



Antioxidant, anti-inflammatory and cytotoxicity properties of medicinal plants used for producing herbal products against hepatic diseases in Ghana

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ABSTRACT

Medicinal plants have become the option for management of liver diseases because of their availability, cost effectiveness and lesser side effects compared to pharmaceutical drugs. Various parts of plants including roots, leaves, stem, bark and seed have been reportedly used to treat liver diseases including jaundice, hepatitis, hepatosteatosis, hepatocellular carcinoma, hepatobiliary disorders and hepatocellular carcinoma. Phytochemicals exhibit anti-oxidant and anti-inflammatory properties due to the presence of acidic polyhydroxyl groups in the phenols. These hydroxyl groups quench free radicals from oxygen, nitrogen and sulphur reactive species thereby inhibiting oxidative stress, inflammation and fibrogenic processes. The objective of the study was to determine the antioxidant, anti-inflammatory and cytotoxicity properties of medicinal plants for the purpose of selecting more efficacious raw materials for herbal products. Ethanolic and methanolic extracts of 13 medicinal plants were test for level of total phenols, flavonoids, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) reducing potential, ferric reducing (FRAP), total antioxidant binding capacity (ABTS), red cell antihemolysis assay and 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) cytotoxicity. Except for DPPH scavenging activity, there was significance of difference between the methanolic and ethanolic extracts, total phenols ($p = 0.03$), flavonoids ($p = 0.024$), FRAP ($p = 0.02$), ABTS ($p = 0.00$) and red cell hemolysis ($p = 0.010$). There was correlation between phytochemicals, phenols and flavonoids and antioxidant markers, DPPH, FRAP, ABTS and red cell hemolysis ($p < 0.05$). *Syzygium aromaticum*, *Curcuma longa*, *Taraxacum officinalis* and *Moringa oleifera* exhibited the highest inhibitory anti-inflammatory and antioxidant properties. The study indicates that, phytochemical, antioxidant and inflammatory properties varied with the kind of plant, concentration of extract, extractant and polyphenol content. These properties should be evaluated in selecting materials for drug formulation.

1. Introduction

Medicinal plants in their raw or processed products have been used to manage various conditions that affect the human liver due to their availability, efficacy and cost effectiveness compared to pharmaceutical

medicines (Nkempu et al., 2021; Akhrai and Okafor, 2021). Medicinal plants parts including roots, stem, bark, fruit, seed, sap, pulp, spike, oil, whole plant and leaves (Aly et al., 2020; Acheampong et al., 2021; Sagar et al., 2022; Salih et al., 2022) have been used to treat jaundice, alcohol hepatitis, hepatosteatosis, hepatobiliary disorders, hepatoma,

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hepatocarcinoma and viral hepatitis (Salama et al., 2016; Sani et al., 2020; Dongsogo et al., 2023).

Hepatoprotective and regenerative capacity of various herbal plants and products have been demonstrated in-vitro against several induced hepatotoxicity in animal models. Oyibo et al. (2021), reported of ethanol extract of *Vitellaria paradoxa* (Gaertn, F) leaves against sodium-arsenite induced toxicity in male Wistar rats. Patel et al. (2019) demonstrated the hepatoprotective effect of methanolic extract of *Syzygium aromaticum* against hydralazine induced toxicity in fresh liver tissue. *Spathodea campanulata* Leaf extract against *Salmonella* infected and paracetamol-induced Swiss albino mice has been reported by Akh-raiyi and Okafor (2021).

The bases for the hepatoprotective and regenerative activity of medicinal plants and their products against liver disorders in in-vitro, animal and clinical setting is the presence of varied phytochemicals and metabolites including saponins, tannins, triterpenes and alkaloids as in *Mormodica charantia* (Zahra et al., 2012; Agyemang et al., 2020), Sulphur-containing compounds as in *Allium sativum* (Jamuna et al., 2018; Ezeigwe et al., 2022), hydroxybenzoic acids, hydroxycinnamic acids, hydroxyphenylpropenols, caffeic acid, ferulic acid, salicylic acid, eugenol, kaempferol, beta-caryophyllene, vanillin, crategolic acid, quercetin, rhamnetin, eugenitin, oleanolic acid, stigmaterol and ellagic acid as in *Syzygium aromaticum* (Jose et al., 2018; Patel et al., 2019), alpha and β -turmerones, demethoxycurcumin, bis-demethoxycurcumin and dihydrocurcumin as in *Curcuma longa* (Sagar et al., 2022; Wang et al., 2022).

Mechanisms underlining the biochemical actions of these plants metabolites against hepatic disorders include inhibition of oxidative stress, inhibition of expression of pro-inflammatory cytokines and agents as well as the supply of micronutrients and vitamins (Verma, 2018; Ejuiwa et al., 2022). Inhibition of the synthesis of triacylglyceride through reduction in mRNA expression of adipocyte transcription factors has also been suggested (Zahra et al., 2012). Phytochemicals also increase activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) (Jamuna et al., 2018; Nwaogu et al., 2022). These cumulatively result in restoration of hepatic membrane integrity, quenching and mopping of free radicals, inhibition of hepatic stellate cells generation (Akbarizare et al., 2020; Dongsogo et al., 2023).

Even though inflammatory and oxidative stress pathways are activated in hepato-pathophysiology, these are often not investigated in selecting medicinal plant raw materials for the manufacturing of liver-targeting herbal products. The objective of the study was to determine the cytotoxicity, inflammatory and antioxidant activities of common medicinal plants used for formulating liver-targeting herbal products in Ghana. This is essential for determining their appropriateness and selection for used as hepatorestorative or protective agents.

2. Materials and methods

2.1. Collection, authentication and solvent extraction of plants materials

The plant materials were obtained from Botanical gardens, Water works Tamale, Ghana and identified by Botanist at the Faculty of Agriculture, Food and Consumer Sciences, University for Development Studies, Tamale Ghana with voucher number CL/JU09/23. Fresh mature leaves of *Pepper guineese*, *Taraxacum officinalis*, *Vernonia amygdalina*, *Azadirachta indica*, *Moringa olifera*, *Khaya senegalensis*, *Biden pelosa* and *Trema orientalis* were harvested early mornings. Bulbs and Rhizomes of *Allium sativum*, *Szgcium aromaticum*, *Zingibar officinalis*, *Curcuma longa* and whole plant of *Sida acuta* were also obtained.

For the hydroethanolic and methanolic extracts, 100 g of powdered shade-dried of the plant was soaked in 70 % ethanol and methanol respectively for 72 h with regular vortexing. The mixture was filtered with Whatman filter paper (Swastik Scientific Company, Mumbai, Maharashtra, India) after which the filtrate was concentrate with Rotar

evaporator (Biobase, IKA, infitek, Beijing, China) at 70°C until all the solvent evaporates. The condensate was dried in oven at 50°C till a solid extract was obtained for analysis. Duration of the study was from June 2022 to August 2023 at Kumasi, Ghana.

