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The Anti-inflammatory Activity and the Antioxidant Effect of Some Natural Ethanolic Plant Extracts at the Molecular Level in Rat Liver Lysosomes *In-vitro*

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Abstract This study was to evaluate the effect of *Aloe Vera*; *Carica Papaya* (leaves), *Zingiber officinale* (rhizomes), *Solanum melongena* (Fruits), *Asparagus racemosus* (Aerial part) by (25, 50, 100 µg/ml) of each compared with *Rutin* as standard on DPPH(2,2-diphenyl-1-picrylhydrazyl) radical assay *in-vitro*. Liver lysosomes were isolated from rat by ultra-cooling centrifuge, and the lysosomal fractions were incubated for 30 min with *Aloe Vera*, *Zingiber officinale* by (1, 5, and 10 mg/ml) to determine the activity of the released enzymes(acid phosphatase; β-galactosidase; β-N-acetylglucosaminidase), also, the labializing and stabilizing effects on the membrane permeability. In addition, the effect of the combination between *Aloe* and *Zingiber* by the three doses was investigated. The results revealed that the release rate of the three lysosomal enzymes appeared to be significantly decreased ($P<0.05$) as compared to control under the effect of the extracts and their mixtures, different percentage values of inhibition were observed. A low dose exerted highly inhibition, while a high dose revealed a less stabilizing effect on membrane permeability and this stabilizing effect was dose-dependent. It was concluded that the protective effect of each flavonoid was varied according to dose and enzyme type. The most potent inhibitory effect was observed for *Zingiber officinale* followed by *Aloe Vera*, then mixtures.

Keywords DPPH, Aloe vera, Zingiber officinale, Lysosomal enzymes, Rutin, Fe^{++}/Ascorbate

1. Introduction

The plant kingdom offers a wide range of natural antioxidants. In the group of secondary plant metabolites, polyphenols, such as phenolic acids and flavonoids are strong antioxidants and are commonly found in a variety of fruits, vegetables, herbs, and seeds [1].

Aloe Vera is a unique plant which is a rich source of many chemical compounds and plays an important role in the international market. Chemistry of the plant revealed the presence of more than 200 different biologically active substances including: Vitamins, minerals, enzymes, phenolic compounds, lignin, saponins, sterols and amino acids [2]. Biological activities of *Aloe Vera* have been established by large number of studies because of its demand, it is cultivated in large quantities in many parts of the world, and it has multiple constituents possessing potential biological activities [3]. The *Aloe Vera* plants have been used worldwide due to its medicinal properties as: Anti-inflammatory; wound healing; antiviral and antitumor and helps in immune system and burn. *Aloe Vera* has enjoyed a long history as a herbal remedy and is most popular herbal plant. Major value added products from *Aloe* are gel and juice. Plants belonging to genus *Aloe* particularly *Aloe Vera* (A. barbadensis) has been known for their medicinal properties for many centuries [4], Aloe *Vera* is used as anti-septic, germicidal, blood purifier and in chronic ulcers to stimulate healing. These *Aloe* species are currently listed in the pharmacopoeia of many countries in form of pain *Aloe* extract and powder. There are many reports showing the anti-inflammatory effect of *Aloe Vera* gel [5, 6], *Aloe* contains the enzymes carboxypeptidase which has been shown to decrease inflammation and swelling and beneficial effects of plant extracts on skin, there are some evidences that show *Aloe Vera* extract may be useful in the treatment of diabetes [7]. *Aloe* gel has few side effects the Handbook of Medicinal herbs [8] has given *Aloe* the lowest ranking for toxicity. Studies by [9] shown that the aqueous and the ethanol extracts of *Aloe Vera* powder have anti-mutagenic and anti-leukemic activities. Antioxidants effects have been studied in many studies by [9, 10]. A study used an assay system for free radicals to confirm the antioxidant action of *Aloe Vera* extract [10].

Zingiber officinale the rhizome of ginger is one of the most widely used food species of the ginger family [11], recent evidence revealed the potential of ginger for treatment of diabetes mellitus, data from *in-vitro and in-vivo* and

