Volume 11 Number 19, 22 May, 2017
ISSN 1996-0816

Academic Journals

ABOUT AJPP

The **African Journal of Pharmacy and Pharmacology (AJPP)** is published weekly (one volume per year) by Academic Journals.

African Journal of Pharmacy and Pharmacology (AJPP) is an open access journal that provides rapid publication (weekly) of articles in all areas of Pharmaceutical Science such as Pharmaceutical Microbiology, Pharmaceutical Raw Material Science, Formulations, Molecular modeling, Health sector Reforms, Drug Delivery, Pharmacokinetics and Pharmacodynamics, Pharmacognosy, Social and Administrative Pharmacy, Pharmaceutics and Pharmaceutical Microbiology, Herbal Medicines research, Pharmaceutical Raw Materials development/utilization, Novel drug delivery systems, Polymer/Cosmetic Science, Food/Drug Interaction, Herbal drugs evaluation, Physical Pharmaceutics, Medication management, Cosmetic Science, pharmaceuticals, pharmacology, pharmaceutical research 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 published in AJPP are peer-reviewed.

Contact Us

Editorial Office: ajpp@academicjournals.org

Help Desk: helpdesk@academicjournals.org

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

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

Editors

Himanshu Gupta
Department of Pharmacy Practice
University of Toledo
Toledo, OH
USA.

Prof. Zhe-Sheng Chen
College of Pharmacy and Health Sciences
St. John's University
New York,
USA.

Dr. Huma Ikram
Neurochemistry and Biochemical
Neuropharmacology Research Unit,
Department of Biochemistry,
University of Karachi
Karachi-75270
Pakistan

Dr. Shreesh Kumar Ojha
Molecular Cardiovascular Research Program
College of Medicine
Arizona Health Sciences Center
University of Arizona
Arizona,
USA.

Dr. Vitor Engracia Valenti
Departamento de Fonoaudiologia
Faculdade de Filosofia e Ciências,
UNESP
Brazil.

Dr. Caroline Wagner
Universidade Federal do Pampa
Avenida Pedro Anunciação
Brazil.

Dr. Ravi Shankar Shukla
Macromolecule and Vaccine Stabilization Center
Department of Pharmaceutical Chemistry
University of Kansas
USA.

Associate Editors

Dr. B. Ravishankar
SDM Centre for Ayurveda and Allied Sciences,
SDM College of Ayurveda Campus,
Karnataka
India.

Dr. Natchimuthu Karmegam
Department of Botany,
Government Arts College,
Tamil Nadu,
India.

Dr. Manal Moustafa Zaki
Department of Veterinary Hygiene and Management
Faculty of Veterinary Medicine,
Cairo University
Giza,
Egypt.

Prof. George G. Nomikos
Takeda Global Research & Development Center
USA.

Prof. Mahmoud Mohamed El-Mas
Department of Pharmacology,
Faculty of Pharmacy
University of Alexandria,
Alexandria,
Egypt.

Dr. Kiran K. Akula
Electrophysiology & Neuropharmacology Research Unit
Department of Biology & Biochemistry
University of Houston
Houston, TX
USA.

Editorial Board

Prof. Fen Jicai
School of life science, Xinjiang University,
China.

Dr. Ana Laura Nicoletti Carvalho
Av. Dr. Arnaldo, 455, São Paulo, SP.
Brazil.

Dr. Ming-hui Zhao
Professor of Medicine
Director of Renal Division, Department of Medicine
Peking University First Hospital
Beijing 100034
PR. China.

Prof. Ji Junjun
Guangdong Cardiovascular Institute, Guangdong General Hospital, Guangdong Academy of Medical Sciences,
China.

Prof. Yan Zhang
Faculty of Engineering and Applied Science,
Memorial University of Newfoundland,
Canada.

Dr. Naoufel Madani
Medical Intensive Care Unit
University hospital Ibn Sina, Univesity Mohamed V Souissi, Rabat,
Morocco.

Dr. Dong Hui
Department of Gynaecology and Obstetrics, the 1st hospital, NanFang University,
China.

Prof. Ma Hui
School of Medicine, Lanzhou University,
China.

Prof. Gu HuiJun
School of Medicine, Taizhou university,
China.

Dr. Chan Kim Wei
Research Officer
Laboratory of Molecular Biomedicine,
Institute of Bioscience, Universiti Putra,
Malaysia.

Dr. Fen Cun
Professor, Department of Pharmacology, Xinjiang University,
China.

Dr. Sirajunnisa Razack
Department of Chemical Engineering, Annamalai University,
Annamalai Nagar, Tamilnadu,
India.

Prof. Ehab S. EL Desoky
Professor of pharmacology, Faculty of Medicine
Assiut University, Assiut,
Egypt.

Dr. Yakisich, J. Sebastian
Assistant Professor, Department of Clinical Neuroscience R54
Karolinska University Hospital, Huddinge
141 86 Stockholm ,
Sweden.

Prof. Dr. Andrei N. Tchernitchin
Head, Laboratory of Experimental Endocrinology and Environmental Pathology LEEPA
University of Chile Medical School,
Chile.

Dr. Sirajunnisa Razack
Department of Chemical Engineering,
Annamalai University, Annamalai Nagar, Tamilnadu,
India.

Dr. Yasar Tatar
Marmara Unıversıty,
Turkey.

Dr Nafisa Hassan Ali
Assistant Professor, Dow institude of medical technology
Dow University of Health Sciences,Chand bbi Road, Karachi,
Pakistan.

Dr. Krishnan Namboori P. K.
Computational Chemistry Group, Computational Engineering and Networking,
Amrita Vishwa Vidyapeetham, Amritanagar, Coimbatore-641 112
India.

Prof. Osman Ghani
University of Sargodha,
Pakistan.

Dr. Liu Xiaoji
School of Medicine, Shihezi University,
China.

African Journal of Pharmacy and Pharmacology

Table of Contents: Volume 11 Number 19 22 May, 2017

ARTICLES

***In vitro* antioxidant activity of diazenyl schiff base molecules** 233
Sarangi P. K. N., Paidesetty S. K. and Mohanta G. P.

Antioxidant and anticholinesterase activities of the essential oil of *Eugenia dysenterica* DC. 241
Chistiane Mendes Feitosa, Alisson dos Reis Barbosa, Cassio Herbert Santos de Melo, Rivelilson Mendes Freitas, José Eraldo do Nascimento Fontes, Emmanoel Vilaça Costa, Khaled Nabih Zaki Rashed and Joaquim Soares da Costa Júnior

academicJournals

Vol. 11(19), pp. 233-240, 22 May, 2017
DOI: 10.5897/AJPP2017.4770
Article Number: 935D61464395
ISSN 1996-0816
Copyright © 2017
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

In vitro antioxidant activity of diazenyl schiff base molecules

Sarangi P. K. N.[1]*, Paidesetty S. K.[2] and Mohanta G. P.[3]

[1]Department of Pharmaceutical Chemistry, Sri Jayadev College of Pharmaceutical Sciences, Bhubaneswar, India.
[2]Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Bhubaneswar, India.
[3]Department of Pharmacy, Annamalai University, Annamalainagar, Tamil Nadu, India.

Received 21 March, 2017; Accepted 7 April, 2017

A series of several diazenyl schiff base molecules were designed and synthesized through azo coupling of diazotised primary amines with the novel synthesized schiff base ligand *(E)-N-((2-chloroquinolin-3-yl)methylene)-4-phenylthiazol-2-amine*. All the synthesized molecules have been characterized by different spectral techniques for their structural confirmation. The results of *in vitro* antioxidant activity of the molecules by 2,2-diphenyl-1- picryl hydrazyl (DPPH) assay method revealed that the molecules (*NZ)-N-(((4-chlorophenyl) diazenyl) (2-chloroquinolin-3-yl) methylene) -4- phenylthiazol -2-amine* (5A) and *4-(((Z)-(2-chloroquinolin-3-yl)(4-phenylthiazol-2-ylimino)methyl)diazenyl)phenol* (5E) have shown potential free radical scavenging activity.

Key words: Schiff base, diazenyl, spectral, antioxidant.

INTRODUCTION

Oxidative stress is an imbalance between the reactive oxygen species (ROS) and the detoxifying biological process. Oxygen is one of the most essential molecules for life. As a strong oxidizing agent, it facilitates most of the metabolic processes in the body and in due process it generates free radicals. But when our endogenous supply of the antioxidants are insufficient, then the level of free radicals gets increased in our body causing internal cellular damage (Dubey and Batra, 2009). Free radicals are also generated by the external sources of environmental pollutants such as toxic metals, cigarette smoke and pesticides, which damage our body (Aseervatham et al., 2013). Accumulation of the free radicals leads to degenerative diseases such as Alzheimer's disease, Parkinson's disease, atherosclerosis, cancer and other aging problems (Ali et al., 2015). Antioxidants are capable of slowing and preventing the oxidation to build a control over the free radicals generation. Though the endogenous antioxidants are helping to reduce the accumulation of the free radicals,

*Corresponding author. E-mail: pknsarangi@gmail.com. Tel: +91 9437493396.

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

most of the time they are not sufficient to give protection against the reactive oxygen species, so we need to take the antioxidant rich foods such as fruits, vegetables, yogurt and green tea every day. In case of ageing and some disease conditions the dietary supplements with the endogenous antioxidants are unable to prevent oxidative stress. There comes the need for some potent synthetic antioxidants which play a vital role to prevent the production and accumulation of the reactive oxygen species (Raghavendra et al., 2013). On the basis of literature survey, the present investigation is mainly focused on the study of free radical scavenging activity of the reported diazenyl schiff base molecules.

MATERIALS AND METHODS

This experimental work includes the use of synthetic and analytical grade of chemicals procured from Sigma Aldrich, Hi Media Laboratories Pvt. Ltd. and Merck specialties Ltd. (Mumbai, India). The thin layer chromatographic (TLC) study of the synthesized molecules was done with appropriate solvent system to monitor the progress of reaction. The spectroscopic analysis of the synthesized molecules were performed by Fourier Transform/ InfraRed (FT/IR) (JASCO FT/IR 4100 Spectrophotometer) using KBr pellets, Liquid chromatography-mass spectrometry (LC-MS) (Shimadzu-Mass spectrophotometer) and ^{1}H NMR (Bruker^{1}HNMR 400 *MHz*) using tetramethylsilane as an internal standard. The elemental analysis for C, H, N and S were carried out on Perkin Elmer model 2400 CHNS/O analyzer. The melting points were determined by open capillary method (Elico). The solvatochromic analysis of the synthesized molecules was done using different solvents by UV-Vis spectrophotometer (JASCO V-630 Spectrophotometer). The chemical structures of the synthesized molecules were made using Chem Draw ultra 10.0 software.

Scheme

Synthesis of diazenyl schiff base derivatives (5A-E)

Synthesis of schiff base ligand (3) was prepared as per the procedure suggested by Hussain et al. 2014. To a solution of aromatic primary amine (3 mmol) and water (5 ml), a few drops of of concentrated H_2SO_4 (8-9 mmol) was added on an ice bath. The drop wise addition of a cold solution of $NaNO_2$ (0.207 g, 3 mmol) was made to it by maintaining the temperature of the reaction up to 5° c. To complete the diazotization reaction, the solution was kept for 15 min with occasional stirring. The above prepared ice cold solution of schiff base (3 mmol) with ethanol and 10% of 20 ml of aqueous NaOH, individual diazotised aromatic primary amines were added. The resultant mixture was stirred well and allowed to stand in an ice bath for 1 h by maintaining the pH at 5 to 6 with occasional and controlled addition of dilute HCl. Then the final products (5A-5E) obtained were filtered, washed repeatedly with water, dried and recrystallized of with ethanol (Sahoo et al., 2015).

