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Antioxidant and Hepatoprotective properties of *Helianthus annuus* seed extract against paracetamol-induced liver toxicity

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ABSTRACT

This experiment aimed to evaluate the antioxidant and hepatoprotective effect of methanolic extract of *Helianthus annuus* (sunflower) seeds against paracetamol induced liver injury. Four groups of rats ($n = 3$) were used and administered orally once daily with *H. annuus* methanolic extract (100, 300, and 500 mg/kg) for 7 days, followed by the induction of hepatotoxicity using acetaminophen. The blood and liver samples were subjected to liver function biochemical and lipid peroxidation assay. The extract was also subjected to in-vitro antioxidant study using the 2, 2-diphenyl-1-picrylhydrazyl radical scavenging assay. The total phenolic content and total flavonoid content were also evaluated. The extract was found to have a total flavonoids of 7.72 ± 2.3 mg/g quercetin equivalence and total phenolic content of 40.60244 ± 1.14 mg/g Gallic acid equivalence. *H. annuus* methanolic extract exhibited a significant decrease ($P < 0.0001$) in Alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase enzyme levels. The extract exhibited antioxidant activity and contained high total phenol content. *H. annuus* methanol extract was found to possess a potential hepatoprotective activity that could be partly attributed to its antioxidant activity and high phenolic content.

Keywords: Hepatoprotective, Antioxidant, *Helianthus annuus*, Acetaminophen, DPPH, Lipid peroxidation.

INTRODUCTION

Good and strong health is the priority for every individual; however, it is difficult attaining it by most people due to the existence and increasing number of new infectious diseases with no effective therapies available. Waning of effective drugs currently used in disease management and development of resistant microorganism which has been stated as one of the greatest threats to human health by the World Health Organization [1]. These have made it a necessity to research for new potent drugs to solve these problems to help improve the health of people. Medicinal plants serve as the world's major source of drug and alternative in solving health problems including degenerative effects of reactive radicals, combating antimicrobial resistant and management of existing and emerging diseases [2-4]. Hence it is significant to explore new chemicals of plant origin to help combat the emergence of resistant microorganisms in the management of diseases.

Oxidative stress results from the production of large quantities of reactive chemical species (oxygen and nitrogen reactive radicals) in the body to exceed the endogenous antioxidant capacity. Reactive free radicals' productions are inevitable in the body. The effect of these radicals in humans is known to be the root causes of many diseases including liver cirrhosis, sclerosis, diabetes, multiple sclerosis, inflammatory bowel disease, scleroderma etc. and autoimmune diseases like systemic lupus erythematosus and rheumatoid arthritis [5].

According to Hassler *et al.* [6], oxidative stress generated from metabolism of chemicals including drugs used to treat tuberculosis, pain medications especially acetaminophen and drugs for cancer chemotherapy etc., contributes to liver damage. Thus, substances that are hepatotoxic cause's damage to the liver through the production of high concentrations of reactive oxygen species hence an antioxidant can help harness and protect the liver against oxidants [7-8].

Sunflower seeds have been used for different purposes among different people and countries and have gained attention due to the substantial contribution in health [9-11]. Sunflower seeds serves as an edible oil crop use as snack food in a form of a 'nutmeats' or in a form of candy. The oil obtained from the seeds is used to produce margarine, salad and frying oil. It is employed traditionally to treat microbial infections and other diseases. It is use as an antipyretic, anti-inflammatory, vermifuge, diabetes mellitus and stomach problems [11]. Currently, there is the need to search for more potent natural antioxidants with less side effects to replace the few available synthetic antioxidant with numerous unwanted side effects.

Identification of free radical scavengers of natural origin are important in prevention and treatment of human diseases caused by these reactive radicals and their disruptive effect on normal human health [12]. Sunflower has been reported to possess effective radical scavenging property. In line with this, the antioxidant and hepatoprotective effect of the extract of sunflower seed were evaluated in-vivo models.

MATERIALS AND METHOD

Plant Collection and Extraction

The *Helianthus annuus* seeds used in the experiment were purchased from Herbs Ghana limited at Kumasi in the Ashanti region on 20th November, 2019. The commercial seeds were authenticated at the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST). A sample (KNUST/HMI/2019/5001) was deposited at the Faculty of Pharmacy and Pharmaceutical Sciences Herbarium, KNUST.