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clinical trials has demonstrated the antihyperglycaemic effect of ginger. The mechanisms underlying these actions are associated with insulin release and action and improved carbohydrate and lipid metabolism [12]. The most active ingredients in ginger are the pungent principles gengerols, and shogaol, ginger has shown protective effects on diabetic liver, kidney, eye, and neural system complications. The pharmacokinetics, bioavailability, and the safety issues of ginger are also discussed [13]. Ginger is used as anti-inflammatory, antioxidant and a cholesterol- lowering herb [14]. Ginger contains a number of pungent constituents and active ingredients, steam distillation of powdered ginger products ginger oil, which contains a high proportion of sesquiterepene hydrocarbons, predominantly zingiberene [11]. The major strong compounds in ginger from studies of the lipophilic rhizome extracts have yielded potentially active gingerols, which can be converted to shogaols, zingerone and paradol. The compound 6-gingerol appears to be responsible for its characteristic taste. Zingerone and shogaols are found small amounts in fresh ginger and in larger amounts in dried or extracted products [15]. The analyzed chemical composition of aqueous extracts of ginger root (*Zingiber officinale*) was polyphenols, vitamin C, B, β-carotene, flavonoids and tannis [11, 16]. While the HPLC analysis of *Zingiber officinale* ethanolic extract were shogaol and gingerol, moreover, based on recent observations that 6-shogaol may have more potent bioactivity than 6-gingerol [11, 12, 17]. Several studies documented antioxidant effect of *Zingiber officinale* extracts in rats fed a high fat diet, intoxicated rats by paracetamole, radiation and arsenic, where ginger supplementation provide significant, raising tissue concentrations of superoxide dismutase, catalase, and reducing glutathione [18-20], respectively. Moreover, the hypolipdemic effect of *Zingiber officinale* extracts have been investigated in high fat feed diet rats by [21, 22] documented by lowering blood level of cholesterol, triglycerides, while HDL was not significant change when compared with high fat diet fed [11]. Aqueous extract of *Zingiber officinale* rhizomes were studied to evaluate their anti-diabetic effects on protein glycation and on the diffusion of glucose *in-vitro* in the study of [23-25]. *Zingiber officinale* "ginger" rhizome is one of the classic examples of an herb used for not only culinary preparation but also for unique therapeutic significance owing to its antioxidant, antimicrobial, anti-inflammatory and chemo protective potential [26-32]. Although several studies have mentioned anti-diabetic activity of *Zingiber officinale* [31, 33-36], *Zingiber officinale* can lower the blood glucose, improve activities of mitochondrial enzymes and could be used as a nephro-protective supplement particularly to reverse diabetic complications [35, 36]. The potential effects of Zingiber *officinale* in terms of protein glycation and glucose diffusion inhibition were evaluated. It was found that *Zingiber officinale* rhizomes with anti-diabetic/hyperglycemic properties might provide a viable approach, either food based or pharmacological in the treatment of diabetic complications *9250*. The rhizome of the ginger is generally used as a spice in food. Gingerols and their corresponding dehydration products shogaols were considered as the active principles of ginger and these constituents are responsible for strong antioxidant activity [37, 38]. Ginger posses various pharmacological activity including hypoglycemia; anticancer; anticardic; antirenal; hepatoprotective and antioxidant [39]. Ginger has many antioxidant compounds; these compounds may either mitigate or prevent generation of free radicals in toxic conditions. The active ingredients of ginger include gingerols, shagogals, phytochemicals and other compounds show antioxidant activity in various models [40]. It has varied pharmacological activities including antioxidant, anti-inflammatory, anticancer [41], and analgesic [42, 43].

Eggplant ***(Solanum melongena L.)*** is an important and widely consumed vegetable crop of India grown, the constitutive defenses of plants include structural barriers such as the plant cell wall, as well as inhibitory compounds including phenolics [44-46]. However, there have been no reports on induction of resistance in eggplant against *R. solanacearum* by elicitors [44, 47, 48].

Aqueous extract of ***Asparagus racemosus*** was evaluated for lead detoxification from the hepatic tissue by the oral administration route in mice. The toxic effects of lead were studied simultaneously on hepatic biochemical and also on histopathological parameters; the hepatic system showed hepatocyte pycnosis, vaculation, blood congestion and high lymphocytic infiltration around the control vein, results suggested that beneficial effect of aqueous extract may be probably due to its antioxidant properties [49]. ***Asparagus racemosus*** is recommended for prevention and treatment of various human ailments. The decoction of root has been used in blood diseases diarrhea, dysentery, cough, bronchitis and general debility [50-52]. Reports indicated that the pharmacological activities of extract include antinuclear [53], anti-tissue [54], antioxidant [55], and anti-bacterial activities [56]. It was investigated that, mice consumed the given amount of lead nitrate showed a significant lower body weight and liver weight, it may be due to the interruption in absorption and metabolism with ***Asparagus racemosus*** on growth performance of body and organ (liver) weight, this study was similar to the observation of [49) who recorded body weight gain in experimental model after ***Asparagus racemosus*** administration.

Carica papaya L. (Papaya) is belongs to caricaceae family, it has been used empirically as food or as medication for kidney stones, hypertension, urinary tract disorders, abdominal pain during menstruation, analgesic, dysentery, diarrhea, fever [57]. The anti-inflammatory activity of an ethanolic extract of *carica papaya* was investigated in rats using carrageenan induced paw edema, the ulcerogenic activity of the extract was also investigated, and the extract also produced slight mucosal irritation at high dose. The study of [58] establishes the anti-inflammatory activity of *carica papaya* leaves, many parts of the plant are employed in the treatment of several ailments for example the seed is used for expelling worms; the seeds and the roots are used as

abortifacient agent; the leaves (especially fallen ones) are used variously for the treatment of fever, diabetes, and inflammation [59, 60], and several other studies [58]. Powdered leaves of *Carica papaya L*. were extracted with ethanol; partitioned in chloroform and distilled water, the extracts and the fractions were tested for antibacterial activity against clinical isolates of Escherichia coli and pseudomonas species using disc diffusion and micro broth dilution technique, the extracts and fractions were further subjected to phytochemical tests for the presence of secondary metabolites using standard procedures [61]. The extract of *C. papaya* can inhibit sub-chronic inflammation in which various types of cellular migration are (e.g. fibroblast) involved [62]. The juice of *C. papaya* has lowering the blood pressure in renal [63].

Anti-inflammatory activity of the three flavonoids: Rutin, quercetin and hesperidin were investigated in rats using the model of acute and chronic inflammation, interaperitoneal administration of the three flavonoids given daily at doses equivalent to 80mg/kg, inhibited both acute and chronic phases of this experimental model of inflammation. Rutin was the most active in the chronic phase, the antihyperglycemic and the antioxidant effect of Rutin, apolyphenolic flavonoid, in normal and streptozotocin-induced diabetic Wister rats [64], diabetes as induced in rats by an intraperitoneal injection of streptozotocin, *Rutin* was orally administered to normal and diabetic rats for 45 days. Fasting plasma glucose, glycosylated haemoglobin, thiobarbituric acid reactive substances and lipid hydroperoxides was significantly increased, whereas insulin, c-peptide, total haemoglobin, protein levels, non-enzymatic antioxidants (glutathione, vitamin C and vitamin E) were decreased significantly in diabetic rats. The result showed that *Rutin* exhibits antihyperglycemic and antioxidant activities in streptozotocin-induced diabetic rats. Also, it was found that the metabolic and pharmacological properties of Rutin underlying the *Rutin*-mediated amelioration of the rat colitis. Apparent partition coefficients of *Rutin* and its aglycone quercetin were compared. The biochemical/chemical stability of *Rutin* was examined in the contents of various segments of gastrointestinal tracts of rats [65]. Inflammatory effect was determined in the colitis rats after oral administration of *Rutin* or rectal administration of quercetin. In human colon epithelial cells, the effect of quercetin on tumor necrosis factor (α-TNF)-induced nuclear factor Kappa B (NF Kappa B) activation was examined. Rutin acted as a quercetin deliver to the large intestine and its anti-inflammatory action in TNBS-induced colitis rats may be through quercetin mediated inhibition of TNF-α-induced NF kappa B activation [65].

