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The Antioxidant and Hematopoietic Effects of the Methanolic Extract Fractions of *Ocimum basilicum* in Acetaminophen-Induced Albino Rats

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ABSTRACT

This study investigated the antioxidant properties of the fractions of the methanolic extract of *Ocimum basilicum* (OB) and their effects on the hematological parameters of acetaminophen-induced albino rats. Using vacuum liquid chromatography, the methanolic extract of OB was separated into four fractions (i, ii, iii, and iv) and studied using the DPPH and FRAP assays. Selected fractions ii and iv with the highest antioxidant activities for the animal study. Forty-two albino rats were divided into seven treatment groups (GP) of six animals each, subdivided into three replicates with two rats each. For seven days, rats in GP1 and GP2 were administered water and silymarin (1000mg/kg), gave those in GP4 and GP5 500mg/kg and 1000mg/kg of fraction ii, respectively, while gave those in GP6 and GP7 500mg/kg and 1000mg/kg of fraction iv respectively. Rats in GP3 were administered 750mg/kg of acetaminophen (per os) on day eight only. The results showed that, in comparison with the control groups (1, 2, and 3), rats in GRP4 showed increased ($P<0.05$) hemoglobin concentration and total WBC count while GRP5 had increased ($P<0.05$) PCV and MCV. Rats in GRP 6 showed increased ($P<0.05$) MCV and MCHC compared to the control group, while those in GRP7 showed decreased ($P<0.05$) PCV and MCV compared with the positive control. It was concluded that the free radical scavenging activity of methanolic extract fractions of OB increased in a dose-dependent manner and high doses (1000mg/kg) of fraction ii showed increased hematopoietic activity.

Key words: Antioxidant, Blood constituents, Radical scavenging, *Ocimum basilicum.*

INTRODUCTION

Free radicals are generated endogenously as byproducts of physiological, pharmacological, and pathological processes. They play crucial roles in several physiological processes such as cell signaling, ion transport, apoptosis, phagocytosis (Li et al. 2017). The excessive generation of free radicals and the deficient detoxification by the body's antioxidant system results in oxidative stress (Tan et al. 2018). Oxidative stress-induced genotoxicity and/or cytotoxicity are key pathological factors in many disease processes such as tissue injury and inflammation. (Zengin et al. 2011; Pizzino et al. 2017). Antioxidants act by either inhibiting the formation or propagation of free radicals and consequently, oxidative stress is combated and the immune system is improved (Tan et al. 2018).

Synthetic antioxidants have wide applications in the food, pharmaceutical and cosmetic industries (Ruirui et al. 2019; Kebede and Admassu 2019). However, due to concerns about the toxicity and carcinogenic effects of synthetic antioxidants, their use has been largely discouraged and the search for naturally occurring substances (especially those from plant-derived sources) have been intensified (Hirose et al. 1998; Huang 2018; Lourenço et al 2019). Consumption of antioxidant-rich fruits, vegetables, and many medicinal plants has thus been promoted as a means of avoiding the harmful effects of oxidative stress. (Yashin et al 2017; Meitha et al 2020).

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In both Ayurvedic and Unani system of medicine, the leaves and flowers of OB are commonly used as a tonic and vermifuge (Muralidharan and Dhananjayan 2004). Its antioxidant activities have been well established and this has been linked with the phenolic compounds it contains (Jayasinghe et al. 2003). Studies have shown that OB possesses free radical scavenging activity which acts by transforming DPPH to its reduced form, DPPH-H and also neutralizes nitric oxide radicals (Kaurinovic et al. 2011). Studies (Khosro et al. 2012; Calderón et al., 2021) have shown that the ethanolic extract of OB showed more antioxidant activity when compared to standard antioxidants and display significant nitric oxide and superoxide radical scavenging activity. In another study, it was observed that aqueous and ethanolic extracts from OB increased the O6-methylguanine-DNA methyl transferase, glutathione S-transferase-P1 (Niture et al. 2006) and hepatic glutathione reductase, superoxide dismutase, and catalase are responsible for antioxidant effects in human cells. The methanolic extract of OB have also been proven to have significant hepatoprotective and antioxidant activities (Asala et al. 2021). However, studies on the bioactivities of the fractions of OB are sparse. As a result, the goal of this study is to look into the antioxidant activities of the fractions of the methanolic extract of OB *in vitro* and analyze their impacts on health using acetaminophen-induced albino rats.

