Phytochemical profile, Antimicrobial Activity, Antioxidant Capacity, and Anti- Cyclooxygenase Activity of Green Kohlrabi plant extract

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Abstract

Phytochemical compounds were extracted from Green Kohlrabi (GK) tubers plant using soxhlet apparatus with three different solvents including ethanol, water and ethyl acetate. The ethanolic extract showed higher antimicrobial activity than the other extracts. Gas Chromatography-Mass Spectroscopy (GC-MS) analysis found that the ethanolic extract of GK was a rich source of phytochemicals (approximately 32 compounds). whereas analysis with Atomic Absorption Spectrometry (Flame and flameless mode) exhibited that the concentration of trace elements was 9.34×10^{-4} g mL^{-1} Cu, 37.5×10^{-4} g mL^{-1} Zn, 8.3×10^{-4} g mL^{-1} Pb, 0.081×10^{-4} g mL^{-1} Cd, 0.053×10^{-4} g mL^{-1} Cr, 0.007×10^{-4} g mL^{-1} Ni and 0.014×10^{-4} g mL^{-1} Mn, respectively. Qualitative analysis revealed the presence of alkaloids, tannins, terpenoids, saponins, glycosides, resins, and flavonoids. Total phenolic content was evaluated in the range of 0.2-1 g mL^{-1} and the concentration was equal to 0.969 g mL^{-1} indicating the high phenolic acid levels in GK ethanolic extract. The spectrophotometric method was used to measure the radical scavenging activity (Folin-Ciocalteu and 2,2- diphenylpicrylhydrazyl (DPPH)) and compared with a new technique called paper-based analytical device (μPADs) and the results obtained indicate a strong and effective antioxidant DPPH scavenging affects 85.14 %, while the μPADs was used for semi-quantitative analysis. The GK extract could inhibit cyclooxygenase (COX-2) enzyme activity better than an anti-inflammatory drug (Aspirin) in sera of thyroid cancer patients. As a result, our study establishes that GK ethanolic extract might be useful as a natural potent antimicrobial, antioxidant, and anti-inflammatory agent.

Keywords: Green Kohlrabi plant extract; microfluidic paper-based analytical device (μPADs); antimicrobial activity; antioxidant activity; Cyclooxygenase (COX-2).

Introduction

Kohlrabi (Brassica oleracea var. gongylodes) is a significant species in the Brassicaceae family with a European origin and due to its short growing season and the possibility to export, it can be an alternative crop to other Brassicaceae families including cauliflower, Broccoli and Brussels sprouts[1- 3]. It is an annual or a biennial plant that is grown as round swollen steam at the base of the plant standing quite above the ground with branched roots system and leaves[4,5]. Red Kohlrabi (RK) and green Kohlrabi (GK) are the two well-known types of Kohlrabi plants depending on the cultivar[5]. The edible parts of Kohlrabi are generally consumed either as a cooked or raw vegetable and possess anti-diabetic, anticancer, antioxidant, antimicrobial, and anti-inflammatory activities [6,7].

Kohlrabi contains a treasure of primary nutrients which is responsible for the physiological processes in human (reproduction, growth, and development) such as magnesium, potassium, phosphorus, calcium, and vitamins A, C, E, K, dietary fiber and are low in calories and fat [8-10]. It is considered one of vitamin C top plants where one cup of this plant consists of more than 100 % of the everyday dose that is recommended for a person's consumption[9]. Epidemiological studies strongly recommended the routine intake of Kohlrabi as a member of the Brassicaceae family to reduce the risk of chronic diseases, such as cardiovascular, diabetes, and cancer disease [10]. This can be related to the natural presence of secondary phytochemicals (disease-fighting compounds) including glucosinolates derived from amino acids and glucose that are hydrolyzed by the endogenous enzyme myrosinase to many bioactive compounds which reduce the risk of the stomach, lungs, prostate, breasts, rectum, colon, and pancreas diseases [13,14]. Carotenoids are a group of orange, yellow, and red pigments such as lutein and zeaxanthin that have a significant impact on age-related macular degeneration prevention [12]. Also, Anthocyanins are bioactive compounds that have health-related properties[12]. In addition, the Kohlrabi plant possesses antioxidant properties due to the presence of natural compounds called antioxidant phytochemical compounds such as flavonoids, and polyphenols which scavenge the free radicals formed within the redox reactions and prevent the free radical chain reaction[13]. Therefore, Kohlrabi is well known for its medicinal, pharmaceutical, nutritional, and economic value[9].