In many pathological conditions, changes in the state of lysosomes take place, the loss of the stability of lysosomal membrane has been observed in the leakage of enzymes from lysosomes [66-68]. The direct effects of the test compounds on the lysosomal membrane was to determine the relative stabilizing or labializing effects within the limits of antioxidant concentration level and the exposure period of times [69]. Lysosomes have a central role in cellular homeostasis as sites for digestion of foreign materials and for degradation of intracellular components undergoing autolytic processing [70]. Autophagy provides cells with oxidizable substrate when nutrients become scarce but also that it can provide protection against aging and a number of pathologies such as cancer, neuro-degeneration, cardiac disease, diabetes, and infections [71]. It was supported that the intracellular release of the lysosomal enzymes proceeds cellular death by initiating the cellular injury process ultimately causing tissue necrosis. The compounds which antagonize the effect of labilizers and prevent or reduce the release of the lysosomal enzymes are designated stabilizers [72, 73]. Lysosomes are membrane –bonded structures containing hydrolytic enzyme capable of degrading most of the cellular constituents, play an important role in secretion and transport processes. Leakage of lysosomal enzymes accounts for many tissue and target organopathies [74], lysosomes are found in all animal cells and in disease-fighting cells. The major lysosomal enzymes according to their importance as liver lysosomal markers are: Acid phosphatase (ACP), β-galactosidase (β-GAL), β-N-acetyl glucosaminidase and β-glucuronidase (β-NAG) [75].

The purpose of the present study was to investigate the antioxidant activity of five natural ethanolic plant extracts as: ***Aloe Vera*** (leaves); ***Zingiber officinale*** (rhizome); ***Solanum melongena*** (fruits); ***Asparagus racemosus*** (aerial part), and ***Carica papaya*** (leaves) on DPPH (2,2-diphenyl-1-picrylhydrazyl) radical assay *in-vitro*. Also, the anti-inflammatory effects of ***Aloe Vera*** (leaves); ***Zingiber officinale*** (rhizome) and their mixtures on the release rates of the three lysosomal acid hydrolases "ACP, β-GAL, β-NAG" in rat *in-vitro* were performed.

Table 1. The families; Latin name; Arabic name; English name; part used, and sources of plant under investigation

No.	Latin name	Family	English name	Arabic name	Part used	Sources
1	**Aloe Vera**	Liliaceae	Aloe	الصبار	Leaves	ESFA
2	**Zingiber officinale**	Zingibraceae	Common Gingiber	الزنجبيل	Rhizome	Market
3	**Solanum Melongena**	Solanceae	Egg plant	الباذنجان الأسود	Fruits	Market
4	**Asparagus racemosus**	Asparagaceae	Asparagus	الأسبرجس	Aerial parts	ESFA
5	**Carica papaya**	Caricaceae	Papaya	الباباز	Leaves	ESFA

ESFA: Experimental station of faculty of Agriculture Cairo University.

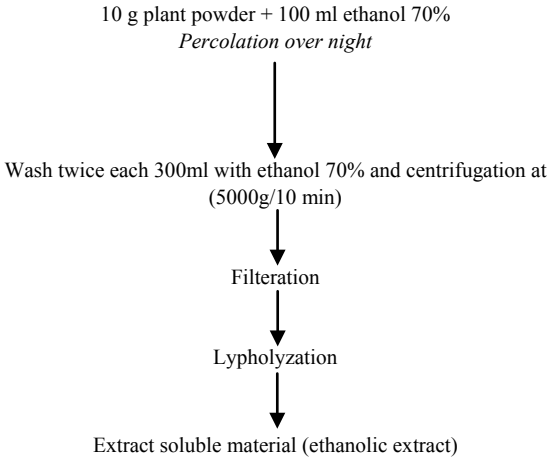
2. Materials and Methods

❖ ***Plants***

The families; Latin name; Arabic name; English name; part used [76], and source of plant samples are listed in Table 1. All plant samples were authenticated via plant herbarium of Orman Garden, Ministry of Agriculture.

❖ ***Preparation of ethanolic extracts***

All collected plant samples were cleaned, dried in lypholyzer, and then grinded to fine powder before extraction. Such powdered samples were kept in dark glass bottles ready for further investigation [77].



❖ ***2,2-Diphenyl-1-picrylhydrazyl assay (DPPH)***

The free radical scavenging effect of the five ethanolic natural plant extracts was assessed by the discoloration of a methanolic solution of DPPH according to [78-79].

❖ ***Preparation of lysosomal fraction***

Twenty male albino rats weighing about 200-270g were used. After decapitation and bleeding, the liver was perfused *in situ* with 0.25 M ice-cold sucrose medium via portal vein at a rate of approximately 15 ml/minute according to the method of [80]. The tissue was cutting into small pieces and dispersed in 0.25 M sucrose buffer pH (7.4) placed in CAT (R18) homogenizer. After homogenization, the volume was adjusted to 6.0 ml sucrose buffer 0.25 M contains 1.0g wet tissue of liver.