2.2. Test for flavonoids

The protocol for the test follows Sani et al. (2020), with slide modifications. The test samples and quercetin (standard) were dissolved in methanol and made to a final concentration of 100 mg/mL. One mL of the extract was treated with 1 mL of 2 % aluminum chloride in ethanol in a volumetric flask, and volumes were made up to 10 mL with methanol. After keeping this solution for an hour at room temperature, the absorbance was measured at 415 nm using a spectrophotometer (Thermo Scientific, Waltham, MA, USA). A quercetin standard graph was plotted and used to estimate the flavonoid concentration. The content of flavonoids in the extracts was read in triplicates and expressed in terms of quercetin equivalents.

2.3. Test for phenols

Estimation was done following Folin-Ciocalteu method as applied by Sani et al. (2020). Using gallic acid as standard, 0.5 mL of the extract solution was mixed with 2.5 mL of 1.0 N Folin-Ciocalteu reagent and incubated for 5 min. Afterwards, 2 mL of 75 g/L sodium carbonate was added followed by distilled water. After incubation at room temperature for 2 h, absorbance of reaction mixture was measured at 760 nm against methanol as blank. The total phenolic content was expressed in μ g of gallic acid equivalent (GAE) of dry plant material.

2.4. Test for DPPH scavenging activity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to measure the free radical scavenging capacity of extracts as described by Sarfo-Antwi et al. (2018) and Sani et al. (2020). A volume of 3 mL of each of the diluted extracts was placed in a test tube and 1 mL of a methanol solution of DPPH (0.1 mM) added. The mixture was then kept in the dark at room temperature for 30 min and absorbance read at 517 nm against a blank and vitamin C as the standard. The equation below was used to determine the percentage of the radical scavenging activity of each extract:

$$\text{Scavenging effect (\%)} = 100 \times ((A_s - A_o) / A_s)$$

Where, A_o is the absorbance of the blank and A_s is the sample absorbance.

2.5. Determination of red cell hemolysis inhibition

Freshly collected human red blood cells were taken and washed three time with sterile phosphate buffer (PBS, NaCl (150 mM), NaH_2PO_4 (1.9 mM), and Na_2HPO_4 (8.1 mM), pH 7.4) at room temperature and resuspended in PBS four times its volume. Each washing step was carried out by centrifuging the cells at 3000 rpm, 7 min, RT, discarding the supernatant after each wash. Three different concentrations (120 mg, 250 mg, 500 mg) of extracts were mixed with 200 mL of RBC solutions and the final reaction mixture volume was made up to 1.0 L by adding sodium phosphate buffer. The reaction mixture was then placed in water bath for 1 h at 37 °C. After the incubation time the reaction mixture was centrifuged again at 2500 rpm for 15 min. The supernatant was collected and the absorbance read at 541 nm using a multiwell plate Bio-Rad ELISA (Sigma-Aldrich, Urbana, IL, USA). The experiment was done in triplicate and mean $\pm$ S.D. was calculated. Aspirin was used as standard.

2.6. Determination cytotoxicity of ethanolic and methanolic extracts

The cytotoxicity of the extracts against HEK-293 was determined as described by Goh et al. (2021). Cells were harvested at 70–80 % confluency and centrifuged at 1200 rpm at 25 °C for 3 min. The cells were counted under a microscope before seeded into 96 well plates at 1×10^5 cells per mL. Incubation was set to 5 % CO₂ at 37 °C for 24 h to allow for cell attachment. After that, the unattached cells were removed carefully. Cells were then treated with 100 µL of 1 mg/mL of extract and made up to a final volume of 200 µL and further incubated for 20 h with incubator (Thermo Fisher Scientific, UK). Twenty µL MTT reagent (5 mg/mL) was then added into each well and incubated at 5 % CO₂, 37 °C for 4 h. The mixture of cell media and MTT were removed, and the purplish formazan crystals were dissolved in dimethyl sulfoxide (DMSO), further incubated in a dark environment for 15 min at 25 °C. The absorbance was read on Microplate Reader (Thermo Scientific, Waltham, MA, USA) at 570 nm wavelength. All experiments were performed in triplicate. Cell viability was determined using the formula below: Cell Viability = (AbsTreated cells/AbsUntreated cells) × 100.

The same procedure was repeated for the determination of cytotoxicity of the methanolic extracts of the plant species.

2.7. Determination of ferric reducing antioxidant properties (FRAP)

The protocol described by Jose et al. (2018) was used to determine the FRAP activity. A reaction mixture containing 1 mL of a solution of 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of potassium ferricyanide (30 mM), and the extract at different concentrations (50–250 µg/g), was incubated at 50 °C for 20 min. Thereafter, 2.5 mL of trichloroacetic acid (TCA; 600 mM) was added to the reaction mixture and centrifuged at 3000 rpm for 10 min. The supernatant of about 2.5 mL was collected and mixed with 2.5 mL of distilled water and 0.5 mL of FeCl₃ (6 mM). The absorbance was then determined at a wavelength of 700 nm. The blank contained all the reactants except the extract. Ascorbic acid was used as a standard. All tests were run in triplicates.

2.8. Total antioxidant binding capacity (ABTS)

The protocol described by Jose et al. (2018) was used to determine the ABTS reducing potential. A 5 mL of a 4.9 mM potassium persulfate solution was added to 5 mL of a 14 mM ABTS solution and kept for 16 h in the dark. Different concentrations of extract (50–700 µg/g) were added to the above activated pregenerated ABTS solution. This solution was suitably diluted with distilled water to yield an absorbance of 0.70 at 734 nm and then used for antioxidant assay. Ascorbic acid (50 µg/mL) was used as reference compound. A 50 µL was added to 950 µL of ABTS solution and vortexed for 10 s and after 6 min and then reduction in absorbance was recorded at 734 nm, using distilled water as a blank. Same volume of test solutions of each extract was also taken in similar manner. The result was compared with control (only ABTS solution) having absorbance 0.712 ± 0.032 .

2.9. Determination of the half-maximum inhibition (IC₅₀) concentration of the extracts

The percentage inhibition concentrations of the extracts for DPPH, ABTS, FRAP and red cell antihemolysis were calculated using the formula; Percentage Inhibition (%) = $100 \times ((A_0 - A_s)/A_0)$. Where A₀ is the standard absorbance, A_s is the extract absorbance. Standard graphs of percentage Inhibition against concentration of extracts were plotted. The IC₅₀ was calculated as the extract concentration that at 50 % of inhibition.

2.9.1. Statistical analysis

The data was subjected to analysis with IBM SPSS software version 27 (IBM Group, NewYork). Difference between ethanolic and

methanolic extracts as well as extracts concentration were analysed using analysis of variance (ANOVA). Correlation of flavonoids and phenols with antioxidants and inflammatory makers were analysed using correlation test and Pearson co-efficient test. A p-value less than 0.05 was deemed statistically significant. Continuous variables are expressed as mean ± standard deviation while categorical data are expressed in percentages.

