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Research Article

Novel Anti-ulcerogenic Effects of Total Extract and Isolated Compounds from *Cakile arabica*

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Abstract

Background and Objective: Natural products take a special interest in the discovery of new anti-ulcerogenic drugs. Family Brassicaceae is one of the most important families in use for many investigations. *Cakile arabica* (Velen) was used in folk medicine in treatment of gastric disorder with no scientific approve, so the aim of this study was to investigate its contents and prove their biological activities. **Materials and Methods:** Different chromatographic equipment and instrument were used for isolation such as column chromatography. Identification of compounds were done by several spectroscopic methods; ¹H-NMR. The ¹³C-NMR and DEPT COSY, HSQC and HMBC. Absolute ethanol-induced ulcer model in rats were used to evaluate the anti-ulcerogenic activity using reference standard drug ranitidine (100 mg kg⁻¹). **Results:** Ten compounds were isolated from *C. arabica* for the first time and were identified as: (1) α -amyrin, (2) β -sitosterol, (3) Tetracosanoic acid, (4) 4-hydroxy-5-n-butoxy-5-oxopentanoic acid, (5) Scopoletine, (6) Umbelliferone, (7) Esculetin, (8) Kaempferol, (9) Quercetin and (10) Quercetin-3-O-rutinoside. The total alcohol extract (1000 mg kg⁻¹) and the isolated compounds (50 mg kg⁻¹) showed anti-ulcerogenic activity with different potentials. The total alcohol extract was the most effective agent in this study, as it showed 98.5% protection of control ulcer followed by compounds tetracosanoic acid and 4-hydroxy-5-n-butoxy-5-oxopentanoic acid, which produced 80 and 55.5%, respectively while standard ranitidine produced 46.2%. The total alcohol extract was safe up to 4000 mg kg⁻¹ and showed no side effects on liver and kidney functions when administrated orally for 15 consecutive days at dose 1000 mg kg⁻¹. **Conclusion:** The cytoprotective mechanism may explain the potent anti-ulcerogenic activity of the *Cakile arabica* extract and the isolated compounds. Compounds tetracosanoic acid and 4-hydroxy-5-n-butoxy-5-oxopentanoic acid could be the main active constituents as they showed highest activities between compounds. But it is clear that there was a synergistic activity between compounds as the activity of the total extract exceeds the activity of any compound alone.

Key words: Anti-ulcerogenic, sub-chronic toxicity, non-polar contents, liver and kidney functions, LD₅₀

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Natural products are now a days receiving a great deal of concern from scientists all over the world. The Arab world has not heritage in the field of medicinal plants which goes back as far as the Chinese heritage, if not even older. However, in China as well as in Japan, they were able by systematic studies and applying modern analytical technology to explore their natural resources of medicinal plants so that more than 80% of their diseases are now treated by what they call traditional medicine¹.

Medicinal plants from traditional healers revealed 73 plant species being used as wound healing remedies, according to the definitions of wounds given by the healers themselves. The plants, belonging to 34 plant families are used as first aids in the washing of wounds, extraction of pus, as coagulants as well as for infected wounds². Some others can be a rich source of potential antiviral compounds³.

Brassicaceae is a large family includes vegetable crops, medicinal and nutritional plants. Plants of this family are used medically in many purposes as antidiabetic, antibacterial, antifungal, anticancer, antirheumatic and insecticidal⁴. Nutrition benefit of Brassicaceae species are important sources of oil and proteins for animal and human nutrition. In addition, they constitute a very rich source of health-promoting phytochemical like phenols, flavonoids, phenylpropanoids, vitamins, glucosinolates^{4,5}, fibers, soluble sugars, fats and carotenoids. Besides this, there is growing evidence that a higher intake of Brassica vegetables (e.g., broccoli, cabbage, kale, mustard greens, brussels-sprouts and cauliflower) could help to reduce the risk of cancer, atherosclerosis, chronic inflammation and reducing serum levels of glucose in *Diabetes mellitus*⁶⁻⁸.

Peptic ulcer is a common gastrointestinal disorder in modern era. It becomes a common global health problem affecting a large number of people worldwide. Different classes of drugs have been used in the treatment of peptic ulcer but serious side effects like, arrhythmias, gynaecomastia, arthralgia and hypergastrinemia are exhibited by most of these drugs⁹.

Natural products take a special interest in the discovery of new anti-ulcerogenic drugs. Family Brassicaceae is one of the most important families in use for many investigations. *Cakile arabica* was used in folk medicine in treatment of gastric disorder with no scientific approve, so the aim of this study was to investigate its contents and prove their biological activities.