MATERIALS AND METHODS

Ethical Approval

All appropriate international, national, and/or institutional animal care and use guidelines were adhered to. All procedures used in animal experiments were compliant with the ethical requirements of the institution or practice where the research was done. The tests were carried out in compliance with the ethical principles outlined in the Declaration of Helsinki of 1964 and subsequent amendments.

The study was divided into two experiments

1. *Invitro* antioxidant assay of fractions of methanolic extract of OB
2. The evaluation of the effect of various dosages of fractions of OB's methanolic extract on haematological parameters in acetaminophen induced albino rats.

Experiment 1: Invitro antioxidant assay of methanolic fractions of whole OB plant

Experimental Location: Central laboratory, University of Ibadan, Nigeria.

Plant Collection and Preparation of Extracts

Whole plants of *Ocimum basilicum Labiatae* were collected from Molete Bode, Ibadan Nigeria and identified at the Botany Department of the Faculty of Life Sciences at the University of Ibadan. The plants were air dried and pulverized. Solvent to solvent extraction of homogenized dried matter of the plants was done using the various fractions of the methanolic solvents. The percentage yield of the methanolic extracts was 2.12%.

Vacuum Liquid Chromatography (VLC) Analysis

The methanolic extract of whole OB plant was partitioned using vacuum liquid chromatography (VLC) analysis (Pelletier et al. 2004) with a column of (5 × 18.7cm) packed with silica Gel/60G. In order of increasing polarity, hexane, ethyl acetate, and methanol were utilized as eluents. The following were the four fractions obtained, i) 30% hexane and 70% ethyl acetate, ii) 70% hexane and 30% ethyl acetate, iii) 50% each of ethyl acetate and methanol, and iv) 100% methanol. The concentrations of eluents used were as described by Narasimha and Chandrasekar (2019). The methanolic extract fractions obtained were then subjected to further *in vitro* antioxidant studies and assayed using DPPH and FRAP antioxidant studies. The antioxidant activity of the fraction was compared to the standard, ascorbic acid, as determined by its ability to scavenge DPPH free radicals. The fractions were diluted, and the assay repeated.

Ability to scavenge on DPPH (2,2-diphenyl-1-picry-lhydrazyl) radical

The ability of the OB methanolic fractions to scavenge free radicals was evaluated according to the technique outlined by Sarikurkcu et al. (2008). Fifty percent v/v of methanol was used to dissolve 0.5mL of OB methanolic fractions (basal solutions). Thereafter, 10% v/v of methanol was also used to dissolve 0.1, 0.08, 0.06, and 0.04mL of basal solutions in 10mL volumetric flasks. Three milliliters of 610-5 M/l methanolic solution of the DPPH radical was combined with 0.1mL of previously prepared extract dilutions. The reaction mixture was kept at room temperature in the dark. After 30 minutes, the absorbance at 517nm was measured to quantify the scavenging activity on the DPPH radical. The inhibitory activity was determined as follows:

IC50 (%) = 100 x (A0-A1)/A0 Where;

A0 denotes the control's absorbance, and
A1 denotes the extract/standard's absorbance,
The extract's IC50 (percentage of free radical inhibition) was calculated. The control was ascorbic acid. All tests were carried out in triplicates.

Ferric Reducing Power Activity (Iron (III) to iron (II) reduction) (FRAP)

The FRAP was determined using the Zengin et al. (2011) technique. Phosphate buffer (2.5 mL, 0.2M, pH 6.6) and potassium ferricyanide (2.5mL, 1%) was mixed with 22.5mL of extract fraction solutions. The mixture was incubated for 20 min at 50°C. After the incubation, 2.5mL trichloroacetic acid (10%) was added to the mixture. The reaction mixture (2.5mL) was mixed with ferric chloride (0.5mL, 0.1%) and distilled water (2.5mL). At 700nm, the solution's absorbance was measured. A rise in absorbance at 700nm suggests a boost in reducing power. Increased absorbance of the reaction mixture indicated a high reducing power.