3. Results

3.1. Phytochemical content of ethanolic and methanolic extracts of the plants extracts

The total phenolic compounds and flavonoids of ethanolic and methanolic extracts of the plant materials are presented in Table 1. For the flavonoids of ethanolic extracts, *Sida acuta* recorded the highest total flavonoid content (541.41 ± 1.62 mg/g) followed by *Azadirachta indica* (343.13 ± 1.68 mg/g). *Khaya senegalensis* had the lowest total flavonoid content (12.27 ± 0.98 mg/g). For flavonoids of methanolic extracts, *Bidens pilosa* had the highest total flavonoid content (331.46 ± 1.53 mg/g) with *Trema orientalis* containing the lowest total flavonoid content (4.92 ± 0.26 mg/g). For total phenolic compounds, *Curcuma longa* had the lowest phenols 6.07 ± 0.15 mg/g whereas *Sida acuta* recorded the highest 56.93 ± 1.52 mg/g phenolic content among the methanolic extracts. *Pepper guineese* had the highest phenolics concentration among the ethanolic extracts 64.78 ± 2.51 mg/g with *Trema orientalis* recording the lowest 14.89 ± 0.81 mg/g. There was significant difference between the ethanolic and methanolic extracts for phenols ($p = 0.03$), Flavonoids ($p = 0.024$).

3.2. Antioxidant binding capacity (ABTS) capacity of the extracts

The antioxidant marker ABTS inhibition potential of the ethanolic and methanolic extracts are presented in Fig. 1a, Fig. 1b and b respectively. For ABTS, among the ethanolic extracts, *Szygium aromaticum* exhibited the highest concentration of 1.83 mg/g when extract concentration was 125 mg/g. Increasing the extract concentration to 250 mg/g, *Allium sativum* and *Szycium aromaticum* exhibited the highest binding capacity of 0.67 mg/g, whereas *Szycium aromaticum* exhibited the highest capacity of 0.65 mg/g when extracts concentration was further increased to 500 mg/g. For the methanolic extracts, *Sida acuta* exhibited the highest binding capacity of 1.28 mg/g when concentration was 125 mg/dl while at 250 mg/dl and 500 mg/dl extracts concentrations, *Szygium aromaticum* and *Sida acuta*, *Bidens pilosa* had the highest capacity of 0.87 mg/g and 0.56 mg/g respectively. There was significance of differences between binding capacity of extracts at different concentrations but same solvent ($p = 0.00$). There was also significance of difference between methanolic and ethanolic extracts at same concentration ($p = 0.010$).

3.3. Ferric iron antioxidant reducing potential (FRAP) of the extracts

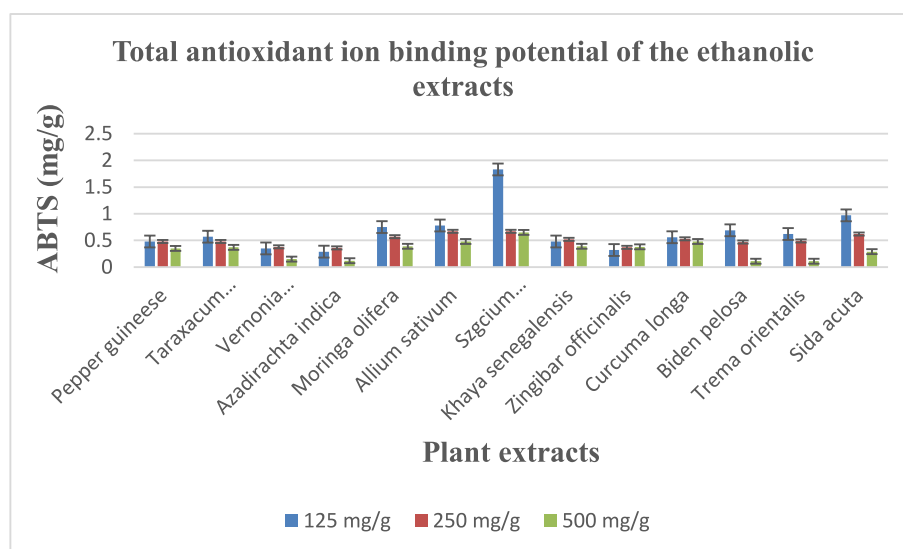
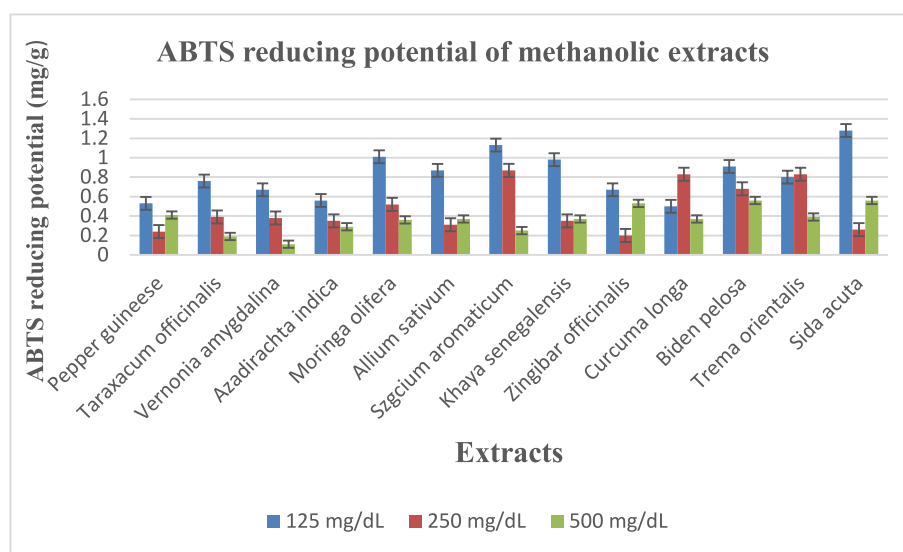
The ferric ion antioxidant reducing potential (FRAP) of the ethanolic and methanolic extracts are presented in Fig. 2a, Fig. 2b and b respectively. For the ethanolic extracts, at 125 mg/dl, *Azadirachta indica* had the highest reducing power of 0.92 mg/g while at 250 mg/dl, *Pepper guineese* exhibited the highest reducing potential of 3.86 mg/g. For the methanolic extracts, *Taraxacum officinalis* recorded 0.64 mg/g and *Pepper guineese* recorded 3.74 mg/g as the highest antioxidant reducing potential at 125 mg/dl and 250 mg/dl respectively. There was significance of difference between the methanolic extracts at 125 mg/dl and 250 mg/dl ($p = 0.02$). Similarly, there was significance of difference between the ethanolic extracts at 125 mg/dl and 250 mg/dl ($p = 0.012$). There was also significance of difference between ethanolic and ethanolic extracts at same concentration ($p < 0.05$).

DPPH inhibition capacity of ethanolic and methanolic extracts.

Table 1

Total flavonoids and phenolic compounds concentration of plants extracts.

