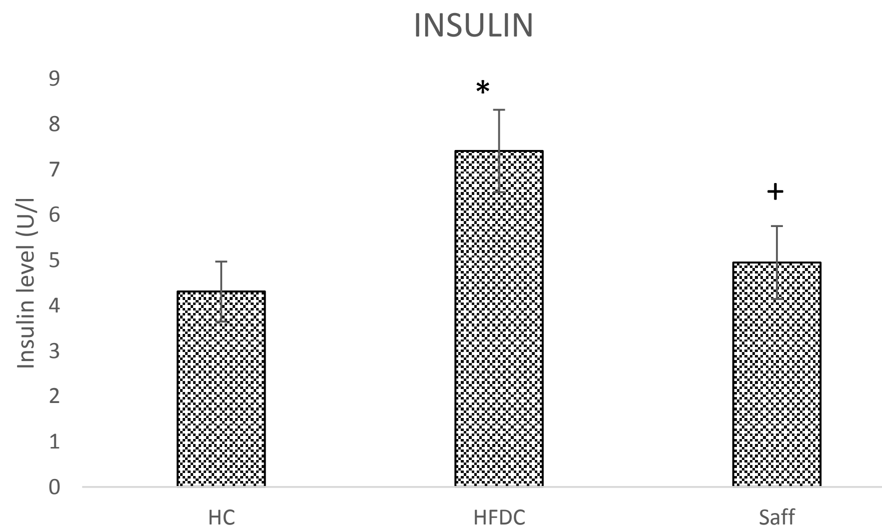


Figure 3. Effects of saffron on insulin in High fat diet treated rats. Values are presented as means ± SD (n=10). Tukey's test Significant values:* p<0.05 from control HC group, + p<0.05 from HFDC group.

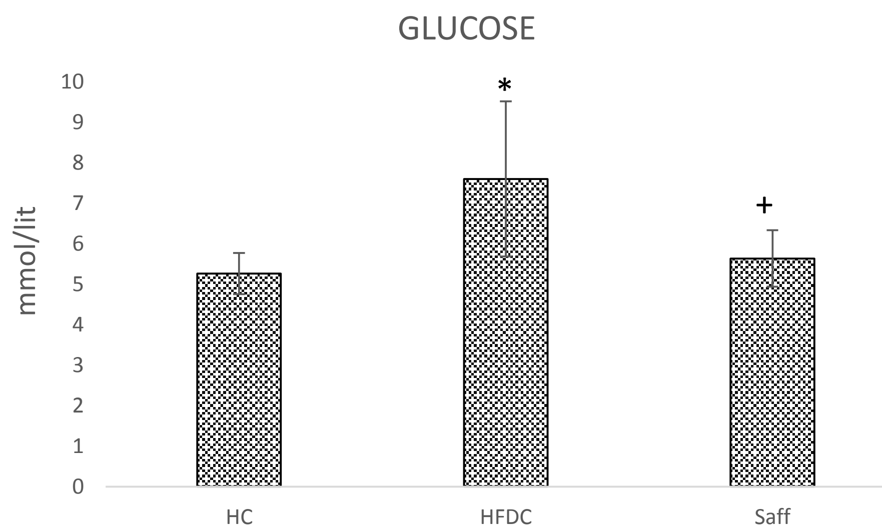


Figure 4. Effects of saffron on glucose in High fat diet treated rats. Values are presented as means ± SD (n=10). Tukey's test Significant values:* p<0.05 from control HC group.

one-way ANOVA revealed the effect of saffron ($F_{2.27}$ = 67.3, p<0.05) was significant. The *post hoc* Tukey's test revealed that the consumption of high fat diet reduced the BDNF level in HFDC group as compared to normal diet treated rats (HC) (p<0.05). The long term administration of saffron showed significantly (p<0.05) increased BDNF level in HFDC group and provided the antidepressant effects.

DISCUSSION

Insulin resistance, diabetes, obesity, and CVDs are all linked to a high-fat diet, particularly trans-fats. CVDs are becoming a serous life threatening cause of modern ages and they offer a challenging task for clinical managements. Beside the chronic metabolic diseases the behavioral deficits may also appear which mainly include depressive symptoms. The aim of the presented study was to be evaluated the effects of methanolic extract of saffron to treat high fat diet induced metabolic and neuropsychological deficits.

The long term consumption of high fat diet reduces the brown adipocytes which inhibit the synthesis of fatty acid while increasing white adipocytes which stimulates the synthesis of fatty acid and dysregulate the levels of

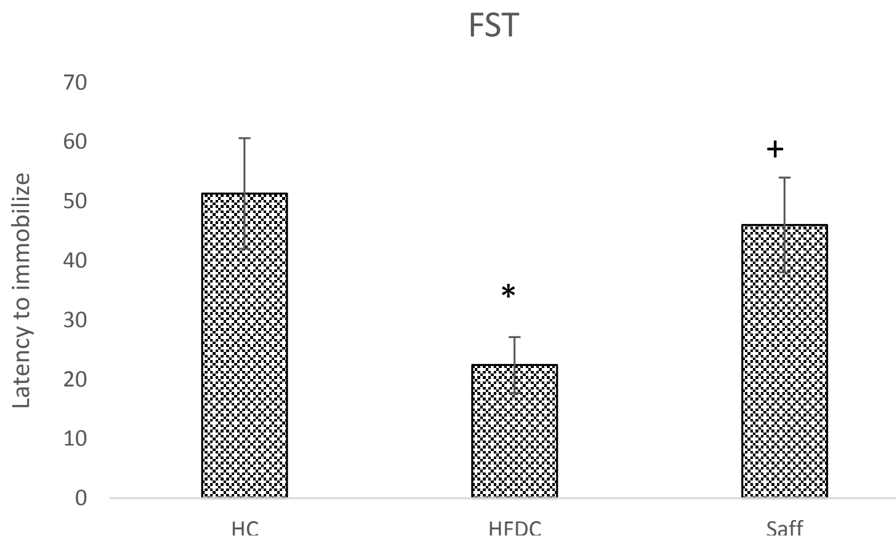


Figure 5. Effects of saffron on FST in High fat diet treated rats. Values are presented as means ± SD (n=10). Tukey's test Significant values:* $p<0.05$ from control HC group.

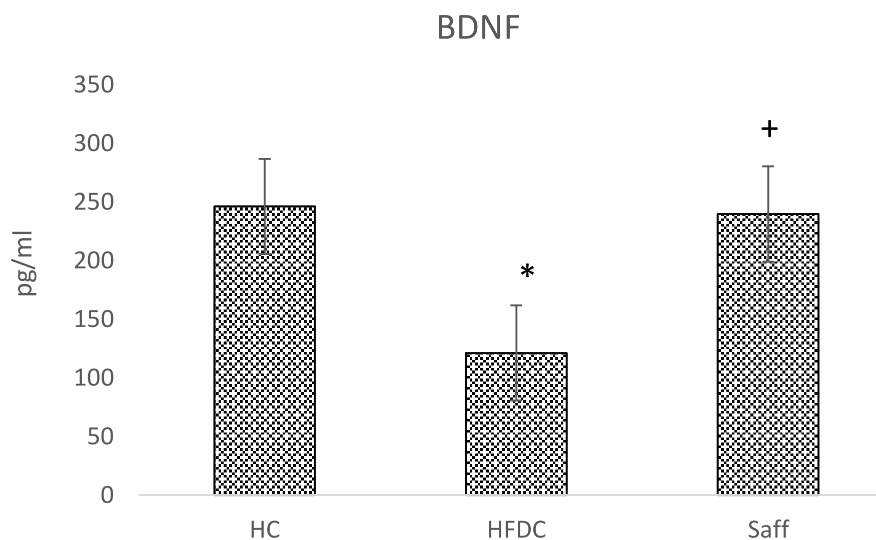


Figure 6. Effects of saffron on BDNF in High fat diet treated rats. Values are presented as means ± SD (n=10). Tukey's test Significant values:* $p<0.05$ from control HC group.

insulin. The pathological alterations of high fat diet in terms of hyperglycemia and hyperlipidemia reflect altered psychological behavior of animals. Variations in glycemic parameters occur when the insulin become unable to exert its affects. Excessive calories due to obesogenic diet decline the insulin synthesis and functions by the accumulation of fat in pancreatic cells. Instead of adipocytes, saturated fats accumulate in other organs, interfering with glucose metabolism. Excess fat buildup in organs causes oxidative stress by raising lipid peroxidation levels due to mitochondrial dysfunction.