The seeds were washed and further dried under the sun for 5 days. It was coarsely powdered with a hammer mill and cold macerated with methanol. The filtrate was concentrated using a rotary evaporator (R-114, Buchi, Switzerland) and oven dried at 30°C. The resulted paste was stored in an amber container and kept at 20 °C.

Reagents/Chemicals

Quercetin, Folin-Ciocalteu, Gallic acid, 2, 2-diphenyl-1-picrylhydrazyl, 96 well plate (Sigma Aldrich LTD, UK), AlCl₃·6H₂O, NaOH (Eurostar Scientific LTD, UK), ascorbic acid and sodium carbonate (Finkem LTD, UK), NaNO₂ and Methanol (Fisher Scientific limited, UK).

Animals

Sprague-Dawley rats purchased from Nugochi Memorial Institute, and they were housed in stainless steel cages of dimensions; 34 cm x 57 cm x 40 cm at a population of 10 rat per cage. Standard rodent feed and clean water were provided *ad libitum*. The *vivarium* temperature was maintained at about 29 °C and a 12-hour light-dark cycle was maintained using overhead incandescent illumination. Daily cage maintenance was done and the animals were checked daily for normal behavior until the experimental day. The experimentation was done in accordance with the National Institute of Health guidelines for care and use of laboratory animals (NIH Department of Health Services publication number: 83-23, revised 1985; Committee, 2011) and was approved by the Ethics Committee, Department of Pharmacology, College of Health Sciences of the Kwame Nkrumah University of Science and Technology.

Phytochemical screening

Phytochemical analysis was carried out using standard protocols [13] to determine the presence or absence of the secondary plant metabolites. The presence of alkaloids, tannins (polyphenols), reducing sugars, plant sterols, flavonoids and triterpenoids was qualitatively investigated.

Antioxidant effects of *Helianthus annuus* extract

DPPH free radical scavenging activity

The *in vitro* antioxidant activities of the methanol extract of *Helianthus annuus* seeds extract was determined by the 2, 2-diphenyl-1-

picrylhydrazyl (DPPH) scavenging method described by Esmaeili *et al.* [14], with slight modification. 1 ml of different methanolic concentrations of the test extract (1, 0.5, 0.25, and 0.125 mg/ml) were prepared by the serial dilution method. To each concentration, 2 ml of 0.5 mM DPPH solution was added. The mixtures were allowed to stand in the dark for 30 minutes at room temperature and absorbance was measured at 517 nm afterwards. Same approach was used with same concentrations for ascorbic acid (vitamin C) which was used as the standard. A blank experiment was performed with 2.0 ml of the 0.5 mM DPPH solution and 1.0 ml of methanol as the control. The antioxidant activity was calculated as %DPPH Scavenging using the formulae below.

$$\% \text{DPPH Scavenging effect} = \frac{\text{ABSORBANCE}_{\text{CONTROL}} - \text{ABSORBANCE}_{\text{TEST}}}{\text{ABSORBANCE}_{\text{CONTROL}}} \times 100$$

Total phenolic content

The extract was assayed for the total phenolic content by using Folin-Ciocalteu method described by Esmaeili *et al.* [14]. Serial dilution was used to prepare 1, 0.5, and 0.25, mg/mL concentrations of the sunflower seeds extract using methanol in different test tubes followed by addition of 2mL of 2% (w/v) sodium carbonate solution. 100µL of Folin-Ciocalteu reagent was added after allowing the mixture to stand for about 5 minutes. Absorbance was measured at 750 nm after keeping the mixture at room temperature for about 30 minutes. Absorbance of varying concentrations of Gallic acid (100, 50, 25, 12.5, and 6.25 µg/ml) were also measured as described for the standard curve. The total phenolic content was calculated as mg Gallic acid equivalent (GAE) per gram of dried mass of the sample.

Total flavonoid content

The method described by Esmaeili *et al.* [14] was used to assay the flavonoid content of the sunflower extract. Varying concentrations (1, 0.5, and 0.25mg/mL) of the extract were prepared using methanol. 300 µL of each concentration was measured into separate test tubes. 3.4 mL methanol, 150µL of 0.5M NaNO₂, and 150µL of 0.3M AlCl₃·6H₂O were added to each test tube. The mixture was allowed to stand for about 5 minutes and 1mL of NaOH (1M) was added afterwards. Absorbance for each concentration at 510 nm was measured. Same approach was used for quercetin at varying concentrations of 100, 50, 25, 12.5 and 6.25µg/ml. A standard curve was drawn from the quercetin absorbance and used to calculate the total flavonoid content in milligram of quercetin equivalents (QE) per gram dry matter of extract.