(*E*)-N-[(2-chloroquinolin-3-yl)methylene]-4-phenylthiazol-2-amine, (3): Pale yellow color powder; Yield 82%; R_f: 0.6; m.p.: 207-10°C; UV-Vis (λ max, ethanol): 419 nm; IR (KBr, cm^{-1}): 1612 (C=N str.), 1527 (C=C str.), 1013(C-S str.), 717 (C-Cl str.), 3157 (C-H str. of azomethine); ^{1}H NMR (DMSO-d_6, δppm, 400 MHz): δ 7.43-7.79 (m, 5H, Ar H), 9.33 (s, 1H, Quinolinyl H-4), 8.07 (d, 1H, Quinolinyl H-5), 7.59 (m, 1H, Quinolinyl H-6), 7.78 (m, 1H, Quinolinyl H-7), 8.00 (d, 1H, Quinolinyl H-8), 8.13 (s, 1H, thiazolyl H-5), 9.005 (-CH=N-); LC-MS (RT, % area): 1.685, 93.62; *m/z*: 349.13 (M+1); Analysis for $C_{19}H_{12}ClN_3S$: Calcd: C,65.23; H, 3.46; N, 12.01; S, 9.17 Found: C, 65.28; H, 3.43; N, 12.09; S, 9.16%.

(NZ)-N-(((4-chlorophenyl) diazenyl) (2-chloroquinolin-3-yl) methylene) -4- phenylthiazol -2-amine (5A): Coffee red color powder; Yield 93%; R_f: 0.9; m.p.: 126-30°C; UV-Vis (λ max, Ethanol): 425 nm; IR (KBr, cm^{-1}): 1028 (C-S), 1617 (C=N str.), 1441 (-N=N-), 753 (C-Cl), 826 (1,4 disubstitution); 1H NMR (DMSO-d6, δppm, 400 MHz): 9.12 (s, 1H, Quinolinyl H-4), 8.03 (d, 1H, Quinolinyl H-5), 7.61 (m, 1H, Quinolinyl H-6), 7.78 (m, 1H, Quinolinyl H-7), 7.99 (d, 1H, Quinolinyl H-8), 7.52-7.76 (m, 5H, Ar H), 7.26-7.50 (m, 4H, diazenylAr H), 8.26 (s, 1H, thiazolyl H-5); LC-MS (RT, % area); 2.801, 91.72; m/z: 488.41 (M); Analysis for $C_{25}H_{15}Cl_2N_5S$: Calcd: C, 61.48; H, 3.10; N, 14.34; S, 6.57 Found: C, 61.23; H, 3.03; N, 14.39; S, 6.69%.

(NZ)-N-((2-chloroquinolin-3-yl)((4-nitrophenyl)diazenyl)methylene)-4-phenylthiazol-2-amine (5B): Yellowish brown color powder; Yield 97%; R_f: 0.6; m.p.: 116-19°C; UV-Vis (λ max, DMSO): 427 nm; IR (KBr, cm^{-1}): 998 (C-S), 1528, 1394 (NO_2 str.), 1444 (-N=N-), 753 (C-Cl), 840 (1,4 disubstitution); ^{1}H NMR (DMSO-d6, δppm, 400 MHz): 9.15 (s, 1H, Quinolinyl H-4), 8.03 (d, 1H, Quinolinyl H-5), 7.63 (m, 1H, Quinolinyl H-6), 7.79 (m, 1H, Quinolinyl H-7), 7.93 (d, 1H, Quinolinyl H-8), 7.55- 7.73 (m, 5H, Ar H), 7.29-8.15 (m, 4H, diazenylAr H), 8.19 (s, 1H, thiazolyl H-5); LC-MS (RT, % area); 1.821, 87.72; m/z: 498.71 (M); Analysis for $C_{25}H_{15}ClN_6O_2S$: Calcd: C, 60.18; H, 3.03; N, 16.84; S, 6.43 Found: C, 60.11; H, 3.11; N, 16.87; S, 6.39%.

(NZ)-N-((2-chloroquinolin-3-yl)((4-methoxyphenyl)diazenyl)methylene)-4-phenylthiazol-2-amine (5C): Brown color powder; Yield 88%; R_f: 0.6; m.p.: 123-27°C; UV-Vis (λ max, DMSO): 579 nm; IR (KBr, cm^{-1}): 1617 (C-N str.), 2922 (-CH2- str.), 1490 (-N=N-), 1028 (C-O-CH3), 751 (C-Cl); ^{1}H NMR (DMSO-d6, δppm, 400 MHz): 9.42 (s, 1H, Quinolinyl H-4), 7.99 (d, 1H, Quinolinyl H-5), 7.60 (m, 1H, Quinolinyl H-6), 7.78 (m, 1H, Quinolinyl H-7), 7.97 (d, 1H, Quinolinyl H-8), 7.41- 7.80 (m, 5H, Ar H), 6.90-7.15 (m, 4H, diazenylAr H), 8.22 (s, 1H, thiazolyl H-5), 3.70 (s, 3H, OCH3); LC-MS (RT, % area); 1.726, 87.72; m/z: 483.7 (M); Analysis for $C_{26}H_{18}ClN_5OS$: Calcd: C, 64.52; H, 3.75; N, 14.47; S, 6.63 Found: C, 64. 47; H, 3.83; N, 14.28; S, 6.49%.

(NZ)-N-(((4-bromo-3-methylphenyl)diazenyl) (2-chloroquinolin-3-yl) methylene)-4-phenylthiazol-2-amine (5D): Reddish brown color powder; Yield 91%; R_f: 0.7; m.p.: 115-17°C; UV-Vis (λ max, DMSO): 376 nm; IR (KBr, cm^{-1}): 1611 (C=C str./ C-N str.), 1480 (-N=N-), 862 (Tri substitution), 755 (C-Br); ^{1}H NMR (DMSO-d6, δppm, 400 MHz): 9.32 (s, 1H, Quinolinyl H-4), 8.09 (d, 1H, Quinolinyl H-5), 7.65 (m, 1H, Quinolinyl H-6), 7.73 (m, 1H, Quinolinyl H-7), 7.93 (d, 1H, Quinolinyl H-8), 7.49- 7.79 (m, 5H, Ar H), 7.03-7.48 (m, 3H, diazenylAr H), 8.14 (s, 1H, thiazolyl H-5), 2.45 (s, 3H, CH3); LC-MS (RT, % area); 1.926, 93.72; m/z: 546.17 (M); Analysis for $C_{26}H_{17}BrClN_5S$ Calcd: C, 57.10; H, 3.13; N, 12.81; S, 5.86 Found: C, 56.93; H, 3.21; N, 12.87; S, 5.74%.

4-(((Z)-(2-chloroquinolin-3-yl)(4-phenylthiazol-2-ylimino)methyl)diazenyl)phenol (5E): Yellowish brown color powder; Yield 81%; R_f: 0.8; m.p.: 168-70°C; UV-Vis (λ max, DMSO): 350 nm; IR (KBr, cm^{-1}): 3181 (OH str.) 1519 (C=C str str.), 1435 (-N=N-), 1273 (C-O str.), 756 (C-Cl); ^{1}H NMR (DMSO-d6, δppm, 400 MHz): 9.53 (s, 1H, OH), 9.28 (s, 1H, Quinolinyl H-4), 8.01 (d, 1H, Quinolinyl H-5), 7.58 (m, 1H, Quinolinyl H-6), 7.80 (m, 1H, Quinolinyl H-7), 7.99 (d, 1H, Quinolinyl H-8), 7.27- 7.75 (m, 5H, Ar H), 7.13-7.22 (m, 3H, diazenylAr H), 8.25 (s, 1H, thiazolyl H-5); LC-MS (RT, % area); 3.056, 93.72; m/z: 468.5 (M); Analysis for $C_{25}H_{16}ClN_5OS$: Calcd: C, 63.89; H, 3.43; N, 14.90; S, 6.82 Found: C, 63.91; H, 3.21; N, 14.83; S, 6.73%.

***In vitro* antioxidant activity of diazenyl schiff base molecules by DPPH assay method**

The free radical scavenging activity of the selected newly synthesized molecules were measured by 2,2-diphenyl-1- picryl hydrazyl (DPPH) assay method (Sahoo and Kumar, 2015). The different concentrations of the synthesized molecules were prepared with methanol. The final volume of each test sample was adjusted up to 3 mL with methanol. To each of the test sample, 1 mL of freshly prepared 0.1mM DPPH in methanol was added. The test samples were vigorously shaken and kept in dark for 30 min. One milliliter of 0.1 mM of methanolic solution of DPPH was considered as control and 3 mL methanol was taken for blank. The antioxidant activity of synthesized molecules was compared with standard ascorbic acid. The optical density was measured at 517 nm and the inhibition concentration was calculated:

$$\% \text{ of inhibition} = \frac{A_{cont} - A_{test}}{A_{cont}} \times 100$$

Where A_{cont} = absorbance of control and A_{test} = absorbance of the test sample. All the experiments were carried out in triplicate and the values were expressed as mean ± SD.

Statistical analysis

The observed data on mean percent of inhibition for antioxidant activity of different synthesized molecules were subjected to one way- analysis of variance (ANOVA) for comparison of group means of different molecules. In the study of antioxidant activities of two of the molecules (**5A** and **5E**) of the scheme is given Figures 5 and 6 were compared with the standard ascorbic acid and among them through Post Hoc Bonferroni test. The test of significance, cut off value of p was taken as < 0.05. The statistical analysis was done using SPSS 16.0 software.

RESULTS AND DISCUSSION

The mixture of two reactants (**1)** and (**2)** in the presence of glacial acetic acid in ethanol gave schiff base (E)-N-((2-chloroquinolin-3-yl) methylene)-4- phenyl thiazol-2-amine (**3**) by nucleophilic addition reaction. The electron rich azomethine group of the schiff base ligand was undergone azo coupling reaction with a series of five diazotized primary aromatic amines (**4A-4E**) which act as electrophiles and gave some new diazenyl schiff based derivatives (**5A-5E**). The synthetic scheme is presented in Figure 1. The structures of prepared intermediates and final molecules have been confirmed by FT/IR, ^{1}HNMR, UV, LC-MS and elemental analysis. The short medium absorption band in all the compounds (**5A**-**5E**) appeared at the range of 1490 to 1435 cm^{-1} assigned to –N=N- group. The FT/IR spectral image of the compound **5E** is given in Figure 2.

The ^{1}H NMR analysis of the synthesized compounds showed the Quinolinyl H-4 singlet at a range of δ 8.83 to 9.42 ppm, thiazolyl H-5 singlet at a range δ 8.13 to 26 ppm and attached diazenyl aromatic protons at a range of δ 6.90 to 8.15 ppm. The ^{1}H NMR spectra of the compound **5E** is illustrated in Figure 3.

The predicted molecular weight of the synthesized compounds was confirmed by LC-MS. The compound (NZ)-N-((2-chloroquinolin-3-yl) ((4-methoxyphenyl)diazenyl)methylene)-4-phenylthiazol-2-amine (**5c)** having molecular ion peak 483.7 (M) showed in Figure 4 strongly reveals the predicted molecular formula $C_{26}H_{18}ClN_5OS$.

In vitro antioxidant activity of diazenyl schiff base molecules

The *in vitro* antioxidant activity of the diazenyl schiff base molecules **5A-5E** is presented in Tables 1 and 2.