The active chemical ingredient of saffron is crocin, which contains four analogues: crocin 1, crocin 2, crocin 3 and crocin 4. Crocin is mainly known to relieve the pain and to improve the blood circulation. Crocin is bearing multiple therapeutic activities including antioxidant, cardioprotective, antidepressant and anticonvulsant effects. The extract of saffron is known to protect against the damage of lipid peroxidation, the present study provided the positive effects of saffron on CVDs by lowering the Castelli's risk index (Figures 1 and 2). The doses of saffron also found to produce antidiabetic effects by augmentation of insulin level, the mechanism behind the antidiabetic effects is the stimulation of

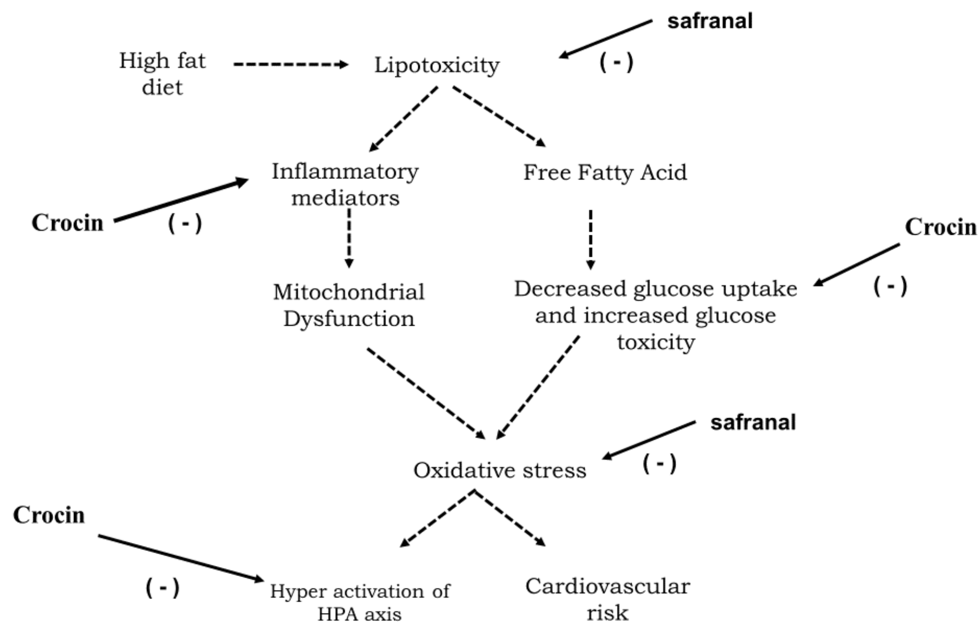


Figure 7. Summarized effects of saffron extract on high fat diet and its manifestations. (-) signs shows the inhibitory effects of saffron ingredients.

peripheral glucose uptake and liver glycogen storage. The effects of chemical constituents of saffron displayed the potential radical scavenging properties and protective effects of vital tissues as well as regenerative activity which may trigger the pancreas to produce insulin for maintaining the glucose homeostasis (Figures 3 and 4).

Long-term consumption of saturated fats disrupts the hippocampal receptor that regulates inflammatory cytokines, which are important for detecting pathogenic causes (Hanke and Kielian, 2011). Previous study has also shown that the HFD causes the creation of triglycerides, which are harmful to brain health and cause neuropsychiatric illnesses, and are detected in people with bipolar disorder (van Dooren et al., 2013). Saffron has been shown to lower oxidative stress and boost mitochondrial activity (Fathimoghadam et al., 2019). In numerous *in-vitro* and *in-vivo* investigations, prolonged doses of saffron were linked to improved mental health, demonstrating higher antidepressant and cognitive function after saffron administration in mice. Depression is also linked to an increase in insulin resistance and the Castelli's index, which is a powerful predictor of coronary artery disease (Kianbakht and Mozaffari, 2009). Depression is also linked to changes in the expression of neurotrophic factors, particularly brain derived neurotrophic factors (BDNF), which play a key role in neuronal growth and plasticity. The levels of BDNF draw broad attention in many clinical and s studies which indicate that the progression of depression is inversely related with BDNF expression and most of the antidepressant reduces the depression by increasing the levels of BDNF (Sanati et al., 2018). The measuring parameters of depression in rodents are mainly involve the time to show behavioral despair, the forced swim test (FST) is the most widely used to observe the severity of depression in rodents. The level of neurotrophic factors are indicators of healthy brain functioning however, the low levels of BDNF describe the severity of depressive symptoms. The induction of high fats in animals might interrupt the activity of HPA axis, the dysregulated HPA is often seen to increase the depressive symptoms (Figure 5), the effects of saffron in FST demonstrated the anti-depressive profile of the treatment (Dourado et al., 2020). The long term doses of saffron successfully increased the level of BDNF and ensure the antidepressant effects (Figure 6). It has been concluded from the present study that the ethanolic extract of saffron has significant pharmacological effects (Figure 7) with curative and protective potentials in CVDs and diabetes. Further experimental studies are necessary to demonstrate the molecular mechanisms.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are thankful to the Department of the Biological and Biomedical Sciences, the Aga Khan University, Karachi, Pakistan for all the support during the research. This work was supported by the Dean’s Fund

of Aga Khan University.

REFERNCES

De Souza J, Mente A, Maroleanu A, Cozma I, Ha V, Kishibe T, Anand S. (2015). Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. British Medical Journal 351 p.

Dourado NS, Souza CD, de Almeida MM, Bispo da Silva A, Dos Santos BL, Silva VD, De Assis AM, da Silva JS, Souza DO, Costa MD, Butt AM (2020). Neuroimmunomodulatory and neuroprotective effects of the flavonoid apigenin in in vitro models of neuroinflammation associated with Alzheimer's disease. Frontiers in Aging Neuroscience 12:119.

Faridi S, Delirezh N, Froushani SMA (2019). Beneficial effects of hydroalcoholic extract of saffron in alleviating experimental autoimmune diabetes in C57bl/6 mice. Iranian Journal of Allergy, Asthma and Immunology 18(1):38-47.

Fathimoghadam H, Farbod Y, Ghadiri A, Fatemi R (2019). Moderating effects of crocin on some stress oxidative markers in rat brain following demyelination with ethidium bromide. Heliyon 5(2):e01213.

Gwarzo MY, Ahmadu JH, Ahmad MB, Dikko AU (2014). Serum glucose and malondialdehyde levels in alloxan induced diabetic rats supplemented with methanolic extract of tacazzea apiculata. International journal of biomedical science 10(4):236.

Hanke ML, Kielian T (2011). Toll-like receptors in health and disease in the brain: mechanisms and therapeutic potential. Clinical Science 121(9):367-387.

Kianbakht S, Mozaffari K (2009). Effects of saffron and its active constituents, crocin and safranal, on prevention of indomethacin induced gastric ulcers in diabetic and nondiabetic rats. Journal of Medicinal Plants 8(29):30-38.

Li Y, Hruby A, Bernstein AM, Ley SH, Wang DD, Chiuve SE, Sampson L, Rexrode KM, Rimm EB, Willett WC, Hu FB (2015). Saturated fats compared with unsaturated fats and sources of carbohydrates in relation to risk of coronary heart disease: a prospective cohort study. Journal of the American College of Cardiology 66(14):1538-1548.

Liu CS, Carvalho AF, Mansur RB, McIntyre RS (2013). Obesity and bipolar disorder: synergistic neurotoxic effects? Advances in Therapy 30(11):987-1006.

Lopresti AL, Drummond PD (2014). Saffron (Crocus sativus) for depression: a systematic review of clinical studies and examination of underlying antidepressant mechanisms of action. Human Psychopharmacology: Clinical and Experimental 29(6):517-527.

Mäkinen VP, Civelek M, Meng Q, Zhang B, Zhu J, Levian C, Huan T, Segrè AV, Ghosh S, Vivar J, Nikpay M (2014). Integrative genomics reveals novel molecular pathways and gene networks for coronary artery disease. PLoS Genetics 10(7):e1004502.

Mayberry LS, Gonzalez JS, Wallston KA, Kripalani S, Osborn CY (2013). The ARMS-D out performs the SDSCA, but both are reliable, valid, and predict glycemic control. Diabetes Research and Clinical Practice 102(2):96-104.

Sanati S, Razavi BM, Hosseinzadeh H (2018). A review of the effects of Capsicum annuum L. and its constituent, capsaicin, in metabolic syndrome. Iranian Journal of Basic Medical Sciences 21(5):439.

van Dooren FE, Nefs G, Schram MT, Verhey FR, Denollet J, Pouwer F (2013). Depression and risk of mortality in people with diabetes mellitus: a systematic review and meta-analysis. PloS ONE 8(3):e57058.

Vargas HO, Nunes SO, Barbosa DS, Vargas MM, Cestari A, Dodd S, Venugopal K, Maes M, Berk M (2014). Castelli risk indexes 1 and 2 are higher in major depression but other characteristics of the metabolic syndrome are not specific to mood disorders. Life Sciences 102(1):65-71.

Vol. 16(1), pp. 19-23, January-March 2022
DOI: 10.5897/AJBR2021.1132
Article Number: 1B5024F68598
ISSN 1996-0778
Copyright © 2022
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJBR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biochemistry Research

Full Length Research Paper

Amino acids and carbohydrates composition in breast milk of lactating mothers of different age group from Aleto Health Center, Rivers State

Ndidi NWACHOKO*, Udiomine B. AKURU, Fatima M. ODEJAYI and Jack Gbenenee TETAM

Department of Biochemistry, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt P. M. B 5080, Rivers State, Nigeria.

Received 1 September, 2021; Accepted 6 December, 2021

Breast milk is the white liquid produced by women to breast-feed their babies. It has many benefits for infants, including reduced risk of gastroenteritis and respiratory infection. This study investigated the amino acids and carbohydrates composition in breast milk of lactating mothers. Eighteen (18) lactating mothers between the ages of 16-45 years participated in this study. Amino acid content was analysed with amino acid analyser, while the carbohydrate content was analysed with high performance liquid chromatography (HPLC) and generated data was analysed using one-way analysis of variance (ANOVA). The result showed that values of ribose, arabinose, fructose, sorbitol and mannitol were statistically ($p<0.05$) different in younger mothers when compared with middle and older mothers. Values for HMF, glucose and maltose were statistically different than that of other age groups. Also, sucrose level for older mothers was 2.65 ppm, which was statistically different when compared with the values for other age groups. The amino acid composition showed that younger mothers had the highest concentration of aspartate (2.43 mg/100 g) though not statistically significant with the values of other age groups. Proline value (4.20 mg/100 g) was statistically different in middle aged mothers when compared with other age groups. Values of valine, alanine, arginine and serine were statistically higher when comparing older mothers to other age groups. The varying concentrations of both amino acid and carbohydrate composition in these mothers might be due to the nutritional status of the lactating mothers.