Plant species	T.PHENOLS (GAE mg/g)		T.FLAVONOIDS (QE mg/g)	
	Ethanollic extract	Methanolic Extract	Ethanollic	Methanol extract
Pepper guineese	64.78 ± 2.51	34.24 ± 0.62	86.75 ± 0.61	8.17 ± 0.49
Taraxacum officinalis	41.96 ± 1.95	29.09 ± 0.41	153.72 ± 0.67	88.53 ± 1.63
Vernonia amygdalina	36.36 ± 0.46	31.10 ± 3.78	66.63 ± 1.49	96.67 ± 2.67
Azadirachta indica	29.14 ± 1.18	25.19 ± 2.19	343.13 ± 1.68	39.10 ± 2.21
Moringa olifera	32.28 ± 0.32	17.19 ± 1.11	20.92 ± 0.69	22.29 ± 1.19
Allium sativum	25.07 ± 1.15	22.11 ± 2.52	43.18 ± 1.19	15.18 ± 1.18
Szgcium aromaticum	20.42 ± 1.27	12.48 ± 0.05	59.49 ± 2.07	20.68 ± 1.30
Khaya senegalensis	15.16 ± 1.27	29.78 ± 2.01	12.27 ± 0.98	48.44 ± 1.56
Zingibar officinalis	30.87 ± 2.11	49.24 ± 1.62	34.78 ± 2.34	47.51 ± 1.97
Curcuma longa	32.18 ± 2.89	6.07 ± 0.15	48.19 ± 1.56	6.98 ± 2.08
Biden pelosa	49.25 ± 0.67	13.09 ± 1.06	48.03 ± 2.08	331.46 ± 1.52
Trema orientalis	14.89 ± 0.81	9.79 ± 0.27	151.73 ± 0.51	4.92 ± 0.26
Sida acuta	29.33 ± 0.54	56.93 ± 1.52	514.41 ± 1.62	106.58 ± 0.13

**Fig. 1a.** ABTS inhibition capacity of ethanolic extracts of the plant species.**Fig. 1b.** ABTS inhibition capacity of methanolic extracts of the plant species.

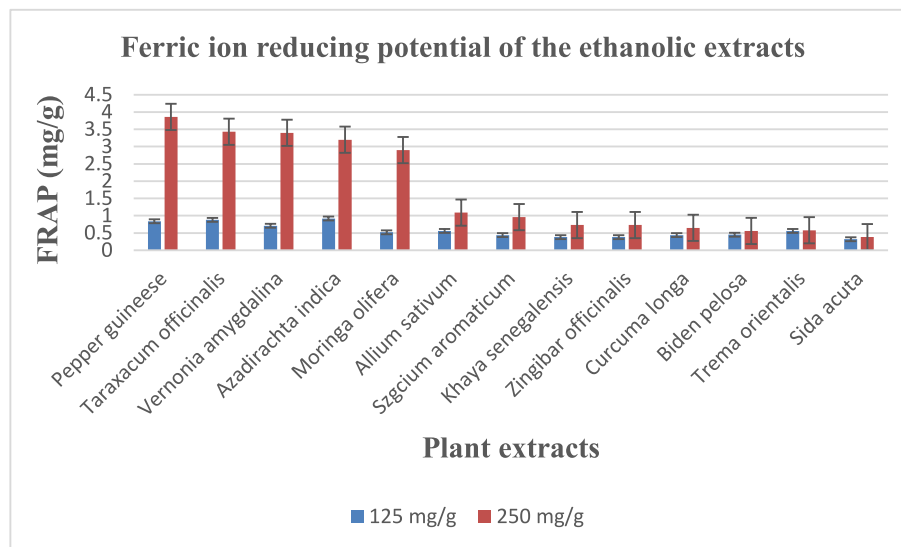


Fig. 2a. Ferric ion reducing potential of the ethanolic extracts.

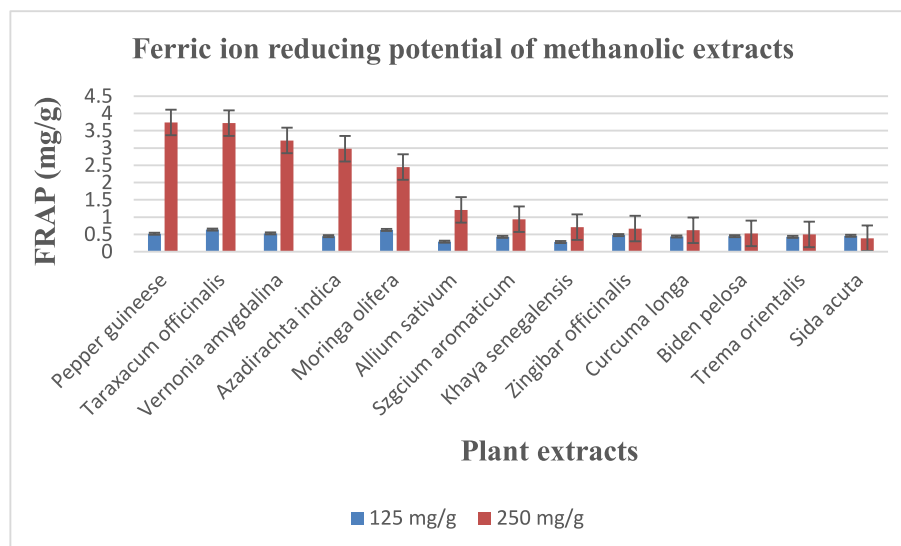


Fig. 2b. Ferric ion reducing potential of methanolic extracts.

The DPPH scavenging potential of the ethanolic and methanolic extractions are presented in Fig. 3. *Pepper guineese* exhibited highest DPPH scavenging activity of 71.57 ± 0.15 %. While *Zingibar officinalis* exhibited the highest DPPH scavenging activity of 75.50 ± 0.06 % among the ethanolic and methanolic extracts respectively. There was no significant difference between methanolic and ethanolic extracts.

3.3.1. Antihemolysis capacity of suspended human red blood cells by extracts

The percentage inhibition of the ethanolic and methanolic extracts at various concentrations against inflammation induced hemolysis of wash human red blood cells is indicated in Fig. 4a, Fig. 4b and b respectively (see Fig. 5a, Fig. 5b, Fig. 5c).

For the methanolic extracts, at extracts concentration of 125 mg/dl, *Szygium aromaticum* had the highest inhibition potential of 67.86 % while *Sida acuta* (52.68 %) and *Moringa oleifera* (50.89 %) exhibited the highest inhibition of hemolysis at 250 mg/dl and 500 mg/dl extracts concentration respectively.

For the ethanolic extracts, at extracts concentration of 125 mg/dl and 250 mg/dl, *Taraxacum officinalis* had the highest inhibition rate of

55.67 % and 56.76 % respectively. *Trema orientalis* (46.87 %) exhibited the highest inhibition of hemolysis at 500 mg/dl extracts concentration. There was significance of difference between the levels of inhibition of hemolysis at various extracts concentration $p = 0.037$ for ethanolic concentrations and ($p = 0.014$) among the methanolic concentrations. There was significance of difference between the ethanolic and methanolic extracts ($p = 0.01$).

MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) reducing cytotoxicity of ethanolic extracts against HEK-239.