Experiment 2: Animal Studies

Location of experiment: The experiment was carried out at the Animal house of the Veterinary Physiology Department at the University of Ibadan, Oyo state, Nigeria.

Experimental Animals Management

Forty-two albino rats of both sexes and weighing between 180-220g were acquired from the small experimental station of National Veterinary Research Institute Vom, Plateau State, Nigeria and acclimatized for two weeks at room temperature. They were housed in the animal house of the Veterinary Physiology Department at the University of Ibadan, Oyo state, Nigeria, fed at *ad libitum* with vital animal feed and water throughout the experiment. Animal housing and handling were ethically considered.

Pilot toxicity Studies

The limit test for acute toxicity was carried out on five rats using the crude extract of OB by administering a single dose of 3000mg/kg. No mortality was observed in the rats 14 days after the treatment.

Design of Experiment

A total of 42 albino rats were randomly assigned to seven different treatment groups (GP) of six animals each, each replicated 3 times with 2 rats per replicate. The treatment groups and drugs administered were as follows:

GP1: Negative control (Water only)
GP2: Standard control (1000mg/kg Silymarin)
GP3: Positive control (750mg/kg per os Acetaminophen)
GP4: low dose (500mg/kg) methanolic fraction ii (Ethyl acetate: Methanol)
GP5: High dose (1000mg/kg) methanolic fraction ii (Ethyl acetate: Methanol)
GP6: Low dose (500mg/kg) Methanolic fraction iv (100% Methanol)
GP7: High dose (1000mg/kg) Methanolic fraction iv (100% methanol)

All rats in the different treatment groups were administered water, silymarins and methanolic fractions (as applicable), respectively for 7 days while 750mg/kg per os acetaminophen was administered on day 8

Hematological Analysis

About 2 mL blood from each albino rats (n=42) were collected by cervical venipuncture into vials containing Ethylene Diamine Tetraacetic Acid (EDTA). Hematological analysis was done using the ABACUS Junior auto-hemoanalyser which works on two basic principles of the coulter counter method of white blood cells, red blood cells, platelets, and colorimetric method of determining hemoglobin.

Statistical Analysis

All data was presented as mean±SEM. One-way analysis of variance (ANOVA) was used to compare the results statistically using graph pad prism 5.0. data analysis. The statistical significance level was set at 0.05.

RESULTS

Phytochemical Constituent

The qualitative screening of the phytochemical constituents of the methanolic extract of OB whole plant revealed the presence of flavonoids, tannin, terpenes, steroids, and cardiac glycosides. Alkaloids, saponins and anthraquinones were not detected.

DPPH

The capacity of the four fractions of the OB methanolic extract to scavenge DPPH radicals is depicted in Fig. 1. The extract fractions' ability to scavenge DPPH radicals was lower than that of ascorbic acid (92%) at 0.1mg/mL, according to the results. However, the percentage inhibition seen in all doses (01, 0.08, 006, and 0.04mg/mL) of fraction ii was the closest to the control. This was successively followed by fractions iv, and fraction iii. The least scavenging activity was observed in fraction i. It was also observed that the radical scavenging ability of DPPH of fraction i, ii, iii, and iv increased dose-dependently.

FRAP

The FRAP assay of the methanolic extract of *O. basilicum* as presented in Figure 2 indicated that different amounts of OB extract fractions have different antioxidant properties. The FRAP activity was highest in fraction ii (100% ethyl acetate) and fraction iv (100% methanol). The trend revealed the highest FRAP activities in fraction ii followed closely by fraction iv, and then fraction iii. The least FRAP activity was seen in fraction i. The FRAP antioxidant properties of the fractions were also discovered to rise in a dose-dependent manner. Against this backdrop, fractions ii and iv were adjudged as the ones with highest antioxidant properties and were then used for the animal trial.