Key words: Breast milk, amino acid, carbohydrate, lactating mothers.

INTRODUCTION

Breastfeeding, also called nursing, is the process of feeding a mother's breast milk to her infant, either directly from the breast or by expressing (pumping out) the milk from the breast and bottle-feeding it to the infant (Raj et al., 2020). The milk is produced by the gland located in the breasts of females which is responsible for lactation or the production of milk. Both males and females have glandular tissue within the breasts, however, females glandular tissues begin to develop after puberty in response to oestrogen release (Collins et al., 2016; Yu et

*Corresponding author. E-mail: ndididence@gmail.com.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

al., 2019). Mammary glands produce milk after childbirth, during pregnancy progesterone and prolactin are released. The progesterone interferes with prolactin, preventing the mammary glands from lactating. During this period, small amounts of a pre-milk substance called colostrum are produced (Collins et al., 2016; Yu et al., 2019). This liquid is rich in antibodies and nutrients to sustain an infant during the first few days of life. After childbirth, progesterone levels decrease and the levels of prolactin remain high. This signals the mammary glands to begin lactating. Each time a baby is breastfed, the milk is emptied from the breast and immediately the mammary glands are signalled to continue producing milk. As a woman approaches menopause, the tissues of the ductile system become fibrous and degenerate. This causes shrinkage of the mammary gland, the gland then loses the ability to produce milk (Collins et al., 2016; Yu et al., 2019).

During the first few weeks of life, babies may nurse roughly every two to three hours, and the duration of a feeding is usually ten to fifteen minutes on each breast while older children feed less often (Victora et al., 2016). Breastfeeding has a number of benefits to both mother and baby, mothers may pump milk so that it can be used later when direct breastfeeding is not possible (Ip et al., 2009). The benefits of human milk include reduced risk of neonatal necrotizing, enterocolitis, gastroenteritis, respiratory infection and improved later cognitive development (Schanler and Atkinson, 1999). Human milk provides non-immune protection and the bioactive substances play important roles in the non-nutritional effects of human milk on the development of the infant (Lönnerdal, 2003).

Newborn gastrointestinal tract undergoes maturational changes in the first weeks after birth and human milk has been shown to stimulate gastrointestinal mucosal proliferation and maturation in animal models and is thought to protect the neonatal infant from harmful environmental factors by affecting and promoting the mucosal barrier (Takeda et al., 2004). The growth factors in human milk [epidermal growth factor (EGF), transforming growth factor alpha (TGF) and insulin-like growth factors (IGFs)], stimulate the proliferation of intestinal cells and the formation of the mucosal barrier (Wagner and Forsythe, 2000). EGF is thought to have the most significant effect on the proliferation of cells that line the intestine and the promotion of the covering mucosal layer (Wagner and Forsythe, 2000).

The lactation period is a major source of concern in developing countries because of its positive impact on the health and nutrition of infants. Lactating women from developing countries are considered nutritionally vulnerable groups because this period places a high nutritional demand on the mother. Inadequate maternal diet during this period will lead to poor secretion of nutrients in breast milk and this can have long term impact on the child's health (Jones et al., 2010). Some studies have looked at correlations between diets, baby's age and genetic factor and nutritional composition of maternal breast milk. However, there is paucity of studies on correlation of maternal age and nutrients composition in breast milk, this research examines the composition of amino acids and carbohydrates in breast milk of lactating mothers of different age group and possible variations among different age groups.

MATERIALS AND METHODS

Collection of breast milk samples

Breast milk samples (10 ml) each were collected from eighteen (18) lactating mothers aged 16-45 years from Aleto Health Centre, Rivers State, Nigeria. The subjects, six in each group were categorised into different age groups younger mothers (16-25) years, middle mothers (26-35) years, and older mothers (36-45) years. The samples were expressed with a manual pump into sterile containers and placed on ice and transported to the laboratory at 4°C.

Amino acid analysis

Hydrolysis

0.5 g of lyophilized breast milk was weighed into a test tube and 15 ml of 6N HCl was added. The tube was flushed with N_2, capped and placed in an oven at 110°C for 24 h. The tube was then brought out and the content filtered. The filtrate was made up to 25 ml with water. An aliquot of this solution was further filtered with 0.50 µm pore-size membrane and a standard solution containing 1.25 µmol/ml of each amino acid in 0.1 N HCl was made (Elkin et al., 1985).

Derivatization

A standard solution of the sample (20 µL) was pipette into a tube and dried in vacuum at 65°C. 30 µL of methanol-water-phenylisothiocianate (2:2:1 (v/v)) was added to the formed residue, the tube was agitated and allow to stand at room temperature for 20 min.

The solvents were then removed under a nitrogen stream, and the tube was sealed and stored at 4°C (Elkin et al., 1985).

Chromatographic procedure for amino acid analysis

Prior to injection, 150 µL of diluent (5 mM sodium phosphate with 5% acetonitrile) was added to derivatized sample.

Analysis of carbohydrate with HPLC

Hydrolysis/Derivatization

10 mg of lyophilized sample was weighed into a tube and dissolved in 1 mL of 3 M triflouroacetic acid (TFA) in a 5 ml ampoule, the mixture was then incubated at 130°C for 2 h. The mixture was cooled and centrifuge at 2000 rpm for 5 min and evaporated to dryness under reduced pressure to allow removal of TFA. The hydrolyzed and dried sample was re-dissolved in 1 ml of water.

Thirty microliters of NaOH (0.3 M) trichloromethane were added

to the sample before derivatization and the mixture was incubated at 70°C for 60 min, cooled at room temperature and further neutralized with 30 µL of HCl (0.3 M). 1 ml of trichloromethane was added and mixed properly, the organic phase was carefully removed and discarded. The aqueous phase was filtered before injection (APHA, 1998; Kuanget al., 2011).

Statistical analysis

The statistical analysis for this research was done with the aid of Statistical Package for Social Sciences (SPSS) for windows (SPSS Inc., Chicago, Standard version 21.0), it was used to determine difference between means using ANOVA. Data was reported as mean ± standard error of mean (SEM) and the level of significance were set at P< 0.05.

RESULTS

The result in Table 1 showed that the compositions of glutamate and leucine were more when compared with the compositions of all the other amino acids. The values of glutamate, leucine and phenylalanine showed no significant difference in all the age groups. The values of phenylalanine in all the groups were different than the value of the FAO/WHO standard. The values of lysine for mothers of 26-35 and 36-45 differ significantly when compared with the value for mother of 16-25 years old.

The result in Table 2 showed that the compositions of glucose, maltose and fructose were more than the other saccharides present; maltose is a disaccharide of two glucose units. Glucose and fructose are both dietary monosaccharides used for generation of energy (ATP) and other high energy yielding compound (NADH). The maltose and glucose values for mothers of 26-35 years old were significantly different when compared with the values for mothers of 16-25 and 36-45 years old. Also the value of fructose for mothers of 16-25 years old was significantly different when compared with the values for mothers of 26-35 and 36-45 years old.

DISCUSSION

Living things require food for existence, food provide macronutrients such as proteins and carbohydrates. These nutrients serve as cellular fuel, support growth, repair of tissues and other metabolic activities. Breast milk protein is a key nutrient which supports cellular growth and organ development during the first few months of life. The function of a protein is dependent on its amino acids composition. The result in Table 1, showed the values of amino acids component in breast milk of mothers of different age groups. The result showed that glycine, alanine, serine, valine, lysine, glutamate, phenylalanine, histidine, and arginine values for mothers of age 36-45 years were different than that for 16-25 and 26-35 years old. Valine, arginine and serine, values for this group (36-45 years) old were significantly higher when compared with values for other groups. Also glycine and alanine values for mothers of 36-45 years old had a significantly increase when compared with the values for middle age mothers. Aspartate value for mothers of age 16-25 years differ when compared with its values for other groups. Middle aged mothers had higher values of cysteine and proline than that for other groups. Methionine, cysteine, tyrosine, and phenylalanine values in all the groups were different than the recommended standard (WHO/FAO/UNU, 1985), while the values for proline, valine, threonine, isoleucine, leucine, aspartate and lysine, irrespective of the age group were less than the standard recommended by WHO/FAO/UNU (1985). Alanine and serine values for mothers of age 36-45 were different than the recommended standard (WHO/FAO/UNU, 1985).

Tyrosine contains hydroxyl and aromatic group, it is an essential component for the production of several important brain chemicals called neurotransmitters, including epinephrine, norepinephrine, and dopamine. It also contributes to the inherent fluorescence of proteins (Lakowicz, 1999). This study showed that the breast milk of these mothers were rich in tyrosine. The values were higher than the recommended. Cysteine is a component of glutathione (an antioxidant) that function in reducing oxidative stress (Larsson et al., 2015); the concentration of cysteine was above the WHO/FAO/UNU standard for all the groups. The values of proline for mothers of 36-45 years old, lysine for mothers of 26-35 and 36-45 years old, Methionine for mothers of 26-35 years old and phenylalanine and arginine for mothers of 16-25 years old were similar to that reported by Ogechi and Irene (2013). Methionine plays an important role in the immune system as its catabolism leads to an increase in the production of glutathione. Also, studies have shown that methionine can chelate lead and remove it from tissues, thus decreasing oxidative stress (Martínez et al., 2017). Furthermore like tyrosine, phenylalanine also contributes to the inherent fluorescence of proteins (Lakowicz, 1999) and this study showed that the samples were rich in phenylalanine.

