Gas Chromatography-Mass Spectrometry-based Phytochemical Analysis and *In-Vitro* Anti-Lipid Peroxidation, Cyclooxygenase Inhibition Activities of Saudi *Eruca sativa* Leaves

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Summary: *Eruca sativa* is a wholesome yearly shrubbery herb in Saudi Arabia. *Eruca sativa* leaves are a conventional food and are consumed raw in salads. The present research reports the phytochemical analysis, *in-vitro* anti-lipid peroxidation, total anti-oxidant capability, cyclooxygenase-1, and -2 (COX1 and COX2) inhibition activities of Saudi *Eruca sativa* leaves water decoction (EWD). Gas chromatography-mass spectrometry (GC-MS) of EWD revealed seventeen constituents of six different chemical groups: phenolics (23.60%), aromatic and aliphatic esters (16.97%), terpenoids (31.91%), heterocyclics (14.83%), sulfur containing organics (11.25%), and silyl compounds (1.44%). The presence of Astaxanthin (1.96%), Clionasterol (12.81%), Ingol-12-acetate (4.77%), and Phytol (12.37%) in EWD indicated that the decoction technique was effective in extracting thermostable terpenoids. This research is the first report on *Eruca sativa* unraveling the thermostable phytochemicals. The EWD exhibited a straight-line relationship between liver lipid peroxidation inhibition and 150 to 400 µg/ml concentrations. The % anti-lipid peroxidation effect produced by EWD and Quercetin was statistically significant. At the highest 400 µg/ml dose, EWD exhibits 68.46 ± 0.01% anti-lipid peroxidation activity. The demonstrated IC_{50} of EWD and Ascorbic acid concerning the total anti-oxidant capability is 217.90 µg/ml and 74.91 µg/ml. The *in-vitro* assay protocols delineated the modes of inhibition of the biological oxidation processes by *Eruca sativa*, indicating transfer of hydrogen ion and metal ions reduction. The COX inhibitory potential was screened using Ellmann's reagent. The IC_{50} values for COX1 and COX2 inhibitions were 152.31µg/ml and 146.4 µg/ml, respectively, indicating that EWD has a potent COX inhibitory potential compared to Indomethacin. The COX2/COX1 ratio of inhibition, less than one, suggested that EWD phytochemicals would be preferential inhibitors of COX2. The current investigation justifies *Eruca sativa* leaves as a beneficial health food by establishing the chemical composition of water decoction that contributed to the anti-oxidant and COX inhibition activity.

Keywords: COX1, COX2, *Eruca sativa***,** GC-MS, *In-vitro*, Lipid peroxidation.

Introduction

Eruca sativa, Brassicaceae, is a popular plant in the Arabian diet and is consumed worldwide [1]. *Eruca sativa* has the following vernacular names: Arugula, Gerger, Jarjeer, Rocket salad, and Taramira [2]. In Saudi, gerger salads contain fresh leaves. Vegetarian dishes containing cooked *Eruca sativa* leaves, flowers, and sprouted seeds are familiar in Saudi Arabia [3]. Marked characters of *Eruca sativa* leaves are their peculiar penetrating peppery flavor and bitter taste [4]. *Eruca sativa* leaves have good market value as a vegetable due to their extensive global consumption, converting them into valuable crops [5]. Scientists in food and pharmaceuticals are continuously engaged in research to explore the nutritional and therapeutic benefits of consuming *Eruca sativa* as a whole or its leaves or seeds. Vitamins like A and C, essential microminerals such as calcium for bone development and maintenance, magnesium for healthy functioning of the physiological system, iron for the synthesis of hemoglobin, sulfur for DNA synthesis and maintenance of skeletal system, and potassium for regulating fluid volume, and proteins are available in high quantity in *Eruca sativa* leaf [6]. Anti-carcinogenic constituents like Glucosinolates, isothiocyanates, and indole compounds are extensively studied and contribute to the characteristic flavor of *Eruca sativa* [4, 7, 8]. Eruca *sativa* leaves also contain phytoconstituents like flavanol glycosides with a protective effect on the heart [9] and anti-carcinogenic properties [10]. The fatty acids of *Eruca sativa* leaves induced adipogenic activity, thereby valuable for diabetes mellitus [11]. Research indicated that the methanolic extract of *Eruca sativa* leaves exhibits a beneficiary anti-oxidant activity in rats when screened *in-vivo* [12]. The ethanolic *Eruca sativa* leaf extract improved the reproduction rate in male rats [13]. The anti-oxidant compounds in ethanolic extract possess excellent free radical scavenging activity in *in-vitro* tests [14]. Erucin, the chief constituent of *Eruca sativa* leaves'

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essential oil, is an isothiocyanate compound that possesses anti-bacterial, anti-cancer, and anti-oxidant properties [15, 16]. Anti-ulcer, astringent, antiphlogistic, diuretic, and laxative effects of *Eruca sativa* leaves are also beneficial [6].

The studies mentioned above utilized polar organic solvents like Ethanol or Methanol to extract *Eruca sativa* leaves. People consume *Eruca sativa* in day-to-day life in a raw form or cooked or steamed with water. Therefore, it would be meaningful to use water as a solvent to extract *Eruca sativa* leaves. Water is also suitable as a solvent for extraction due to its non-toxic, polar nature, and screening water extract of *Eruca sativa* leaves would explore the health benefits in its consumed form. Besides, there are no reports about the phytochemical or biological profiles of the *Eruca sativa* variety belonging to Saudi Arabia.