MATERIALS AND METHODS

Plant material: Aerial parts of *Cakile arabica* were collected during flowering stage in March, 2013 from Riyadh territory, the sample was identified by Dr. Jacob Thomas; assistant professor of taxonomy, Botany and Microbiology Department, Faculty of Science, King Saud University. Specimen was kept in the herbarium of Chemistry Department. Samples of the aerial parts were air dried in shade, grinded to fine powder and kept for phytochemical and biological investigation.

Animals: Swiss albino mice of both sex (26-30 g) and male Wister rats (180-200 g) were supplied by the animal house of King Saud University, KSA. Animals were housed in standard polypropylene cages with wire mesh top and maintained under standard conditions (temperature $23 \pm 1.0^\circ\text{C}$, humidity $55 \pm 10\%$, 12 h light/12 h dark cycle). They fed with a standard pellet diet with water *ad libitum* and were allowed to adapt to the laboratory environment for one week before experimentation.

Extraction and isolation: Air-dried 2 kg of *C. arabica* was extracted with 96% ethanol (4×5 L) for 2 h, filtered off. The combined filtrates were separately evaporated under reduced pressure and low temperature. The obtained residue 210 g was kept for further phytochemical investigation. The previous extract was dissolved in hot distilled water (300 mL), filtered over cotton while hot to remove non-polar contents (chlorophyll and fatty matters). The non-filtered materials were washed with hot water, dried, weighed 70 g then kept for further investigation of the lipid substances.

To investigate the polar content of *C. arabica* the aqueous extract lifted was re-concentrated by lyophilization and through the precipitation with excess MeOH. Finally, the collected filtrates were separately dried under reduced pressure to obtain 90 g. The sample was separately dissolved in distilled water (200 mL), extracted successively by diethyl ether, chloroform, ethyl acetate and n-butanol. The extract was filtered over anhydrous sodium sulphate and dried in vacuum to give 4, 7, 20 and 35 g for diethyl ether, chloroform, ethyl acetate and n-butanol respectively.

Investigation of non-polar contents: Twenty grams from *C. arabica* was separately saponified by boiling with 150 mL. About 10% alcoholic potassium hydroxide and 5 mL benzene on boiling water bath under reflux for 18 h. The saponified liquid was distilled off almost to dryness under vacuum, then

the residue was mixed with 100 mL distilled water and extracted with ether (peroxide free) till complete extraction of the USP matter (6×3100 mL). The combined ethereal extracts were washed from traces of alkali with distilled water, dehydrated over anhydrous sodium sulphate and the solvent distilled off. The residues were dried in desiccators over anhydrous calcium chloride and kept for isolation and identification of USP matter (15.5 g). The aqueous mother liquor was then acidified with concentrated hydrochloric acid (using litmus paper) and the liberated fatty acids was extracted using ether (4×100 mL). The combined ethereal extracts were washed with distilled water till neutral conditions, dried over anhydrous sodium sulphate, distilled off. The residue obtained was 3.5 g was kept for further analysis as SP matter. Fatty acids composition of the SP matter was determined after derivatization to the corresponding methyl ester according to the published procedure¹⁰ and investigated using gas liquid chromatography adopting the following condition: Column coiled glass (1.5 m×4 mm) stationary phase, 10% PEGA on diatomite (100-120 mesh), programming temperature from 25-190°C for 45 min detector temperature 300°C, injector temperature 280°C, nitrogen flow rate 30 mL min⁻¹, nitrogen flow rate 33 mL min⁻¹, air flow rate 330 mL min⁻¹ and chart speed 2 cm min⁻¹. Identification was carried out by direct comparison of R_t values with those of available authentic that analysed under the same experimental conditions.

The USP was subjected to isolation of their contents separately by applying 10 g of each extract on polyamide 300 g column (2×120 cm length) eluted gradually using benzene increased with ethyl acetate. Hundred fractions (50 mL each) were collected, reduced to 4 mean fractions (according to colour and R_f of the spots), each fraction was concentrated under reduced pressure and subjected to further purification using multiple column and solvent fractionation from methanol to get 36.8, 100.8, 220 mg of compounds 1, 2 and 3 respectively.