Table 1: Hematological parameters of rats administered with methanolic fractions ii and iv of Vacuum Liquid Chromatography

Parameters	Units	GP1	GP2	GP3	GP4	GP5	GP6	GP7
PCV	%	53.5±3.13	55.6±2.81	61.0±2.63	32.8±2.45abc	71.9±0.07abc	50.1±3.19b	52.7±3.58b
Hb	g/dL	12.9±0.69	13.4±0.50	13.3±0.42	17.9±2.68ab	14.8±2.29	13.4±0.5	14.1±0.53
RBC	$\times 10^6$ /cm^3	6.75±0.42	7.98±1.11	7.98±1.11	8.80±1.34a	8.01±1.61	6.8±0.88	7.14±0.14
WBC	$\times 10^3$ /cm^3	6.22±0.02	3.91±0.94	7.50±0.10	13.1±0.013bc	28.8±0.28	6.1±0.48a	6.6±0.14
PLT	$\times 10^2 \mu L^{-1}$	948.4±201	642.6±69	724.3±44	2146±212	1086±51	1058±212	897±55
MCV	Fl	77.7±3.35	71.9±2.00	70.7±4.16	69.5±2.12a	82.3±5.bc	71.9±2.00	67.6±2.21a
MCH	Pg	18.6±0.65	18.0±0.50	18.5±0.90	19.2±0.78	18.7±1.02	19.0±0.49	18.8±0.69
MCHC	g/dL	25.7±0.45	25.6±0.49	25.9±0.32	25.4±0.1	22.6±0.40abc	27.4±0.87abc	25.4±0.30

Values (Mean±SD) bearing a, b and c differ significant (P<0.05) when compared with negative control group, positive control group and standard control, respectively. PCV=Packed cell Volume; Hb=Hemoglobin; RBC=Red blood cells; WBC=White blood cell; PLT= Platelet count; MCV=Mean corpuscular volume; MCV=Mean corpuscular hemoglobin, and MCHC=Mean corpuscular hemoglobin concentration. GP1: Negative control (Water only), GP2: Standard control (1000mg/kg Silymarin), GP3: Positive control (750mg/kg per os Acetaminophen), GP4: low dose (500mg/kg) methanolic fraction ii (Ethyl acetate: Methanol), GP5: High dose (1000mg/kg) methanolic fraction ii (Ethyl acetate: Methanol), GP6: Low dose (500mg/kg) Methanolic fraction iv (100% Methanol), and GP7: High dose (1000mg/kg) Methanolic fraction IV (100% methanol).

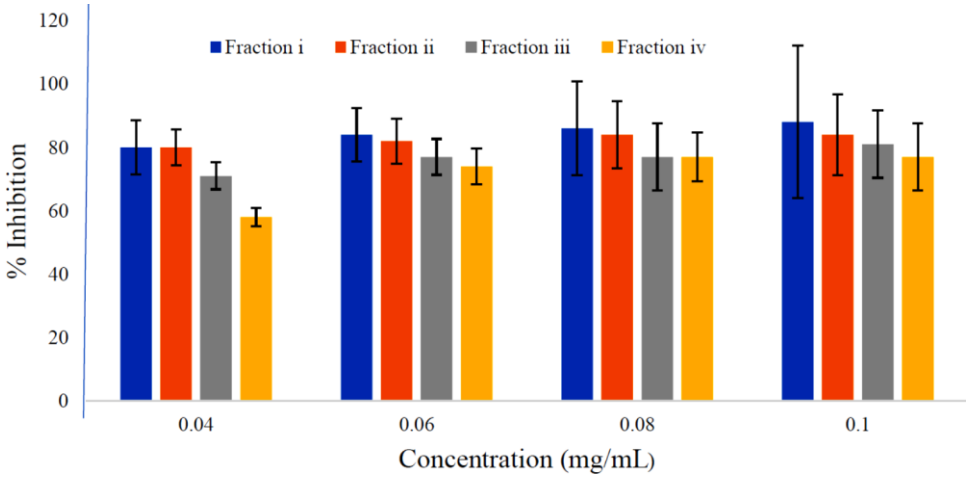

Fig. 1: Scavenging effects of *Ocimum basilicum* extract fractions on the DPPH free radical.