The anti-oxidant properties of *Eruca sativa* leaves are well known, though the leaves' anti-lipid peroxidation activity is not investigated enough. Lipid peroxidation is one of the major causes of oxidative stress in humans [17]. COX2 is a pro-inflammatory enzyme involved in the synthesis of prostaglandins which induces inflammation. COX2 is an attractive molecular target for discovering analgesics and anti-inflammatory compounds without adversely affecting the gastrointestinal tract. Hence, this study aims to justify Saudi *Eruca sativa* leaves as a functional food by exploring the phytochemical, anti-lipid peroxidation, and COX inhibition activity of its water decoction applying gas chromatography-mass-spectrometry (GC-MS) and *in-vitro* techniques.

Experimental

Eruca sativa was gathered from agricultural land in Jazan province, Saudi Arabia, between February and March 2020. The Herbarium Department of Jazan University authenticated the plant. A sample of the plant is available in the herbarium. GC-MS (Thermo Scientific, USA) was used for the phytochemical analysis of EWD. The GC-MS has a T.R. 5MS column of inside diameter 30m x 0.25mm, coated with a 0.25µm thick film of hydrophobic silica. The chromatographic column was hyphenated to a Thermo Scientific mass spectrometer equipped with an ISQ detector. The colorimetric absorbances were measured in a UV-1800 240V (Shimadzu, Japan) spectrophotometer. Refrigerated tabletop centrifuge (Sigma, Germany) and homogenizer (Thermo Scientific, USA) were used to centrifugate and homogenize hepatic tissue-sample mixtures in lipid peroxidation studies. The *in-vitro* cyclooxygenase inhibition assays were measured by the MultiskanFC microplate photometer (Thermo Scientific).

Lab-made distilled water was used as the solvent for the preparation of leaves decoction. All the chemical compounds, reagents, and solvents for GC-MS, anti-lipid peroxidation, and total anti-oxidant studies were of Merck, analytical grade purchased from Ejadah Medical Supplies, Jeddah, Saudi Arabia and were used without further purification. The inhibitory effect of *Eruca sativa* decoction on COX was determined using the Cayman (United States of America) ready-to-use COX (ovine/human) inhibitor assay kit. Ultrapure water (Cayman, United States of America) was used to dissolve buffer and Ellmann's reagent. *Eruca sativa* decoction was dissolved in Ethanol for cyclooxygenase inhibition assays.

Preparation of Saudi Eruca sativa leaves water decoction (EWD)

Fresh leaves of *Eruca sativa*, 600g, were milled for 50 sec using a domestic mixer grinder. The decoction was prepared in a beaker by boiling shredded leaves of *Eruca sativa* with 600ml of water for 30 min on a conventional hot-water bath [18]. After cooling to room temperature, the decoction was filtered using a Buchner funnel. The resultant decoction was a viscous green solution. The decoction, when dried at 100 ℃, 1000 mbars pressure for 10 minutes in a vacuum oven (Thermo scientific), yielded shining black amorphous powder. The dried EWD was stable for more than two months when stored in a closed amber-colored glass container. The phytochemical analysis by GC-MS was carried out on a freshly prepared dried EWD. The i*n-vitro* studies for anti-oxidant and cyclooxygenase inhibition potential of EWD required around 50 days, during which the dried decoction did not show any visible signs of deterioration.

GC-MS analysis of Saudi Eruca sativa leaves water decoction

A 1in 10 dilutions of EWD in Methanol was used for the GC-MS-based phytochemical analysis. Helium at a flow rate of 1.2ml/min carried 2µl of EWD sample injected in a splitless mode, which allows the whole sample to pass into the column for vaporization and then for detection. The sample injection channel was maintained at 270℃besides 40 ℃ of oven temperature for a minute. The chromatographic column with the capillary line for transferring ions to the detector was heated to 290 ℃ whereas the oven was programmed for a constant increase of temperature from 70℃ to 270 ℃, retaining the temperatures for 5min at 70 ℃, 140℃, 200℃, 250℃, and 270 ℃. Later, the oven was held at 270 ℃ throughout the analysis. The total time of chromatographic analysis was 70 min. The phytochemicals were detected in electron ionization mode of the MS, having the ion source at 270 ℃, 70eV ionization energy, scan range 60-800 atomic

mass units, and time for ionization maximum was 0.6min. Set the equilibration time 10 minutes prior to starting the chromatogram to avoid the solvent peaks in the spectrum. The mass spectra obtained were interpreted using Xcalibur software. The phytoconstituents of EWD were confirmed by comparing their complete ion fragmentation patterns with the reference spectra in NIST, MAINLIB, and REPLIB libraries. The composition of each constituent of EWD was obtained by dividing the corresponding peak area by the sum of peak areas followed by conversion to percentage. Moreover, while reporting, the compounds showing peaks with occurrence probability greater than 90% and having match factor (SI) and reverse match factor (RSI) threshold values above 900 compared to the standard library spectrum were considered.

In-vitro anti-lipid peroxidation studies

Lipid peroxidation is central to oxidative stress and is an important biological marker [19]. Hence, we aimed to determine the anti-lipid peroxidation capacity of EWD. The majority of herbs contain phenolics like flavanols and derivatives, terpenes, carotenoids, catechins, and tannins known for their anti-oxidant and anti-inflammatory properties [20]. For this reason, EWD was screened for total anti-oxidant activity. Most of the compounds, as mentioned earlier, can function as anti-inflammatory compounds through probable inhibition of pro-inflammatory cyclooxygenase enzymes. Thus, EWD was subjected to cyclooxygenase inhibition assay.