❖ ***Enzyme substrates***

1. p-nitrophenyl phosphate (Sodium salt) was used for acid phosphatase (EC 3.1.3.2) "ACP".
2. p-nitrophenyl-ß-D-galactopyranoside was used for ß-galactosidase (EC 3.2.1.23)"β-GAL".
3. p-nitrophenyl-2-acetamido-2-deoxy-ß-D-glucopyranoside was used for N-acetyl-ß-glucosaminidase (EC 3.2.1.30)"β-NAG".

All these substrates were purchased from Sigma Chemical Co. U.S.A.

❖ ***Isolation of lysosomal fraction***

The liver homogenate was then centrifuged at 2500 r.p.m. in a Beckman refrigerating ultracentrifuge (model J2-21) for 15 minutes. The pellet was washed under the same conditions, and then the first supernatant and the wash were combined together. The whole lysosomal fraction was prepared by centrifuging the last supernatant at 14,000 r.p.m. for 15 minutes. The pellet was washed and resuspended in the 0.25 M. sucrose buffer and this step was repeated three times for isolating pure lysosomal fraction. After washing and purification, the pellet was resuspended in the 0.25M sucrose buffer medium to give 1.0g liver weight per 1.25 ml sucrose buffer [80].

❖ ***Incubation of lysosomes with the antioxidant compounds***

Incubation mixtures consisted of 1.0 ml of lysosomal fraction and 1.0 ml of antioxidant solution, the total volume was completed to 3.0ml by the addition of sucrose buffer solution. The tubes were incubated in a shaking water bath at 37°C/30min., Tubes of each antioxidant concentration were removed and centrifuged at 19000 r.p.m./15 min. The resulting supernatant was subjected to enzyme assay to determine the activity of released enzymes [81].

❖ ***Methods of enzyme assay***

For determination the total enzymatic activity, some culture tubes containing 1ml antioxidant compounds+1ml lysosomal fraction + 1ml TritonX-100 (0.1%) were exposure to thawing and freezing for three times, then centrifuged at 19000r.p.m/15min. The resulting supernatant was also subjected to each enzyme assay for determination the total enzymatic activities of each by lysosomal enzyme. The activities were measured spectrophotometerically according to the method of [82] with slight modifications described by [83].

❖ ***Determination of individual phenolic compounds by HPLC***

Determination of phenolic compounds was performed according to the method outlined by [84, 85], Identification of individual phenolic compounds by using an Agilent HPLC series 1090 (Agilent, Waldron, Germany) apparatus. HPLC method was performed for the photochemical screening and identification of active ingredients (major and minor components with bioactive).

❖ ***Statistical analysis of the results***

All values are mean ± S. E. obtained from eight animals. For statistical analysis, one way ANOVA [86] with Duncan's variance (SPSS 10) was used to compare groups. In all the cases a difference was considered significant when ρ was < 0.05.

3. Results and Discussions

1) DPPH radical-scavenging activity

The effect of antioxidant activity on DPPH radical scavenging is thought to be due to their hydrogen donating ability: DPPH• + AH → DPPH− H + A• . DPPH is a stable free radical and accepts an electron or hydrogen radical to

become a stable diamagnetic molecule, the reduction capability of DPPH radicals was determined by the decrease in its absorbance induced by antioxidants, it is visually noticeable as a discoloration from purple to yellow colour [87].

The three concentrations of each ethanolic extract were used (25, 50, and 100 µg/ml) in scavenging test, the antioxidant activity of Rutin as standard antioxidant at high concentration (100µg/ml) appeared to be the highest value of inhibition by (96.0%), followed by the extracts: Aloe Vera (leaves; 91.0%), *Zingiber officinale* (rhizome; 83.0%), *Solanum melongena* (fruits; 70.5%), *Asparagus racemosus* (aerial parts; 62.0%) and *Carica papaya* (leaves; 59.4%), and the decrease could be directly attributed to the increase of the phenolic contents of each plant. The close correlation between antioxidant activity and phenolic content obtained from various natural sources has been already demonstrated [88, 89]. It was also reported that the solvent used in extraction may be important in antioxidant activity of the extract depending on phenolic content (phenolic and flavonoid contents of an *endophytic xylaria* species higher in methanol than in hexane extracts [88, 90] (Table 2 and Fig. 1).

Rutin exhibited a high scavenging effect toward DPPH radicals, while ethanolic extracts of *Aloe Vera and zingiber officinale* exhibited nearer effects on the activity as compared to *Rutin* [91], these results indicated that *Rutin* has a noticeable effect on scavenging free radicals; free radical scavenging activity was also increased with an increasing of the concentration [92]. These data clearly indicated that ethanolic extracts of *Aloe Vera and Zingiber officinale* has powerful free radical inhibitors or scavenger as compared to *Rutin* at high dose (100µg/ml), these results were in agreement with [9, 10, 11, 39].

The results of Eggplant (*Solanum melongena* L.) is in agreement with [44, 46] they reported that consumed of vegetable crop of India grown round the year, the more constitutive defenses of plants include structural barriers, such as the plant cell wall, as well as inhibitory compounds including phenolics groups.

Our results are in agreement with [50-52] they reported that reported that *Asparagus racemosus* is recommended for prevention and treatment of various human ailments in blood diseases diarrhea, dysentery, cough, bronchitis and general debility. Also the reports of [53] indicated that the pharmacological activities of extract include antinuclear and anti-tissue [54]; antioxidant [55], and anti-bacterial activities [56].