The cytotoxicity of the ethanolic extracts of the plants against human embryonic kidney cells-293 at extract concentrations of 125, 250 and 500 mg/dl are indicated in Fig. 5a, b, c respectively. At concentration of 125 mg/dl and incubating for 24 h, *Biden pilosa* had the highest toxicity to the cells at 84.04 %, after 48 h of incubation, *Pepper guineese* exhibited the highest activity. *Biden pilosa* again exhibited the highest cytotoxicity when the concentration was increase to 250 mg/dl for 24 h, whereas, *Vernonia amygdalina*, had the highest cytotoxicity, at concentration of 500 mg/dl for 24 h. There was significance of difference between cytotoxicity levels at the various durations of incubation ($p = 0.02$) and at the various concentration of the extracts ($p = 0.032$).

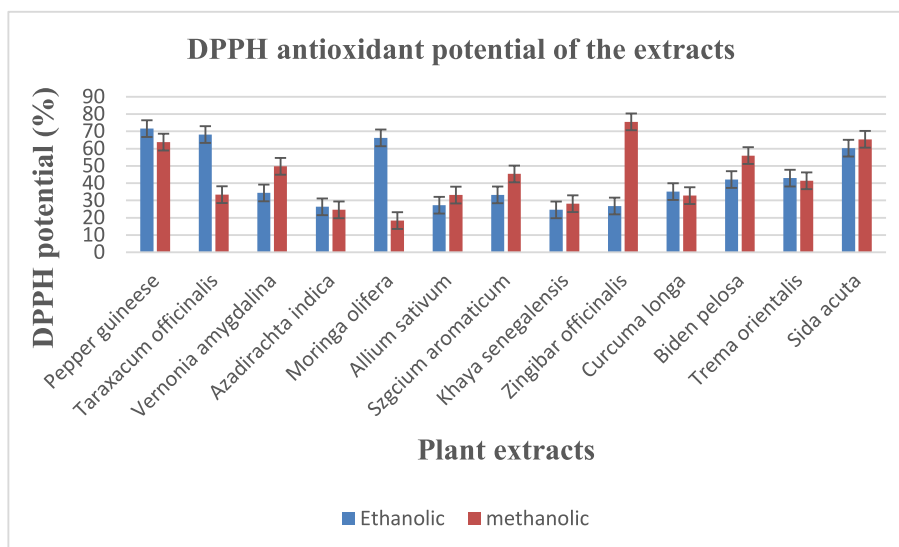


Fig. 3. DPPH inhibitory potential of the methanolic and ethanolic extract.

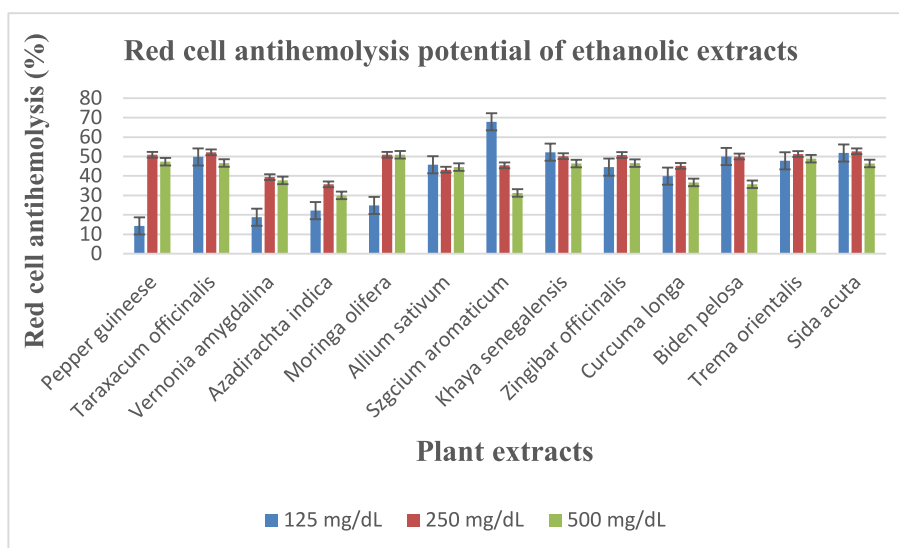


Fig. 4a. Red cell anti-hemolysis of ethanolic extracts. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.4. Pearson correlation co-efficient values of total phenolic content and total flavonoid content with anti-inflammation and antioxidant markers

The Pearson co-efficient values of the correlation between ethanolic and methanolic extracts with DPPH, red cell hemolysis, FRAP and ABTS are indicated in Table 3 (see Table 2). There was positive correlation between methanolic and ethanolic extracts of phenols and flavonoids with DPPH, FRAP, ABTS and red cell hemolysis ($p < 0.05$). Except red cell hemolysis, there was positive correlation (positive R-values) between phenols and flavonoids with the inflammation and antioxidant markers. Generally, methanolic extracts show strong correlation with these markers than ethanolic extracts (ethanolic extracts were closer to 1.00).

3.4.1. Half-maximum inhibition (IC_{50}) concentrations of the extracts

For the ethanolic extracts, *Zingibar officinalis* exhibited the highest inhibitory of 143.64 mg/g and 212.88 mg/g using DPPH and ABTS measurements. *Azadirachta indica* (182.03 mg/g) and *Taraxacum officinalis* (142.88 mg/g) exhibited the highest minimum inhibitory

concentrations using FRAP and RBC hemolysis. For the methanolic extracts, *Szygium aromaticum* (142.85 mg/g), *Curcuma longa* (173.13 mg/g), *Taraxacum officinalis* (175.35 mg/g) and *Moringa oleifera* (134.46 mg/g) exhibited the highest minimum inhibitory concentration using DPPH, ABTS, FRAP and RBC hemolysis respectively. There was significance of difference between the methanolic and ethanolic extracts minimum inhibitory concentration ($p = 0.021$).

4. Discussion

The objective of the study was to determine the phytochemical composition, cytotoxicity, inflammatory and antioxidant potentials of most commonly used medicinal plants for formulating liver targeting herbal medicinal products in Ghana in two different solvent extraction modules, ethanol and methanol. These solvents differ in polarity which influences the kind and quantity of phytochemical that will be isolated. The kind and quantity of phytochemicals directly influence the biological activities of the extract *in vitro* and *in vivo*.

For the two solvents used, there was significant difference between

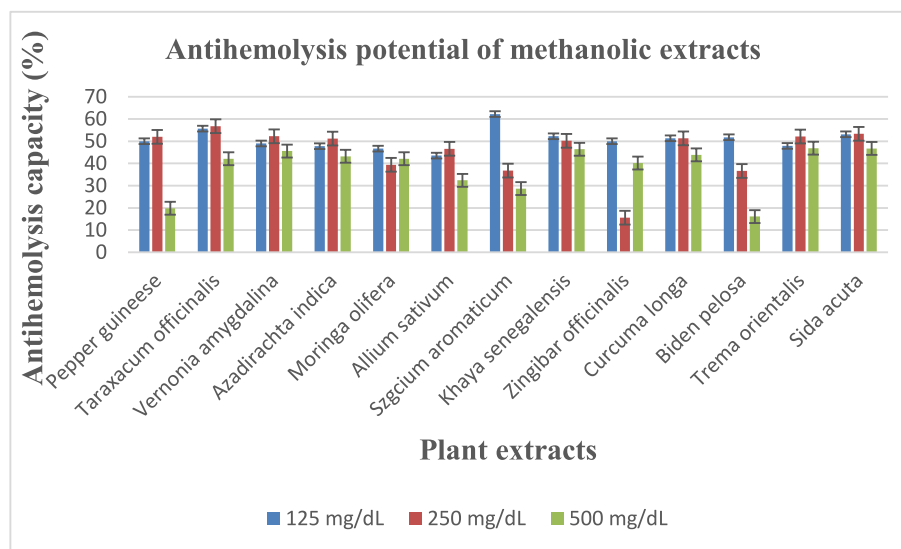


Fig. 4b. Red cell anti-hemolysis of methanolic extracts. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