Carbohydrates are biological molecules which consist of poly hydroxyl aldehyde and ketone; it is one of the nutrients required by the body in large amount and constitutes the major source of energy in human diet. The result in Table 2 showed that the values of ribose, arabinose, rhaminose, fructose, galactose, sorbitol, and mannitol, for mothers of age 16-25 were above the values for ages 26-35 and 36-45. HMF, ribose, arabinose, fructose, maltose, glucose, sorbitol, and mannitol values for mothers of age 26-35 were significantly increased when compared with the values for mothers of age 36-45. Ribose, fructose, galactose, sorbitol, mannitol, and sucrose values for mothers of age 16-25 were significantly increased when compared with values for mothers of age 26-35. Ribose, arabinose, fructose, maltose, galactose, glucose, sorbitol and

Table 1. Amino acid composition in the breast milk of lactating mothers from Aleto Health Center.

S/N	Amino acid (mg/100 g)	16-25 years (group 1)	26-35 years (group 2)	36-45years (group 3)	FAO/WHO Standard
1	Histidine	0.88±0.13[d]	1.11±0.42[c]	2.42±1.25[c]	2.6
2	Tryptophan	0.89±0.87	1.13±0.57	1.00±0.13	-
3	Phenylalanine	4.38±1.42	4.16±0.87	4.77±0.74	3.2
4	Methionine	2.58±2.16	2.90±2.38	1.31±0.21	1.3
5	Valine	1.70±0.17[ad]	1.41±0.30[ad]	4.71±0.17*[bc]	9.4
6	Threonine	3.24±1.36	4.13±0.51	4.13±0.29	8.6
7	Isoleucine	3.94±1.13[bc]	1.73±0.21*[ad]	3.89±0.83[bc]	4.0
8	Leucine	7.17±0.50	7.73±0.10	7.25±2.00	8.9
9	Lysine	3.37±1.99	6.01±1.03	6.50±3.14	11.1
10	Glycine	3.63±0.23[bc]	1.36±0.12*[ad]	3.78±0.42[bc]	-
11	Alanine	3.52±0.78[ac]	1.73±0.21[ad]	4.73±1.36[bc]	4.2
12	Glutamate	13.35±1.36	14.33±0.52	14.95±0.84	-
13	Aspartate	2.43±1.86	1.62±0.23	0.79±0.31	6.8
14	Cysteine	1.64±0.83	2.66±1.60	1.75±0.74	1.2
15	Arginine	3.89±1.07[ad]	2.59±1.39[ad]	6.20±1.09[bc]	3.9
16	Tyrosine	3.02±0.27	3.02±0.27	3.08±0.19	2.6
17	Serine	1.51±0.37[ad]	1.57±0.18[ad]	4.32±0.16*[bc]	3.2
18	Proline	1.36±0.33[bd]	4.20±0.55*[ac]	3.79±0.66*[ac]	10.2

Values are expressed as mean ± standard error of mean (SEM) for n=6 at 95% confidence level. Values with super script * differ significantly when comparing age range 16-25years with others Values with different superscript ab differ significantly when comparing age range 26-35 years with other ages. Values with superscript cd differ significantly when comparing age range 36-45 years with other ages.

Table 2. Carbohydrate composition in the breast milk of lactating mothers from Aleto health center.

S/N	Carbohydrates (ppm)	16-25 years (group 1)	26-35 years (group 2)	36-45 years (group 3)
1	HMF	0.35±0.11[b]	0.853±0.25*[a]	0.537±0.10[a]
2	Ribose	6.10±0.01[bd]	2.68±0.16*[ad]	1.77±0.19*[bc]
3	Arabinose	8.55±4.08[ad]	4.193±1.50[ca]	2.157±0.18*[ac]
4	Raminose	1.39±1.20	0.55±0.00	0.99±0.01
5	Fructose	10.23±0.01[bd]	6.54±0.18*[ad]	3.37±0.01*[bc]
6	Maltose	7.62±0.44b[bd]	12.01±0.21*[ad]	5.15±0.01*[bc]
7	Galactose	2.41±0.08[bd]	1.27±0.08*[ac]	1.23±0.06*[ac]
8	Glucose	17.51±0.327[bd]	21.78±2.16*[ad]	8.21±2.01*[bc]
9	Sorbitol	6.44±0.53[bd]	3.73±0.19*[ad]	0.00±0.00*[bc]
10	Mannitol	7.85±0.46[bd]	4.27±0.15*[ac]	3.57±1.37*[ac]
11	Sucrose	1.89±0.01[bd]	1.20±0.01*[ad]	2.55±0.04*[bc]

Values are expressed as mean ± standard error of mean (SEM) for n=6 at 95% confidence level. Values with super script * differ significantly when comparing age range 16-25 years with others. Values with different superscript ab differ significantly when comparing age range 26-35 years with other ages. Values with superscript cd differ significantly when comparing age range 36-45 years with other ages.

mannitol values of mothers of age 16-25 had significant increase when compared with values for mothers of age 36-45. Carbohydrates assist in the complete metabolism of fats and emptying of bowel. Pyruvate which is the end product of glycolysis is usually carboxylated to yield oxaloacetate when there is a drop in its cellular level, this reaction ensures continuous oxidation of acetylCoA generated during fatty acid oxidation. Some studies have shown that constituents of breast milk could reflect the diet state of the individual, Garcia-Rodenas et al. (2016) noted that nutrients in breast milk is an evolutionary conserved trait largely independent of geographical, ethnical or dietary factors. However, similar work by Nwachoko et al. (2021) noted that breast milk of younger

mothers (16-26) years old contain high carbohydrates content than that in the breast milk of older mothers. Fields et al. (2017) stated that carbohydrates are of major importance, particularly with regard to oligosaccharides. In animals, oligosaccharides have been reported to influence brain function through different mechanisms such as sialylation of oligosaccharides which contribute sialic acid to gangliosides for brain signaling, and greater abundance of sialylated gangliosides enhances learning ability (Gridneva et al., 2019). According to Berger et al. (2020), there is a growing body of evidence showing breast milk composition is affected by genetic factors, with the greatest impact on oligosaccharides. The profile of oligosaccharides in breast milk is largely determined by maternal blood antigen (secretor status) which is genetically determined.

Conclusion

This work showed that the breast milk of these mothers were rich in phenylalanine, tyrosine, cysteine and arginine. Also, mothers of age 36-45 years had the highest total concentration of amino acids while mothers of age 16-25 had the highest total carbohydrate content. Older mothers (36-45 years) had the highest concentration of both essential amino acids (35.98 mg/100 g) and non-essential amino acid (43.39 mg/100 g). This research showed that there could be maternal age influence on the amino acids and carbohydrate content in breast milk.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Garcia-Rodenas CL, Affolter M, Vinyes-Pares G, De Castro CA, Karagounis LG, Zhang Y, Wang P, Thakkar SK (2016). Amino acid composition of breast milk from urban Chinese mothers. Nutrients 8(10):606.

Collins CT, Gillis J, McPhee AJ, Suganuma H, Makrides M (2016). Avoidance of bottles during the establishment of breast feeds in preterm infants. The Cochrane Database of Systematic Reviews 10:5252.

Fields DA, George B, Williams M, Whitaker K, Allison DB, Teague A, Demerath EW (2017). Associations between human breast milk hormones and adipocytokines and infant growth and body composition in the first 6 months of life. Pediatric Obesity 12(1):78-85.

Gridneva Z, Rea A, Tie WJ, Lai CT, Kugananthan S, Ward LC, Murray K, Hartmann PE, Geddes DT (2019). Carbohydrates in human milk and body composition of term infants during the first 12 months of lactation. Nutrients 11(7):1472.

Ip S, Chung M, Raman G, Trikalinos TA, Lau J (2009). A summary of the Agency for Healthcare Research and Quality's evidence report on breastfeeding in developed countries. Breastfeeding Medicine 4(S1):S-17.

Jones KD, Berkley JA, Warner JO (2010).Perinatal nutrition and immunity to infection. Pediatric Allergy and Immunology 21(4p1):564-576.

Lakowicz JR (1999). Principle of Fluorescence Spectroscopy.2nd Edition, Kluwer Academic /Plenum Publisher, New York.

Larsson SC, Håkansson N, Wolk A (2015). Dietary cysteine and other amino acids and stroke incidence in women. Stroke 46(4):922-926.

Lönnerdal B (2003). Nutritional and physiologic significance of human milk proteins. American Journal of Clinical Nutrition 77(6):1537S-1543S.

Martínez Y, Li X, Liu G, Bin P, Yan W, Más D, Valdivié M, Hu CA, Ren W, Yin Y (2017). The role of methionine on metabolism, oxidative stress, and disease. Amino Acids 49(12):2091-2098.

Nwachoko N, Ejiowhor O, Tonkiri A (2021). Carbohydrate and amino acids composition in breast milk of lactating mothers from Rumuolumeni Health Center, Rivers State. RSU Journal of Biology and Applied Science 1(2):54-60.

Berger PK, Plows JF, Demerath EW, Fields DA (2020) Carbohydrate composition in breast milk and its effect on infant health. Current Opinion in Clinical Nutrition and Metabolic Care 23(4):277.

Raj N, Parmar BP, Joseph R (2020). A Quasi Experimental Study to Evaluate the Effectiveness of Revised Nursing Care Standard Operative Procedures on Knowledge & Practice Regarding Infant Feeding among Students of Selected Nursing College, Vadodara. Indian Journal of Public Health Research and Development 11(2):86-89.