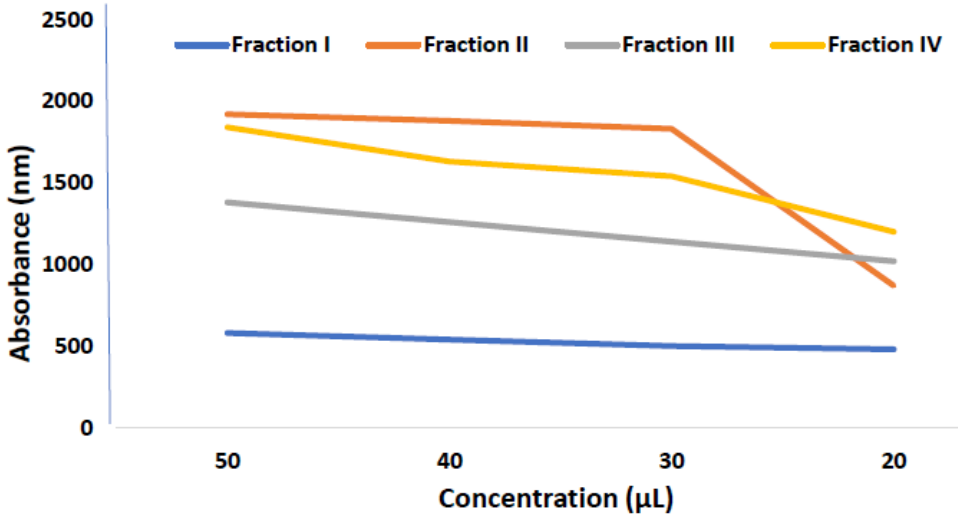

Fig. 2: Scavenging effects of *Ocimum basilicum* extract fractions on the FRAP free radical.

Hematological Parameters

The effect of methanolic fractions ii and iv on the hematological parameters of rats is presented in Table 1. The following trends were observed. Rats on GP4 (low dose fraction ii) showed that hemoglobin concentrations increased significantly ($P<0.05$), and total leucocyte count, and decreased ($P<0.05$) MCV and PCV compared to the control groups. Rats on GP 5 (high dose fraction ii) had significantly increased ($P<0.05$) PCV, MCV, and lower MCHC when compared to the positive and standard control groups. Amongst the rats on GP6 (500mg/mL fraction iv), PCV was found to be significantly lower ($P<0.05$) when compared to positive control. However, there was a significant increase in MCV and MCHC compared to all control groups. Rats on GP7 (high dose Fraction iv) showed a significant decrease in PCV and MCV compared with the positive control.

DISCUSSION

The presence of flavonoids in the methanolic extract of OB suggests antioxidant activity. Flavonoids are a class of natural polyphenols which are secondary plant metabolites that contain the hydroxyl group and serve as powerful antioxidants by donating hydrogen and forming stable radical intermediates to combat oxidative stress (Panche et al. 2016: Ziemlewska et al. 2021).

The antioxidant studies indicated that fractions ii have the ability to scavenge DPPH radicals close to that of the standard antioxidant. This observation suggests that the extract fraction ii has a proton donating ability, acting as a primary antioxidant inhibiting or scavenging free radical and an alternative natural replacement for synthetic antioxidants such as ascorbic acid. This observation can be attributed to the fact that the solvent used (70% hexane: 30% ethyl acetate) for fraction ii helps to release more alkaloids, glycosides, flavonoids, and several other classes of compounds with antioxidant mechanisms related biological properties (Zhang et al. 2009; Sobhy et al. 2021). Hence, the observed antioxidant activity in the result agreed with the work of Jayasinghe et al. (2003) and Bilal et al. (2013) who showed that *Ocimum basilicum* possesses antioxidant properties that aid it's the ability to relieve oxidative stress in rats.