Anti-lipid peroxidation assay

The imbalance in liver metabolism and hepatic damage is mainly due to the stress induced by the imbalanced biological oxidation process [21]. Oxidative damage to cellular membranes or critical cellular components is due to hyper lipid peroxidation states. Liver mitochondria are the top sites for hepatic lipid peroxidation [22]. So, the inhibitory effect of EWD on liver lipid peroxidation was determined. Malondialdehyde is a prominent biological marker to quantify lipid peroxidation [19]. Malondialdehyde, the end product of peroxidation of lipids, reacts with thiobarbituric acid to form a chromophore that is pink in color. This reaction forms the basis for the quantitative determination of malondialdehyde by colorimetry [23]. Therefore, the anti-lipid peroxidation effect of EWD was estimated using the thiobarbituric acid-reactive substance screening that involved the colorimetric estimation of malondialdehyde [24].

Beef liver is rich in lipids, so it is a suitable lipid source for studying the peroxidation effect. The beef liver was purchased from the local market. A test solution and standard solution containing 1mg/ml of EWD in distilled water and Quercetin in distilled water and methanol, respectively, were prepared. Beef liver homogenate containing 10 g in 100 ml of phosphate-buffered saline, pH 7.4, was centrifuged applying 2880 x g of force at 4°C for 10 min. A 500µl of the supernatant obtained was treated with 100 µl of phosphate-buffered saline. Ferrous chloride 0.04 M, 50 µl was added. Peroxidation was induced by adding 50 µl of 0.1mM solution of Ascorbic acid. Test or standard solutions containing 150 to 400 µg/ml of EWD or Quercetin were added to the reaction mixture and were incubated at 37°Cfor 1 hr. Chromophore was developed by adding 2 ml of Thiobarbituric acid solution containing 0.6 g in 100 ml of 1M Sodium hydroxide followed by the addition of 0.9 ml of distilled water. The reaction mixture was heated for 30 min in an electric water bath to facilitate the interaction of lipid metabolic intermediates with the reagent. To the cold reaction mixture, added 5ml of n-butanol. We centrifuged the obtained slurry at 4°C for 30 min. Then, the absorbance in the separated organic layer was measured at 532 nm.

Absorbance measurements were made in triplicate and the % inhibition presented as mean ± S.E.M. (Standard error of the mean). The % anti-lipid peroxidation activity of EWD was obtained from the following equation,

$$\% \text{ anti-lipid peroxidation} = (Abs_{control} - Abs_{sample}) / Abs_{control} \times 100$$

The Abs_{sample} is the absorbance of EWD and $Abs_{control}$ is the absorbance of the blank solution containing the reagent.

Total anti-oxidant capability assay

Phosphomolybdenum assay for estimating the total anti-oxidant capability of EWD applies the principle of colorimetric determination of Molybdenum (V) complex [24]. The anti-oxidant phytochemicals function as reducing agents and reduce Molybdenum (VI) present in the Phosphomolybdate reagent to Molybdenum (V). The resulting complex of phosphomolybdate has a green color that can be measured spectrophotometrically at 692 nm. Briefly, incubated a mixture of EWD or Ascorbic acid at concentrations of 100 to 500 µl with 3ml of Phosphomolybdate reagent (Ammonium molybdate, 4mM added to 28 mM of Sodium phosphate in 06M Sulfuric acid) at 95°C for 90min. After cooling, the absorbance was measured at 692 nm. Measurements were made in triplicate, which also included a blank solution containing the reagent.

Reported results are the mean of triplicate readings ± S.E.M. (Standard error of the mean). The %

total anti-oxidant capability was calculated from the below equation.

% Total anti-oxidant capability = (1-$Abs_{sample} - Abs_{control}$) X 100

The regression curves were obtained from a plot of log % of total anti-oxidant capability Vs. Concentration in µg/ml. The straight-line plot was used to calculate the IC_{50}, the concentration of EWD or Ascorbic acid necessary for producing 50% inhibition of oxidation.

In-vitro cyclooxygenase inhibition assay

The ability of EWD to inhibit COX1 and COX2 enzymes was determined using the Cayman kit (Item. No 560131) specifically designed for antioxidant compounds. The COX-inhibitory assay is based on the measurement of the concentration of $PGF_{2\alpha}$ generated by the Stannous chloride reduction of PGH_2 derived from the substrate arachidonic acid by the action of COX enzymes [25]. Measurement of $PGF_{2\alpha}$ concentration involves the colorimetric assay of yellow color produced by 5-thio-2-nitrobenzoic acid resulting from the hydrolysis of Ellmann's reagent. Ellmann's reagent was used to quantify the activity of prostaglandin-tracer acetylcholinesterase. Acetylcholinesterase and $PGF_{2\alpha}$ compete for a fixed amount of antiserum added during the procedure. The antiserum forms complexes with acetylcholinesterase and $PGF_{2\alpha}$ which in turn binds with the antibodies precoated to the walls of assay plates. Hence, the colorimetric absorbance is proportional to acetylcholinesterase concentration bound to the antibodies attached to the well, which is inversely proportional to $PGF_{2\alpha}$.