Schanler RJ, Atkinson SA (1999).Effects of nutrients in human milk on the recipient premature infant. Journal of Mammary Gland Biology and Neoplasia 4(3):297-307.

Takeda T, Sakata M, Minekawa R, Yamamoto T, Hayashi M, Tasaka K, Murata Y (2004). Human milk induces fetal small intestinal cell proliferation-involvement of a different tyrosine kinase signaling pathway from epidermal growth factor receptor. Journal of Endocrinology 181(3):449-457.

Ogechi UP, Irene II (2013). Protein and amino acid composition of breast milk of mothers in Umuahia, Urban Nigeria. European Journal of Experimental Biology 3(3):605-608.

Victora CG, Bahl R, Barros AJ, França GV, Horton S, Krasevec J, Murch S, Sankar MJ, Walker N, Rollins NC, Group TLBS (2016). Breastfeeding in the 21st century: epidemiology, mechanisms and lifelong effect. The Lancet 387(17):475-490.

Wagner CL, Forsythe DW (2000). Effect of human milk and recombinant EGF, TGF alpha, and IGF-I on small intestinal cell proliferation. Advances in Experimental Medicine and Biology 847(4):373-374.

WHO/FAO/UNU (1985). Human Energy Requirements. Expert Consultation. Food and Nutrition report series.

Yu H, Dilbaz S, Coßmann J, Hoang AC, Diedrich V, Herwig A, Harauma A, Hoshi Y, Moriguchi T, Landgraf K, Körner A (2019). Breast milk alkylglycerols sustain beige adipocytes through adipose tissue macrophages. The Journal of Clinical Investigation 129(6):2485-2499.

Vol. 16(1), pp. 24-31, January-March 2022
DOI: 10.5897/AJBR2017.0927
Article Number: 8AD4B5168613
ISSN 1996-0778
Copyright © 2022
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJBR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biochemistry Research

Full Length Research Paper

Changes in lipid class and fatty acid composition during the development of African pear (*Dacryodes edulis*) fruit pulp

Uhunmwangho E. S.[1*] and Omoregie E. S.[2]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Medical Sciences, Ondo City, Ondo State, Nigeria.
[2]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

Received 7 January, 2017; Accepted 19 April, 2017

The pulp of the African pear (*D.edulis*) fruits were investigated for its oil compositions, major lipid classes and constituents fatty acids from 4 weeks after anthesis (WAA) to fruit maturation. The oil was extracted with n-hexane using soxhlet extractor and characterised by gas chromatography. Fatty acid profile showed a saturated acid content of between 19.5 and 36.5% and unsaturated fatty acid content of 63.5 to 80.5%. Fractions of different fatty acids were synthesised at different stages of fruit development and the predominant fatty acids were palmitic acid (31.0%), stearic isomer (20.9%), oleic acid (7.1%) and linoleic acid (43.8%) at matured stage (20 WAA) of fruit development and 10.4, 12.9, 22.7 and 39.8% respectively at the immature stage (4 WAA) of fruit development. Polyunsaturated fatty acids were not detected in the African pear pulp oil throughout maturation. The major phospholipids were phosphatidylcholine (65.2%), phosphatidylinositol (25.8%) and phosphatidylethanolamine (8.9%), while phosphatidylserine (PS) and lysophosphatylcholine (LPC) remains as traces throughout fruits maturation. The pulp major sterol lipids were sitosterol accounting for about 71.3% and campesterol 12.4% of the total sterol lipids. The immature fruit pulp glyceride lipids were diacylglycerides (DAG) and triacylglyceride (TAG) which accounted for 70.2 and 18.6% respectively. In mature fruit pulp, TAG had a dramatic increase to 72.5% while DAG decreased to 22.1%. Major changes occurred in the TAG with fruit maturity with increased in concentration to 72.5% at 20 WAA. Based on these changing patterns of lipid fractions with fruit maturity, possible pathways of TAG synthesis have been proposed. In conclusion, the results at the 18 to 20 WAA showed that high quantities of essential fatty acids are present in the African pear pulp oil at mature stage of the fruits.

Key words: *Dacryodes edulis,* anthesis, n-hexane, gas chromatography, triacylglyceride.

INTRODUCTION

African pear (*D. edulis*) is well known plants in West Africa. The fruits are edible and the bark, leaves, stem and roots are used in ethno medicinal practice for treatment of diseases (Ajibesin et al., 2008). *D. edulis* is an indigenous fruit tree in the humid low lands and plateau regions of West, Central African and Gulf of Guinea countries. It belongs to the Burseraceae family, an evergreen tree indigenous to the Central Africa and Gulf of Guinea regions. The genus name is derived from the Greek word '*Dakruon*'(a tear) in reference to the resin

droplets that appears on the bark surface of its species (Burkill, 1985; Edem et al., 2009). The species-specific name *edulis* means edible (Anonymous, 2011a). The genus *Dacryodes* comprises about 40 species occurring in the American, Asian and African tropics. In Africa, about 20 species have been described (Anonymous, 2011b). In South-East Nigeria, the trees are grown around homesteads and flowering takes place from January to April. The major fruiting season is between May and October (Kengue and Nyagatchou, 1990).

D. edulis is a tree cultivated widely for its edible and nutritious fruits. Generally, the fruit may be soaked in hot water, or roasted/baked in an oven at about 50°C. The roasted fruit can be eaten with maize, plantain, cassava, cocoyam and bread (Sofowora, 2008).

The entire plant has pharmaceutical properties that are variously exploited by many African communities (Kengue, 2002). For instance, in the Western parts of Cameroon, the bark is crushed and used in concoctions against dysenteries while in Central Cameroon the bark is used to treat toothache. The leaves are boiled in combination with *Lantana camara, Cymbopogon citratus* and *Perseaamericana* to yield a steam bath taken to treat fever/headaches and malaria in Republic of Congo. The leaves and seed are used in Nigeria for animal feed (Ajibesin et al., 2008); the resin from the bark has long been reported to be effective against parasitic skin diseases and jiggers.

D. edulis is a versatile plant in African ethnomedicine as its various parts are employed to treat several diseases. The bark of the plant has long been used to cicatrize wound in Gabon (Adebayo-Tayo and Ajibesin, 2008). In Democratic Republic of Congo, the plant is employed for the treatment of leprosy, tonsillitis and dysentery, anaemia, spitting blood, pains and stiffness and skin diseases (Adebayo-Tayo and Ajibesin, 2008). In Congo Brazzaville, the leaves are boiled with those of *Lanata camara*, *Cymbopogon citretus* in water to form a decoction for treating malaria (Ikhuoria and Maliki, 2007). The bark resin is used in Nigeria to treat parasitic skin diseases and jiggers. When applied in lotions and creams, the resin smoothens and protects the skin. The leaves are often crushed and the juice used to treat generalized skin diseases such as scabies, ringworm, rashes and wounds, while the stems are employed as chewing sticks for oral hygiene (Ajibesin et al., 2008).

The essential oil of the plant has been shown to possess potent antibacterial activity against *Staphylococcus aureus, Bacillus aurens, Escherichia coli* and *Proteus minibillis* (Koudou et al., 2008; Okwu and Nnamdi, 2008). No part of *D. edulis* is known to be toxic (Ajibesin et al., 2008; Dike, 2010).

Fruit pulps form a major part of the diet of Nigerians, consumed as a meal as well as ingredients of local soups. Despite the increased popularity of this fruit pulp, *Dacryodes edulis* have not been used to produce oil on an industrial scale, or cultivated systematically because of lack of basic chemicals and biological knowledge of their values. The present work was carried out with a view to studying the oil potential and determining the suitability of *D. edulis* pulp oil for edible and/or industrial purposes.

MATERIALS AND METHODS

Plant materials

Matured fruits of African pear were collected from private farm land in Okada Town of Ovia North-East LGA of Edo State, Nigeria. The fruits were authenticated by the Department of Botany, Faculty of Sciences, University of Medical Science, Ondo City, Nigeria. A voucher specimen of each plant was there after deposited in the herbarium of the same Department.

Preparation of sample

Forty fruits were collected randomly from each of the studied trees at biweekly intervals starting from the fourth week after fruit set until senescence. The collected fruits were cleaned with a moist soft cotton wool and then the seeds were carefully separated from the fruits. Part of the separated pulp and nut were immediately used for the moisture content and oil extraction, while the remaining part was dried at 65°C for 4 h in an oven, crushed with a laboratory mortar and pestle and kept in a well labelled air tight polythene bags or screw-capped bottles at 4°C for subsequent biochemical analysis.

All reagents used were of analytical grade purchased from Sigma Chemicals Co, London, and BDH Chemicals Ltd., England.

Extraction of oil

The soxhlet extraction method was employed. The sample (5 g) was weighed into a weighed filter paper and folded neatly. This was placed inside the pre-weighed thimble (W_1). The thimble with the sample (W_2) was inserted into the soxhlet apparatus and extraction under reflux was carried out with the n-hexane (40-60°C boiling range) for 6 h. At the end of extraction, the thimble was dried in the oven for about 30 min at 100°C to evaporate off the solvent and was cooled in a desiccator and later weighed (W_3). The fat extracted from a given quantity of sample was then calculated.