The results clearly showed that the extract fractions' scavenging capability, as evaluated by the two techniques (DPPH and FRAP), increases in a dose-dependent manner. When using the DPPH and FRAP techniques, fraction ii and iv showed a higher level of antioxidant activity than fractions iii and I for all concentrations. These findings could indicate that the solvent utilized for this fraction released more flavonoids and/or total alkaloids than other solvents. Such antioxidant capacity could be influenced by a variety of factors. (Then et al. 2003; Maji and Banerji 2015; Degla et al. 2022)

Rats on low and high dose fraction ii caused an increase in RBC when compared to the control. This

observation agrees with the findings of Saha et al. (2012) in male swiss albino mice after benzene induced toxicity and Ali et al. (2017) in male albino mice administered *Ocimum basilicum* extract after $AlCl_3$-induced toxicity. This observation suggests that the secondary metabolites of *O. basilicum* L. methanolic fractions ii might contain an erythropoietin-like agent which may enhance the production of erythrocytes (Ali et al. 2017). An increase in hemoglobin concentration seen in rats on low dose fraction ii compared to control is contrary to the reports of Jimoh et al. (2008) who found that the administration of the aqueous extract of *Ocimum gratissimum* led to a decrease in hemoglobin. This difference in observations may be due to the difference in the solvent used for extraction of plant, part of plant used, and the specie of *Ocimum* used in the different studies (Kobus-Cisowska et al. 2020). The observation also suggests that the administration of low dose fraction ii may have ameliorating effect on be hematological abnormalities induced by acetaminophen. The increase in MCV and PCV in rabbits on high dose fraction ii is contrary to the findings of Ephraim et al. (2000) using aqueous leaf Extract of *Ocimum gratissimum*. Differences in observations may also be linked to differences in specie of *Ocimum* used and solvent of extraction.

Conclusion

The DPPH radical scavenging capacity and FRAP antioxidant properties of fraction I, ii, iii, and iv increased dose-dependently. Based on DPPH and FRAP results fractions ii and iv had the highest antioxidant properties. The methanolic fractions of OB possesses hematopoietic properties especially at high doses of 1000mg/kg of the fractions ii and iv

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Author's Contribution

Oluwole AM, Titilayo AM, and Moradeke BOO, designed the experiment and carried out the animal trials and blood sampling. Adebola SS and Titilayo AM wrote the manuscript and also performed data analysis. Ocheja BO, Gabriel A and Chidinma OA reviewed the manuscript. All authors revised and approved the final version.

REFERENCES

Ali AA, Qasim M, Aftab MN, Azam SM and Iqbal FA, 2017. Effects of *Ocimum basilicum* extract on hematological and serum profile of male albino mice after AlCl3 induced toxicity. Pure and Applied Biology 6: 505-510. http://dx.doi.org/10.19045/bspab.2017.60050

Asala TM, Abatan M, Salami SA, Baba-Onoja OM, Akanbi O, Rowaiye A, Ocheja O and Ada G, 2021. The ameliorative effect of the solvent extracts of *Ocimum basilicum* against acetaminophen-induced liver damage in albino rats. Journal of Phytomedicine and Therapeutics 20: 581-597. https://dx.doi.org/10.4314/jopat.v20i1.4

Bilal A, Jahan N, Ahmed A, Bilal SN and Habib S, 2013. Antifertility activity of hydroalcoholic extract of *Ocimum basilicum Linn* leaves on female Wistar rats. Journal of Reproduction and Contraception 24: 45-54. https://doi.org/10.1016/S1001-7844(13)60020-6

Calderón BH, Vera Céspedes, N, Zura-Bravo L and Muñoz, LA, 2021. Basil seeds as a novel food, source of nutrients and functional ingredients with beneficial properties: A review. Foods 10: 1467. https://doi.org/10.3390/ foods10071

Degla LH, Kuiseu J, Olounlade PA, Attindehou S, Hounzangbe-Adote MS, Edorh PA and Lagnika L, 2022. Use of medicinal plants as alternative for the control of intestinal parasitosis: Assessment and perspectives. Agrobiological Records 7: 1-9. https://doi.org/10.47278/journal.abr/2021.011

Ephraim KD, Salami HA and Osewa TS, 2000. Effect of aqueous leaf extract of *Ocimum gratissimum* on haematological and biochemical parameters in rabbits. African Journal of Biomedical Research 3: 175-179.