Briefly, 10 µl of EWD (1000 µg dissolved in Ethanol) was added to reaction tubes containing 10 µl of Ovine COX1 and human recombinant COX2 containing 10 µl of Heme in reaction buffer and incubated at 37 °C for 20 min. The COX reaction was initiated by adding 10 µl of Arachidonic acid, after which the reaction mixture was incubated for 2 min at 37 °C. The COX reaction was halted by adding 30 µl of Stannous chloride, then vortexed, and incubated at 27 °C for 5 min. The resultant cloudy reaction mixture was added to130 µl of ready-to-use buffer solution. This solution was then pipetted at different dilutions of 10 to 50 µl, into 96-well plates precoated with antibodies. Acetylcholinesterase prostaglandin tracer 50 µl and antiserum 50 µl was added to each well. The plate was then covered with a plastic sheet and incubated on an orbital shaker at room temperature for 18 hrs. All unreacted components were discarded and washed the plate with buffer. Then added 200 µl of Ellmann's reagent into each well. Covered the plate and incubated it on an orbital shaker in the dark for 90 min. The absorbance of the developed plate was measured at 412 nm.

The plate was also maintained to have two wells each for blank (90 µl of buffer + 10 µl of Ethanol), background control (inactivated COX1 and COX2 without sample), total activity (without antiserum, and sample), maximum binding (100µl of buffer), 100% initial activity (contains all reagents without sample). Indomethacin was also screened at concentrations of 50 to 200 µM. All measurements were made in duplicate. The IC_{50}(concentration required to produce 50% COX inhibition) value was determined from the regression plots of % inhibition against the logarithm of the concentration of samples. % COX inhibition was calculated using the following equation.

% inhibition = ($Abs_{sample} - Abs_{initial\ activity}$) / $Abs_{initial\ activity}$ X 100

Statistical analysis

A one-way analysis of variance followed by multiple comparisons test was applied to evaluate the significant differences between groups. A value of $P<0.05$ is statistically significant.

Results and Discussion

The dried Saudi *Eruca sativa* leaves water decoction was obtained as a black amorphous powder with a yield of 1.75 %. The GC-MS chromatogram of EWD is shown in Fig. 1. The GC-MS of EWD was effective, and it separated seventeen chemical substances. Each peak characteristic implying the nomenclature and molecular formula of identified natural compounds is represented in Table-1. The percentage of each phytoconstituent of EWD is directly related to the peak area presented in Table-1. All the phytochemicals reported herein the EWD, though they are already known compounds, are reported for the first time in *Eruca sativa* leaves. Table-2 shows the classification of phytoconstituents of EWD confirming six chemical groups of natural compounds. The biological significance of EWD needs to be interpreted concerning its phytochemical composition. Hence, existing knowledge about these chemical compounds was gathered from the PubChem database (www. pubchem.ncbi.nlm.nih.gov). Dr. Duke's phytochemical and ethnobotanical database (www.phytochem.nal.usda.gov) was used to retrieve the pharmacological uses of EWD's constituents when the information was unavailable in PubChem. The biological activity was declared "Activity unknown" on the non-availability of pharmacological data. Table-3 is the collection of data about the beneficial health effects of constituents of EWD.

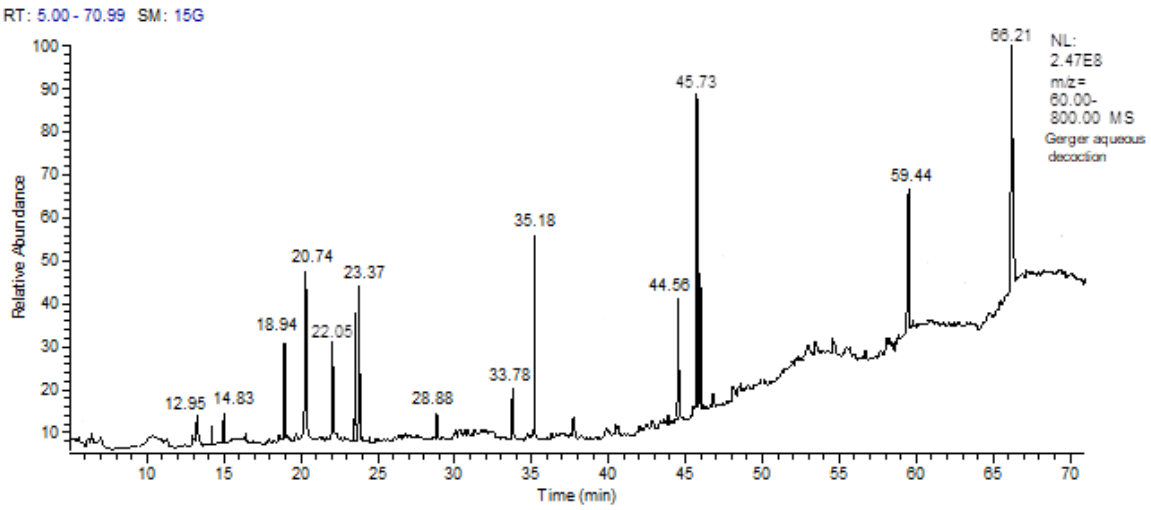
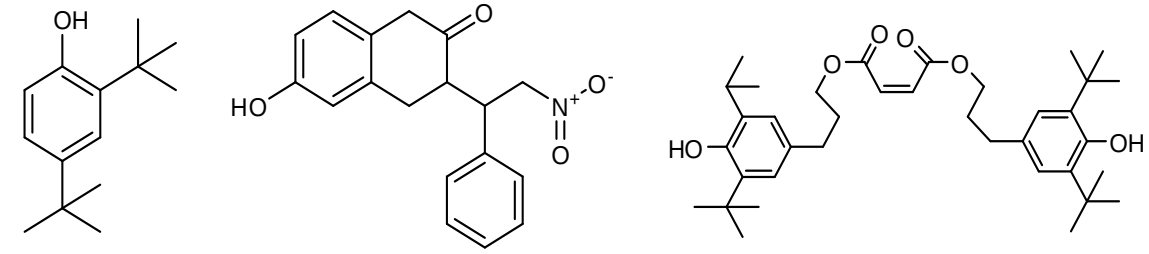


Fig. 1: GC-MS chromatogram of Saudi *Eruca sativa* leaves water decoction.