Investigation of polar contents: Both of ether and chloroform extracts showed same chromatographic behavior (R_f-values, colour and/or fluorescence in UV-light and its change on spraying with specific spray reagents). Accordingly, they are collected and gave 11 g. In similar manner, both of ethyl acetate and n-butanol extracts were collected 50 and 20 g extract, due to their similar constitutive of spots. For isolation of compounds from ether and chloroform extracts column chromatography was used. An amount of 8 g of this extract was dissolved in chloroform and mixed with silica gel G for column chromatography. The solvent was evaporated on water bath with continuous trituration to form a free flowing

dry powder. The powdered homogenate was introduced to the top of a glass column (2×100 cm) packed with 160 g silica gel G. Elution was carried out using chloroform-methanol in gradual increasing polarity. Several columns and re-columns were used for final purification of plant contents to obtain 320, 36 and 40 mg for compounds 4, 5 and 6 respectively.

An amount of 20 g from ethyl acetate n-butanol extract was dissolved in methanol and applied on the top of a column (150×5 cm) packed with 200 g. Sephadex LH-20 and methanol was used as an eluent. Hundred fractions were obtained (100 mL each), similar fractions were collected together and concentrated. For final purification, preparative TLC was used on silica gel G plates (20×20 cm), using solvent system A (benzene: Ethyl acetate 86:14 v/v.) Thereafter, each band corresponding to the major compound was scratched and eluted using methanol. The elutes were dried and reapplied on the top of silica gel column packed with silica gel G. Elution was proceeded using ethyl acetate-methanol with gradual increasing polarities. The individually collected sub-fraction were dried under reduced pressure at about 35°C and re-crystallized from methanol to yield 250, 10, 45 and 20 mg of pure samples of compounds 7, 8, 9 and 10 respectively.

α-amyrin (1): Compound obtained as white needle crystals from methanol (36.8 mg), R_f (in system C (ethyl acetate-methanol-water 90:5:4 v/v/v) was 0.62, (mp 185-187°). By comparing the obtained spectroscopic data analysis (¹H-NMR, ¹³C-NMR and DEPT COSY, HSQC and HMBC) with published one¹¹.

β-sitosterol (2): Whitish crystal residue (100.8 mg) R_f 0.45 (in system benzene: Ethyl acetate 90:10 v/v). ¹HNMR δ (CDCl₃) showed signals at δ 5.34 ppm (1H d, J = 5.4 Hz, H-7), singlet at δ 3.51 ppm (1H, s, -OH), at δ 2.26 ppm (2H q, J = 11.1, 4.8 Hz H-3) nearest from -OH, triplet at δ 1.98 ppm (2H, t, J = H-5 and H-8), at δ 1.83 ppm (3H, t, H-28), sextet at 1.63 ppm (1H, s, H-18), singlet at 1.57 ppm (8H, s, H-1, H-2, H-15 and H-16) at 1.33 ppm (5H, m, H-9, H-11 and H-12), multiple t at 1.14 ppm (6H, m, H-4, 24, 21, 17 and 22) at 1.12 ppm (6H, d, H-29 and H-30), at 0.91 ppm (4H, H-19 and H-20), singlet at 0.81 ppm (9H, s, H-24, H-25 and H-26), singlet at 0.66 ppm (3H, s, H-23). ¹³C-NMR (CDCl₃) showed 30 carbons. The HMQC, DEPT-135 and HMQC confirmed the structure.

Tetracosanoic acid (3): It was isolated as white amorphous powder (220 mg); mp. 78-79°C; R_f-value 0.56 (system: Benzene-ethyl acetate, 90:10 v/v). IR ν_{max} (KBr, cm⁻¹) 3415,

2918, 2849, 1709, 1463, 720; ¹H NMR δ (500 MHz, CDCl₃): δ ppm 2.32 (2H, t, J = 7 Hz, H-2), 1.61 (2H, pentet-like, J = 7 Hz, H-3), 1.23 (40H, br s, H-4 to H-23), 0.86 (3H, t, J = 6.5 Hz, H-24); ¹³C NMR (125 MHz, CDCl₃): δ ppm 177.0 (C-1), 34.0 (C-2), 31.9 (C-3), 29.7 (C-4-16), 29.6 (C-17), 29.5 (C-18), 29.4 (C-19), 29.3 (C-20), 29.1 (C-21), 24.7 (C-22), 22.7 (C-23), 14.2 (C-24).