Calculations

% Crude Fat (W/W) = [Loss in Weight Sample/Original Weight of Sample] × 100

*Corresponding author. E-mail: esosasam@yahoo.com. Tel: +2348128819787.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

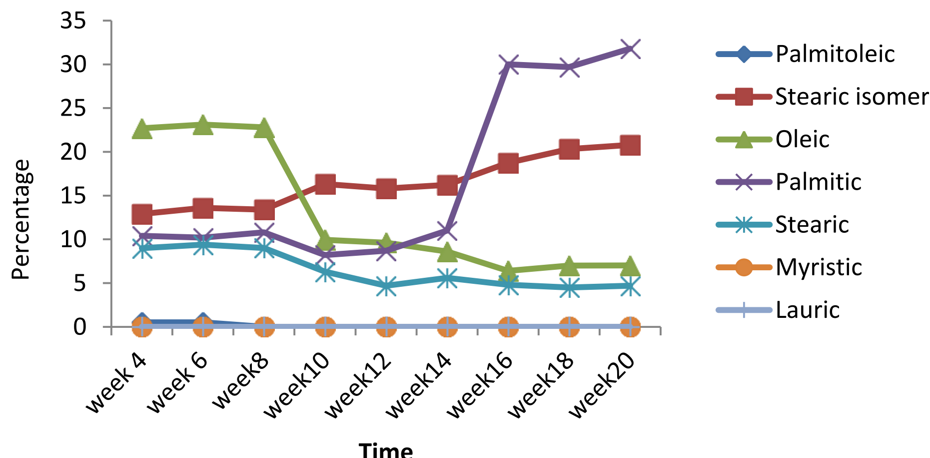


Figure 1. Saturated fatty acids composition in *D. edulis* pulp oil 4-20th WAA of fruits development. Values are mean ± SEM.

Determination of fatty acids

Fatty acids were determined according to the method of Manni and Caron (1995) as reported by Siedlecka et al. (2008). The lipids were converted to methyl esters by refluxing for 1 h with methanolic H_2SO_4 at 70°C. Fatty acid esters (FAME) were analysed on a 6820 gas chromatography (GC) system (Agilent Technologies). A FFAP capillary column (185°C temperature) was used, 30 m × 250 µm × 0.25 µm (Quadrex Corporation). Carrier gas - nitrogen, flow 1.0 ml/per min, detector - FID, temperature programme used: 60 to 200°C (20°C/min, 10 min), injector, 250°C, detector, 300°C. The samples were dosed by a HT 300A automatic dosing device at an injection size of 1 µl of 5 to 10% heptane of methyl esters using the split method and a 30:1 splitting ratio. The needle was then withdrawn and noted the formation of a small peak on the chart paper due to solvent making start reference point.

The reference standard mixture of known composition was analysed in the operating conditions as those employed for the sample and the retention times for the common fatty esters were measured. The resulting peaks for the sample were identified from the graph. Fatty acids appeared on the chart in increasing number of carbon atoms and increasing unsaturation.

Phytosterol lipids analyses were determined according to the standard method of AOAC 994.10 (1996a), phospholipids were determined according to Raheja et al. (1973) and glycerides analysis followed the procedure of the ASTM (2012).

Statistical data analysis

All values are expressed as the means ±SEM of triplicate determinations. All statistical analyses are performed using Graph Pad Prism version 6. To test for differences between the group means, one way analysis of variance (ANOVA) is employed. Significant differences between the means are determined by Duncan's multiple range tests, and P values < 0.05 are regarded as significant (Kyari, 2008).

RESULTS

Nineteen FAs were determined in *D.edulis* fruits oil extracted biweekly intervals starting from the fourth week after fruit set until senescence is shown in (Figures 1 to 5). The notable FAs contained in extracted oil from *D. edulis* pulp fruits were palmitic, stearic, stearic isomer, oleic and linoleic acid. The pattern of oil accumulation in *D. edulis* fruits at different physiological maturity stages is presented in Figures 4 and 5.

The results (Figure 4) revealed the presence of monoglyceride, diglyceride and triglyceride.

DISCUSSION

Determination of oil content in plants is important because it predicts the profitability of given plants as potential source of oil. High oil content in plant seeds implies that processing them for oil would be economical (Ikhuoria et al., 2008). Fatty acids are consumed in a wide variety of end use industries as food, medicine, rubber, plastics, detergents and cosmetics (Gunstone, 1996). The fatty acid composition of *D. edulis* (Table 1) showed palmitic acids as the major saturated fatty acids. Stearic isomer and linoleic acid were the major unsaturated fatty acids in the matured fruits (20 WAA). This is in agreement with earlier reports by Umaru and Dere (1986) who reported high palmitic acid and linoleic acids in *D.edulis* pulp oil. The concentration of linoleic acid in *D. edulis* pulp oil fell within the range (38.2 to 43.8%) (Table 2) which is higher than what is found in common edible oil, such as cotton seed, grape seed, canola oil, soybean oil, corn oil and sunflower oil (Dubois et al., 2007). In the early stages of maturity, oleic acid was highly concentrated, but declined as the fruit matures. This was probably used up as energy for growth or for synthesis of other compounds (Ikhuoria et al., 2008). The most important edible oils are those containing palmitic, stearic, oleic and linoleic acids, of which oleic and linoleic acids are most valuable (Wang et al., 2012). Also, this study showed that *D. edulis* fruit oils

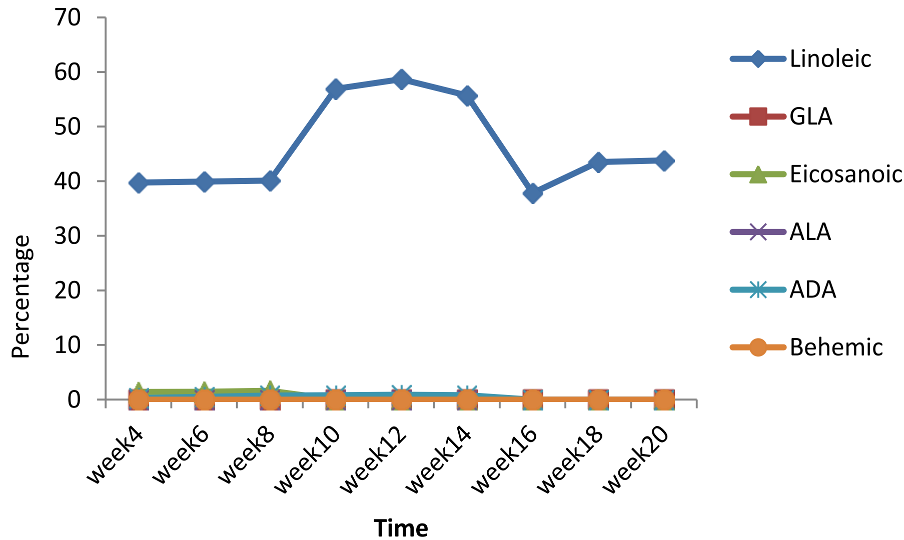


Figure 2. Composition of polyunsaturated fatty acids in *D. edulis* pulp oil 4-20th WAA of fruits development. Values are mean ± SEM.

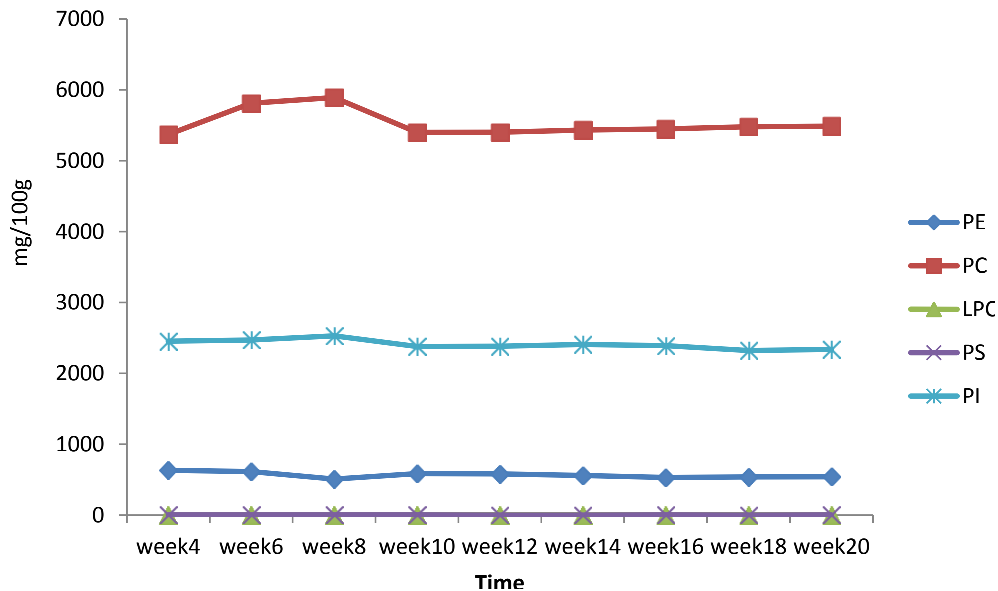


Figure 3. Phospholipids Composition in *D. edulis* pulp oil 4-20th WAA of fruits development. Values are mean ± SEM.

are rich in palmitic acid which is the principal composition of cooking oil (Wang et al., 2012).