The graphical presentation of free radical scavenging activity of diazenyl schiff base molecules (**5A-5E**) is given in Figures 5 and 6.

At each concentration of 5 to 600 µg/ml, there were significant difference among the molecules and the standard ascorbic acid at p<0.05. However with the increase of potency, all the molecules with the standard ascorbic acid registered increase in the mean percentage of inhibition.

At 600 µ/ml the molecule **5E** exhibited the mean percentage of inhibition of 87.88±0.87, which was very close to the performance of the standard ascorbic acid (85.83±0.83) at 50 µg/ml. Similarly **5A:** At 600 µg/ml have the mean percentage of inhibition 82.1 ± 0.87 which was also close to the performance of ascorbic acid (85.83 ± 0.83). Therefore the mean percentage of inhibition of **5A** and **5E** at 600 µg/ml and ascorbic acid (AA) at 50 µg/ml was compared through Post Hoc Bonferroni test. The result of antioxidant evaluation is given in Tables 1 and 2.

The mean percentage of inhibition of **5A** at 600 µg/ml was significantly lower than **5E** (p=0.001) and also lower than ascorbic acid at 50 µg/ml (p=0.005). The mean percentage of inhibition of **5E** at 600 µg/ml and that of ascorbic acid (**AA**) at 50 µg/ml were not significantly different (p=0.547). This implied the molecule **5E** at 600 µg/ml is giving comparable performance to ascorbic acid (**AA**) at 50 µg/ml. Literature survey revealed that phenolic molecules or nitrogen bearing heterocyclic rings have good free radical scavenging activity (Shridhar et al., 2016; Chinnagiri et al., 2013). The molecules (**5A** and **5E**) showed potential antioxidant activity and possess nitogen bearing heterocyclic ring. At the same time the molecule **5E** also possess the phenolic–OH group which may be responsible for exhibiting better antioxidant activity.

Conclusion

This part of research work comprises five diazenyl schiff base molecules (**5A**-**5E**) derived from the molecule (3) as explained in the scheme. The antioxidant activity of the synthesized molecules is investigated by DPPH method. The molecules (*NZ)-N-(((4-chlorophenyl) diazenyl) (2-*

Figure 1. Synthetic scheme of diazenyl schiff base molecules. 5A (R_1=H, R_2=Cl), 5B (R_1=H, R_2=NO_2), 5C (R_1=H, R_2=OCH_3), 5D (R_1=Br, R_2=CH_3), 5E (R_1= H, R_2=OH) Reaction: - i. Ethanol/ Glacial acetic acid reflux 2h, ii. $NaNO_2/H_2SO_4$ (0-5˚C), iii. 10% NaOH coupling reaction.

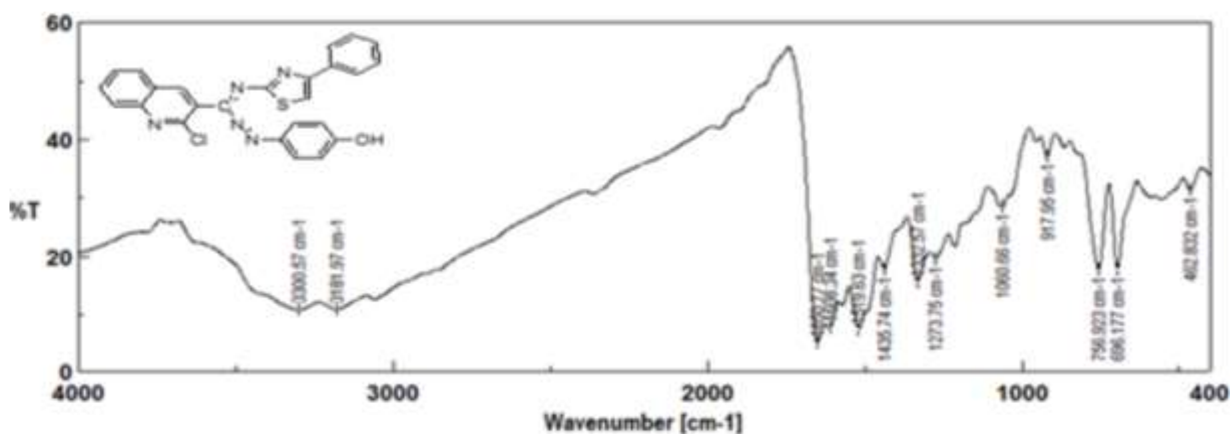

Figure 2. FT/IR spectra of (((Z)-(2-chloroquinolin-3-yl)(4-phenylthiazol-2-ylimino)methyl)diazenyl)phenol (**5E**).

Figure 3. ^{1}H NMR of (((Z)-(2-chloroquinolin-3-yl)(4-phenylthiazol-2-ylimino)methyl)diazenyl) phenol (**5E**).

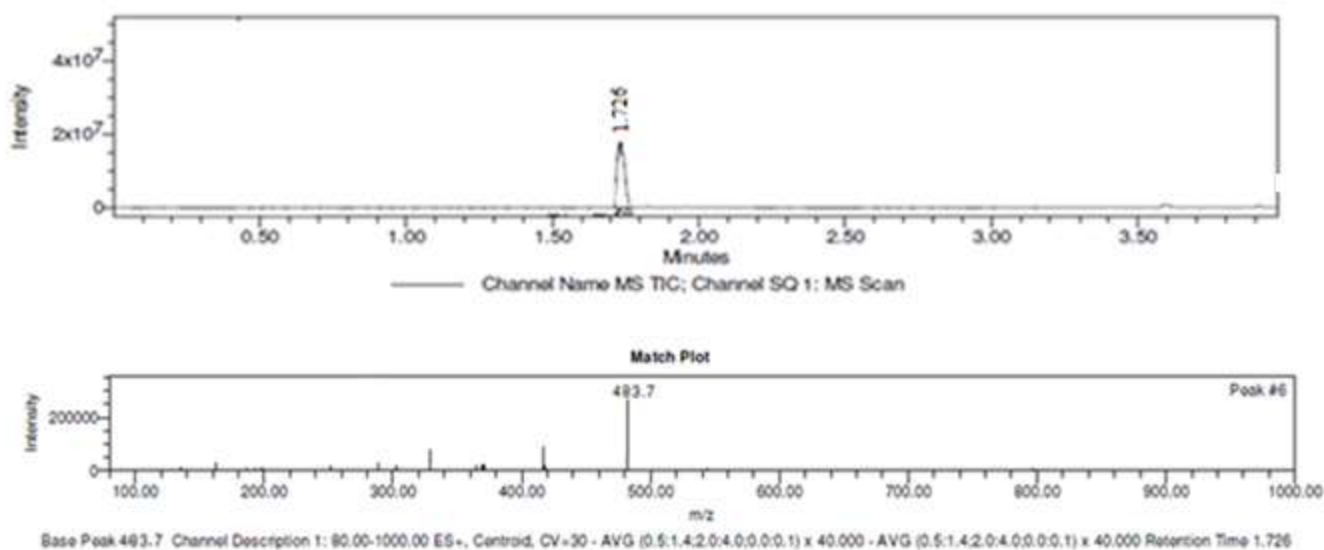

Figure 4. LC-MS of -N-((2-chloroquinolin-3-yl)((4-methoxyphenyl)diazenyl) methylene)-4-phenylthiazol-2-amine **(5C)**

Table 1. *In vitro* antioxidant activity of diazenyl schiff base molecules.

Molecules (n=3)	Concentration (µg/ml)						
	5	10	50	100	200	400	600
	% Inhibition						
5A	41.56±0.87	44.97±0.87	49.25±0.87	54.88±0.87	62.37±0.87	71.89±0.87	82.1±0.87
5B	29.07±0.87	31.9±0.87	35.67±0.87	39.81±0.87	45.38±0.87	56.72±0.87	64.52±0.87
5C	28.51±0.87	29.45±0.87	33.41±0.87	39.16±0.87	46.48±0.87	56.86±0.87	65.78±0.87
5D	27.68±0.87	28.28±0.87	32.01±0.87	38.25±0.87	44.76±0.87	53.35±0.87	61.81±0.87
5E	49.33±0.87	51.26±0.87	56.2±0.87	60.78±0.87	67.41±0.87	77.79±0.87	86.88±0.87
Ascorbic acid	73.37±0.66	79.62±0.7	85.83±0.83	95.96±0.61	99.18±0.27	99.4±0.3	99.48±0.39
ANOVA 'p' value	0.000	0.000	0.000	0.000	0.000	0.000	0.000

$p < 0.05$ indicates significant different among group means at different level of concentration. Data are expressed mean ± SD.

Table 2. *In vitro* antioxidant activity of diazenyl schiff base molecules.

(I) Molecules	(J) Molecules	Mean Difference (I-J) ± SE
5A at 600	5E at 600	-4.78±0.7**
	Ascorbic acid at 50	-3.73±0.7**
5E at 600	Ascorbic acid at 50	1.05±0.7

* p<0.05, ** p<0.01

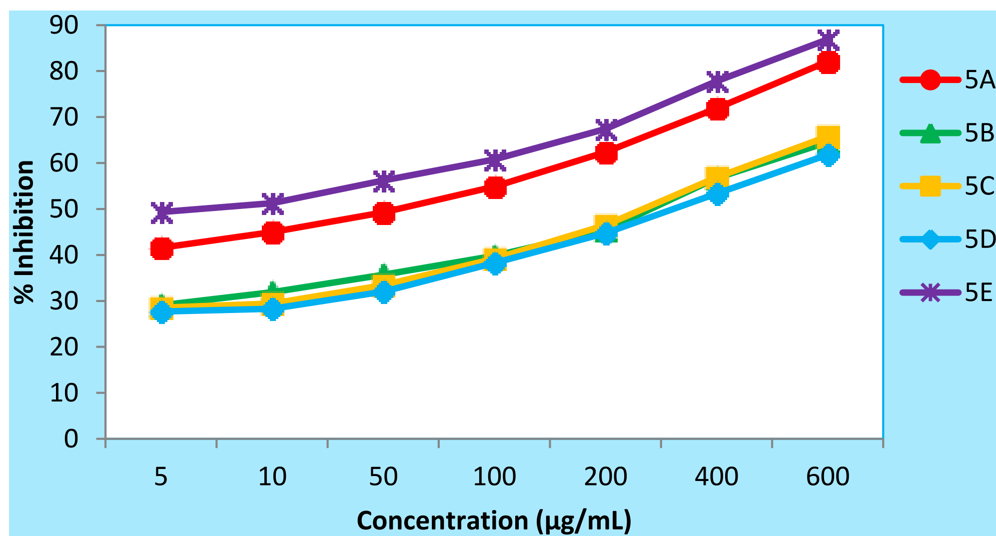

Figure 5. Trend of mean percentage of inhibition of antioxidant activities of diazenyl schiff base molecules (**5A-5E**) in different concentrations. Data are expressed in mean ± 95% CI.

chloroquinolin-3-yl) methylene) -4- phenylthiazol -2-amine (**5A**) and *4-(((Z)-(2-chloroquinolin-3-yl)(4-phenylthiazol-2-ylimino)methyl)diazenyl)phenol* (**5E**) showed potential antioxidant activity which justified that the existence of phenolic -OH group and heterocyclic nitrogen bearing molecules can promote free radical scavenging activity for which these novel molecules may have gone for further investigations to establish them as potent molecules for the treatment of oxidative stress related diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