Table 2. Free radical scavenging activity of ethanolic plant extracts by three concentrations on DPPH radical assay

Samples	[a] DPPH decolouration %		
	25(µg/ml)	50(µg/ml)	100(µg/ml)
Rutin hydrate (st.)	76.7%	91.0%	96.0%
Aloe Vera	57.7%	78.5%	91.0%
Zinger Officinalis	48.0%	71.2%	83.0%
Solanum Melongena	40.4%	53.6%	70.5%
Asparagus Officinalis	38.7%	50.0%	62.0%
Carica Popaya	24.5%	50.1%	59.4%

[a] (%): was expressed as the absorbance of the sample with absorbance of the control
DPPH: Diphenyl picryl hydrazyl radical

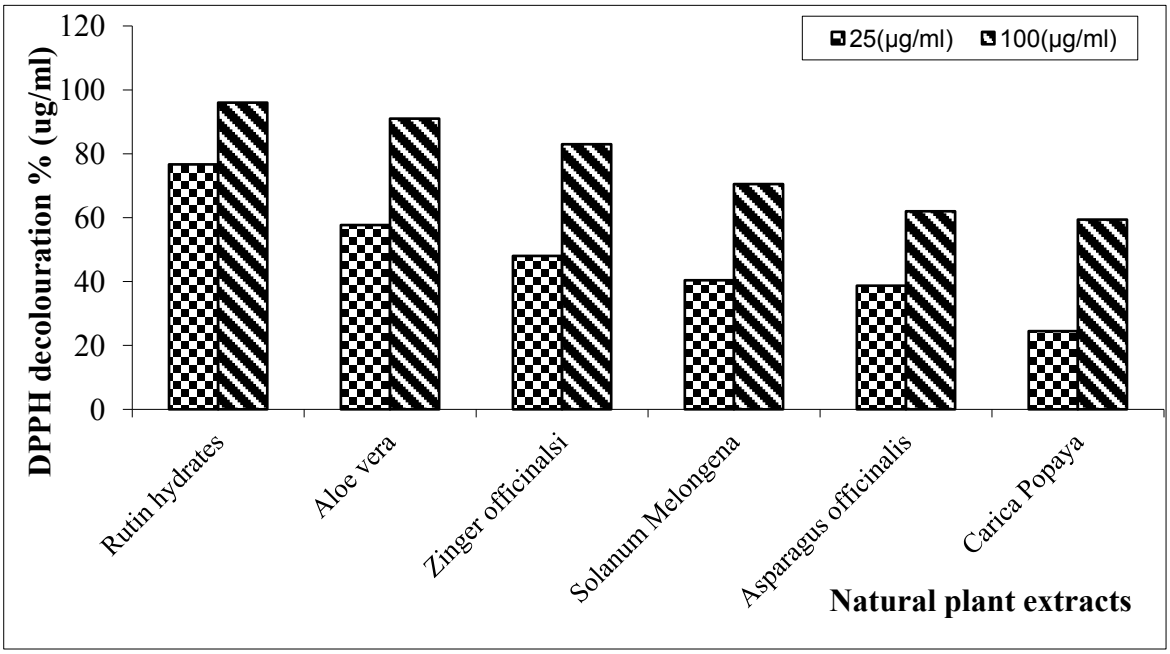


Figure 1. Free radical scavenging activity of the natural plant extracts

The results of *C. papaya* are in agreement with the result of [58], who stated that the ethanolic extract of *C. papaya* can inhibit prostaglandins mediated inflammation since the extract produced marked reduction in the carrageenan induced edema after 3 and 5 hrs of carrageenan injection. Further studies are required before the mechanism of action of the extract or its active constituent can be identified [93, 94]. Phytochemical screening of ethanol, chloroform and water extracts and fractions of *C. papaya* revealed the presence of alkaloids, flavonoids, saponins, steroids and tannis, these metabolites have been reported to possess antimicrobial; anti-inflammatory and antioxidant activities [95]. In particular the flavonoids were reported to be responsible for antimicrobial activity associated with some ethno-medicinal plants [96], in addition to alkaloids and flavonoids, which were reported to be responsible for antimicrobial properties of some ethno-medicinal plants [96].

2) Effect of *Aloe Vera, Zingiber officinale* in compared with *Rutin* as standard antioxidant on the three marker lysosomal enzymatic activities

The results in (Table 3) illustrate some characteristics of the three marker hepatic lysosomal enzymes: ACP, β-GAL and β-NAG, under the effect of some natural flavonoids which have the higher antioxidant activity as: Aloe Vera (leaves) and Zingiber officinale (rhizome) in compared with Rutin as standard. ACP has been considered the marker enzyme of the hepatic lysosomes for measurement of cell viability by its presence in surplus amounts not only in the secondary lysosomes but also in the primary lysosomes [97]. The other lysosomal enzymes β-GAL and β-NAG are very important for liver lysosomal fractions [98]. The effects of the three concentrations of Rutin under investigation on the lysosomal enzymatic releases after 30 min of incubation were observed. It appears to have stabilizing effect on the membrane permeability of lysosomes, this stabilizing effect of the natural flavonoids may be due to its anti-inflammatory activity on membrane permeability. A low dose showed a higher stabilizing effect on membrane permeability than the high dose with regard to control group ($P<0.05$). Total enzymatic activity showed high values for ACP, and lowest ones for β-GAL and β-NAG. This may be due to the enzyme synthesis by rough endoplasmic reticulum and regulatory genetic coding which was accompanied by elevation in enzymatic activities and stabilization mediated by prostaglandin synthetase [99]. Anti-inflammatory activity of Rutin was due to the flavonoids in the chronic phase [100], a number of flavonoids were reported to possess anti-inflammatory activity as: Hesperidin, a citrus flavonoid, possess significant anti-inflammatory effect. Recently, quercetin and Rutin have been reported to exhibit anti-inflammatory activity acting as antioxidants as well as antiviral and anticancer substances.