Phenol,2,4-bis(1,1-dimethylethyl)-

6-hydroxy-3-(2-nitro-1-phenylethyl)-3,4-dihydro-1H-naphthalen-2-one

Bis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)propyl]maleate

Fig. 2: Structures of phenolic compounds from Saudi *Eruca sativa* leaves water decoction.

Table-1: Phytochemicals identified from GC-MS spectrum of Saudi *Eruca sativa* leaves water decoction.

Peak Characters		Compound Name	Mol.Formula
R.T.(min)	Area (%)		
12.95	4.96	Benzoic acid, ethyl ester	$C_9H_{10}O_2$
14.17	7.39	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$
14.83	5.66	Thieno[3,2-c] pyridine	C_7H_5NS
16.95	1.30	Pipradrol	$C_{18}H_{21}NO$
18.94	3.98	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	$C_{12}H_{24}O_3$
20.74	4.77	Ingol 12-acetate	$C_{22}H_{32}O_7$
22.05	7.71	Phenol,2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$
22.36	5.59	S-methyl-, N-(2-methyl-3-oxobutyl)-Dithiocarbamate	$C_7H_{13}NOS_2$
23.02	0.40	5- [3,4-bis [(trimethylsilyl) oxy] phenyl]-3-methyl-5-phenyl-1-(trimethylsilyl)-2,4-Imidazolidinedione	$C_{25}H_{40}N_2O_4Si_3$
23.37	5.77	6-hydroxy-3-(2-nitro-1-phenylethyl)-3,4-dihydro-1H-naphthalen-2-one	$C_{18}H_{17}NO_4$
28.88	1.04	á-D-Galactopyranoside, methyl2,3-bis-O-(trimethylsilyl)-, cyclicbutylboronate	$C_{17}H_{37}BO_6Si_2$
33.78	1.96	Astaxanthin	$C_{40}H_{52}O_4$
35.18	12.37	Phytol	$C_{20}H_{40}O$
44.56	6.14	2(1H)-Isoquinolinecarboximidamide, 3,4-dihydro-	$C_{10}H_{13}N_3$
45.73	8.03	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$
59.44	12.81	Cilonasterol	$C_{29}H_{50}O$
66.21	10.12	Bis[3-(3,5-di-tert-butyl-4-hydroxyphenyl) propyl] maleate	$C_{38}H_{56}O_6$

Table-2: Classification of phytochemicals in Saudi *Eruca sativa* leaves water decoction.

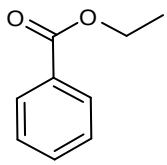
Phytochemical class	Content %
Phenolic compounds	23.60
Aromatic and aliphatic esters	16.97
Terpenoids	31.91
Heterocyclic compounds	14.83
Sulfur containing organics	11.25
Silyl compounds	1.44

Table-3: Health benefits of Saudi *Eruca sativa* leaves water decoction constituents.

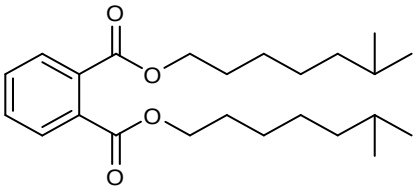
Compound name	Pharmaceutical use
Benzoic acid, ethyl ester	Food additive, flavoring agent*, antibacterial
Dasycarpidan-1-methanol, acetate (ester)	Anti-inflammatory, antimicrobial
Thieno[3,2-c] pyridine	Anti-platelet therapy
Pipradrol	Antifatigue, antidepressant, antiobesity*
Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	Food and drug colorant*
Ingol 12-acetate	Anti-inflammatory
Phenol,2,4-bis(1,1-dimethylethyl)-	Anti-oxidant
S-methyl-, N-(2-methyl-3-oxobutyl)- Dithiocarbamate	Anti-inflammatory (Ref 37)
5- [3,4-bis [(trimethylsilyl) oxy] phenyl]-3-methyl-5-phenyl-1-(trimethylsilyl)-2,4-Imidazolidinedione	Activity unknown
6-hydroxy-3-(2-nitro-1-phenylethyl)-3,4-dihydro-1H-naphthalen-2-one	Activity unknown
á-D-Galactopyranoside, methyl2,3-bis-O-(trimethylsilyl)-, cyclicbutylboronate	Activity unknown
Astaxanthin	The carotenoid pigment in nutraceutical, cosmetics, food and feeds*, anti-oxidant
Phytol	Food additive as flavoring agent*, anti-oxidant
2(1H)-Isoquinolinecarboximidamide, 3,4-dihydro-	Anti-hypertensive
1,2-Benzenedicarboxylic acid, diisooctyl ester	Food additive*, antimicrobial
Cilonasterol	Antidiabetic, hypolipidemic
Bis[3-(3,5-di-tert-butyl-4-hydroxyphenyl) propyl] maleate	Activity unknown

* Retrieved from PubChem. Others were retrieved from Dr. Duke's phytochemical and ethnobotanical databases.