Hepatoprotective activity of *Helianthus annuus* extract

Experimental design

Twelve albino rats were randomly grouped into four groups of three. All the rats were randomly assigned to the following four groups: group 1 received paracetamol, 600 mg/kg, group 2 received 600mg/kg APAP + 100 mg/kg SSE, group 3 received 600 mg/kg APAP + 300 mg/kg SSE, and group 4 received 600mg/kg APAP + 400 mg/kg SSE). Group 2 to 4 received the APAP doses and the SSE concomitantly twice daily orally for 5 days whiles group 1 received only the APAP doses twice daily for 5 days. Twelve hours after the last doses, blood were taken from each rat for lipid peroxidation test and liver function test [15].

Liver function test

Biochemical analyses were performed on serum collected for the determination of the following parameters: fasting blood glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL), total protein, albumin, calcium, urea and creatinine. All analyses were carried out using an automated clinical chemistry analyzer (Flexor Junior®, Vital Scientific, AC Dieren, Netherlands).

Serum Lipid Peroxidation

Serum from the blood samples collected were used to determine the level of lipid peroxidation by assaying for the amount of thiobarbituric acid reactive substance (TBARS) and malondialdehyde (MDA) produced by following the method described by Samuel *et al.* [16]. 1 ml serum of each blood sample was added to 3 ml of a mixture of 20 % TCA and 0.5 % TBA separate test tubes. The mixtures were heated over water bath for 30 minutes and cooled in ice for 5 minutes. The mixture was then centrifuge at 2000 rpm about 10 minutes. Absorbance of the supernatant were determined at 532 nm and then read again at 600 nm with a UV spectrophotometer.

$$\text{nmol MDA/mg protein} = \frac{\text{Absorbance}_{532\text{ nm}} - \text{Absorbance}_{600\text{ nm}}}{155 \times \text{total protein}} \times 10^6$$

Statistics

In the hepatoprotective study, the ordinary one-way (*treatment versus control*) analysis of variance (ANOVA) followed by the Dunnett's comparison test was employed. The data in this study is presented as the mean ± standard deviation (SD)/standard error of mean (SEM).

RESULTS

Phytochemical analysis

Phytochemical analysis of the *H. annuus* extract revealed the presence of the following groups of phytochemicals indicated in *Table 1* below. The extract tested positive for the presence of free alkaloids, flavonoid, phenolics, reducing sugars, saponins, tannins, triterpenoid and Plant sterol.

Table 1: Results of phytochemical screening of the methanolic extract of sunflower seeds.

Test	Results
Tannins	Positive
Phenolics	Positive
Flavonoid	Positive
Saponin	Positive
Alkaloids	Positive
Triterpenoids	Positive
Plant Sterols	Positive

Positive denotes the phytochemical is present.

Antioxidant activity

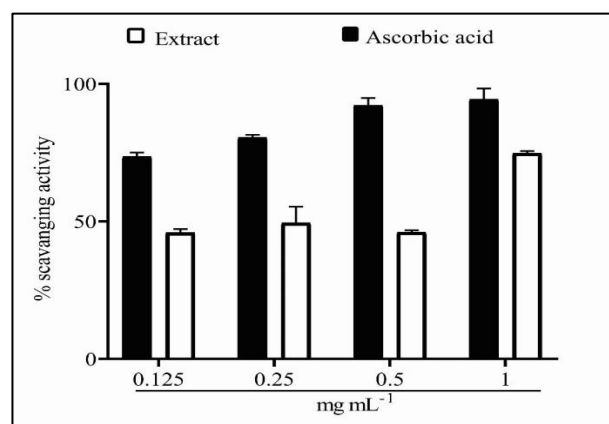


Figure 1: The DPPH radical scavenging effects of *H. annuus* methanolic extract. Data is presented as the mean ± SD, n = 3.