4-hydroxy-5-n-butoxy-5-oxopentanoic acid (4): It was isolated as white crystals (320 mg); R_f-value = 0.45 (system: Chloroform-methanol 90:10 v/v, TLC); bp 99-100°C; IR ν_{max} (KBr, cm⁻¹): 3415, 2918, 2849, 1709, 1463, 720; ¹H NMR δ (500 MHz, CDCl₃): δ ppm 4.21 (1H, dd, J = 7.8, 5 Hz, H-4), 4.10 (1H, t, J = 6.25, 5 Hz, H-1'), 2.45 (1H, m, H-3), 2.31 (1H, m, H-2), 2.16 (1H, m, H-3'), 1.58 (2H, pentet-like, J = 7.1 Hz, H-2'), 1.33 (2H, sextet-like, J = 7.3 Hz, H-3'), 0.88 (3H, t, J = 7.3 Hz, H-4'); ¹³C NMR (125 MHz, CDCl₃): δ ppm 178.5 (C-1), 172.3 (C-5), 65.3 (C-1'), 55.6 (C-4), 30.5 (C-2'), 29.3 (C-2), 24.8 (C-3), 19.0 (C-3'), 13.6 (C-4'). The structure of 2 was established by chemical and physicochemical data (IR, ¹H and ¹³C NMR) given above and confirmed by DEPT and H, H-COSY, HSQC and HMBC 2D-NMR correlation experiments.

Scopoletin (5): Thirty six milligrams white crystals, R_f = 0.33 (system A); m.p. 203-204°C. UV λ max (MeOH): (nm) 229, 255, 263, 297, 346; (NaOAc) 246, 278, 392. IR (KBr) ν_{max} cm⁻¹: 3325, 2969, 2912, 1707, 1626, 1610. ¹H NMR δ (CDCl₃): δ 7.9 (1H, J = 9, H-4); δ 7.2 and 6.75 (2H, 2S, H-5 and H-8, respectively), δ 6.2 (1H, d, J = 9 Hz, H-3), δ 3.8 (3H, s, OCH₃). ¹³C NMR (CDCl₃): δ 161.01 (C-2), 154.67 (C-9), 150.76, (C-7), 146.78 (C-4), 114.29 (C-6), 113.23 (C-10), 113.04 (C-3), 110.50 (C-5), 103.24 (C-8), 56.80 (O-CH₃).

Umbelliferone (6): Forty milligrams white crystals, R_f = 0.75 (system A), mp. 272-274°C. UV λ max, (MeOH): (nm) 244, 257, 320, 324. EI-MS m/z (% rel. int.): 162 (M⁺) (100), 134 (15), 106 (10), 78 (9), 77 (10), 51 (5). ¹H NMR δ (DMSO-d₆): δ 7.79 (1H, d, J = 9.5 Hz, H-4); δ 7.42 (1H, d, J = 8.4 Hz, H-5); δ 6.77 (1H, dd, J = 8.4, 2.2 Hz, H-6); δ 6.71 (1H, d, J = 2.2 Hz, H-8) and δ 6.1 (1H, d, J = 9.5 Hz, H-3). ¹³C NMR (DMSO) 161.101 (C-2'), 121.40 (C-3'), 145.10 (C-4'), 129.78 (C-5'), 113.42 (C-6'), 162.11 (C-7'), 103.10 (C-8'), 146.5 (C-9'), 112.34 (C-10').

Esculetin (7): Two hundred and fifty milligrams, needle crystals, R_f = 0.12 (system A), m.p. 272-274°C. UV λ max (MeOH): (nm), 226, 257, 293 and 346 nm. EI-MS m/z (% rel. int.): M⁺ 179 (100), 150 (20), 132 (3), 122 (22), 121 (18), 94 (20) and 69 (10). ¹H NMR δ (DMSO-d₆): δ 7.9 (1H, d, J = 9.5 Hz, H-4); δ 7.1 (1H, s, H-5); δ 6.79 (1H, s, H-8) and δ 6.2 (1H, d, J = 9.5 Hz,

H-3). ¹³C NMR (DMSO) 161.28 (C-2'), 110.77 (C-3'), 144.60 (C-4'), 112.25 (C-5'), 148.40 (C-6'), 150.33 (C-7'), 102.53 (C-8'), 142.84 (C-9'), 110.77 (C-10').

Kaempferol (8): Yellow crystals (10 mg), R_f = 0.91 (system B (Chloroform-methanol 95:5 v/v), m.p. 277°C, UV, λ max in MeOH: nm 367, 268; (AlCl₃): 265, 350, 420; (AlCl₃/HCl): 265, 350, 420; (NaOA): 275, 300 (sh), 380; (NaOAc/H₃BO₃): 267, 319 (sh), 380; (NaOMe): 285, 322, 430. ¹H-NMR δ (DMSO-d₆): δ 8.0 (2H, d, J = 8 Hz, H2' and H6'); δ 6.9 (2H, d, J = 8 Hz, H3' and H5'); δ 6.4 (1H, d, J = 2.5 Hz, H8) and δ 6.2 (1H, d, J = 2.5 Hz, H6). EI-MS m/z (% re. lent): 285 (M⁺) (100), 258 (15), 229 (16), 184 (8), 121 (22) and 93 (10).