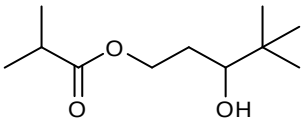
Fig 2 shows the structures of phenolic compounds identified by GC-MS analysis of EWD. These simple phenolic compounds are the second major compounds in EWD. The identified phenolics do not belong to the known plant phenols like flavonoids or tannins. They are small molecules whose pharmacological profiles need investigation in the future. Fig. 3 shows the structures of aromatic and aliphatic esters present in EWD. Fig. 4 showcases the chemical structures of terpenoids identified in *Eruca sativa* leaves decoction. Terpenoids of EWD are the first major group of compounds with a contribution of 31.91 %w/w. Cilonasterol, one of the terpenoids isolated from EWD, is not much studied. Cilonasterol is also known as γ-sitosterol. Cilonasterol chemically is a tetracyclic triterpenoid or steroid or stigmastane derivative [26]. Cilonasterol has been reported as a major phytoconstituent from various plants. Cilonasterol possesses anti-diabetic, anti-hyperlipidemic, anti-oxidant, and anti-cancer activities with a proven safe toxicological profile [27]. Phytol or *Trans*-phytol also is a widely distributed plant terpenoid available in marine organisms [28]. Phytol exhibits anti-cancer, anti-nociceptive, and anti-oxidant properties [29-31]. Ingol-12-acetate, also a terpenoid, has been reported in several plants. The chemical structure of Ingol-12-acetate is known, but scanty information is available about its biological activity. Astaxanthin is a terpenoid related to xanthophylls. Astaxanthin is chiefly available in marine animals like fish but has also been reported from plant sources. Astaxanthin is a beneficial food supplement providing anti-oxidant, anti-cancer, anti-inflammatory, analgesic, and anti-diabetic effects. Astaxanthin can also improve immunity and prevent cardiovascular diseases [32]. Fig. 5 shows the heterocyclic compounds isolated from *Eruca sativa* leaves. Though these compounds are reported from many plants, their biological activities have to be studied. Fig. 6 shows the structures of sulfur containing organics identified in *Eruca sativa* leaves. Brassicaceae plants, such as cruciferous vegetables, usually contain a high proportion of sulfur containing organics. S-methyl-, N-(2-methyl-3-oxobutyl)-Dithiocarbamate, a constituent reported in EWD exhibits anti-inflammatory activity [33]. Two-dimensional structures of naturally occurring silyl compounds isolated from EWD are shown in Fig. 7. Natural products possessing trimethylsilyl sugar structures and trimethylsilyl-linked-heterocyclic rings have been exploited for their antibacterial, antifungal, anti-carcinogenic, and anti-inflammatory activities. Pharmacological importance of 5- [3,4-bis [(trimethylsilyl) oxy] phenyl]-3-methyl-5-phenyl-1-(trimethylsilyl)- 2,4-Imidazolidinedioneand á-D-Galactopyranoside, methyl2,3-bis-O-(trimethylsilyl)-, cyclic butylboronate are unknown. Nevertheless, these two compounds are commonly available in many herbs and seaweeds [34, 35].



Benzoic acid, ethyl ester

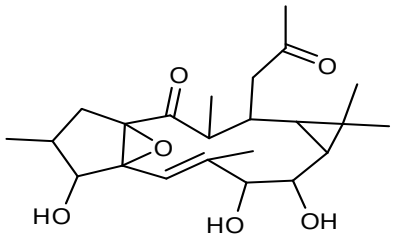


1,2-Benzenedicarboxylic acid, diisooctyl ester

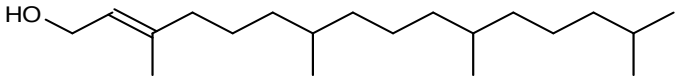


Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester

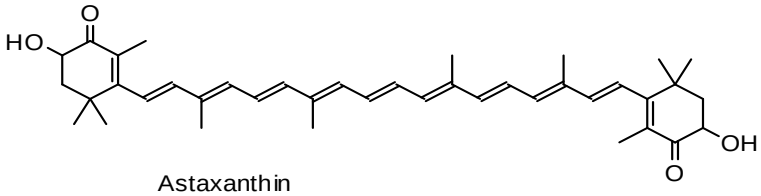
Fig. 3: Structures of aromatic and aliphatic esters of Saudi *Eruca sativa* leaves water decoction.



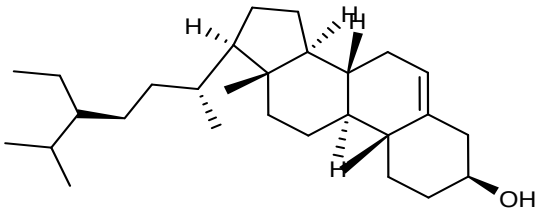
Ingol 12-acetate



Phytol

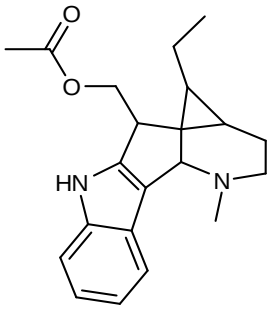


Astaxanthin

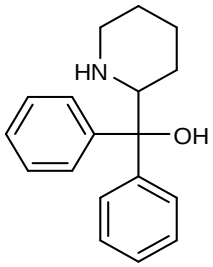


Cilonasterol

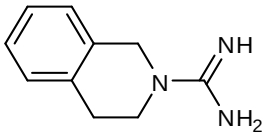
Fig. 4: Structures of terpenoids of Saudi *Eruca sativa* leaves water decoction.



Dasycarpidan-1-methanol, acetate

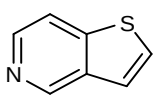


Pipradrol

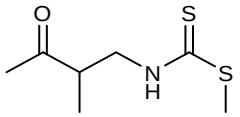


2(1H)-Isoquinolinecarboximidamide, 3,4-dihydro-

Fig. 5: Structures of heterocyclic compounds of Saudi *Eruca sativa* leaves water decoction.