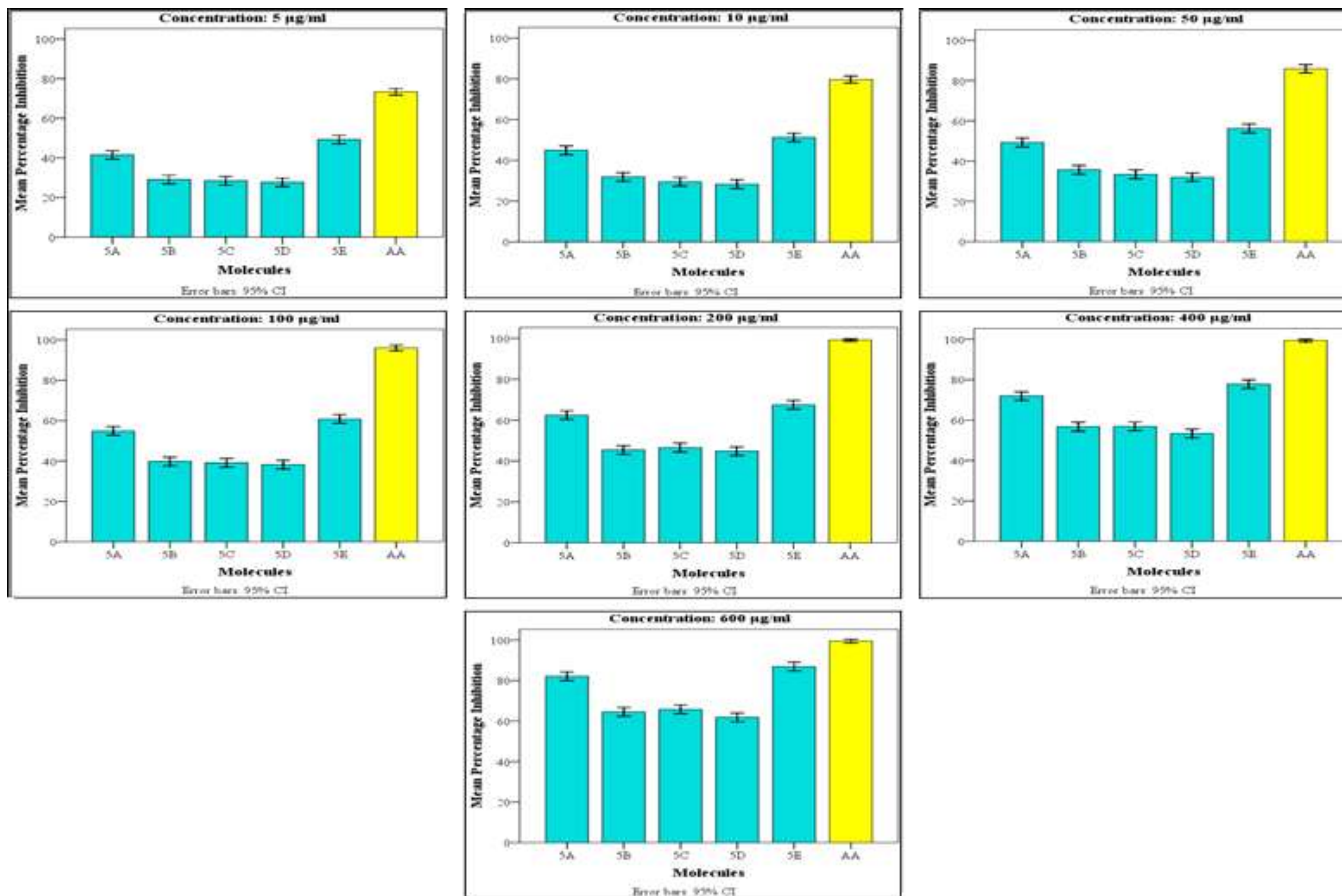

Figure 6. Antioxidant activities of the diazenyl schiff based molecules **5A-5E** and standard Ascorbic acid (**AA**) by DPPH model. Data are expressed as mean percentage of inhibition ± 95% CI at different concentration

REFERENCES

Ali K, Khalid Daoudy K, Marmouzi I, Bourhim M, Faouzi MA, Cherrah Y, Chokairi O, Meddah B (2015). *In vitro* antioxidant and anti-inflammatory activity of extracts of pearl millet (Pennisetum glaucum L.). J. Chem. Pharm. Res. 7(6):1-6.

Aseervatham GS, Sivasudha T, Jeyadevi R, Arul Ananth D (2013). Environmental factors and unhealthy lifestyle influence oxidative stress in humans an overview. Environ. Sci. Pollut. Res. Int. 20(7):4356-4369.

Chinnagiri TKK, Keshavayya J, Rajesh NT, Peethambar KS, Shoukat Ali AR (2013). Synthesis, characterization, and biological activity of 5-Phenyl-1, 3, 4-thiadiazole-2-amine incorporated azo dye derivatives. Org. Chem. Int. 2013:1-7.

Dubey SK, Batra A (2009). Antioxidant activities of *Thujaoccidentalis linn*. Asian J. Pharm. Clin. Res. 2(1):73-76.

Hussain Z, Yousif E, Ahmed A, Altaie A (2014). Synthesis and characterization of schiff's bases sulphamethoxazole. Org. Med. Chem. Lett. 4(1):1-4.

Raghavendra M, Reddy AM, Yadav PR, Raju AS, Siva Kumar L (2013). Comparative studies on the *in vitro* antioxidant properties of methanolic leafy extracts from six edible leafy vegetables of India. Asian J. Pharm. Clin. Res. 6(3):96-99.

Sahoo J, Mekap SK, Paidesetty SK (2015). Synthesis, spectral characterization of some new 3-heteroaryl azo-4-hydroxycoumarin derivatives and their antimicrobial evaluation. J. Taibah Univ. Sci. 9:187-195.

Sahoo J, Paidesetty SK (2015). Antimicrobial, analgesic, antioxidant and *in silico* study of synthesized salicylic acid congeners and their structural interpretation. Egypt. J. Basic Appl. Sci. 2:268-280.

Shridhar AH, Keshavayya J, Peethambar SK, Joy Hoskeri H (2016). Synthesis and biological activities of Bis alkyl 1, 3, 4-oxadiazole incorporated azo dye derivatives. Arab J. Chem. 9:S1643-S1648.

academicJournals

Vol. 11(19), pp. 241-249, 22 May, 2017
DOI: 10.5897/AJPP2015.4438
Article Number: AF8B2F164397
ISSN 1996-0816
Copyright © 2017
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Antioxidant and anticholinesterase activities of the essential oil of *Eugenia dysenterica* DC.

Chistiane Mendes Feitosa[1]*, Alisson dos Reis Barbosa[1], Cassio Herbert Santos de Melo, Rivelilson Mendes Freitas[1], José Eraldo do Nascimento Fontes[2,3], Emmanoel Vilaça Costa[4,5], Khaled Nabih Zaki Rashed[6] and Joaquim Soares da Costa Júnior[7]

[1]Department of Chemistry, Federal University of Piaui, 'Campus Universitário Ministro Petrônio Portela', Bloco 06-Bairro Ininga,64049-550, Teresina, PI, Brazil.
[2]Chemistry Institute, University of Campinas (UNICAMP), CP 6154, 13083-970, Campinas, SP, Brazil.
[3]Department of Chemistry, Federal University of Sergipe, Av. Marechal Rondon, S/N, Jardim Rosa Elze, 49100-000, São Cristóvão, SE, Brazil.
[4]Department of Chemistry, Federal University of Amazonas, Bloco 08, Setor Norte do Campus Universitário Sen. Arthur Virgílio Filho, Av. Rodrigo Otávio, 6.200, Coroado, 69077-000 Manaus, AM, Brazil.
[5]Department of Chemistry, Sergipe Federal University, Av. Vereador Olímpio Grande, S/N, Centro, 49500-000, Itabaiana, SE, Brazil.
[6]National Research Centre, Pharmacognosy Department, Dokki, Giza, Egypt.
[7]Department of Teacher Education, Arts and Sciences Federal Institute of Piauí, Praça da Liberdade n° 1597, Centro Teresina Piauí Brazil.

Received 28 August, 2015; Accepted 5 November, 2015

This paper describes the anticholinesterase and antioxidant activities of *Eugenia dysenterica* DC. (O. Berg. (Myrtaceae) essential oils from leaves (EOED). EOED were obtained by hydrodistillation using a Clevenger-type apparatus and the products were analyzed by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detector (GC-FID). The main constituents of EOED were caryophyllene oxide (66.3%), isoledene (3.9%), 1,3,8-*p*-menthatriene (3.5%), mustakone (3.46%), β-phellandrene (1.7%), and selin-11-en-4-α-ol (1.7%). The antioxidant assay was performed based on the formation of thiobarbituric acid reactive substances (TBARS), hydroxyl radical, and nitric oxide production. By performing the Ellman assay, it was observed that EOED was able to inhibit the enzyme acetylcholinesterase (AChE) with an IC_{50} = 0.92 μg.ml^{-1} promising better value when compared with the drug rivastigmine (IC_{50} = 1.87 μg.ml^{-1}), used in the treatment of Alzheimer's disease. The caryophyllene oxide (the main compound) was tested after purification on the AChE with an IC_{50} = 0.31 μg.ml^{-1}. Caryophyllene oxide (the majority compound) was tested on the AChE and presented the IC_{50} = 0.31 μg.ml^{-1}. At concentrations of 0.9, 1.8, 3.6, 5.4, and 7.2 μg.ml^{-1}, it was found out that EOED prevented lipid peroxidation inhibiting amount of TBARS formed in a similar manner to ascorbic acid. In addition, there was a reduction in the production of hydroxyl radical as well as the production of nitric oxide. To the best of our knowledge, this is the first report on compounds from this species that have activity for potentially preventing neurodegenerative disorders.

Key words: *Eugenia dysenterica*, essential oil, antioxidant, anticholinesterase.

INTRODUCTION

A variety of essential oil of plants has shown acetylcholineterase (AChE) inhibitory activity and may be relevant to the treatment of neurodegenerative disorders, such as Alzheimer's disease (AD). The essential oils of *Cistus* species have functional properties in prevention of neurodegenerative disorders (Loizzo et al., 2013). *Centella asiatica* essential oil and various other essential oils from plant species, for example *Cistus salvifolius* and *Ocimum canum* have shown pharmacological activities relevant to the treatment of cognitive disorders, indicating potential for therapeutic use in disorders, such as AD (Houghton and Howes, 2003). Though recent intensive efforts have been made to understand the mechanism of neurodegeneration involved in AD and to discover new drugs combating the symptoms; at present, there is a deficit in the number of efficient and safe therapeutic agents to treat the disease. No new drugs have been approved by the US Food and Drug Administration (FDA) since 2003, likely because the abnormal brain deposits of Aβ and τ-proteins still cannot be considered causes or by-products of the disease (Buckholtz, 2011). Since the approval of galantamine for the treatment of AD patients, the search for new anticholinesterase alkaloids has escalated, leading to promising candidates, such as huperzine A (Konrath et al., 2013).

Many monoterpenes and sesquiterpenes for example 1,8-cineole, α-pinene, and linalool have been cited in promising research due to their potent anticholinesterase activity (Kiendrebeogo et al., 2013). However, few reports exist that deal with the inhibition of AChE by plant essential oils (Chaiyana and Okonogi, 2012).

Acetylcholinesterase inhibitors (IAChE) have therapeutic applications in AD and in addition, the central cholinergic system is considered one of the more important neurotransmitter systems involved in the regulation of cognitive functions. Cholinergic neuronal loss in the hippocampal area is the major feature of AD and enhancement of central cholinergic activity by using IAChE is presently the mainstay of pharmacotherapy of senile dementia of Alzheimer type (Enz et al., 1993; Siddiqui and Levey, 1999).