Hirose M, Takesada Y, Tanaka H, Tamano SK and Shirai T, 1998. Carcinogenicity of antioxidants BHA, caffeic acid, sesamol, 4-methoxyphenol and catechol at low doses, either alone or in combination and modulation of their effects in a rat medium-term multi-organ carcinogenesis model. Carcinogenesis 19: 207–212. https://doi.org/10.1093/carcin/19.1.207

Huang D, 2018. Dietary antioxidants and health promotion. Antioxidants 7: 9. https://doi.org/10.3390/antiox7010009

Jayasinghe C, Gotoh N, Aoki T and Wada S, 2003. Phenolics composition and antioxidant Activity of Sweet Basil (*Ocimum basilicum L.*). Journal of Agricultural and Food Chemistry 51: 4442−4449. https://doi.org/10.1021/jf034269o

Jimoh OR, Olaore J, Olayaki LA, Olawepo A and Biliaminu SA, 2008. Effects of aqueous extract of *Ocimum gratissimum* on haematological parameters of wistar rats. Biokemistri 20: 33-37. https://doi.org/10.4314/biokem.v20i1.56436

Kaurinovic B, Popovic M, Vlaisavljevic S and Trivic S, 2011. Antioxidant Capacity of *Ocimum basilicum L. and Origanum vulgare L.* extracts. Molecules 16: 7401-7414. https://doi.org/10.3390/molecules16097401

Kebede M and Admassu S, 2019. Application of antioxidants in food processing industry: Options to improve the extraction yields and market value of natural products. Advances in Food Technology and Nutritional Sciences Open Journal 5: 38-49. https://doi.org/10.17140/AFTNSOJ-5-155

Khosro I, Pahlaviani MR, Massiha A, Bidarigh S, Giahi M and Muradov PZ, 2012. Analysis of the phytochemical contents and anti-microbial activity of *Ocimum basilicum L.* International Journal of Molecular and Clinical Microbiology 2: 141-147.

Kobus-Cisowska J, Szczepaniak O, Szymanowska-Powałowska D, Piechocka J, Szulc P and Dziedziński M, 2020. Antioxidant potential of various solvent extract from *Morus alba* fruits and its major polyphenols composition. Ciência Rural, Santa Maria 50: 1. https://doi.org/10.1590/0103-8478cr20190371

Li Z, Xu X, Leng X, He M, Wang J and Cheng S, 2017. Roles of reactive oxygen species in cell signaling pathways and immune responses to viral infections. Archives of Virology 162: 603-610. https://doi.org/10.1007/s00705-016-3130-2

Lourenço SC, Moldão-Martins M and Alves VD, 2019. Antioxidants of natural plant origins: from sources to food industry applications. Molecules (Basel, Switzerland) 24: 4132. https://doi.org/10.3390/molecules24224132

Maji AK and Banerji P, 2015 *Chelidonium majus L.* (Greater celandine): A review on its phytochemical and therapeutic perspectives. International Journal of Herbal Medicine 3: 10-27. https://doi.org/10.10.22271/FLORA.2015.V3.I1.03

Meitha K, Pramesti Y and Suhandono S, 2020. Reactive oxygen species and antioxidants in postharvest vegetables and

fruits, International Journal of Food Science 2020: 1-11. https://doi.org/10.1155/2020/8817778

Muralidharan A and Dhananjayan R, 2004. Cardiac stimulant activity of *Ocimum basilicum Linn* extracts. Indian Journal of Pharmacology 36: 163-166.