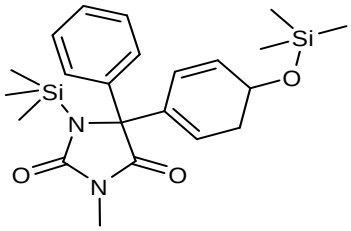


Thieno[3,2-c]pyridine

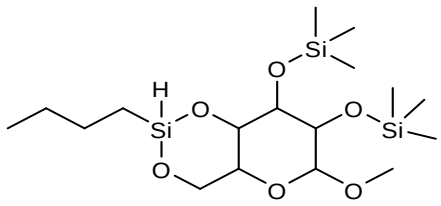


Dithiocarbamate, S-methyl-, N-(2-methyl-3-oxobutyl)-

Fig. 6: Structures of sulfur containing organics from Saudi *Eruca sativa* leaves water decoction.



2,4-Imidazolidinedione, 5-[3,4-bis [(trimethylsilyl) oxy]phenyl]-3-methyl-5-phenyl-1-(trimethylsilyl)-



á-D-Galactopyranoside, methyl2,3-bis-O-(trimethylsilyl)-,cyclic butylboronate

Fig. 7: Structures of silyl compounds in Saudi *Eruca sativa* leaves water decoction.

EWD contains a significant concentration of simple phenolic compounds and natural terpenoids, which possess profound anti-oxidant properties. The extraction of hydrophobic, thermostable phytoconstituents can be effective by decoction method because it applies high temperature [18]. Thus, it is suggested that the simple phenols, terpenoids, and other compounds reported in EWD are thermostable. Astaxanthin, a prevalent tetraterpene; Cilonasterol (γ-Sitosterol), a triterpene; Ingol-12-acetate, a diterpene; *Trans*-Phytol, a diterpene identified in EWD are thermostable, neutral hydrophobic compounds [36].

Eruca sativa leaves are popular in Saudi Arabia and constitute a part of a regular diet. Regardless of their use in conventional diet, the nutritional value or biological value is uninvestigated. *Eruca sativa* has commercial value around the globe and in Saudi Arabia. Consequently, there is a need to prove the anti-lipid peroxidation property of water decoction of Saudi *Eruca sativa* leaves. The *in-vitro* anti-lipid peroxidation assay of EWD suggested that it can significantly inhibit liver lipid peroxidation, and the results are shown in Table-4.

Table-4: *In-vitro* anti-lipid peroxidation assay of Saudi *Eruca sativa* leaves water decoction.

Concentration (µg/ml)	% Inhibition (mean ± S.E.M) Water decoction	% Inhibition (mean ± S.E.M) Quercetin
150	17.12 ± 0.20	49.99 ± 0.03
200	31.98 ± 0.01	55.36 ± 0.06
250	43.02 ± 0.09*	61.09 ± 0.02
300	51.59 ± 0.03*	72.11 ± 0.01
350	59.99 ± 0.02*	82.07 ± 0.01
400	68.46 ± 0.01*	91.26 ± 0.01

* $P < 0.05$ compared to Quercetin.

The EWD inhibited hepatic lipid peroxidation at 250µg/ml to 400µg/ml concentrations. The EWD produced 68.46 % inhibition of lipid peroxidation at the highest screened 400 µg/ml concentration. The assay protocol involved the addition of EWD to the hepatic tissues after inducing lipid peroxidation. The reduction in absorbance indicates the reduction in the amount of dialdehyde, indicating that EWD can decrease the lipid peroxidation rate. Anti-oxidants like the terpenoids Astaxanthin, Clionasterol, Ingol-12-acetate, Phytol, and Phenolic compounds in EWD are the reason for the inhibition of liver lipid peroxidation. EWD exhibited a linear increase in the lipid peroxidation inhibitory activity with the dose. The assay model used revealed the mode of inhibition of lipid peroxidation by EWD. EWD can inhibit lipid peroxidation by hydrogen atom transfer, as suggested by the assay model [20].

Further investigation of the EWD's total anti-oxidant capability by the phosphomolybdenum method confirmed it as a potential natural anti-oxidant. The % total anti-oxidant capability of EWD, when plotted against the log of concentrations, yielded a straight line in Fig. 8 from which the IC_{50} of EWD was obtained. A statistically significant IC_{50} of 217.90µg/ml was observed for EWD. The IC_{50} of ascorbic acid was 74.91 µg/ml. The assay method utilizes phosphomolybdate reagent containing molybdenum (VI) that is reduced by EWD to Molybdenum (V), suggesting that the phytochemicals act as reducing agents, which is a known mechanism of anti-oxidant action by plant products [37]. The

mixed phytochemicals of EWD show a linear relationship between the log of % total anti-oxidant activity and its concentration.

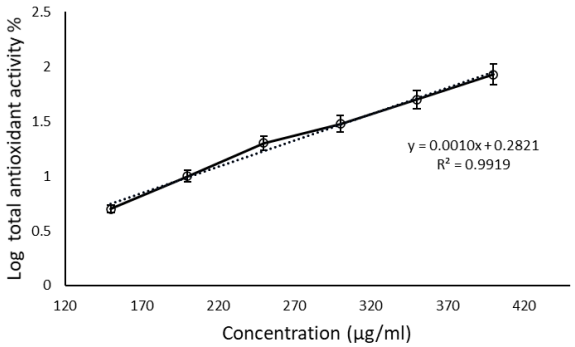


Fig. 8: The total anti-oxidant capability of Saudi *Eruca sativa* leaves water decoction.