The Gallic acid equivalence (GAE) and the Quercetin acid equivalence (QE) in the total phenol content and total flavonoid content assays respectively had a repeated pattern as the DPPH radical scavenging assay. The equivalent amount of phenols and flavonoids were evaluated using the calibration curves of Gallic acid and quercetin respectively (Fig. 2). *H. annuus* extract had a GAE of 40.60 ± 1.14 mg/g and QAE of 7.72 ± 2.3 mg/g. The percentage DPPH radical scavenging activity as shown in figure 1 above indicate that the activity of the extract is concentration dependent with 1 mg/mL producing the highest activity (Figure 1).

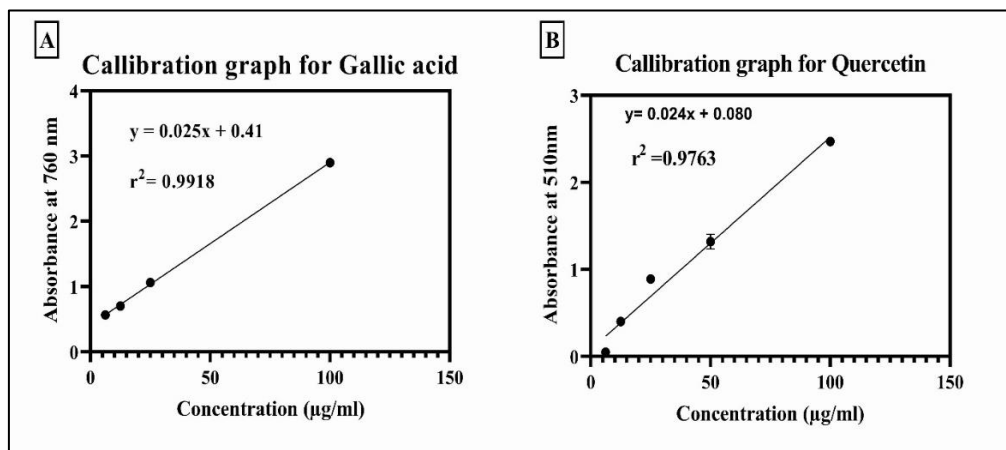


Figure 2: Calibration curves of the Gallic acid and Quercetin

Table 2: Results of the total phenols and total flavonoids content of the methanolic extract of sunflower seed.

Test	Results
Total flavonoids	7.72 ± 2.3 mg/g QE
Total phenolic	40.60 ± 1.14 mg/g GAE

Where QE = Quercetin equivalent and GAE = Gallic acid equivalent

Hepatoprotective effects of *H. annuus* methanolic seed extract

Effects of *H. annuus* extract Lipid peroxidation

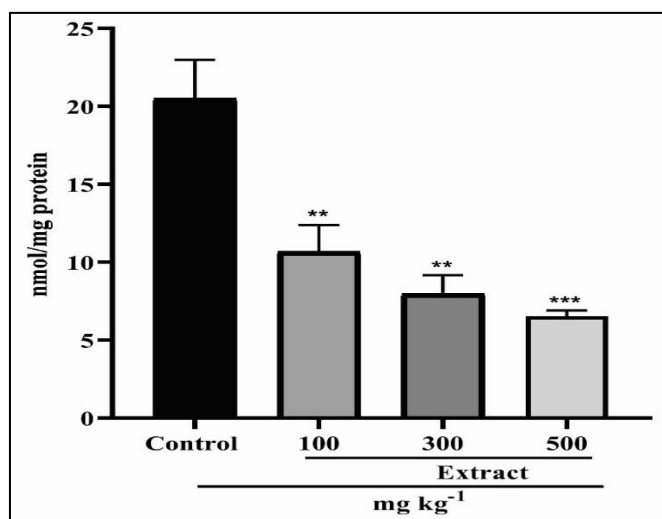


Figure 3: Effects of the extract on lipid peroxidation in acetaminophen-induced toxicity. Data is expressed as mean MDA concentration/mg protein ± SEM (n = 3). ** P < 0.01 and ***P < 0.001 as compared to the acetaminophen group using one-way ANOVA followed by Dunnet’s multiple comparison post hoc test.