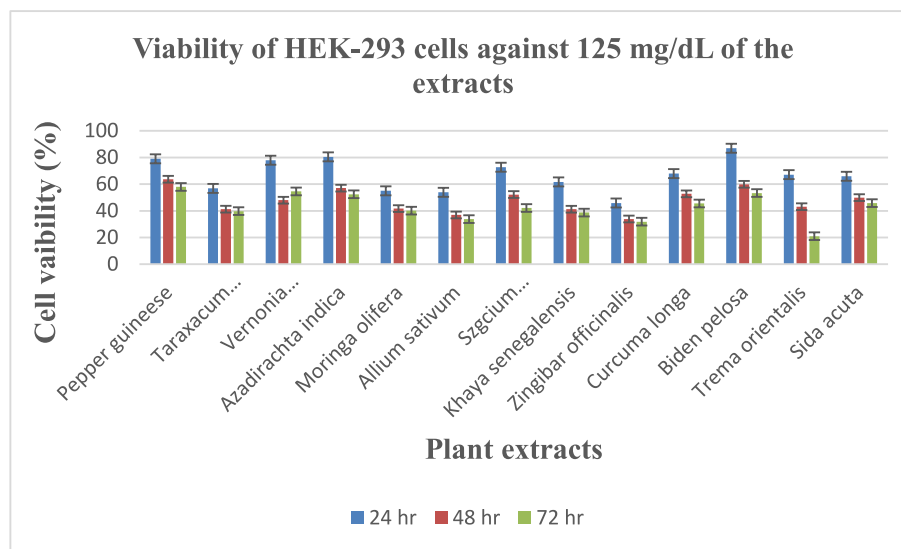


Fig. 5a. MTT reducing cytotoxicity of extracts against HEK-239 at 125 mg/dl

the methanolic and ethanolic extracts in terms of total phenols ($p = 0.03$), flavonoids ($p = 0.024$), FRAP ($P = 0.02$), ABTS ($p = 0.00$) and red cell hemolysis ($p = 0.010$). There was however no significant difference in methanol and ethanol extracts for DPPH scavenging activity. Ethanol has more non-polar hydrogen and carbon atoms in its molecular structure than methanol which make it less non-polar compared to methanol. As a general rule, polar substances dissolve in polar solvents. Therefore, more polar phytochemicals such as free or conjugated polyhydroxylated phenols, phospholipids and lipoproteins will be concentrated in the methanolic solvent while less polar phytochemical such as thanins and proanthocyanins will be isolated with ethanolic solvents. The polarity of the solvents were not sensitive to DPPH levels because DPPH antioxidant reducing power depends on the acidity of the polyhydroxyl phenolic groups in the phytochemical. Therefore, methanol because of its higher comparative polarity may concentrate more polyphenols than ethanol, however, their overall DPPH activity will depend on whether its glycosylated or aglycone form. The presence of steric hindrances in the molecule and electronegativity potential of the bonding cation also influence DPPH antioxidant potential (El-Mannoubi, 2023). El-Mannoubi

(2023), reported of high antioxidant activity using DPPH, FRAP and ABTS levels ($p < 0.03$) in methanolic followed by ethanolic and then acetone extracts in deseeded *Opuntia stricta* fruit.

Within the same solvent model, the total phenolic and flavonoid concentration, the antioxidant activity and antihemolytic capacity followed a dose-dependent effect at extracts concentrations of 125 mg/dl, 250 mg/dl and 500 mg/dl in terms of FRAP ($p = 0.02$), ABTS ($p = 0.00$) and red cell hemolysis ($p = 0.014$). At same reaction conditions, the quantity of the extract is directly proportional to the quantity of concentrated metabolites what will be isolated. Secondary metabolites including phenols follow the principle of pharmacokinetics that, a minimum concentration is required to initiate a biological activity or pathway. Therefore, the higher the extract contention, the higher their bioavailability and the better their biological activities. The antioxidant activities of deseeded *Opuntia stricta* fruit using DPPH, FRAP and ABTS levels also show dose-dependent effect (El-Mannoubi, 2023).

There was positive correlation between the antioxidant markers (DPPH, FRAP, ABTS) with red cell hemolysis ($p < 0.05$) in both methanol and ethanol extracts. There was also positive correlation between