Narasimha SL and Chandrasekar K, 2019. Principles of chromatography method development, biochemical analysis tools - Methods for Bio-Molecules Studies, Oana-Maria B, Cornel B and Nasser SA, IntechOpen. https://doi.org/10.5772/ intechopen.89501

Niture SK, Rao US and Srivenugopal KS, 2006. Chemopreventative strategies targeting the MGMT repair protein: Augmented expression in human lymphocytes and tumor cells by ethanolic and aqueous extracts of several Indian medicinal plants. International Journal of Oncology 29: 1269-1278. https://doi.org/10.3892/ijo.29.5.1269

Panche AN, Diwan AD and Chandra SR, 2016. Flavonoids: an overview. Journal of Nutritional Science 5: 47. https://doi.org/10.1017/jns.2016.41

Pelletier SW, Chokshi H and Desai HK, 2004. Separation of diterpenoid alkaloid mixtures using vacuum liquid chromatography. Journal of Natural Product 49: 02-92, https://doi.org/10.1021/np50047a021

Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D and Bitto A, 2017. Oxidative stress: Harms and benefits for human health. Oxidative Medicine and Cellular Longevity 2017: 1-13. https://doi.org/10.1155/2017/8416763

Ruirui S, Qi W, Lin Z and Zhenyu Y, 2019. Advances on antioxidants in research and applications. E35 Web Conferences 131: 1-5 https://doi.org/10.1051/e3sconf/201913101009

Saha S, Mukhopadhyay MK, Ghosh PD and Nath D, 2012. Effect of methanolic leaf extract of *Ocimum basilicum* on benzene induced hematotoxicity in mice: Evidence based complementary and Alternative Medicine 2012: 176385. https://doi.org/10.1155/2012/176385

Sarikurkcu C, Tepe B, Daferera, D, Polissiou M and Harmandar M, 2008. Studies on the antioxidant activity of the essential oil and methanol extract of *Marrubium globosum subsp. globosum (Lamiaceae)* by three different chemical assays. Bioresource Technology 99: 4239–4246. https://doi.org/10.1016/j.biortech.2007.08.058

Sobhy H, AboElnaga TR, Behour TS and Razin EA, 2021. *In vitro* trypanocidal activity of essential oils of some plants against *Trypanosoma evansi*. International Journal of Veterinary Science 10: 191-195. https://doi.org/10.47278/journal.ijvs/2021.043

Tan BL, Norhaizan ME, Liew WP and Rahman HS, 2018. Antioxidant and oxidative stress: A mutual interplay in age-related diseases. Frontiers in Pharmacology 9: 1162-1173. https://doi.org/10.3389/fphar.2018.01162

Then M, Szentmihályi K, Sárközi A and Szöllôsi V, 2003. Examination on antioxidant activity in the greater celandine (*Chelidonium majus L.*) extracts by FRAP method. Acta Biologica Szegediensis 47: 115-117.

Yashin A, Yashin Y, Xia X and Nemzer B, 2017. Antioxidant activity of spices and their impact on human health: A Review. Antioxidants 6: 70. https://doi.org/10.3390/antiox6030070

Zengin WG, Aktumsek A, Guler GO, Cakmak YS and Yildiztugay E, 2011. Antioxidant properties of methanolic extract and fatty acid composition of *Centaurea urvillei* DC. subsp. Hayekiana. Record of Natural Products 5: 2123-2132.

Zhang WM, Lin HB and Zhang HD, 2009. Antioxidant activities of extracts from *Areca* (*Areca catectu* L.) flower, husk and seed. Electronic Journal of Environmental Agricultural and Food Chemistry 8: 740–748. https://doi.org/10.5897/AJB09.411

Ziemlewska A, Zagórska-Dziok M and Nizioł-Łukaszewska Z, 2021. Assessment of cytotoxicity and antioxidant properties of berry leaves as by-products with potential application in cosmetic and pharmaceutical products. Scientific Reports 11: 3240. https://doi.org/10.1038/s41598-021-82207-2