The present study revealed that increase in the synthesis of triacylglycerols was accompanied by corresponding decrease in the level of diacylglycerides (Table 4) throughout the period of maturation (4 to 20 WAA) of the fruit (Table 3). The pattern of accumulation of triacyglycerides (TAG) indicate possible pathway that may be involved in their synthesis. In the plants, large amounts of free fatty acids (FFA) characterised the immature stage (4 to 12 WAA) with high levels of oleic acid, stearic acid and palmitoleic acid while the amount of TAGs formed was low. However, as the fruits mature, there were decreases in stearic, oleic and palmitoleic acids levels with a dramatic increase in TAG level. The reductions in the levels of FFAs with a corresponding dramatic increase in the TAG content suggest that most of the available FFAs were incorporated into TAG synthesis. This pattern of synthesis and lipid accumulation was observed in previous studies of some tropical oil

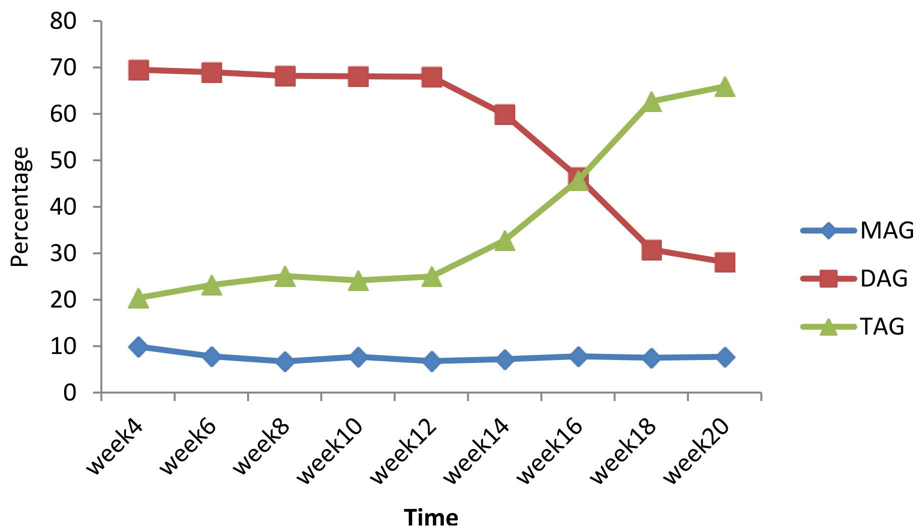


Figure 4. Composition of Neutral lipids in *D. edulis* pulp oil 4-20th WAA of fruits development. Values are mean ± SEM.

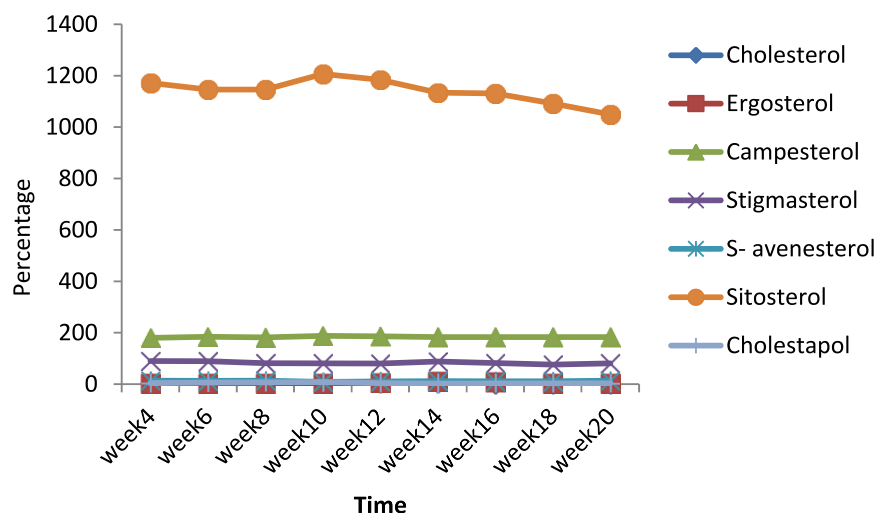


Figure 5. Percent sterol lipids in *D. edulis* pulp oil 4 to 20th WAA of fruits development. Values are mean ± SEM.

Table 1. Fatty acid composition of *D. edulis* at week 4 to 20 of fruit development.

Week	Lauric C12:0	Myristic C14:0)	Palmitic C16:0	Palmitoleic C16:1 Cis9)	Stearic C18:0	Stearic isomer (C18:1 (Cis 6)	Oleic C18:1 (Cis 9)
4	ND	ND	10.41±0.06	0.51±0.01	9.00±0.01	12.91±0.04	22.71±0.06
6	ND	ND	10.22±0.02	0.52±0.03	9.41±0.04	13.60±0.03	23.11±0.08
8	ND	ND	10.85±0.01	ND	9.02±0.02	13.38±0.08	22.81±0.03
10	ND	ND	8.23±0.31*	ND	6.31±0.03*	16.32±0.04*	9.95±0.10*
12	ND	ND	8.68±0.04*	ND	4.71±0.07*	15.81±0.05*	9.62±0.10*
14	ND	ND	11.00±0.03	ND	5.62±0.09*	16.22±0.03*	8.61±0.07*
16	ND	ND	30.90±0.04*	ND	4.80±0.07*	18.72±0.01*	6.40±0.06*
18	ND	ND	29.70±0.06*	ND	4.51±0.08*	20.32±0.08*	7.02±0.09*
20	ND	ND	31.81±0.08*	ND	4.70±0.08*	20.80±0.11*	7.02±0.01*

Values are mean ± SEM (* = P<0.05) compared with week 4. ND = Not detected.

Table 2. Fatty acid composition of *D. edulis* at weeks 4 to 20 of fruit development.

Week	Linoleic C18:2 (Cis9,12)	GLA C18:3 (Cis6,9,12)	Eicosenoic C20:1 (Cis11)	ALA C18:3 (Cis912,15)	EDA C20:2 (Cis 11,14)	Behenic C22:0	% Unsaturation	% Saturation
4	39.76±0.08	ND	1.41±0.01	ND	0.30±0.03	ND	80.50±0.81	19.51±0.031
6	39.92±0.06	ND	1.42±0.06	ND	0.50±0.03	ND	78.88±1.34*	20.10±0.56
8	40.11±0.10	ND	1.60±0.08	ND	0.73±0.04	ND	83.24±0.36*	15.30±0.81*
10	56.92±0.03*	ND	ND	ND	0.80±0.02	ND	84.20±0.611*	14.22±1.31*
12	58.70±0.08*	ND	ND	ND	0.90±0.08	ND	80.12±0.85	17.82±0.08*
14	55.71±0.13*	ND	ND	ND	0.81±0.04	ND	62.90±0.56*	35.29±0.73*
16	37.82±0.04*	ND	ND	ND	ND	ND	70.80±1.76*	28.53±0.61*
18	43.50±0.08*	ND	ND	ND	ND	ND	65.81±0.76*	34.22±1.33*
20	43.80±0.33*	ND	ND	ND	ND	ND	63.50±0.59*	36.52±0.61*

Data are the average of three replicates ± SEM (P>0.05) compared with week 4. ND = Not detected.

Table 3. Phospholipids composition of *D. edulis* at week 4-20 of fruit development.

Week	PE (mg/100 g)	PC (mg/100 g)	PS (mg/100 g)	LPC (mg/100 g)	PI (mg/100 g)
4	633.62±0.91	5370.50±0.10	2.54±0.08	3.61±0.13	2455.72±0.41
6	614.11±0.44*	5811.71±1.31*	2.57±0.04	3.08±0.36	2471.81±0.51*
8	509.86±0.71*	5891.77±0.93*	2.55±0.46	3.16±0.78	2530.04±1.06*
10	586.12±0.36*	5400.96±1.34*	2.31±0.31	3.29±0.07	2380.71±0.81*
12	582.17±0.04*	5402.34±0.64*	2.04±0.36	3.10±0.14	2384.38±0.98*
14	561.71±0.41*	5431.31±0.31*	2.14±0.05	3.44±0.36	2407.19±0.46*
16	531.81±0.49*	5449.16±0.89*	2.21±0.16	3.41±0.04	2391.12±0.76*
18	539.89±0.25*	5478..89±0.38*	2.19±0.35	3.27±0.81	2322.07±0.41*
20	541.12±0.36*	5487.10±0.43*	2.29±0.04	3.38±0.03	2338.23±0.49*

Data are the average of three replicates ± SEM (* = P<0.05) compared with week 4. PC = Phosphatidylcholine. PE = Phoshatidylethanolamine. PI = Phosphatidylinositol. PS = Phosphatidylserine. LPC = lysophosphatidylcholine.

Table 4. Composition of glyceride lipids in *D. edulis* at week 4 to 20 of fruit development.

Week	MAG (%)	DAG (%)	TAG (%)
4	9.91±0.03	69.51±0.20	20.43±0.12
6	7.81±0.438	69.04±0.54	23.17±0.24*
8	6.70±0.09*	68.19±1.04	25.11±0.72*
10	7.72±0.77*	68.14±0.99	24.16±1.01*
12	6.87±0.69*	68.11±1.44	25.02±1.44*
14	7.21±0.21*	59.89±1.00*	32.83±0.86*
16	7.86±0.51*	46.43±0.65*	45.79±0.73*
18	7.56±0.81*	30.80±0.95*	62.67±1.24*
20	7.77±0.31*	28.14±0.31*	65.87±0.60*

Values are mean ± SEM (* = P<0.05) compared with week 4.

seeds and fruits in which the disappearance of the partial glycerides and FFA which characterised the immature stage mesocarplipid coincided with the formation of TAG (Bafor and Osagie, (1988); Wang et al., 2012).

Another possible mechanism for TAG synthesis in the oil palm fruit mesocarp is the glycerol-3-phosphate pathway via phosphatidic acid (PA) acting as a transient intermediate. This is due to the fact that phosphatidic acid

Table 5. Sterol lipids in *D. edulis* pulp oil at week 4 to 20 of fruit development.