Quercetin (9): Forty five milligrams, yellow needle crystals, R_f = 0.7 (system B), mp. 313-315°C. UV λ max, (MeOH): (nm) 255, 269 (sh), 370; (AlCl₃): 270, 290 (sh), 455; (AlCl₃/HCl): 270, 357, 426; (NaOAc): 274 (Dec.), 325; (NaOAc/H₃BO₃): 261, 385; (NaOMe): 246, 330, 398. ¹H NMR δ (DMSO-d₆): δ 8.16 (1H, d, J = 2 Hz, H-6); δ 7.67 (1H, d, J = 2 Hz, H2'); δ 7.6 (1H, dd, J_{6,2'} = 8 Hz, H-6'); δ 6.80 (1H, d, J = 8 Hz, H-5') and δ 6.3 (1H, d, J = 2 Hz, H-8).

Quercetin-3-O-rutinoside (10): Twenty milligrams yellow crystals. R_f = 0.5 (system B), m.p. 190°C. UV λ max, (MeOH): (nm) 256, 265 (sh.), 290, 355; (ALCL₃): 274, 302 (sh.), 330 (sh.), 432; (ALCl₃/HCl): 270, 298, 359, 399; (NaOAc): 272, 324, 398; (NaOAc/H₃BO₃): 263, 292 (sh.), 368; (NaOMe): 272, 310, 410. ¹H NMR δ (DMSO-d₆): δ 8.10 (1H, d, J = 2 Hz, H-2'); δ 7.86 (1H, d, J = 8 Hz, H-6'); δ 6.89 (1H, d, J = 8 Hz, H-5'); δ 6.65 (1H, d, J = 2 Hz, H-8); δ 6.5 (1H, d, J = 2 Hz H6); δ 5.13 (1H, d, J = 7.5 Hz H1"); δ 4.55 (1H, d, J = 1.3 Hz, H""); δ 3.82 (1H, dd, J = 10 Hz, J = 2 Hz H6"); δ 3.65 (1H, dd, H-2'); δ 3.47-3.87 (6H, m, Sugar protons) and d 1.23 (3H, d, J = 6 Hz, CH₃).

Pharmacological activities

Preparation of the TAE: Dried aerial parts of *C. arabica* 300 g plant powder was successively extracted with 95% ethanol (5×11) at room temperature and the total alcohol extracts were separately concentrated under reduced pressure at temperature not exceeding 40°C. The residue obtained for each extract was weighed. Thereafter, a known weight of each extract was freshly prepared by dissolving or suspending in distilled water, just before administration, by the aid of few drops of tween 80 (vehicle).

Determination of median Lethal Dose (LD₅₀): The oral median Lethal Doses (LD₅₀) of the alcohol extract of the two investigated plants (*C. arabica* and *D. acris*) were determined

as described by Walum (1998). Swiss albino mice in groups of six, received one of 500, 1000, 2000 or 5000 mg kg⁻¹ doses of the tested extract. Control animals were received the vehicle and kept under the same conditions. Signs of acute toxicity and number of deaths per dose within 24 h were recorded and the LD₅₀ was calculated as the geometric mean of the dose that resulted in 100% mortality and that caused no lethality at all.

Anti-ulcerogenic activity: Evaluation of the anti-ulcerogenic activity was carried out using absolute ethanol-induced ulcer model as described by Santhoshkumar *et al.*¹².

About 30 Wistar rats were used, they divided into 6 groups each of 6 rats. Group 1 received the vehicle and served as control group, group 2 received ranitidine (100 mg kg⁻¹) and served as standard group, group 3 received the total alcohol extract of the plant under investigation at a dose of 1000 mg kg⁻¹. Groups 4 and 5 received the isolated compounds 1 and 2 at dose 50 mg kg⁻¹, respectively.

Rats of all groups were fasted for 24 h then all medications were administered orally. About 1 h after treatment, the animals received an oral dose of absolute ethanol (1 mL/200 g) and then sacrificed 1 h later, by ether inhalation, the stomachs were rapidly removed, opened along their greater curvature and gently rinsed under running tap water.

Number of lesions: In the glandular part of the stomach were measured under an illuminated magnifying microscope (10x). Long lesions were counted and their lengths were measured. Petechial lesions were counted and then each five petechial lesions were taken as 1 mm of ulcer.

Lesion scores: The mucosal lesions were quantified by the scoring system (0-5) 0: No damage, 1: Local edema and inflammation without ulcers, 2: One ulcer without inflammation, 3: One to two ulcers with inflammation and lesion diameter <1 cm, 4: More than two ulcers with lesion diameter 1-2 cm and 5: Sever ulceration with lesion diameter >2 cm.