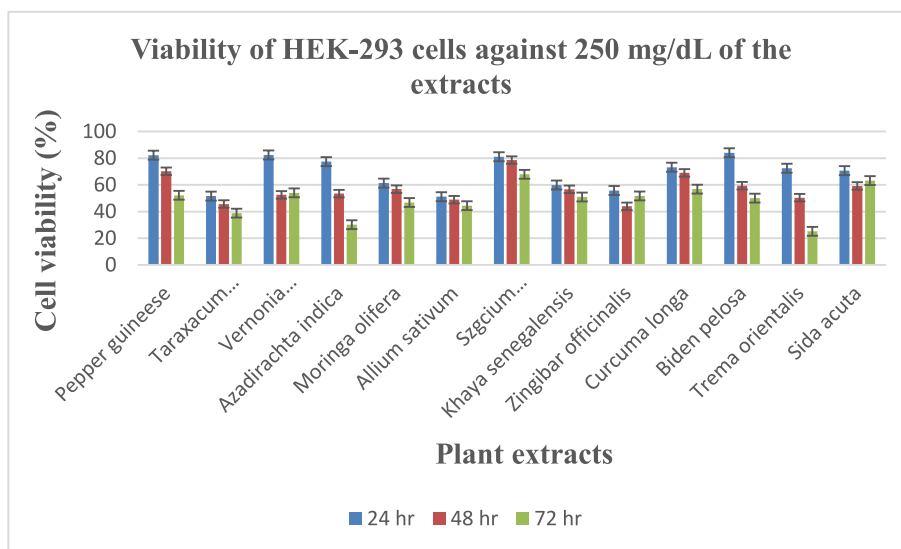


Fig. 5b. MTT reducing cytotoxicity of extracts against HEK-239 at 250 mg/dl

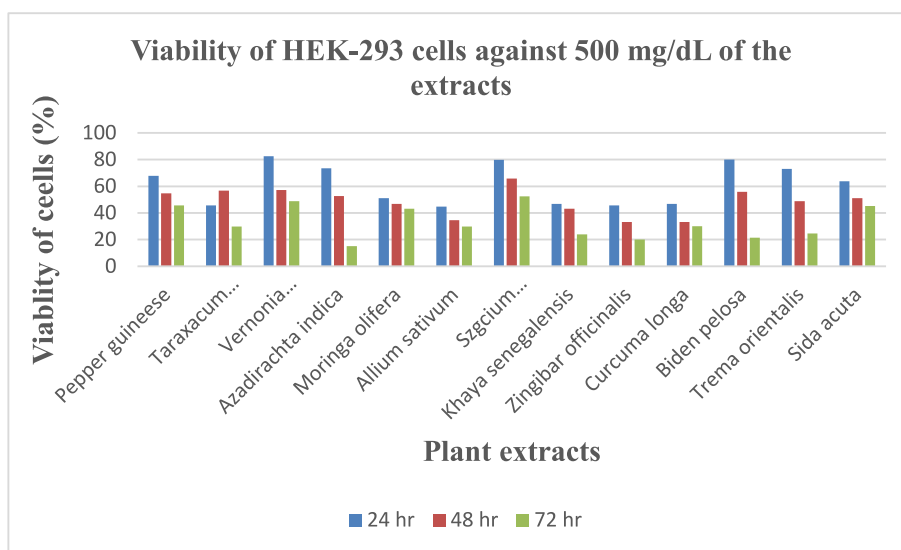


Fig. 5c. MTT reducing cytotoxicity of extracts against HEK-239 at 500 mg/dl

Table 2

Pearson Correlation co-efficient values of Total phenolic content and total flavonoid content with anti-inflammation and antioxidant markers.

	DPPH		RED CELL HEMOLYSIS		FRAP		ABTS	
	ethanol	Methanol	Ethanol	methanol	ethanol	methanol	Ethanol	Methanol
TPE	0.926	0.563	−0.850	−0.654	0.970	0.900	0.902	0.799
TPM	0.678	0.448	−0.670	−0.819	0.785	0.782	0.892	0.721
TFE	0.900	0.879	−0.799	−0.798	0.921	0.817	0.799	0.417
TFM	0.965	0.867	−0.805	−0.799	0.479	0.489	0.902	0.677

P < 0.05. T: total P: Phenolic content M: methanolic E: ethanolic.

the phytochemicals (phenol and flavonoids) with antioxidant markers (DPPH, FRAP, ABTS), except for red cell hemolysis. The positive correlation between the phytochemicals, antioxidant markers and anti-haemolysis is because the driving factor for antioxidant and anti-inflammatory processes is the presence of acidic hydroxyl groups to quench the free radicals from reactive oxygen, nitrogen or sulphur species or inhibit the gene expression of proinflammatory cytokines such as interleukins, tumor necrosis factors and transforming growth factors.

Phenolic compounds are broad spectrum of phytochemicals characterized by hydroxyl, methoxy, galloyl and ortho-hydroxyl groups in their molecular structure. The various acidic hydroxyl groups present in phenolic phytochemicals enable them to initiate various chemical reactions such as substitution or displacement reactions which form the bases of antioxidant and anti-inflammatory activities. Akhrai and Okafor (2021), reported of correlation between total phenols concentration with antioxidant markers in. Works by Nuchuchua and

Table 3
Half-maximum inhibition (IC₅₀) concentrations of the extracts required to initiate the various biological activities.

Plant species	IC ₅₀ Values						
	Methanolic extract			Ethanol extract			
	DPPH	ABTS	RED CELL	DPPH	ABTS	RED CELL	MTT:24 h
Pepper guineese	254.12	258.92	185.92	250	408.19	161.92	142.89
Taraxacum officinalis	253.72	236.53	186.3	250	255.08	142.88	254.62
Vernonia amygdalina	233.2	248.22	142.47	268.17	223.96	155.36	175.63
Azadirachta indica	250	222.24	141.7	228.04	229.32	144.32	199.79
Moringa olifera	247.06	226.7	155.02	279.08	250	134.46	244.45
Allium sativum	281.19	239.05	149.89	219.77	264.92	275.84	272.37
Szgcium aromaticum	341.29	348.32	272.39	278.47	248.53	143.88	288.17
Khaya senegalensis	147.68	272.72	254.06	250	250	250	299.38
Zingibar officinalis	253.17	242.88	146.13	143.64	212.88	200.32	300.8
Curcuma longa	438.78	173.13	141.78	268.04	263.19	157.93	250
Biden pelosa	438.78	230.83	257	318.45	262.25	435	268.03

colleagues, (2024) also show a positive correlation between the phytochemicals, quercetin, kaempferol ant triterpenoid GIA1 with antioxidant activities in *Gymena inodorum*, a green leafy vegetable used in Northern Thai Cuisine.

Szygium aromaticum, *Curcuma longa*, *Taraxacum officinalis* and *Moringa oleifera* had lower IC₅₀ values of 143.88 mg/g, 141.78 mg/g, 142.00 mg/g for red cell anti-hemolysis, and 222.24 mg/g for ABTS respectively. The indicates a high bioavailability at lower concentration of these extracts resulting in pharmacokinetic or biological activity. *Bidens Pilosa*, *Pepper guineese* and *Adzirachta indica* had better MTT cell viability values above 80 % after 48 h in HEK-293 possibly due to their higher phytochemical contention, presence of lower contaminants concentration and bioavailability of their metabolites.

5. Conclusion and recommendation

Medicinal plant species exhibit differences in level of phytochemicals, antioxidant, cytotoxicity and anti-hemolysis properties depending on the polarity of the solvent. Extracts of *Szygium aromaticum*, *Curcuma longa*, *Taraxacum officinalis*, *Moringa oleifera*, *Bidens*, *Pilosa*, *Pepper guineese* and *Azadirachta indica* higher antioxidant, anti-hemolysis and lower cytotoxicity which makes them possible pharmacological agents.

CRediT authorship contribution statement

Dongsogo Julius: Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Larbie Christopher:** Validation, Supervision. **Appiah-Oppong Regina:** Supervision. **Benjamin Obukowho Emikpe:** Supervision. **Daniel Ataanya Abera:** Data curation, Conceptualization. **Yusif Mubarik:** Investigation, Funding acquisition. **Iddrisu Abdul-Mumeen:** Investigation, Conceptualization.

Statement on data availability

Data is availability by request in writing to the corresponding Author.

Declaration of competing interest

I declare on behalf of the Authors that there was no benefit directly or indirectly the influence or independent judgment or scientific factors during the research or writing of the manuscript.

References

Acheampong, D.O., Baffour, K.I., Barku, A.Y.V., Addo, K.J., Essuman, K.M., Boye, A., 2021. *Zanthoxylum zanthoxyloides* alkaloidal extract improves CCL₄-induced hepatocellular carcinoma-like phenotypes in rats. Evid. base Compl. Alternative Med. 2021, 3804379. <https://doi.org/10.1155/2021/3804379>, 16 pages.

Agyemang, A.O., Mensah, L.K., Yamile, R.C., Ocloo, A., Appiah, A.A., Mensah, A.Y., Thomford, K.Y., 2020. Clinical evaluation of the safety and effectiveness of heptonica: a Ghanaian hepatorestorative polyherbal product. Evid. base Compl. Alternative Med. 2020, 9596182. <https://doi.org/10.1155/2020/9596182>.