Table 3. The Effect of Ethanol Extracts of ***Aloe Vera and* Zinger *officinalis*** in compared with ***Rutin*** as Standard antioxidant by three concentrations on extralysosomal enzymatic release of rat liver ***in-vitro*** after **(30)** minutes of incubation period

Concentration / Enzymes		Mean values (x) ± S.E. of enzymatic activities (nmole/ml/hr.)		
		Acid phosphatase (ACP)	β –*Galactosidase* (β -GAL)	β -*N-Acetyl Glucosaminidase* (β -NAG)
Total activity		18097.56 ± 0.0016	1075.10 ± 0.084	2849.34 ± 0.043
Control % change		9260.99 ± 0.0029 -----	747.02 ± 0.0057 -----	1361.99 ± 0.082 -----
Rutin	LC. 1.0mg/ml % change	3670.33 ± 0.085 ↓60.4%*	338.69 ± 0.0046 ↓54.7%*	1034.99 ± 0.019 ↓24%*
	MC. 5.0mg/ml % change	7296.33 ± 0.054 ↓21.2%*	549.75 ± 0.081 ↓26.4%*	1072.0 ± 0.099 ↓21.3%*
	HC. 10.0mg/ml % change	8000.46 ± 0.034 ↓13.6%*	701.98 ± 0.0057 ↓6.01%†	1088.67 ± 0.016 ↓20.1%*
Aloe vera	LC. 1.0mg/ml % change	4521.99 ±1.02x10^{-2} ↓51.2%*	295.20 ± 0.077 ↓60.5%*	339.00 ± 0.014 ↓75.1%*
	MC. 5.0mg/ml % change	8682.33 ±2.76x10^{-2} ↓6.2%†	690.81 ± 0.011 ↓7.5%†	398.0 ± 0.037 ↓70.8%*
	HC. 10.0mg/ml % change	9232.99 ±0.015 ↓0.3%†	711.67 ± 0.099 ↓4.7%†	544.67 ± 0.035 ↓60.0%*
Zinger officinalis	LC. 1.0mg/ml % change	5063.33 ±0.067 ↓45.3%*	283.54 ± 0.053 ↓62.04%*	76.03 ± 0.095 ↓94.4%*
	MC. 5.0mg/ml % change	7473.66 ±0.017 ↓19.3%*	620.45 ± 0.07 ↓16.9%*	118.0 ± 0.039 ↓91.34%*
	HC. 10.0mg/ml % change	7709.33 ± 0.061 ↓16.8%*	701.27 ± 0.08 ↓6.1%†	307.34 ± 0.054 ↓77.4%*

*: The mean difference is significant at the $p< 0.05$ level (LSD).
(L.C. = Low concentration, M.C. = Medium concentration, H.C= High concentration)

Aloe Vera (leaves) has stabilizing effect on membrane permeability after 30min of incubation with regards to the control (low dose with a highly stabilizing effect), all the three concentrations have stabilizing effects on membrane permeability with the three enzymes as compared to control. β-GAL activity shows the highly stabilizing effect on the membrane permeability then β-NAG and ACP by the three concentrations.

Zingiber officinale (rhizome) at low dose exerted highly stabilizing effect on the membrane permeability than medium and high doses. Also, β-NAG exerted the highly stabilizing activity then β-GAL then ACP by the three concentrations on the membrane permeability. ***Zingiber officinale*** by the three concentrations exerted a highly stabilizing effect than *Aloe Vera* extract on the three lysosomal enzymes. This may be due to the active compound [6]-gingerol and the other flavonoids to scavenge the free radical and protect the membrane of lysosomes. Our results are also in agreement with the findings of [42, 101] they revealed that the phenolic and hydroxyl groups could affect enzyme activity and membrane permeability. This effect may be due to the principles active ingredients in ginger which are gingerols, and shogaol. Also, our results in agreement with [11, 12, 17] they reported that HPLC analysis of ethanolic extract of *Zingiber officinale* were shogaol and gingerol, moreover, based on recent observations that 6- shogaol may have more potent bioactivity than 6-gingerol as strong antioxidant, anti-inflammatory,…….and so on.

Variation in the enzyme activity was dependent on the type of the enzyme; it may also be dependent on the enzyme kinetics and the behavior of each enzyme [102]. The enzyme activities seem to be dose-dependent and the differences in the activities may be due to the behavior of each enzyme towards the functional groups of each compound [103]. The change in marker enzyme activity could be attributed to the variability in lysosomal membrane stabilization and labilization, affecting the outward leakage of these enzymes [72].

3) Effect of mixture *Aloe Vera* (leaves) with *Zingiber officinale* (rhizome) in compared with *Rutin* as standard antioxidant on the three marker lysosomal enzymatic activities

The results in (Table 4 and Fig. 2) illustrate the effect of the three marker hepatic lysosomal enzymes: ACP, β-GAL, and β-NAG under the effect of mixture of *Aloe Vera with zingiber officinale*, at the high concentration the effect of the lysosomal enzymes of ACP and β-GAL did not observed, in addition to medium concentration for ACP wasn't observed.

β-NAG revealed the highest stabilizing effect on the membrane permeability by the three concentrations, β-GAL then ACP revealed moderate effects. This may be due to the combination between the active compounds of *Aloe Vera* with *Zingiber officinale* mixtures which affected the membrane permeability than other enzymes. The concentration of the mixture exerted insignificant effect on the activity of ACP (the high and medium concentrations) and the high concentration of β-GAL, this may be due to dose-dependent.