Ulcer index: To calculate the ulcer index (mm), the sum of the total length of long ulcers and petechial lesions in each group of rats was divided by its number. The curative ratio was determined according to the equation:

$$\text{Protection of control ulcer (\%)} = \frac{\text{Control UI} - \text{Test UI}}{\text{Control UI}} \times 100$$

Effect on liver and kidney functions: Male Wister rats were divided into 2 equal groups each of 10 rats. The 1st group was left as a control and administrated water orally, while the 2nd group was orally given the TAE in a dose of 1000 mg kg⁻¹ for 35 days. Blood samples were collected from the orbital plexus of rats, 6 h after the last dose. Samples were left to clot at room temperature for 30 min then centrifuged at 1000 rpm for 20 min.

The collected sera were used for determination of the activity of both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as a liver markers. In addition, levels of blood urea, serum creatinine were also estimated as a kidney markers¹³.

RESULTS AND DISCUSSION

Identification of the isolated compounds: The isolated compounds were identified using different physical and spectral methods, melting point, UV and IR spectra, ¹H-NMR, ¹³C-NMR, DEPT and correlation 2D NMR. Ten compounds were (Fig. 1) isolated from *Cakile arabica* for the first time and were identified as: (1) α-amyrin, (2) β-sitosterol, (3) Tetracosanoic acid, (4) 4-hydroxy-5-n-butoxy-5-oxopentanoic acid, (5) Scopoletine, (6) Umbelliferone, (7) Esculetin, (8) Kaempferol, (9) Quercetin and (10) Quercetin-3-O-rutinoside.

In accordance with our findings of phenolic and flavonoid compounds is the detection of phenolics and flavonoids from *Cakile maritima*¹⁴, however its content were greatly affected by salinity¹⁵. Besides, alkaloids and phytosterols were detected from *Cakile maritima* as a halophyte plant grown in the United Arab Emirates (UAE) it was investigated for the first time for its phytochemical constituents and antibacterial activity. The detection of alkaloids and phytosterols were confirmed by gas chromatography-mass spectrometry¹⁶. Other useful *Cakile* species were in North America as Indians used the powdered root of *Cakile edulenta* (Bigel.) Hook and *Cakile maritima* Scop., as antiscorbutic properties, in baking.

GLC-analysis of fatty acid methyl esters: Quantitative determination of fatty acid methyl ester using GLC revealed the presence of 7 fatty acids in *C. arabica* (Table 1). The most major fatty acids identified in *C. arabica* were heptadecanoic acid (44.40%) and palmitic acid (21.20%). Additionally, the lowest constitutive fatty acid identified in *C. arabica* was myristic acid (0.91%).