Effects of *H. annuus* methanolic extract on the liver biochemistry

In this study, Paracetamol administration caused significant (F (27, 80) = 8.35, P < 0.0001) elevation in the ALT, AST, and ALP serum marker

Malondialdehyde (MDA), a direct product of lipid peroxidation, was elevated significantly (F (3, 8) = 15.01, P = 0.0012) to 20.52 ± 2.47 nmol/mg protein in the acetaminophen treated group (Fig. 3). This evident oxidative stress induced by acetaminophen treatment was significantly (F (3, 8) = 15.01, P = 0.0012) inhibited by *H. annuus* methanolic extract. At 500 mg/kg, *H. annuus* methanolic extract suppressed the elevated MDA level to 6.54 ± 0.37 nmol/mg protein while 100 and 300 mg/kg significantly suppressed the diseased levels of MDA to 10.69 ± 1.70 and 7.99 ± 1.84 nmol/mg protein, respectively (Figure 3).

level in non-treated group (Control) (Table 3). However, oral administration of *H. annuus* (100 - 500 mg/kg) showed an ability to counteract the toxic effect of Paracetamol by decreasing the level of these enzymes.

Table 3: Effects of *H. annuus* on the liver biochemistry

Test	Extract mg/kg							
	Control	SD	100	SD	300	SD	500	SD
Globulins	44.37	2.12	32.00	4.36	46.83	7.02	39.07	10.71
Albumen	28.53	1.05	22.93	1.77	29.07	2.24	24.47	4.20
Total protein	72.90	2.87	54.93	4.15	75.90	7.10	63.53	14.52
ALP	1255.37	420.78	549.30	83.56 ^a	153.43	34.74 ^a	569.87	252.44 ^a
AST	415.73	116.96	225.73	14.58 ^d	274.07	52.94	208.30	74.31 ^d
ALT	331.47	173.82	134.33	21.55 ^d	132.37	35.37 ^d	69.07	29.33 ^c
GGT	<6	0.00	6.00	0.00	6.00	0.00	6.00	0.00
Indirect bilirubin	3.50	0.95	2.20	0.89	0.70	0.10	3.30	2.33
Direct bilirubin	2.83	0.71	2.87	1.80	2.23	1.53	6.03	0.55
Total bilirubin	6.33	1.58	5.07	2.08	2.40	0.70	9.33	2.30

Values are expressed as means ± S.E.M. (n = 3). ^d(* P) < 0.05, ^c**P < 0.01 and ^a**** P < 0.0001 as compared to the acetaminophen group using one-way ANOVA followed by Dunnet’s multiple comparison post hoc test.

DISCUSSION

Plants are endowed with numerous secondary metabolites which are not necessarily produced under all conditions in contrast to primary metabolites that are directly involved in growth, development or reproduction of organisms. The secondary metabolites such as

alkaloids, flavonoids, tannins, glycosides, triterpenoids and plant sterols in various plant extracts have been found to be responsible for their respective pharmacological properties [17, 18]. These biological activities are basically due to the array of phytochemicals that act individually or in synergism and a mixture of such phytochemicals can show a broad spectrum of pharmacological activities including wound

healing properties [19]. Preliminary phytochemical screening of *H. annuus* seed extract for secondary metabolites showed that it contained alkaloids, saponins, tannins, flavonoids and triterpenoids. Tannins, flavonoids, saponins, glycosides, alkaloids and triterpenoids are known to possess therapeutic advantages including antioxidant effects [20, 21, 22]. Therefore, this chemical constituent may be responsible for the hepatoprotective and antioxidant effects exhibited by *H. annuus* methanolic seed extract. The antioxidant test provides information on the interaction of the substances with a stable free radical. The degree of reduction and increase in the optical density is an indication of the radical scavenging and the reducing (antioxidant) power of a test substance [23]. It was observed that the free radical scavenging activity of extracts were significant and comparable to the reports in previous research [9, 10, 11]. The extracts served as free radical scavenger or acting possibly as primary oxidants and inhibited oxidative stress induced by NAPQI in the liver toxicity study.

An overdose of paracetamol will bring about hepatocellular injury that leads to the elevation of levels of serum enzyme that is not supposed to be in circulation or in lesser amount (eg aminotransferases, phosphatases, gamma-glutamyl transferase, dehydrogenase, lactate dehydrogenase) hence levels of these enzymes in serum is used to assess the function and injury of the liver. Acetaminophen is one of the most commonly used agents in whole animal experimental studies of hepatotoxicity. The toxic effects of acetaminophen are largely due to highly reactive toxic metabolite, N-acetyl-para-benzo-quinone imine (NAPQI) [8]. An exaggerated increase in NAPQI concentration causes an oxidative stress via the depletion of glutathione (GSH) levels, generation of peroxynitrite and cessation of ATP synthesis [24, 25]. The free activated radicals bind irreversibly to lipids rich in polyunsaturated fatty acids, thus leading to lipid peroxidation. Malondialdehyde (MDA) is one of the products of lipid peroxidation that cause damage to the cell membrane of mammalian tissues [8]. MDA formation is one of the main causes of liver toxicity, this is evidenced by the elevation of TBARS and a decline in the activity of superoxide dismutase, catalase and GSH [25]. Also, an elevation in the serum enzymes is evidenced in severe liver damage. Taking cognizance of this, an increase in bilirubin levels infers icterus, with an increase in transaminases a clear indication of cellular leakage and a loss of the functional integrity of the hepatic cell membrane. The current study investigated the hepato-protective and the antioxidant effects of *H. annuus*.