Pharmacological activities from plants and their constituents may be relevant for the treatment of cognitive disorders, including enhancement of cholinergic function in the central nervous system, anti-inflammatory, and antioxidant activities (Houghton and Howes, 2003). A variety of plants has been reported to show AChE inhibitory activity and so may be relevant to the treatment of neurodegenerative disorders, such as AD (Mukherjee et al., 2007). Research and interest in essential oils is on the increase. Recently, our research group published studies with essential oils with antioxidant and antinociceptive effects from *Citrus limon* Osbeck as studied on mice (Campelo et al., 2012).

Eugenia dysenterica (DC).O. Berg and several species of *Eugenia* are used in folk medicine with anti-inflammatory, anti-diarrheic, diuretic, and other properties. In Brazil, this species is popularly known as 'cagaiteira', with opposite leaves, simple, ovate or elliptical limbo; has edible white flowers (Rizzini, 1970), holders of laxative properties. Its fruits are consumed raw or in the form of juices, or processed to ice cream and liqueurs and when fermented can produce alcohol and vinegar. Sensory evaluation of fruit wine from cagaita showed over 70% acceptability for colour, flavor, and taste for all cagaita beverages (Oliveira et al., 2011; Oga and Fonseca, 1994). Tea from the leaves is used to combat diarrhea and the bark is used as anti-inflammatory agent. Daily consumption of 'cagaita' (100 g) contributed significantly to the supply of daily requirements of vitamin C (on average 71.0%), vitamin A (on average 7.5%), and folates (on average 7.9%). The 'cagaiteira' has a high pulp yield, reduced total energy value and is considered a source of vitamin C, which is an important role in human health (Cardoso et al., 2011).

This paper describes the anticholinesterase and antioxidant activities of *E. dysenterica* DC. (cagaiteira) essential oil from leaves (EOED). This is the first report on the activities of EOED.

MATERIALS AND METHODS

Plant

E. dysenterica fresh leaves were collected in october 2013 in Uruçuí, Piauí State, Brazil, coordinates [07°14′02″S and 44°33′14″W]. Plant identification was confirmed by Dr. Roseli Farias Melo de Barros, Department of Biology, Piauí Federal University (UFPI), Brazil and a voucher specimen (number 28824) have been deposited at the Graziela Barroso herbarium of the UFPI.

Pure compound, solvents and enzymes

AChE enzyme, (−)-Caryophyllene oxide (95% of purity), ascorbic acid, and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich.

Hydrodistilation of the essential oils

The EOED (3 samples of 300 g each were used) were extracted

*Corresponding author. E-mail: chistiane@ufpi.edu.br.

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

using hydrodistillation for 3 h with a Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulphate and the percentage content was calculated on the basis of the dry weight of the plant material. The essential oils were stored in a freezer (-20°C) until analyzed.

Gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) analysis of the essential oils

GC analyses were carried out using a Shimadzu GC-17A fitted with a FID and an electronic integrator. Separation of the compounds was achieved employing a ZB-5MS fused capillary column (30 m × 0.25 mm × 0.25 µm film thickness) coated with 5% phenyl-arylene and 95% methylpolysiloxane. Helium was the carrier gas at 1.0 $ml.min^{-1}$ flow rate. The column temperature program was: 40°C/3 min, followed by a rate of $4°C.min^{-1}$ to 240°C, then a rate of $10°C.min^{-1}$ to 300°C and then 300°C/3 min. The injector and detector temperatures were 250 and 280°C, respectively. Samples (0.5 µl in CH_2Cl_2) were injected with a 1:50 split ratio. Retention indices were generated with a standard solution of *n*-alkanes (C_8-C_{20}). Peak areas and retention times were measured by an electronic integrator. The relative amounts of individual compounds were computed from GC peak areas without FID response factor correction.

GC/MS analyses were performed on a Shimadzu QP5050A GC/MS system equipped with an AOC-20i auto-injector. A J&W Scientific DB-5MS (coated with 5% phenyl and 95% dimethylpolysiloxane) fused capillary column (30 m × 0.25 mm × 0.25 µm film thickness) was used as the stationary phase. MS were taken at 70 eV with scan interval of 0.5 s and fragments from 40 to 500 Da. The other conditions were similar to the GC analysis.

Identification of constituents

The essential oil components were identified by comparison of (i) their retention times (t_R) with those of the same standard compounds (caryophyllene oxide) analyzed under identical conditions, (ii) their retention indices (RIs), determined on a DB-5MS column relative to the t_R of a series of *n*-alkanes (C_8-C_{20}), according to Van Den Dool and Kratz (1963) with those published in the literature (Van Den Dool andKratz, 1963) and their mass spectra with those listed in the NIST (05, 05s, 21 and 107) and Wiley 8 mass spectral libraries and those published in the literature (Adams, 2007).

AChE inhibition assay

The inhibitory effect of EOED on AChE activity is evaluated by an adaptation of the spectrophotometric method of Ellman (Ellman et al., 1961).

The EOED and caryophyllene oxide were dissolved in methanol to prepare solutions of 10 $mg.ml^{-1}$. Then, 1.5 µl of the methanol EOED was spotted onto silica gel thin layer chromatography (TLC) plated and developed with chloroform: methanol 9:1 after which the enzyme inhibitory activity was detected using Ellman's method "*in situ*" on the plate (Ahmad et al., 2015; Ellman et al., 1961; Rhee et al., 2001). The developed plates were sprayed with 1 mM 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) and 1 mM ATCI in buffer A. The plate was dried for 3 to 5 min and then an enzyme solution of AChE from an electric eel (type VI-s lyophilized, 261 $U.mg^{-1}$ solid, and 386 $U.mg^{-1}$ protein) dissolved in buffer A (500 $U.ml^{-1}$ stock solution) was diluted with buffer A to obtain 5 $U.ml^{-1}$ enzyme and was then sprayed on the plate. A yellow background with white spot for inhibiting EOED was visible after about 5 min. The observation must be recorded within 15 min, because they fade after 20 to 30 min. To observe whether the positive results of the extract in TLC or the microplate assay are due to enzyme inhibition or to the inhibition of the chemical reaction between DTNB and thiocholine (the product of the enzyme reaction), 5 $units.ml^{-1}$ of AChE was premixed with 1 mM acetylthiocholine (ATCI) in buffer A and incubated for 15 min at 37°C. The enzyme-substrate mixture was used as thiocholine spray. The extract was spotted on the silica gel TLC plate developed as described earlier and sprayed with 1 mM solution DTNB followed by the thiocholine spray. White spot on a yellow background was observed for false positive extract.

The inhibitory effect quantitative of EOED on AChE activity was evaluated using and adaptation of the spectrophotometric method of Ellman modified by Rhee (Ellman et al., 1961; Rhee et al., 2001). Six different concentrations were prepared in triplicate, starting from the EOED and caryophyllene oxide (0.9, 1.8, 2.7, 3.6, 5.4, and 7.2 $µg.ml^{-1}$). The reaction is monitored for 5 min at 412 nm inspectrophotometer. Test tube placed in 100 µl of the sample (concentration 0.1% solution in 50 mM Tris-HCl, pH 8, and methanol 10%) was mixed with 100 µl of AChE 0.22 $U.ml^{-1}$ (22U of enzyme diluted in 100 ml of 50 mM Tris-HCl, pH 8, and 0.1% bovine serum albumin (BSA)) and 200 µl of buffer (50 mM Tris-HCl, pH 8, and BSA 0.1%). The mixture was incubating for 5 min at 30°C. Subsequently, was added 500 µl of DTNB acid (concentration of the 3 mM in Tris-HCl, pH 8, 0.1 M NaCl, and 0.02 M $MgCl_2$) and 100 µl of ATCI (4mM in water).

The quantitative inhibitory effect ofv EOED on AChE activity was evaluated using an adaptation of the spectrophotometric method of Ellman modified by Rhee (Ellman et al., 1961; Rhee et al., 2001). Six different concentrations were prepared in triplicate, starting with the EOED and caryophyllene oxide (0.9, 1.8, 2.7, 3.6, 5.4, and 7.2 $µg.ml^{-1}$). The reaction was monitored at 412 nm for 5 min inspectrophotometer. 100 µl of the sample (concentration of 0.1% solution in 50 mM Tris-HCl, pH 8, and methanol 10%) was mixed with 100 µl of AChE 0.22 $U.ml^{-1}$ (22U of enzyme diluted in 100 ml of 50 mM Tris-HCl, pH 8, and 0.1% BSA) and 200 µl of buffer (50 mM Tris-HCl, pH 8, and BSA 0.1%). The mixture is incubated for 5 min at 30°C. Subsequently, was add 500 µl of DTNB (concentration of the 3 mM in Tris-HCl, pH 8, 0.1 M NaCl, and 0.02 M $MgCl_2$) and 100 µl of ATCI (4 mM in water). A blank is prepared by substituting AChE with 100 µl of buffer (50 mM Tris-HCl buffer pH 8, and 0.1% BSA). The reaction is monitored for 5 min at 412 nm and the initial velocity (V_0) recorded. Anticholinesterase activity (%) was calculated using Equation 1 (Kiendrebeogo et al., 2011): Sample V_0 and blank V_0 represents the initial velocities of samples and blank. Inhibition concentration of 50% (IC_{50}) values are obtained using Log-Probit. Rivastigmine (commercial IAChE) is used as positive control at the same concentration of the essential oil.

$$I\ (\%) = (1 - V_0\ Sample/V_0\ Blank) \times 100 \quad (1)$$

Equation 1- Anticholinesteraseactivity(%)

Evaluation of *in vitro* potential against production of thiobarbituric acid reactive substances (TBARS) in essential oil from *E. dysenterica*

Determination of TBARS was performed to quantify the lipid peroxidation level (Esterbauer and Cheeseman, 1990). This method was used to determine the EOED, using homogenized egg yolk as a lipid rich substrate (Guimarães et al., 2010). Briefly, egg yolk was homogenized (1% w/v) in 20 mM phosphate buffer (pH

Table 1. Essential oil composition from the leaves of *E. dysenterica.*

Compound	RI[a]	RI[b]	Peak (%)
β-Phellandrene	1030	1025	1.73±0.10
1,3,8-*p*-Menthatriene	1112	1108	3.51±0.11
Isoledene	1374	1374	3.91±0.10
caryophyllene oxide	1583	1582	66.37±2.10
Selin-11-en-4-α-ol	1659	1658	1.69±0.07
Mustakone	1677	1676	3.46±0.04
Monoterpene identified			5.24
Sesquiterpenes identified			75.43
Total identified			80.67

Data are expressed as mean ± SD of three analyses. RI[a] (calc.), retention indices on DB-5MS column calculated according to Van den dool and Kratz (1963). RI[b] retention indices according to Adams (2007).

7.4). A volume of 1 ml of this homogenate was homogenized with 0.1 ml of EOED, at concentrations of 0.9, 1.8, 3.6, 5.4, and 7.2 $\mu g.ml^{-1}$ of EOED. Lipidperoxidation was induced by adding 0.1 ml of 2,2-azobis-2-midinopropane (AAPH, 0.12 $mol.L^{-1}$). Control was carried out only with the solution (0.05% Tween 80 dissolved in 0.9% saline solution) used to emulsify the substance that was evaluated. Reactions were performed for 30 min at 37°C. After cooling, samples (0.5 ml) were centrifuged with 0.5 ml of trichloroacetic acid (15%) at 1200 g for 10 min. An aliquot of 0.5 ml of the supernatant was mixed with 0.5 ml of thiobarbituric acid (0.67%) and heated at 95°C for 30 min. After cooling, absorbance of the samples was measured on a spectrophotometer at 532 nm. The results were expressed as the percentage of TBARS was formed by AAPH alone (inducedcontrol). Ascorbic acid is used as control in this assay (Ahmad et al., 2015).