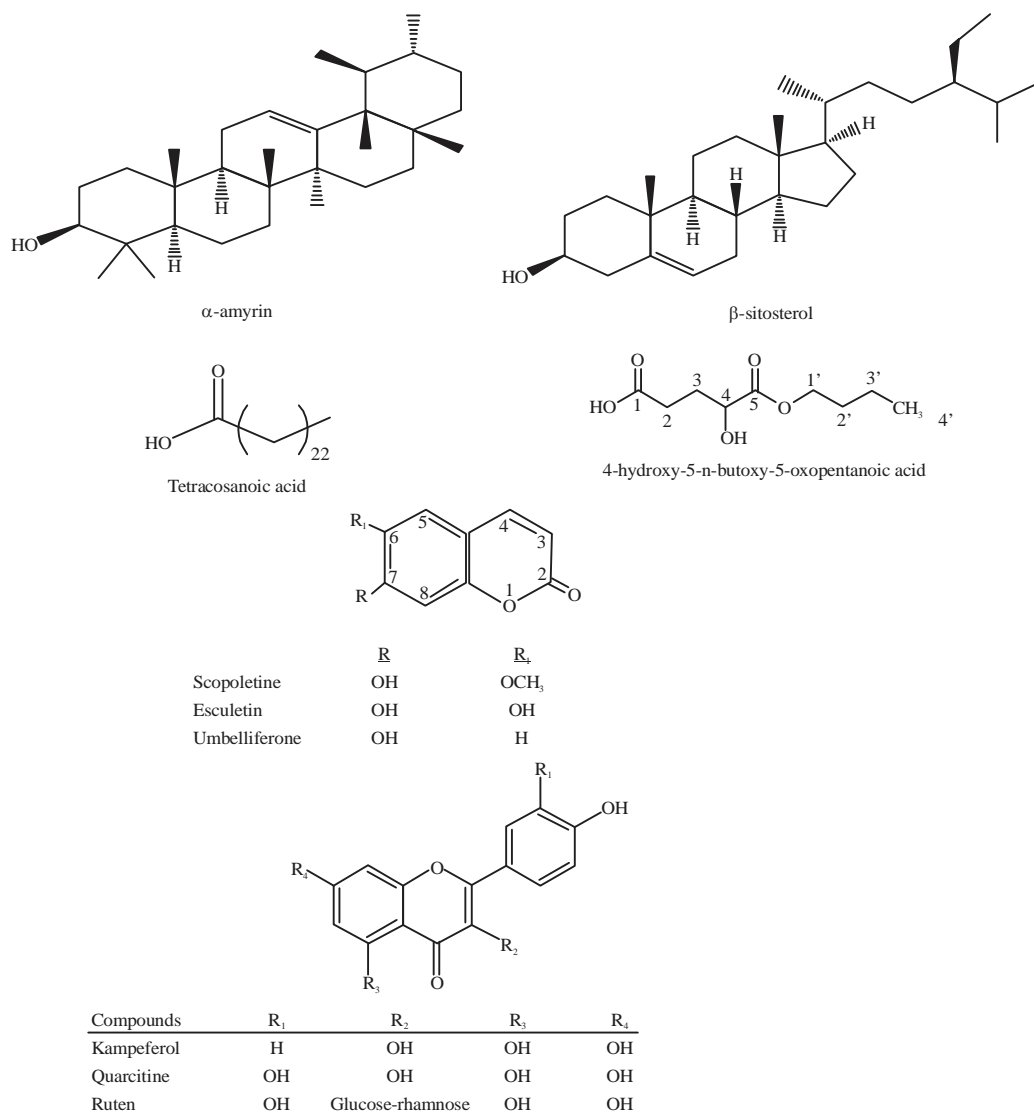


Fig. 1: Structures of the isolated compounds

Table 1: GLC analysis of fatty acid methyl esters of *C. arabica*

| No. of peaks | R _t | RR _t | Authentic methyl ester | Percentage |
|--------------------------|----------------|-----------------|------------------------|------------|
| <i>C. arabica</i> | | | | |
| 1 | 13.12 | 0.72 | Myristic acid | 0.91 |
| 2 | 16.74 | 0.92 | Palmitic acid | 21.20 |
| 3 | 18.10 | 1.00 | Heptadecanoic acid | 44.40 |
| 4 | 19.99 | 1.10 | Oleic acid | 4.86 |
| 5 | 20.36 | 1.12 | Stearic acid | 9.86 |
| 6 | 21.51 | 1.19 | Linoleic acid | 17.15 |
| 7 | 28.13 | 1.55 | Behenic acid | 1.62 |
| 8 | - | - | - | - |

Biological activities

Determination of median Lethal Dose (LD₅₀): The obtained results indicated that the different doses of *C. arabica* alcohol extracts (1000, 2000 and 5000 mg kg⁻¹) did not produce any symptom of acute toxicity and none of the mice died during

24 h observation. All mice did not exhibit any signs of toxicity, diarrhea, hematuria, restlessness, uncoordinated muscle movements or respiratory distress. Accordingly, it is suggested that oral LD₅₀ of the tested extracts were higher than 5000 mg kg⁻¹, hence, the tested extracts are considered to be safe since substances possessing LD₅₀ higher than 50 mg kg⁻¹ are nontoxic¹⁷.

Anti-ulcerogenic activity: Gastric damage induced by absolute ethanol in the current study was characterized by both long ulcers and petechial lesions. The number of ulcers and the ulcer index in the control rats that received ethanol were significantly increased when compared with normal untreated animals. These results were in agreement with the results of Jainu and Devi¹⁸.

Table 2: Effect of *C. arabica* extract, fractional extracts and compounds on absolute ethanol-induced ulcer in rats