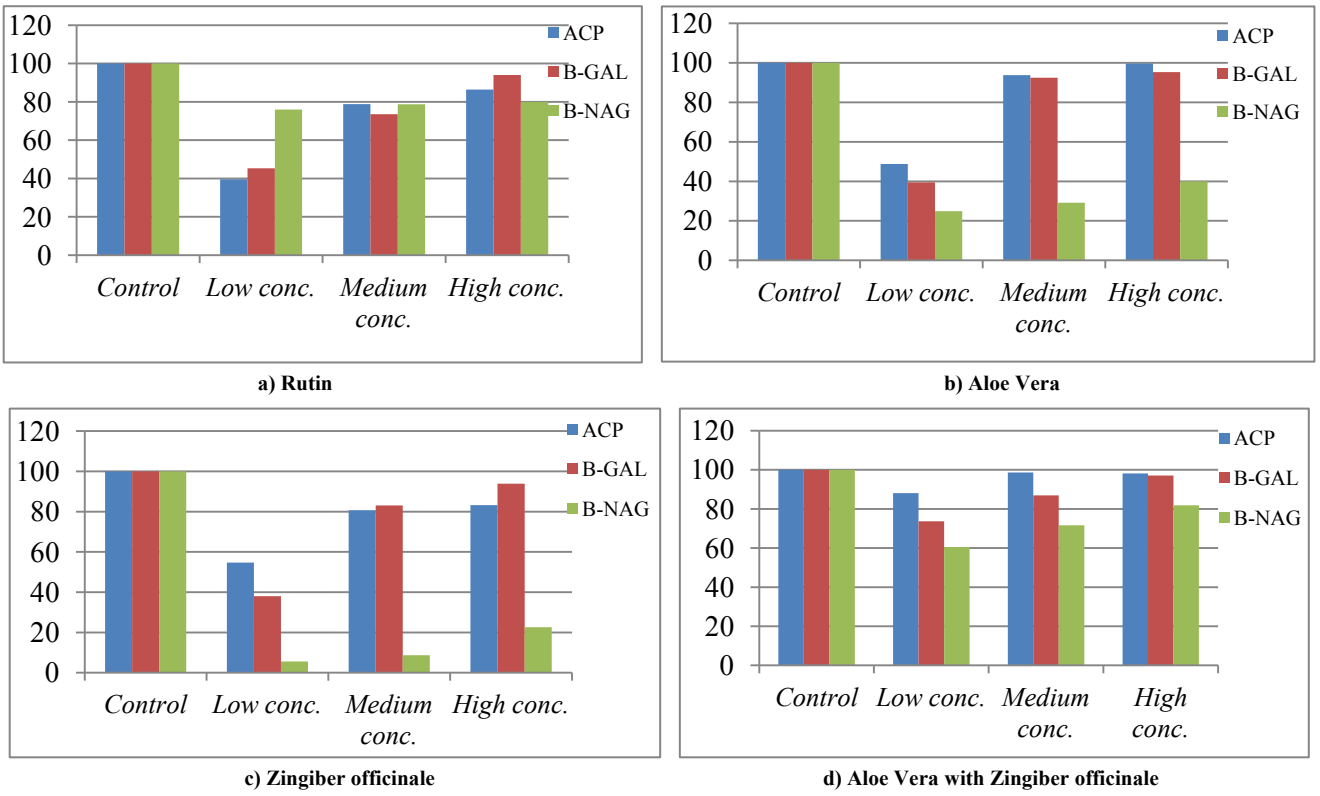
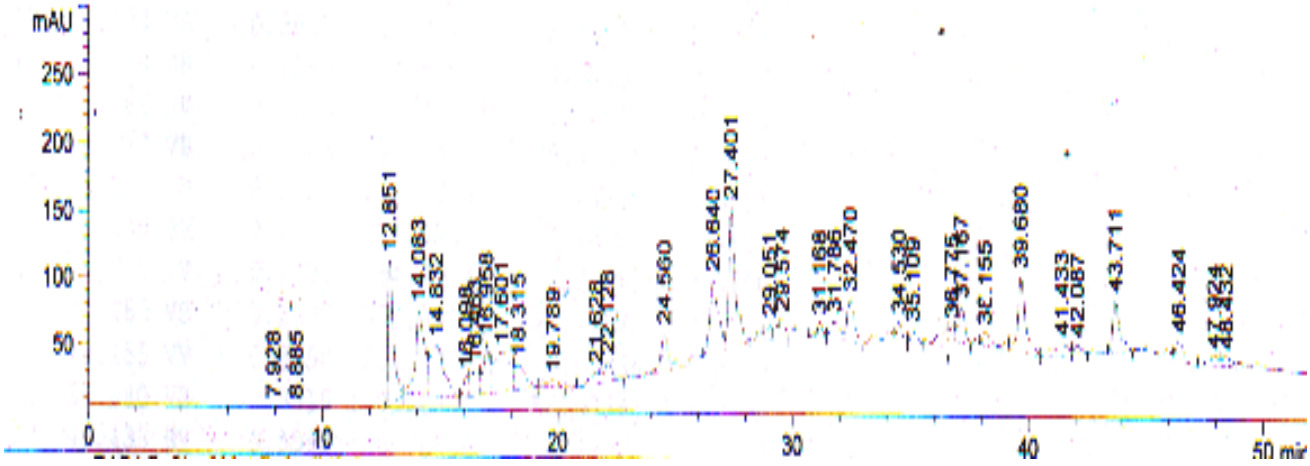


Figure 2. Relative Activity effects of ***Aloe Vera, Zingiber officinale***, and ***their mixtures*** by three concentrations and *Rutin* on extralysosomal relative enzymatic activity of rat liver *in-vitro*