Week	Cholesterol (mg/100 g)	Cholesta-pol (mg/100 g)	Ergos-terol (mg/100 g)	Campesterol (mg/100 g)	Stigmasterol (mg/100 g)	Save-nesterol (mg/100 g)	Sitosterol (mg/100 g)
4	4.94±0.06	4.17±0.01	2.54±0.46	180.88±0.33	89.75±0.46	13.35±0.08	1177.63±0.07
6	3.86±0.03	6.17±0.06•	2.42±0.04	184.71±0.03•	89.16±0.16	12.81±0.07	1146.01±0.18•
8	3.01±0.76*	7.00±0.08•	2.11±0.05	182.01±0.38•	81.40±0.26•	13.41±0.03	1146.28±0.18•
10	3.20±0.65•	8.11±0.05•	1.84±0.81	188.06±0.22•	80.91±0.91•	8.94±0.91•	1207.01±0.23•
12	3.10±0.33•	4.53±0.41	5.10±0.06•	186.14±0.71•	80.14±0.02•	10.81±0.41•	1184.50±0.08•
14	3.12±0.09•	2.29±0.95•	9.95±1.86•	183.01±•0.86•	87.85±0.75•	11.52±0.66•	1134.04±0.15•
16	ND	2.46±0.44•	8.41±0.04•	183.23±1.24•	82.11±0.04•	11.01±0.06•	1131.14±0.18•
18	ND	3.47±0.41•	2.38±0.77	183.65±1.80•	75.87±1.14•	13.47±0.17	1092.06±0.41•
20	ND	2.01±0.31•	2.02±0.31	183.88±1.31•	80.71±0.41•	13.11±0.50	1049.30±0.97•

Data are the average of three replicates ± SEM (* = P<0.05) compared with week 4.

(PA), a major lipid fraction in some immature oil seed, was not detected in the matured oil palm fruit mesocarp during lipids accumulation. The PA is probably hydrolysed by a specific phosphotidate phosphydroxylase enzyme to give a 1, 2-diacylglycerol which is acylated to TAG in a reaction that is catalysed by a 1, 2-diacylglycerol acyltranferase enzyme (Bafor and Osagie, 1988). In support of this is the dramatic drop in the level of diacylglycerides at 20 WAA. This pattern of TAG synthesis has been supported as the most widely occurring pathway in most seed tissues (Bafor and Osagie, 1986; Wang et al., 2012).

The major phospholipids of the immature and matured stages (weeks 4 to 20) were phosphatidylinositol and phosphatidylethanolamine in both fruit oils (Table 3). This agrees with previous findings of Bafor and Osagie (1988) and Ekman et al. (2008) with oil palm. Lysophosphatidylcholine (LPC), phosphatidylserine (PS) and phosphatidylethanolamine (PE) were low throughout fruits maturation. The phospholipids content reported here are in concert with literatures (Bafor and Osagie, 1986, 1988) implicating PC and PE as an intermediate both in the synthesis of membrane phospholipids and that TAG and PA as a metabolites in the biosynthesis of other polar lipids.

In *D. edulis* pulp oil, cholesterol, cholestapol, ergosterol, s-avenasterol and sitosterol were detected at various levels (Table 5). Sitosterol was more abundant a constant level throughout. Bafor and Osagie (1988) reported the importance of free sterols in controlling membrane stability and permeability in beet and barley roots.

Conclusion

The results at the 18 to 20 WAA showed that high quantities of essential fatty acids are present in the African pear pulp in the mature stage and could as well be regarded as the physiological mature stage of the fruits that would present the optimum values of the properties which could be incorporated in food products. Hence, the African pear fruit which is a nutritious food could be harvested at this period for industrial and domestic uses.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors wish to thank the Management of the Nigerian Institute For Oil Palm Research (NIFFOR) for providing the space, extraction apparatus and valuable advice during the extraction of the oil.

REFERENCES

Adebayo–Tayo BC, Ajibesin KK (2008). Antimicrobial activity of *Coula edulis.* Research Journal of Medicinal Plant 2(2):86-91.

Ajibesin KK, Ekpo BJ, Bala DN, Essien EE, Adesanya SA (2008). Ethnobotanical survey of Akwa Ibom state of Nigeria. Journal of Ethnopharmacology 115(3):387-408.

Anonymous (2011a). *Dacryodes edulis*. Agroforestry Tree Database, World Agroforestry Centre P 159.

Anonymous (2011b). *Dacryodes edulis.* Portabase, Plant Resources of Tropical Africa (PROTA).

Association of analytical chemists (AOAC) (1996a). AOAC official method 994.10: Cholesterol in foods- Direct saponification – Gas chromatography method. Association of analytical chemists, Arlington, VA.

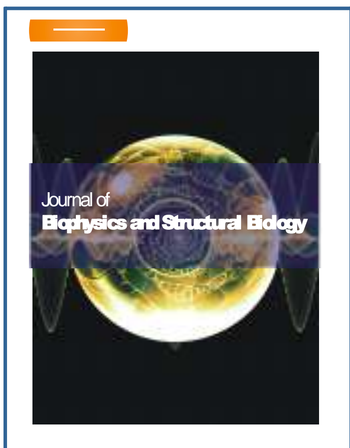
ASTM (2012). ASTM method D6584: The standard test method for the determination of free and total glycerine in B-100 Biodesiel methoyl esters by gas chromatography. Fisher Scientific, Runcorn, Chesire, UK.

Bafor ME, Osagie AU (1986). Changes in lipid class and fatty acid composition during maturation of Mesocarp oil palm (*Elaeis guineesis*) variety Dura. Journal of the Science of Food and Agriculture 37(9):825-832.

Bafor ME, Osagie AU (1988). Changes in some non-polar lipids composition of Deloping oil palm fruit (*Elaeis guineesis*) mesocarp, *Var. Duru.* Journal of the Science of Food and Agriculture 43(4):261-

264.
Burkill HM (1985). The useful plants of West Africa. Royal Botanic Gardens Kew 1:960.
Dike MC (2010). Proximate, phytochemical and nutrient compositions of some fruits, seeds and leaves of some species at Umudike, Nigerians ARPIN. Journal of Agricultural and Biological Science 5(1):7-16.
Dubois V, Breton S, Linder M, Fanni J, Parmentier M (2007). Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. European Journal of Lipid Science and Technology 109(7):710-732.
Edem CA, Dosunmu MI, Bassey FI (2009). Determination of proximate composition, Ascorbic Acid and Heavy metal content of African walnut *Tetracarpidium conophorum*. Pakistan Journal of Nutrition 8(3):225-226.
Ekman Å, Hayden DM, Dehesh K, Bülow L, Stymne S (2008). Carbon partitioning between oil and carbohydrates in developing oat (Avena sativa L.) seeds. Journal of Experimental Botany 59(15):4247-4257.
Gunstone FD (1996). Fatty Acid and Lipid Chemistry. Springer 264 p.
Ikhuoria EU, Maliki M (2007). Characterization of *Avocado* pear (*Paesea 8 ignallin*) and African pear (*Dacryodes edulis*) extracts. African Journal of Biotechnology 6(7).
Ikhuoria EU, Aiwonegbe AE, Okoli P, Idu M (2008). Characteristics and composition of African oil bean seed (*Pentaclethra macrophylla* Benth). Journal of Applied sciences 8(7):1337-1339.
Kengue J (2002). Safou. *Dacryodes edulis*, International Centre for Underutilise Crops, Southampton, UK. RPM Reprographics, Chinchester, England P 147.
Kengue J, Nyagatchou J (1990). Problème de la conservation du pouvoir germinative chez les signal de safoutier (*Dacryodes edulis).* Fruits 45(4):409-412.
Koudou J, Edou P, Obame LC, Bassole IH, Figueredo G (2008). Volatile components, antioxidant and antimicrobial properties of the essential oil of *Dacryodes edulis* from Gabon. Journal of Applied Sciences 8(19):3532-3535.
Manni G, Caron F (1995). Calibration and determination of volatile fatty acids in waste leachates by gas chromatography. Journal of Chromatography A 690(2):237-242.
Okwu DE, Nnamdi DA (2008). Evaluation of the chemical composition of *Dacryodes edulis and Raphia hookeri*. Maun and wendi exudates used in herbal medicine in south eastern Nigeria. African Journal of Traditional, Complementary and Alternative Medicines 5(2):194-200.
Raheja RK, Kaur C, Fingh A, Bhatia IS (1973). New coloromentary method of the quantitative estimation of phospholipids without acid digestion. Journal of Lipid Research 14(6):695-697.
Siedlecka EM, Kumirska J, Ossowski T, Glamowski P, Gołębiowski M, Gajdus J, Kaczyński Z, Stepnowski D (2008). Determination of Volatile Fatty Acids in Environmental Aqueous Samples. Polish Journal of Environmental Studies 17(3):351-356.
Sofowora A (2008). Medicinal plants and traditional medicine in Africa. 2nd Edn., Spectrum books Ltd. Ibadan, Nigeria.
Umaru O, Dere AO (1986). Characrerisation and Composition of the pulp oil and Cake of the African Pear, *Dacryodes edulis (G. Don).* Journal of the Science of Food and Agriculture 38(1):67-72.
Wang X, Ronghua XU, Ruling W, Aizhong L (2012). Transcriptome analysis of Sacha Inchi (*Plukenetia volubilis L.*) seeds at two developmental stages. BMC Genomics 13(716):1471-1483.

Related Journals:



www.academicjournals.org