Akbarizare, M., Ofoghi, H., Hadizadeh, M., Moazami, N., 2020. In vitro assessment of the cytotoxic effects of secondary metabolites from *spirulina*. Egypt. Liver J. 10, 11–18.

Akhraiya, F.O., Okafor, A.C., 2021. Effect of *Spathodea campanulata* ethanol leaf extract on hematology and liver function of *Salmonella*-infected and paracetamol-induced Swiss albino mice. FABAD J. Pharmaceut. Sci. 46 (3), 251–260.

Aly, O., Abouelfadl, D.M.A., Shaker, G.O., Hegazy, G.A., Fayed, A.M., Zaki, H.H., 2020. Hepatoprotective effect of *Moringa oleifera* extract on TNF-α and TGF-β expression in acetaminophen-induced liver fibrosis in rats. Egyptian J. Med. Human Genetics 21, 69. <https://doi.org/10.1186/s43042-020-00106-z>.

Dongsogo, J., Larbie, C., Abdul-Mumin, I., Abera, A.D., Tuuriso, D., 2023. Hepatoprotective activity of plant extracts in non-human models: the toxicants, solvents and diseased models-A review. Am. J. Multidisciplinary Res. Innov. 2 (6), 2832–2854.

Ejuwa, M.C., Obeten, K.E., Mary, O.O., Okim, A.A., Sebastine, O.U., 2022. Hepatotoxic effect of aqueous extract of *Parkia biglobosa* seeds on adult albino wistar rats. SSR Ins. Int. J. Life Sci. 8 (1), 2940–2946.

El-Mannoubi, I., 2023. Impact of different solvents on extraction yield, phenolic composition, in vitro antioxidant and antibacterial activities of deseeded *Opuntia stricta* fruit. J. Umm Al-Qura Univ. App. Sci. 9, 176–184. <https://doi.org/10.10007/s43994-023-00031-y>.

Ezeigwe, O.C., Ani, O.N., Okafor, M.C., 2022. Toxicological studies of *citrus Aurantifolia* fruit juice in wistar rats. Asian J. Biochem. Genetics Mol. Biol. 10 (4), 38–47.

Goh, Y.S., Karunakaran, T., Murugaiyah, V., Santhanam, R., Bakar, A.M.H., Ramanathan, S., 2021. Accelerated solvent extractions (ASE) of *Mitragyna speciosa* korth. (kratom) leaves: evaluation of its cytotoxicity and antinociceptive activity. Molecules 26, 3704. <https://doi.org/10.3390/molecules26123704>.

Jamuna, G., Sharma, A.K., Manimaran, A., Sankar, P., 2018. Hepatoprotective effect of *Allium sativum* and *Withania somnifera* on ochratoxin A-induced toxicity in rats. J. Pharmacogn. Phytochem. 7 (3), 2675–2680.

Jose, P.S., Ratheesh, M., Asha, S., Krishnakummar, I.M., Sandya, S., Girish, K.B., 2018. Hepatoprotective effect of clove bud polyphenol (*syzygium aromaticum*) (clove) by modulating alcohol-induced oxidative stress and inflammation. J. Food Res. 7 (1), 10–17.

Nkempu, A.F., Tembe, E., Edwige, T.M.V., Bayaga, H., Dobgima, J.F., Berinyu, B.E., Nono, B.N., Tabi, Y.O., Ngameni, B., Fokunang, C., 2021. Phytochemical characterization, hepatoprotective activity on alcohol-induced toxicity of the aqueous extract of *Curcuma longa* (Zingiberaceae) in wistar rats. J. Complement. Alternat. Med. Res. 16 (4), 134–149.

Nuchuchua, O., Srinuanhai, W., Chaisak, C., Uthaiwan, C., Piya, T., Nitra, Uracha, R., 2024. Relationship of phytochemicals and antioxidants in *Gymnema inodorum* leaf extracts. Heliyon 10 (1), 2024e23175. <https://doi.org/10.1016/j.heliyon.2023.e23175>.

Nwaogun, J., Sanusi, M., Jega, A., Shafiu Shehu Anka, S.S., 2022. Hepatoprotective effect of *Parkia biglobosa* husk methanol extract against carbon tetrachloride (CCL₄) induced liver damage in albino rats. Int. J. Innov. Sci. Res.Technol. 7 (2), 1073–1083.

Oyibo, A., Gbadegesin, M.A., Odunola, O.A., 2021. Ethanol extract of *vitellaria paradoxa* (gaertn, F) leaves protects against sodium arsenite-induced toxicity in wistar rats. Toxicity Rep. 8, 774–784.

Patel, U.D., Shah, F.C., Jain, N.K., 2019. Hepatoprotective effect of methanolic extract of *syzygium aromaticum* against hydralazine induced toxicity; an invitro study. Pharm. Innov. 8 (6), 537–541.

Sagar, R., Kumar, P., Ahmad, S., Kumar, H., Mandal, S., 2022. Hepatoprotective activity of *Allium cepa* extract in antitubercular drugs induced hepatotoxicity in experimental animals. Europ. J. Pharma. Med. Res. 9 (2), 311–327.

Salama, R.A., Mohammed, M.M., El-Wahab, M.B.A., Shakweer, M.W., 2016. Assessment of effect of *Aloe vera*, *Azadirachta indica* and *Moringa oleifera* aqueous extracts against CCL₄ induced hepatotoxicity in rats. Int. J. Pharm. Pharmaceut. Sci. 8 (4), 83–89.

- Salih, A.I., Saleh, H.M., Sulaiman Khalaf, S., Ayed, S., 2022. Effect of *Moringa oleifera* leaves against hepatotoxicity induced by bisphenol A. *J. Razi Vaccine Serum Res. Inst.* 77 (3), 1083–1089.
- Sani, I., Umar, R.A., Hassan, S.W., Faruq, U.Z., Bello, F., Habiba, Aminu H., Sulaiman, A., 2020. Hepatoprotective effect of *Azadirachta indica* leaf fractionated extracts against snake venom toxicity on albino rats. *Saudi J. Biomed. Res.* 5 (6), 112–117. <https://doi.org/10.36348/sjbr.2020.v05i06.004>.
- Sarfo-Antwi, F., Labie, C., Babatunde, D., 2018. Extracts of *Ageratum conyzoides* L. protects against CCL-4 induced toxicity in rats through inhibition of oxidative stress. *J. Adv. Med. Pharma. Sci.* 19 (2), 1–14.
- Verma, R., 2018. A review on hepatoprotective activity of medicinal plants. *J. Med. Plants Stu.* 6 (1), 188–190.
- Wang, Y., Gou, Y., Zhang, L., Li, C., Wang, Z., Liu, Y., Geng, Z., Shen, M., Sun, L., Wei, F., Zhou, J., Gu, L., Jin, H., Ma, S., 2022. Levels and health risk of pesticide residues in Chinese herbal medicines. *Front. Pharmacol.* <https://doi.org/10.3389/fphar.2021.818268>.
- Zahra, K., Malik, M.A., Mughai, M.S., Arshad, M., Sohail, M.I., 2012. Hepato-protective role extracts *Momordica charantia* L. in acetaminophen-induced toxicity in rabbits. *J. Animal Plant Sci.* 22 (2), 273–277.