Evaluation of EOED *in vitro* potential against production of hydroxyl radical (OH•)

Production of hydroxyl radical (OH•) was quantified by the Fenton reaction. During this reaction, the *in vitro* effect of EOED against the production of OH•, produced by the oxidative degradation of 2-deoxyribose, was determined (Lopes et al., 1999). The principle of the test is to quantify the degradation product of 2-deoxyribose, malonaldehyde (MDA), by its condensation with TBA. Briefly, the reactions were initiated by the addition of Fe^{2+}($FeSO_4$) with 6 $mmol.L^{-1}$ final concentration for solutions containing 2-deoxyribose 5 $mmol.L^{-1}$, H_2O_2 100 $mmol.L^{-1}$ and phosphate buffer 20 $mmol.L^{-1}$ (pH 7.2). Concentrations of 0.9, 1.8, 3.6, 5.4, and 7.2 $\mu g.ml^{-1}$ of EOED were added to the system before the addition of Fe^{2+} in order to determine EOED *in vitro* antioxidant activity against hydroxyl radical formation. The reactions were performed for 15 min at room temperature and they were stopped by the addition of phosphoric acid at 4% (v/v), followed by addition of TBA (1% v/v in NaOH 50 $mmol.L^{-1}$). The solutions were heated in a water bath for 15 min at 95°C. The absorbance was measured at 532 nm and results were expressed as equivalents of MDA formed by $Fe^{2+}$and H_2O_2.

Evaluation of EOED *in vitro* potential against production of nitrite ion (NO_2)

Nitric oxide was generated from the spontaneous decomposition of sodium nitroprusside (SNP) in 20 mM phosphate buffer (pH 7.4). Once generated, NO interacts with oxygen to produce nitrite ions, which were measured using the Griess reaction (Tsikas, 2007). The reaction mixture (1 ml) containing 10 mM SNP in phosphate buffer and EOED evaluated at concentrations of 0.9, 1.8, 3.6, 5.4, and 7.2 $\mu g.ml^{-1}$ concentrations was incubated at 37°C for 1 h. A 0.5 ml aliquot was taken and homogenized with 0.5 ml Griess reagent. The absorbance of the chromophore formed was measured at 540 nm. The extent to which the nitric oxide generated was inhibited was measured by comparing the absorbance values of negative controls (only 10 mM SNP and blank) and assay preparations. Results were expressed as percentages of nitrite formed by SNP alone.

RESULTS AND DISCUSSION

Chemical composition

Hydrodistillation of the leaves of *E. dysenterica* gave a light-yellowish crude essential oil (EOED), with a yield of 1.45±1.48% (w/w), in relation to the dry weight of the plant material. As shown in Table 1, it was possible to identify 6 compounds (80.67% of the total composition) and the sesquiterpenes (75.4%) were the majority (Figure 1 and Table 1). The major compounds identified were caryophyllene oxide (66.4%) (Figure 2), isoledene (3.9%), 1,3,8-*p*-menthatriene (3.5%), mustakone (3.5%), β-phellandrene (1.7%), and selin-11-en-4-α-ol (1.7%)

These results show a high content of caryophylleneoxide (66.37%) and there are also new constituents different from what have been found in other species of *E. dysenterica* collected in another region of Brazil as reported by Costa et al. (2000). β-caryophyllene (14.8%), α-humulene (10.9%), α-terpineol (6.1%), limonene (5.5%), α-thujene (5.6%), caryophyllene oxide (5.4%), and sabinene (3.9%).

In addition to the major constituent, caryophyllene oxide (66.37%) and β-caryophyllene have been reported in the essential oils of several other species of *Eugenia* (higher than 20%), indicating that this species is a typical

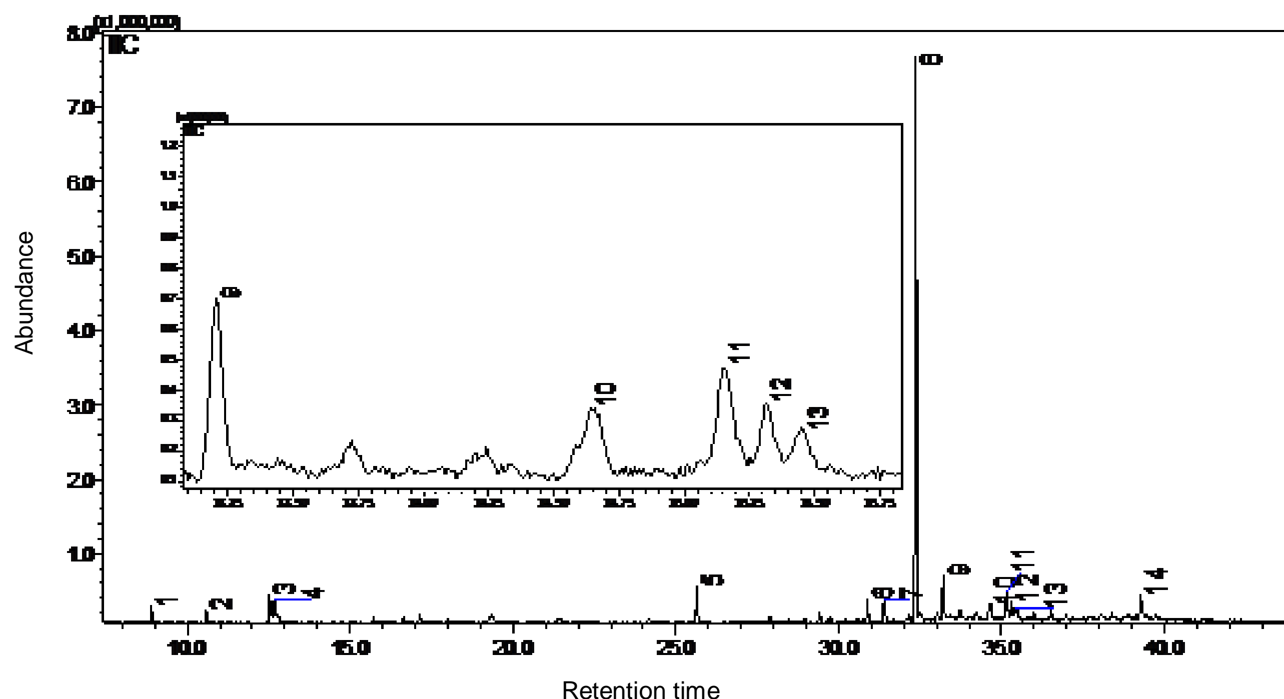

Figure 1. GC-MS of the essential oil of *E. dysenterica*.(1) β-Phellandrene, (3) 1,3,8-*p*-Menthatriene, (5) Isoledene, (8) Caryophyllene oxide, (10) Selin-11-en-4-α-ol, (11) Mustakone, 2,4,6,7,9,12,13, (14) Unknown.

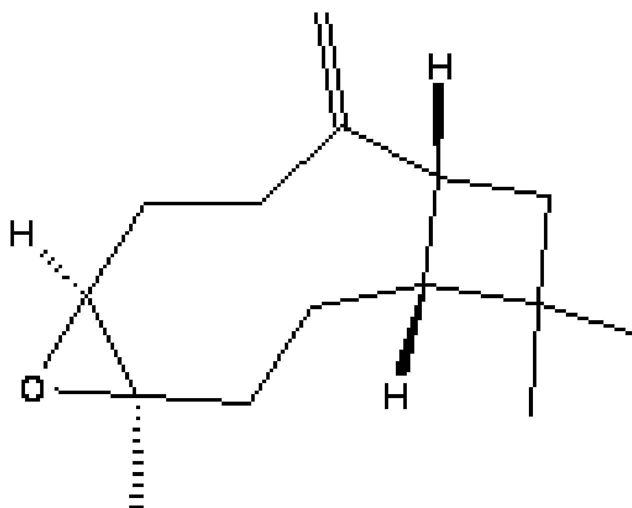

Figure 2. Chemical structure of the caryophyllene oxide.

The member of the Myrtaceae family (Costa et al., 2000). substances isoledene (3.91%), 1,3,8-*p*-menthatriene (3.51%), mustakone (3.46%), β-phellandrene (1.73%), and selin-11-en-4-α-ol (1.69%) were identified for the first time as chemical constituents of EOED.

Inhibition of AChE activity

The qualitative results of inhibition of enzyme AChE in TLC showed that the EOED and caryophyllene oxide inhibited the enzyme by the appearance yellow

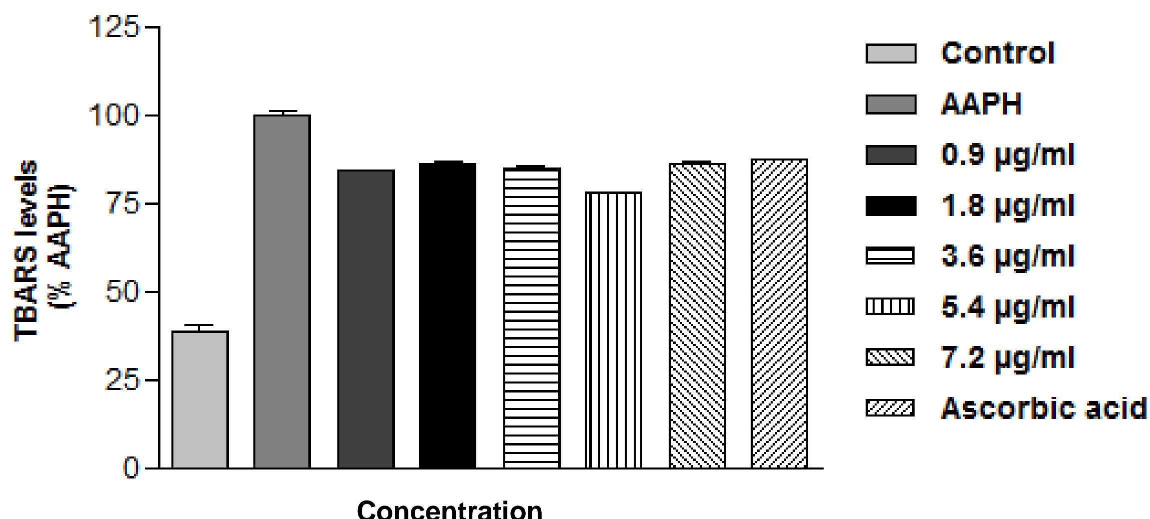

Figure 3. Antioxidant capacity of *E.dysenterica* essential oil in different concentrations (0.9, 1.8, 3.6, 5.4 and 7.2 µg.ml^{-1}) by the reduction of TBARS levels. The results represent the mean ± standard error of mean (SEM) of the values of *in vitro* inhibition, n = 3, of the experiments in duplicate. Ascorbic acid (140 µg.ml^{-1}) was used as standard antioxidant. *p < 0.05 vs. control (0.05% Tween 80 dissolved in 0.9% saline) (ANOVA and t-Student–Neuman–Keuls as post hoc test). $^{a}p < 0.05$ vs. to AAPH (100% of TBARS levels) (ANOVA and Student–Newman–Keuls as post hoc test).

backgrounds with white spots for inhibiting compounds were visible after about 5 min. This are the results of the first tests, yellow backgrounds with white spots for inhibiting compounds were visible after about 5 min.