Administration of acetaminophen caused an increase in ALT, AST and ASP indicating liver injury. Even though these enzymes are not only found in the liver, an elevation of serum ALT is noted for liver injury in higher organism. Lipid peroxidation occurs when an amount of free radicals generated in the body exceeds the antioxidant capacity of the body. The remaining free radicals then react with macromolecules of the liver cells (mostly membrane PUFA) resulting in the cell damage. The damaging effect of paracetamol overdose is mediated via peroxidation of the lipid components of the hepatocytes [25]. From the study, the serum MDA levels revealed that the extract was able to decrease lipid peroxidation in a dose depending manner. MDA level was higher in rats that received only paracetamol while the level decreased drastically with those that received the extract. The liver is the main source of blood protein synthesis especially albumin (with the exception of the immunoglobulins) and its damage leads to reduction in blood plasma protein levels. Half-life of albumen is 21days hence blood protein can be normal in the early phase of liver injury. This may be the reason why the effect on total protein and albumen are very similar and inconsistent. Chronic studies need to be conducted to know the actual effects on albumen and total protein level. Bilirubin was

determined to assess the hepatoprotective effect of the extract. Paracetamol administration caused an increased level of indirect bilirubin in the rats that received only the paracetamol possibly due to liver injury. However, there was a significant decrease in indirect bilirubin in the rats that treated with the extract at 100 and 300 mg/kg. More so, there was a significant decrease in ALT, AST and ASP in the rats that received the extract together with the paracetamol indicating that, the extract protects the liver under oxidative stress conditions induced by acetaminophen. The ALT, AST and ASP level decreases with an increase in the extract concentration indicating that, the extract's protective effect increases with increasing concentration.

CONCLUSION

In conclusion, this study suggests that, the methanolic seed extract of *Helianthus annuus* (Sunflower) possesses hepatoprotective and antioxidant effects.

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Conflict of interest

There is no conflict of interest.

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REFERENCES

1. Smith R. D. and Coast J. Antimicrobial resistance: a global response. *Bulletin of the World Health Organization*. 2002; 2:126-133.
2. Carmona F and Pereira AMS. Herbal medicines: Old and new concepts, truths and misunderstandings. *Brazilian Journal of Pharmacognosy*. 2013; 23(2):379-385.
3. Moreira D de L, Teixeira SS, Monteiro MHD, De-Oliveira ACAX and Paumgarten FJR (2014) Traditional use and safety of herbal medicines. *Brazilian Journal of Pharmacognosy*. 2014; 24(2):248-257.
4. Wodah D and Asase A. Ethnopharmacological use of plants by Sisala traditional healers in northwest Ghana. *Pharmaceutical Biology*. 2012; 50(7):807-815.
5. Norma F. S, Raúl S, Claudia V, and Beatriz H. Antioxidant Compounds and Their Antioxidant Mechanism. *IntechOpen*. 2019; 1-20.
6. Hassler SN, Johnson KM and Hulsebosch CE. Reactive oxygen species and lipid peroxidation inhibitors reduce mechanical sensitivity in a chronic neuropathic pain model of spinal cord injury in rats. *Journal of Neurochemistry*. 2014; 131(4): 413-417.
7. Tian Z, Liu H, Su X, Fang Z, Dong Z, Yu C and Luo K. Role of elevated liver transaminase levels in the diagnosis of liver injury after blunt abdominal trauma. *Experimental and Therapeutic Medicine*. 2012; 4(2):255-260.
8. Yoon E, Babar A, Choudhary M, Kutner M and Pysropoulos N. Review Article Acetaminophen-Induced Hepatotoxicity: a Comprehensive Update. *Journal of Clinical and Translational Hepatology*. 2016; 4:131-142.