| Groups | Dose (mg kg ⁻¹) | Score | No. of ulcers | Ulcer index | Protection (%) |
|--|-----------------------------|-------|---------------|--------------|----------------|
| Ulcer control | - | 4 | 14.00±1.05 | 13.00±1.00 | - |
| Ranitidine | 100 | 2.2 | 7.20±0.84*** | 7.00±1.05** | 46.2 |
| Total | 500 | 1.2 | 4.60±0.82*** | 7.20±1.02** | 44.6 |
| Total | 1000 | 0.2 | 0.20±0.45*** | 0.20±0.45*** | 98.5 |
| Butanol | 250 | 2.6 | 7.80±1.07** | 8.20±0.84** | 36.9 |
| Chloroform | 250 | 2.2 | 3.60±0.54*** | 6.80±0.92** | 47.7 |
| Ether | 250 | 1.4 | 2.00±0.71*** | 4.80±0.84*** | 63.1 |
| Ethyl acetate | 250 | 1 | 1.40±0.19*** | 2.20±0.92*** | 83.1 |
| Lipid | 250 | 1.4 | 1.80±0.05*** | 2.80±0.08*** | 78.5 |
| Tetracosanoic acid | 50 | 1 | 2.00±0.10*** | 2.60±0.58*** | 80.0 |
| 4-hydroxy-5-n-butoxy-5-oxopentanoic acid | 50 | 2 | 3.60±0.90*** | 5.80±1.01** | 55.3 |
| β-sitosterol | 50 | 1.8 | 6.80±1.00*** | 7.00±1.12** | 46.2 |
| α-amyrin | 50 | 1.6 | 3.40±0.72*** | 5.40±1.07** | 58.4 |

Data are expressed as Mean±SD, n = 6, *p≤0.05, **p≤0.01, ***p≤0.001 significantly differ from ulcer control, <https://www.google.com/patents/US20150072028>

Table 3: Effect of *C. arabica* extract on liver and kidney functions in rats

| Groups | ALT (U L ⁻¹) | AST (U L ⁻¹) | Blood urea (mg dL ⁻¹) | Serum creatinine (mg dL ⁻¹) |
|---|--------------------------|--------------------------|-----------------------------------|---|
| Control | 4.80±0.37 | 5.20±0.37 | 45.50±1.36 | 0.82±0.02 |
| <i>C. arabica</i> (1000 mg kg ⁻¹) | 5.10±0.22 | 5.30±0.39 | 46.35±1.9 | 0.84±0.02 |

Data are expressed as Mean±SD, n = 1, <https://www.google.com/patents/US20150072028>

Ulcers caused by ethanol are due to superficial damage to mucosal cells¹⁸. The ethanol-induced ulcers are predominant in the glandular part of stomach. It was reported that, ethanol stimulates the formation of mast cell secretory products¹⁹ and reactive oxygen species resulting in the damage of rat gastric mucosa²⁰.

Ethanol-induced gastric ulcers model have been widely used for the evaluation of gastro protective activity. It has been found that oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration in the gastric mucosa and scavenging these free radicals can play an appreciable role in healing these ulcers²¹.

Family Brassicaceae include some plants with antiulcerogenic activity. For example *Brassica oleracea*^{22,23} and *Schouwia thebaica*²⁴.

The present results showed that the plant extract at doses of 500 and 1000 mg kg⁻¹ possessed a potent anti-ulcerogenic activity against ulcer-induced by absolute alcohol. It produced a percent protection of control ulcer by 87.1 and 98.5% for the high doses of *C. arabica*. The isolated compound tetracosanoic acid showed significant anti-ulcerogenic activity, represented by 80% as a protection percent to control ulcer, followed by kaempferol (78.9%) and quercetin (72.9%), which were more effective than ranitidine that produce 46.2% as a reference drug (Table 2).

Liver and kidney functions: The non-toxic nature of *C. arabica* alcohol extract in acute toxicity study is well

supported by the biochemical data following 35 days treatment period in rats. In the present study, oral dosing of the tested extracts to rats in a dose of 1000 mg kg⁻¹ for 35 days did not show any significant effect on the activity of ALT and AST in the sera as compared to control (Table 3). The serum transaminase level is most widely used as a measure of hepatic injury, due to its ease of measurement and high degree of sensitivity. It is useful for the detection of early damage of hepatic tissue. Since the activity of ALT and AST are specific assayable liver enzymes, their normal levels in serum of experimental groups of rats treated for 35 days means that the *C. arabica* alcohol extract are not hepatotoxic.

The tested extract showed significant change in the mean values of urea and creatinine in sera of rats after 35 days of administration at 1000 mg kg⁻¹ dose when compared with the control rats (Table 3). Urea and creatinine are the most sensitive biochemical markers employed in the diagnosis of renal damage. In kidney damage, there will be retention of urea and creatinine in the blood²⁵. Therefore, marked increase in serum urea and creatinine are indications of functional damage to the kidney²⁶. By these indicators, the alcohol extracts of *C. arabica* are therefore, not nephrotoxic in rats.

CONCLUSION

It can be concluded that cytoprotective mechanism is very useful in showing the anti-ulcerogenic activity of the isolated compounds and *C. arabica*.

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