The best anti-AChE activity was found for EOED (IC_{50} = 0.92 µg.ml^{-1}) when compared with *Eucalyptus camaldulensis* (IC_{50} = 18.98 µg.ml^{-1}), *Ocimum canum* (IC_{50}=36.16 µg.ml^{-1}) and *Cistus salvifolius* (IC_{50} = 58.10 µg.ml^{-1}) (Kiendrebeogo et al., 2011).

When starting the Ellman assay, it was observed that EOC was able to inhibit the enzyme AChE with an IC_{50} = 0.92 µg.ml^{-1} (Means from independent experiments were then expressed as means ± standard deviation (SD).

For all statistical analyses, p<0.001 was considered as statistically significant with a promising value when compared with drug rivastigmine (IC_{50} = 1.87 µg.ml^{-1}), the conventional AChE inhibitor used in treatment of AD.

In another study, *Eugenia sulcata* essential oil contained monoterpenes known for their anticholinesterase activity (for example α-cubebene and β-copaene), with an inhibitory capacity of the enzyme AChE and value of IC_{50} = 4.66 µg.ml^{-1} (Lima et al., 2012).

In research, the caryophyllene oxide pure was tested on the enzyme AChE and showed a value for CI_{50} = 0.31 µg.ml^{-1}. In studies, there are reports of analgesic and anti-inflammatory activities for caryophyllene oxide, major constituent of the EOED (Chavan et al., 2010).

Testing of antioxidants TBARS

To evaluate the antioxidant activity of EOED, two other methods were adapted which are based on the ability of a substance to scavenge free radicals through direct interaction with a substance reactive molecules, converting the less reactive free radical species and therefore more stable (Hoelzl et al., 2005).

Homogenized egg yolk as a lipid rich substrate was used to determine the antioxidant activity of EOED (Guimarães et al., 2010). TBARS is a complex formed by APPH (soluble water-azo compound is used as free radical generator) and TBARS.

From the methods used *in vitro*, it was demonstrated that EOED was able to reduce the production of free radicals at all concentrations tested, with a better performance in antioxidant TBARS test, which is a method used to quantify the peroxidation which corresponds to a lipid in the cell membrane damage caused by oxidative stress. The EOED, at all concentrations tested, 0.9, 1.8, 3.6, 5.4 to 7.2 µg.ml^{-1}, were capable of preventing lipid peroxidation inhibiting the amount of TBARS 16.07, 14.11, 15.02, 22.07, and 13.95%, respectively, as shown in Figure 3.

Similar results were obtained with ascorbic acid as antioxidant used provided standard 12.67% inhibition of TBARS formed. It was also found that 50% inhibitory concentration (IC_{50}) of the oil is 1.2 µg.ml^{-1} against the formation of TBARS with variation margin on the effective concentration 0.3 to 5.8 mg.ml^{-1} (with 95% confidence interval).

There is evidence to indicate that free radicals cause oxidative damage to lipids, proteins, and nucleic acids (Uttara et al., 2009). AD is the most common form of and

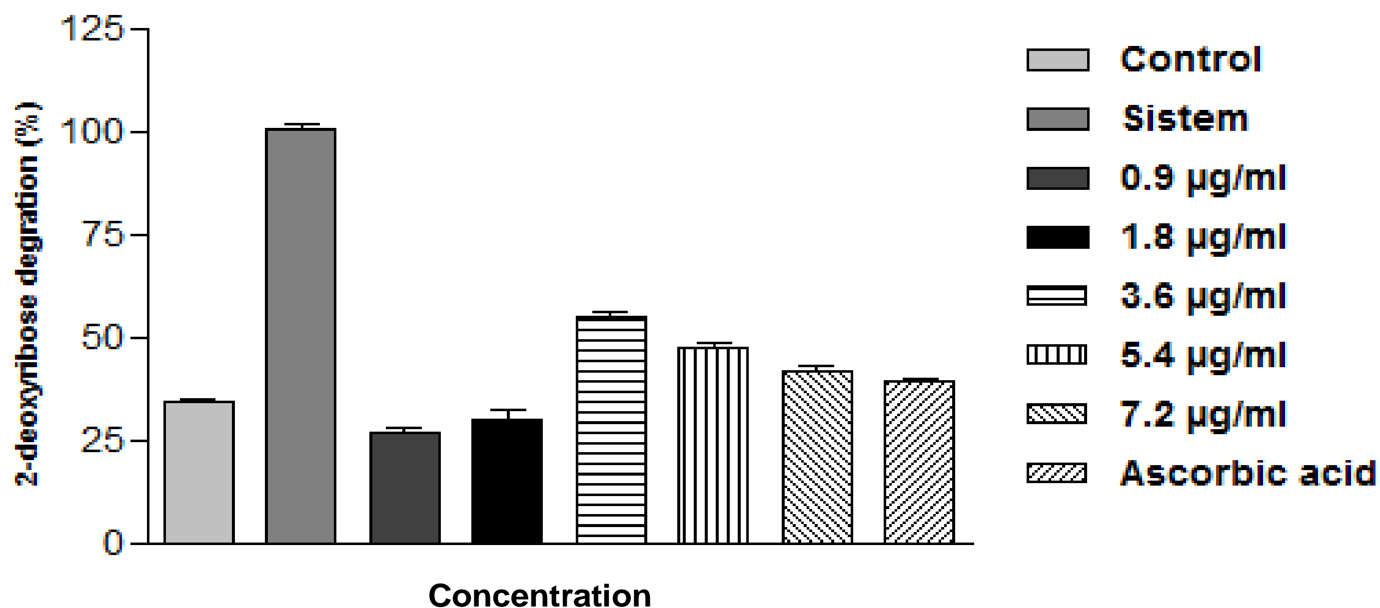

Figure 4. Antioxidant capacity *Eugenia dysenterica* essential oil in different concentrations (0.9, 1.8, 3.6, 5.4 and 7.2 $\mu g.ml^{-1}$) in the inhibition of 2-deoxyribose degradation by removal of hydroxyl radical. The results represent the mean ± standard error of mean (SEM) of the values of *in vitro* inhibition, n = 5, of the experiments in duplicate. Ascorbic acid (140 $\mu g.ml^{-1}$) was used as standard antioxidant. *p < 0.05 vs. control (0.05% Tween 80 dissolved in 0.9% saline) (ANOVA and Student–Newman–Keuls as post hoc test), $^{a}p < 0.05$ vs. (100% of hydroxyl radical) (ANOVA and Student–Newman–Keuls as post hoc test).

dementia, characterized by progressive neurodegeneration. Pathogenetic mechanisms, triggered by β-amyloid (Aβ) accumulation, include oxidative stress, deriving from energy homeostasis deregulation involving mitochondria and peroxisomes. At severe pathological stages, when senile plaques disrupt cortical cytoarchitecture, antioxidant capacity is gradually lost. Porcellotti et al. (2015) reported that oxidative stress occurs during the progression of β-amyloid pathology in the neocortex of the Tg2576 mouse model of AD, suggesting early therapeutic intervention in AD, also targeting peroxisomes.

In small quantities, antioxidants have great therapeutic potential for some conditions caused by free radicals such as arthritis, AD, heart disease, aging, cancer, among other (Halliwell et al., 1995). Antioxidants may thus be used as neuroprotectors agents, for example: aryl amines and indoles-carotene, lycopene polyenes-carotene, lycopene, and retinol selenium containing compounds ebselen, polyphenols-favonoids, stilbenes, and hydroquinone monophenols: tocopherols (vitamin E), 17-estradiol (estrogen), 5-hydroxytryptamine (serotonin), since oxidative damage may be observed before the formation of β-amyloid specific pathological signs (Behl et al., 1992).

The use of antioxidants has been explored in an attempt to slow AD progression and neural degeneration. Given the complex pathology of AD, current strategies for the development of new agents focus on compounds with various powers and plants are a major source of these compounds (Konrath et al., 2012).

Evaluation of EOED *in vitro* potential against production of hydroxyl radical (OH•)

The radical OH• is a more toxic moiety known, since they can oxidise non-specifically all classes of biological macromolecules, including lipids, proteins, and nucleic acids with virtually limited diffusion rates (Imlay and Linn, 1988). Therefore, OH• may result in oxidative damage that gives rise to various diseases, including arthritis, atherosclerosis, cirrhosis, diabetes, cancer, AD, emphysema, and aging (Ozyurek et al., 2008).

In this procedure OH• generated from a Fenton reaction with deoxyribose produce malondialdehyde (MDA) and similar substances (Halliwell and Gutteridge, 2007). After antioxidants testing in vitro was observed at all concentrations tested, 0.9, 1.8, 3.6, 5.4, and 7.2 µg.ml-1 to hydroxyl radical removal capacity in 73.46, 69.90, 45.09, 52.65, and 58.1%, respectively, as shown in Figure 4, results similar to that of ascorbic acid removed60.96% of the produced hydroxyl radical. IC50 was established at 5.814 µg.ml-1 against the formation of the hydroxyl radical with a variation range of 253 to 10.39

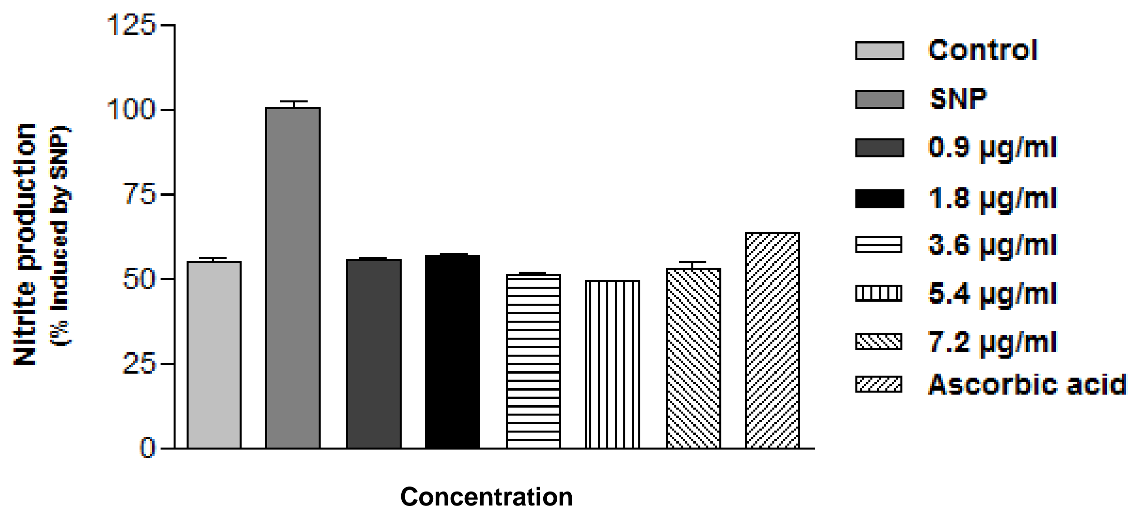

Figure 5. Nitric oxide (NO) scavenging assay. Antioxidant capacity of *E. dysenterica* essential oil in different concentrations (0.9, 1.8, 3.6, 5.4 and 7.2 μg.ml^{-1}) against the formation of nitrite ions generated. The results represent the mean ± standard error of mean (SEM) of the values of *in vitro* inhibition, n = 5, of the experiments in duplicate. Ascorbic acid (140 μg.ml^{-1}) was used as standard antioxidant. *$p < 0.05$ vs. control (0.05% Tween 80 dissolved in 0.9% saline) (ANOVA and Student–Newman–Keuls as post hoc test), $^{a}p < 0.05$ vs. SNP (100% of nitrite ions) (ANOVA and Student–Newman–Keuls as post hoc test).