Methanolic extract of Pakistani *Eruca sativa* prepared by Soxhlet extraction contains phenolic anti-oxidants, namely Catechol, Resorcinol, and Vanillin [38]. An RP-HPLC method for determining Quercetin in the methanolic extract of Saudi *Eruca sativa* has been reported [39]. A comparison study between Bulgarian and Italian *Eruca sativa* leaves ethanolic extract revealed that the Bulgarian variety has a higher total phenolic content and more significant anti-oxidant capability than the Italian variety [15]. The chemical nature of compounds separated and the degree of the extract's anti-oxidant capability depends on the extraction method [40]. Phenolic anti-oxidants identified in the methanolic extract of *Eruca sativa* leaves were lacking in EWD. The lack of hydrophilic phenolic compounds, that is, Catechol, Resorcinol, and Vanillin, in EWD may be due to increased temperatures in decoction preparation.

Nonetheless, lipophilic compounds, namely the higher terpenoids Astaxanthin, Cilonasterol, Ingol-12-acetate, and Phytol possessing anti-oxidant activity, were efficiently extracted by the decoction method, which led to prominent inhibitory effects on cellular oxidation. Metabolic dysfunction and several disease conditions have a solid link to oxidative stress. Functional foods can reduce oxidative stress, and anti-oxidant foods alleviate cancer development, diabetes mellitus, hypertension, inflammation, and obesity [1, 12, 16]. Therefore, the consumption of *Eruca sativa* would prevent oxidative stress.

The *in-vitro* COX inhibition assay revealed that EWD could significantly inhibit COX1 and COX2 enzymes. The log dose-response curve, Fig. 9, was plotted to calculate the IC_{50} values of EWD against both COX isoforms. The IC_{50} of EWD against COX1 was 152.31 µg/ml and for COX2 was 146.4 µg/ml. Indomethacin, a non-specific COX inhibitor, possessed IC_{50} values against COX1 and COX2 as 3.4 µM/ml and 180 µM/ml, respectively. The COX2/COX1 inhibitory activity ratio for EWD was 0.96. The ratio of COX2/COX1 inhibition less than 1 suggests that the decoction is a preferential COX2 inhibitor [41]. The relatively lower IC_{50} values for COX1 and COX2 inhibition indicate that *Eruca sativa* leaves contain potent COX inhibitors, which could be exploited for anti-inflammatory and analgesic properties. The selectivity for COX2 is recommended over non-selective COX inhibitors because of their reduced gastrointestinal tract side effects. COX2 inhibitors may also play a beneficial role in the therapy of epilepsy by decreasing COX2 induced neuroinflammation. Selective COX2 inhibition is also implicated in the treatment of colon and liver cancer. Synthetic COX2 inhibitors may increase the risk of heart and renal failure. Therefore, discovering alternative natural COX2 inhibitors as therapeutic molecules would be beneficial in managing pain and inflammation. The organic compounds present in the water decoction of *Eruca sativa* shall contribute to the COX1 and COX2 inhibitory activity. Further investigations are needed to prove the *in-vivo* anti-inflammatory, analgesic, and COX inhibitory activities.

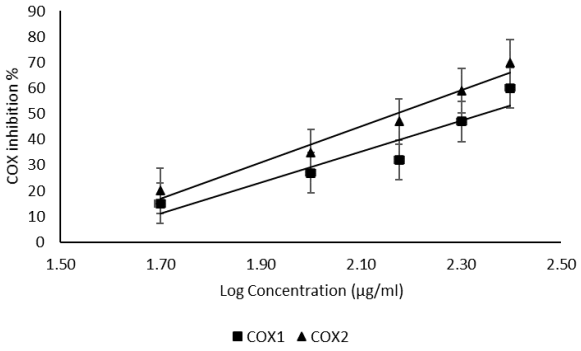


Fig 9: COX-inhibitory activity of Saudi *Eruca sativa* leaves water decoction. Values are the mean of two determinations ± S.E.M.

Regular consumption of fresh *Eruca sativa* leaves by the Saudi and global community could provide beneficial health effects due to the anti-lipid peroxidation and COX inhibitory potential. *Eruca sativa* leaves' safety is conclusive as it has been consumed for since long as a part of a regular diet. Dietary recommendations about the amount of daily intake are required. The demonstrated anti-lipid peroxidation and COX inhibitory capacity of *Eruca sativa* leaves validated the claim that it is a beneficial health food. The nutritional and biological benefits of Eruca leaves, the phytochemical

characterization of several extracts and fractions are to be determined. Also, extensive pharmacological screening to delineate the anti-oxidant modes of action and other biological activities is necessary.

Conclusions

Saudi *Eruca sativa* leaves possess significant hepatic anti-lipid peroxidation, total anti-oxidant, and COX inhibition activity. The GC-MS analysis of Saudi *Eruca sativa* leaves water decoction identified seventeen compounds, chemically classified into six groups: phenolics, aromatic and aliphatic esters, terpenoidsheterocyclics, sulfur containing organics, and silyl compounds. The water decoction contains higher amounts of terpenoids and phenolic compounds. Due to the high temperature during the decoction process, the water decoction has extracted exclusively thermostable compounds. The phytoconstituents of *Eruca sativa* can preferentially inhibit the COX2 enzyme making it a suitable plant for further pre-clinical and clinical studies involving COX2 as the target. Overall, *Eruca sativa* has the potential to reduce oxidative stress. The current investigation supports the use of *Eruca sativa* as a beneficial health food.

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