9. Ahn JH, Kim YP, Seo EM, Choi YK and Kim HS. Antioxidant effect of natural plant extracts on the microencapsulated high oleic sunflower oil. *Journal of Food Engineering*. 2008; 84(2):327-334.
10. Hrádková I, Merkl R, Šmidrkal J, Kyselka J and Filip V. Antioxidant effect of mono- and dihydroxyphenols in sunflower oil with different levels of naturally present tocopherols. *European Journal of Lipid Science and Technology*. 2013; 115(7):747-755.
11. Karamać M, Kosińska A, Estrella I, Hernández T and Dueñas M. Antioxidant activity of phenolic compounds identified in sunflower seeds. *European Food Research and Technology*. 2012; 235(2):221-230.
12. Panahi KE, Ahmadi K, Sadeghi H, Sadeghi H, Dadgary F, Danaei N and Aghamaali MR. Hepatoprotective effect of *Stachys pilifera* ethanol extract in carbon tetrachloride-induced hepatotoxicity in rats. *Pharmaceutical Biology*. 2017; 55(1):1389-1393.
13. Senguttuvan J, Paulsamy S and Karthika K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities. *Asian Pacific Journal of Tropical Biomedicine*. 2014; 4(1):S359-S367.
14. Esmaili, A. K., Taha, R. M., Mohajer, S., & Banisalam, B. Antioxidant Activity and Total Phenolic and Flavonoid Content of Various Solvent Extracts from In Vivo and In vitro Grown *Trifolium pratense*. *BioMed research international*, 2015.
15. Avwioro G, Iyiola S and Aghoghovwia B. Histological and biochemical markers of the liver of Wistar rats on subchronic oral administration of green tea. *North American Journal of Medical Sciences*. Medknow. 2010; 2(8):376-380.
16. Samuel O, E, Eze V. C, Onoja, M.I and Eze U.A. (2014). Evaluation of *in vitro* and in vivo Antioxidant Potentials of *Aframomum melegueta* methanolic seed extract. *Journals of tropical medicine*, 2014; 1-5.
17. Mbagwu HOC, Anene RA and Adeyemi OO. Analgesic, antipyretic and anti-inflammatory properties of *Mezoneuron benthamianum* Baill (Caesalpiniaceae). *Nigerian quarterly journal of hospital medicine*, 2007; 17(1):35-41.
18. Maganha EG, da Costa Halmenschlager R, Rosa RM, Henriques JAP, de Paula Ramos ALL and Saffi J (2010). Pharmacological evidences for the extracts and secondary metabolites from plants of the genus *Hibiscus*. *Food Chemistry*, 2010; 118(1):1-10.
19. Kubmarawa D, Khan ME, Punah AM and Hassan M. Phytochemical screening and antimicrobial efficacy of extracts from *Khaya senegalensis* against human pathogenic bacteria. *African Journal of Biotechnology*. 2008; 7(24):4563-4566.
20. Somava LI, Shode FO, Ramnanan P, Nadar A. Antihypertensive, antiatherosclerosis and antioxidant activity of triterpenoids isolates from *Olea europaea* subspecies of *Africaca* leaves. *Journal of Ethnopharmacology*. 2003; 84(2, 3):299-305.
21. Taguri T, Tanaka T and Kouno I. (2004). Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. *Biological & pharmaceutical bulletin*, 2004; 27(12):1965-1969.
22. Gülçin I, Küfrevioğlu Öİ, Oktay M and Büyükkuroğlu ME. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). *Journal of Ethnopharmacology*, 2004; 90(2):205-215.
23. Prior, R.L., Wu, X. and Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*. 2004; 53:4290-4302
24. Bekhouche K, Ozen T, Boussaha S, Demirtas I, Kout M, Yildirim K, Zama D, Benayache F and Benayache S. Hepatoprotective effects of the n-butanol extract from *Perralderia coronopifolia* Coss. against PCP-induced toxicity in Wistar albino rats. *Environmental Science and Pollution Research*. Springer Verlag 2019; 26(30):31215-31224.
25. Okokon JE, Simeon JO and Umoh EE. Hepatoprotective activity of the extract of *Homalium letestui* stem against paracetamol-induced liver injury. *Avicenna journal of phytomedicine*. Mashhad University of Medical Sciences 2017; 7(1):27-36.

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