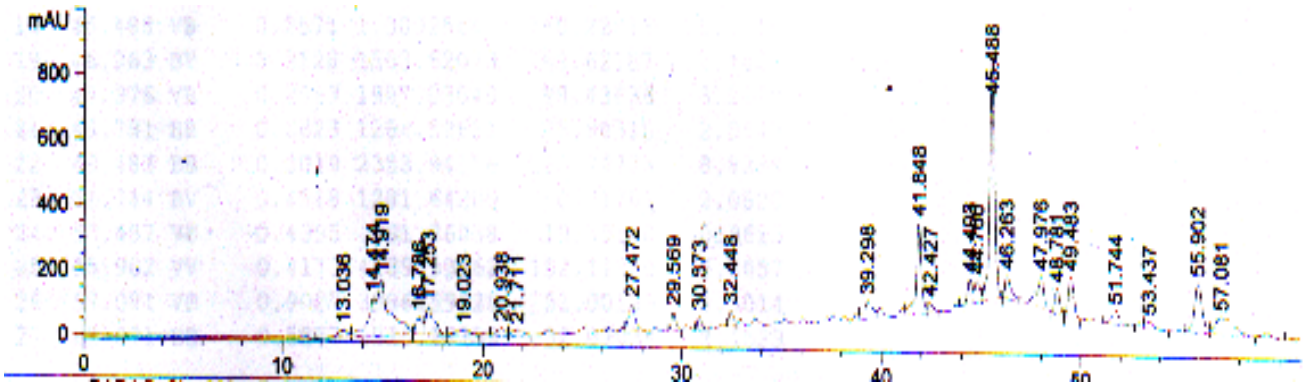
Table 4. The Effect of Ethanol Extracts of mixture ***Aloe Vera with Zinger officinalis*** in compared with ***Rutin*** as Standard antioxidant by three concentrations on extralysosomal enzymatic release of rat Liver ***in-vitro*** after **(30)** minutes of incubation period

Enzymes / Concentration		Mean values (x) ± S.E. of enzymatic activities (nmole/ml/hr.)		
		Acid phosphatase (ACP)	β –*Galactosidase* (β -GAL)	β -*N-Acetyl Glucosaminidase* (β -NAG)
Total activity		18097.56 ± 0.0016	1075.10 ± 0.084	2849.34 ± 0.043
Control % change		9260.99 ± 0.0029 -----	747.02 ± 0.0057 -----	1361.99 ± 0.082 -----
Rutin	LC. 1.0mg/ml % change	3670.33 ± 0.085 ↓60.4%*	338.69 ± 0.0046 ↓54.7%*	1034.99 ± 0.019 ↓24%*
	MC. 5.0mg/ml % change	7296.33 ± 0.054 ↓21.2%*	549.75 ± 0.081 ↓26.4%*	1072.0 ± 0.099 ↓21.3%*
	HC. 10.0mg/ml % change	8000.46 ± 0.034 ↓13.6%*	701.98 ± 0.0057 ↓6.01%†	1088.67 ± 0.016 ↓20.1%*
Aloe vera and Zinger officinalis	LC. 1.0mg/ml % change	8157.33 ± 0.086 ↓11.92%*	550.81 ± 0.051 ↓26.3%*	824.33 ± 0.072 ↓39.5%*
	MC. 5.0mg/ml % change	9132.66 ± 0.022 ↓1.4%†	649.10 ± 0.030 ↓13.1%*	976.00 ± 0.026 ↓28.3%*
	HC. 10.0mg/ml % change	9088.66 ± 0.021 ↑1.86%†	725.15 ± 0.036 ↓2.9%†	1115.00 ± 0.045 ↓18.1%*

*: The mean difference is significant at the p< 0.05 level (LSD).
(L.C. = Low concentration, M.C. = Medium concentration, H.C= High concentration)



a) Aloe Vera



b) Zingiber officinale

Figure 3. HPLC chromatogram of *Aloe Vera* (leaves) (a) and *Zingiber officinale* (rhizome) (b)

4) HPLC investigation of *Aloe Vera* (leaves) and *Zingiber officinale* (rhizome)

The HPLC chromatogram of *Aloe Vera* (leaves) revealing the presence of phenolic compounds: Pyrogallic acid (36.539 mg; high) and Ferulic acid (0.264 mg; low). The HPLC chromatogram of *Zingiber officinale* (rhizome) shows Pyrogallic (66.708 mg; high), and apigenin (0.478 mg; low). All these HPLC results agreed with *(104)* (Table 5 and Fig. 3).

Table 5. HPLC analysis of the *Aloe Vera (leaves) and Zingiber officinale* (rhizome) under investigation

Phenolic compounds	*Aloe Vera*	*Zingiber officinale*
	mg / 100 g extract	
Pyrogallic acid	36.539	66.708
Gallic acid	5.737	10.221
Chlorogenic acid	5.188	10.078
Salicylic acid	4.370	0.000
P-hydroxy benzoic acid	2.835	0.000
Phenol	1.908	2.074
Eugenol	1.723	24.405
5,7-dihydroxy-4-methoxy isoflavone	0.746	0.000
Apigenin	0.390	0.478
Luteoline	0.383	0.000
Caffeic acid	0.300	1.260
Quercetin	0.296	0.000
Ferulic acid	0.264	1.004

4. Conclusions

It was concluded that all the plant extracts under investigation show a highly antioxidant effect and potent anti-inflammatory activities on the lysosomal acid hydrolases such as: ACP; β-GAL and β-NAG in rat liver *in-vitro* which have a stabilizing effect on membrane permeability and also, its ability to scavenge free radical to protect the cell membrane. Also, it was concluded that *Aloe Vera and Zingiber officinale* are recommended as a spice in food plants and due to their medicinal properties as: Anti-inflammatory; antioxidant and so on…., because they posses various pharmacological activities.

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