(with a 95% confidence interval).

Antioxidant potential evaluation in nitric oxide removal (NO)

In our studies, *in vitro*, was also possible to determine the 50% inhibitory concentration (IC_{50}) of the sample 0.1684 μg.ml^{-1} against the formation of nitrite radical, with variation margin on the effective concentration from 0.06630 to 0.4276 μg.ml^{-1} with 95% confidence interval, was observed at all concentrations tested, 0.9, 1.8, 3.6, 5.4, and 7.2 μg.ml^{-1} to remove nitrite metabolic capacity in 44.62, 43.45, 48.97, 51.09, and 47.05% as shown in Figure 5 results similar to that of ascorbic acid removed 36.73%.

Antioxidants comprise a broad and heterogeneous family of compounds that share the common task of interfering with stopping, retarding, or preventing the oxidation or autoxidation of an oxidizable substrate (Savelev et al., 2003). Numerous physiological and biochemical processes in the human body may produce oxygen containing free radicals and other reactive oxygen or nitrogen species as by-products (Chavan et al., 2010). Overproduction of such radicals can cause oxidative damage to biomolecules, eventually leading to many diseases, such as atherosclerosis, cancer, diabetes, or inflammatory conditions and pain (Jeon et al., 2011).

Oxidative stress is implicated in the pathophysiology of a wide variety of neurodegenerative disorders, including Parkinson'sdisease, AD, Friedreich's ataxia, amyotrophic lateral sclerosis, and stroke. This is the first report of biological properties of *E. dysenterica*, a candidate to be a source of antioxidant and anticholinesterase compounds for use as drugs.

Conclusion

The main constituents of *E. dysenterica* DC. (cagaiteira) essential oil from a water extract of leaves were caryophyllene oxide (66.37%), isoledene (3.91%), 1,3,8-*p*-menthatriene (3.51%), mustakone (3.46%), β-phellandrene (1,73%), and selin-11-en-4-α-ol (1,69%). The pure caryophyllene oxide (the main compound) was tested on the AChE and presented the IC_{50} = 0.31 μg.ml^{-1}. This is the first report, that the essential oil from leaves *E. dysenterica* exhibits antioxidant effects preventing lipoperoxidation and AChE activity. The results are encouraging and it is suggested that they should be followed up with *in vivo* testing.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are grateful to CNPq (Grant no. 310064/2014-7) and FAPEPI, for financial support.

REFERENCES

Adam RP (2007). Identification of essential oil components by Gas Chromatography Mass Spectrometry. 4th ed. Carol Stream, Illnois - USA: Allured Publishing Corporation.

Ahmad S, Ullah F, Ayaz M, Sadiq A, Imran M (2015). Antioxidant and anticholinesterase investigations of *Rumexhastatus* D. Don: potential effectiveness in oxidative stress and neurological disorders. Biol. Res. 48:20.

Behl C, Davie J, Cole GM, Schubert D (1992). Vitamin E protects nerve cells from amyloid protein toxicity. Biochem. Biophys. Res. Commun. 186:944-950.

Buckholtz NS (2011). Perspective: In search of biomarkers. Nature. 475:(7355)S8.

Campelo LML, De Almeida AA, Freitas RM, Cerqueira GS, de Sousa GF, Saldanha GB, Feitosa CM, de Freitas RM (2012). Antioxidant and antinociceptive effects *of Citrus limon* essential oil in mice". J. Biomed. Biotechnol. 2011(2011):1-8.

Cardoso LM, Martino HSD, Moreira AVB, Ribeiro SMR, Pinheiro-Sant'Ana HM (2011). Cagaita (*Eugeniadysenterica* DC.) of the Cerrado of Minas Gerais, Brazil: Physical and chemical characterization, carotenoids and vitamins. Food Res. Int.. 44(7):2151-2154.

Chaiyana W, Okonogi S (2012). Inhibition of cholinesterase by essential oil from food plant. Phytomedicine 19(8-9):836-839.

Chavan MJ, Wakte PS, Shinde DB (2010). Analgesic and anti-inflammatory activity of Caryophyllene oxide from *Annonasquamosa* L. bark. Phytomedicine. 17(2):149-151.

Costa TR, Fernandes OFL, Santos SC, Oliveira CM, Lião LM, Ferri PH, Paula JR, Ferreira HD, Sales BH, Silva MR (2000). Antifungal activity of volatile constituents of *Eugenia dysenterica* leaf oil. J. Ethnopharmacol. 72:111-117.

Ellman GL, Courtney DK, Andres VJR, Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7(2):88-90.

Enz A, Amstutz R, Boddeke H, Gmelin G, Malanowski J (1993). Brain selective inhibition of acetylcholinesterase: a novel approach to therapy for Alzheimer's disease. Progress Brain Res. 98:431-438.

Esterbauer H, Cheeseman KH (1990). Determination of aldehydic lipid peroxidation products:Malonaldehyde and 4-hydroxyno- nenal. Methods Enzymol. 186:407-421.

Guimarães AG, Oliveira GF, Melo MS, Cavalcanti SC, Antoniolli AR, Bonjardim LR, Silva FA, Santos JP, Rocha RF, Moreira JC, Araújo AA, Gelain DP, Quintans-Júnior LJ (2010). Bioassay-guided evaluation of antioxidant and antinociceptive activities of carvacrol. Basic Clin. Pharmacol. Toxicol. 107(6):949-957.

Halliwell B, Gutteridge JMC (2007). Free Radicals in Biology and Medicine (4nd edition). Oxford University Press, New York, 704p.

Halliwell B, Aeschbach R, Lölinger J, Aruoma OI (1995). The characterization on antioxidants.Food Chem. Toxicol. Oxford 33(7):601-617.

Hoelzl C, Bichler J, Ferk F, Simic T, Nersesyan A, Elbling L, Ehrlich V, Chakraborty A, Knasmüller S (2005). Methods for the detection of antioxidants which prevent age related diseases: a critical review with particular emphasis on human intervention studies. J. Physiol. Pharmacol. 56(2):49-64.

Houghton PJ, Howes MJR (2003). Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. Pharmacol. Biochem. Behav. 75:513-527.

Imlay J, Linn S (1988). DNA damage and oxygen radical toxicity. Science. 240(4857):1302-1309.

Jeon S, Bose S, Hur J, Jun K, Kim YK, Cho KS, Koo BS (2011). A modified formulation of Chinese traditional medicine improves memory impairment and reduces Abeta level in the Tg-APPswe/PS1dE9 mouse model of Alzheimer's dis*ease. J. Ethopharmacol. 137 91):*783-789.

Van Den Dool H, Kratz PD (1963). A Generalization of the Retention Index System including Linear Temperature Programmed Gas—Liquid Partition Chromatography. J. Chromatogr. 11:463-471.memory impairment and reduces Abeta level in the Tg-APPswe/PS1dE9 mouse model of Alzheimer's disease. J. Ethopharmacol. 137(91):783-789.

Kiendrebeogo M, Coulibaly AY, Nebie RCH, Zeba B, Lamien CE, Lamien-Meda A, Nacoulma OG (2011). Antiacetylcholinesterase and antioxidant activity of essential oils from six medicinal plants from Burkina Faso. Braz. J. Pharmacogn. 21(1).

Konrath EL, Neves BM, Lunardi PS, Passos CS, Simões F, Ortega MG, Gonçalves CA, Cabrera JL, Moreira JCF, Henriques AT (2012). Investigation of the *in vitro* and *ex vivo*acetylcholinesterase and antioxidant activities of traditionally used Lycopodium species from South America on alkaloid extracts. J. Ethnopharmacol.139(1):58-67.

Konrath EL, Passos CS, Klein-Júnior LC, Henriques AT (2013). Alkaloids as a source of potential anticholinesterase inhibitors for the treatment of Alzheimer's disease. J. Pharm. Pharmacol. 65:1701-1725.

Lima BG, Tietbohl LAC, Fernandes CP, Cruz RAS, Botas GS, Santos MG, Silva-Filho MV, Rocha L (2012). Chemical Composition of Essential oils and Anticholinesterasic Acticity of *Eugenia sulcata* Spring ex Mart. Lat. Am. J. Pharm. (formerly Acta Farmacéutica Bonaerense), 31(1):152-155.

Loizzo MR, Jemia MB, Senatore F, Bruno M, Menichini F, Tundis R (2013). Chemistry and functional properties in prevention of neurodegenerative disorders of five *Cistus* species essential oils. Food Chem. Toxicol. 59:586-594.

Lopes GKB, Schulman HM, Lima MH (1999). Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions. Biochim. Biophys. Acta - General Subjects. 1472(1-2):142-152.

Mukherjee PK, Kumar V, Mal M, Houghton PJ (2007). Acetylcholinesterase inhibitors from plants. Phytomedicine 14:289-300.

Oga M, Fonseca CEL (1994). A fast methodto estimate leafarea incagaiteiraseedlings (*Eugenia dysenterica* D.C.). BrazilianAgricultural Research.Brasília. 29(4):571-577.

Oliveira MES, Pantoja L, Duarte WF, Collela CF, Valarelli LT, Schwan R, Dias DR (2011). Fruit wine produced from cagaita (*Eugenia dysenterica* DC) by both free and immobilised yeast cell fermentation. Food Res. Int. 44(7):2391-2400.

Ozyurek M, Bektasoglu B, Guclu K, Apak R (2008). Hydroxyl radical scavenging assay of phenolics and flavonoids with a modified cupric reducing antioxidant capacity (CUPRAC) method using catalase for hydrogen peroxide degradation. Anal. Chim. Acta, Istanbul, Turkey. 616(2):196-206.

Porcellotti S, Fanelli F, Fracassi A, Sepe S, Cecconi F, Bernardi C, Cimini AM, Cerù MP, Moreno S (2015). Oxidative stress during the progression of β-amyloid pathology in the neocortex of the Tg2576 mouse model of Alzheimer's disease. Oxidative Med. cellular longevity. 1-18.

Rhee IK, Van de Meent M, Ingkaninan K, Verpoorte R (2001). Screening for acetylcholinesterase inhibitors from Amaryllidaceae using silica gel thin-layer chromatography in combination with bioactivity staining. J. Chromatogr. A915(1-2):217-223.

Rizzini C (1970). Efeito tegumentar na germinação de *Eugenia dysenterica*DC (Myrtaceae). Braz. J. Biol. 30(3):381-402.

Savelev S, Okello E, Perry NSL, Wilkin RM, Perry EK (2003). Synergistic and antagonistic interactions of anticholinesterase terpenoids in *Salvia lavandulaefolia* essential oil. Pharmacol. Biochem. Behav. 75 (3):661-668.

Siddiqui MF, Levey AI (1999). Cholinergic Therapies in Alzheimer's Disease.Drugs Future. 24(4):417-444.

Tsikas D (2007). Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: Appraisal of the Griess reaction in the L-arginine/nitric oxide area of research. J. Chromatogr. B851(1-2):51-70.

Uttara B. Singh AV, Zamboni P,Mahajan RT(2009). Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options. Curr Neuropharmacol. 7:65-74.

Related Journals Published by Academic Journals

- Journal of Medicinal Plant Research
- African Journal of Pharmacy and Pharmacology
- Journal of Dentistry and Oral Hygiene
- International Journal of Nursing and Midwifery
- Journal of Parasitology and Vector Biology
- Journal of Pharmacognosy and Phytotherapy
- Journal of Toxicology and Environmental Health Sciences

academicJournals