Cyclooxygenase (COX-2) is identified as an isoform different from COX-1 Although both isoforms of this enzyme convert arachidonate to prostaglandins,

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there are significant differences in their distribution in the body and their roles in health and disease [16]. The basis for these important differences lies in the genes for COX-1 and COX-2 and the regulation of these genes. COX-1, the predominantly constitutive form of the enzyme, is expressed throughout the body and provides certain homeostatic functions, COX-2 mRNA rises rapidly in response to inflammatory and mitogenic stimuli and increased expression of COX-2 mRNA and protein occurs at sites of inflammation in tumors and ulcerated gastric tissue [17]. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the activity of COX-1 and COX2. While most conventional NSAIDs show little selectivity and inhibit both isoenzymes to a comparable extent [18]. Thyroid cancer development and progression via COX-2-mediated downstream signaling pathways. Interestingly, the COX-2 inhibitor celecoxib inhibited thyroid tumor growth in animal experiments. COX-2 plays a role in tumor development and progression[14].

To the best of our knowledge, no previous work has been done for GK tubers extracts (ethanol, water and ethyl acetate) compounds, growing in Iraq. Thus, the paper aims to determine the complete profile and content of GK extract compounds, antimicrobial, antioxidant properties, and cytotoxicity of tubers organic extracts of GK.

2. Material and methods

2.1 Chemical and standards

2.2 Plant material and extraction

In September 2020, green Kohlrabi (GK) tubers were purchased from Karrada, Baghdad, Iraq local market (originated from Karbala governorate, Iraq). The plant tubers were rinsed with distilled water, peeled, chopped into small parts using a knife, dried, ground to a fine powder using an electric blender and transferred to a screw-top vial and subsequently to the extraction procedure. One hundred mg of fine powder was extracted with 100 mL of three solvents including ethanol, water and ethyl acetate, individually by soxhlet apparatus at 78 °C with ethanol and ethyl acetate and 100 °C with water for eight hours. The mixture was filtered through Whatman Grade No.18 filter paper and then dried at 40°C under a vacuum. The stock solution(0.05)g mL^{-1} was prepared by weighing an aliquot of fife gram of extracted plant and completed to the mark with ethanol, water and ethyl acetate, respectively into a 100 mL volumetric flask. Secondary stock solution (0.00077, 0.0015, 0.00312, 0.00625 and 0.0158) g mL^{-1} were prepared by serial dilution.

2.3 Antibacterial Activity

The antimicrobial properties of GK extracts (ethanol, water and ethyl acetate) were studied by the agar well diffusion method[15]. The selected bacterial strains *Staphylococcus aureus* and *Staphylococcus epidermidis* as gram-positive bacteria, *Escherichia coli* and *Klebsiella sp.* as a gram-negative bacteria and *Candida albicans* (yeast) which was kindly provided by the Department of Biology Laboratories, Mustansiriyah University were grown on Muller Hinton agar. After incubation at 37°C for 18 hours, 3-5 colonies from each strain were touched using a sterile loop and added to a fresh Muller Hinton broth (5 ml). The suspension turbidity was compared with the 0.5 Mcfarland turbidity standard. The test organism was seeded evenly on a fresh Muller Hinton agar, air-dried for 3-5 minutes and agar wells (5mm) were prepared by using a sterilized cork borer according to Kirby-Bauer. After removing agar discs, 100 microliters from each (0.00077, 0.0015, 0.00312,0.00625,0.0158, 0.05) g mL^{-1}were added to each well and left at room temperature to allow diffusion of the GK extract. All plates were incubated at 37 °C for 24 hours. On the next day, the inhibition zones were measured by mm recorded. This experiment was repeated in triplicate and the solvent was used also as a control[16].

2.4 Qualitative and quantitative

detection of GK extract Chemicals: Qualitative chemicals determination including alkaloids, tannins, terpenoids and steroids, saponins, carbohydrates, resins, and flavonoids were performed by applying standards procedure[17]. On the other hand, quantitative trace elements determination was done using the acid digestion method, an Atomic absorption spectroscopy instrument was used for elemnts detection[18], while a Gas chromatography-mass spectroscopy instrument was used for chemical compounds analysis of GK extract[19].

2.5 Ash and moisture determination

The GK tubers' moisture was determined by adopting O'Kelly procedure[20] while the GK tubers' ash content was determined following Nielsen procedure[21].

2.6 Determination of total phenolic content (TPC)

For the determination of GK ethanolic extract total phenolic content, the Folin-Ciocalteu reagent was used [2]. An aliquots of 100 µl of GK ethanolic extract (0.25) g mL^{-1} were mixed with 46 ml distilled water and 1 ml of Folin–Ciocalteu reagent and neutralized with 3 ml of 2% Na_2CO_3. The reaction mixture was then incubated for 2 hours at 25°C. The absorbance was measured at 760 nm and the results were determined from the linear equation of the gallic acid (GAE) standard curve. Gallic acid (0.2–1 g mL^{-1} gallic acid) [15,24,25].

2.7 Determination of DPPH radical scavenging activity

In the present study two different techniques (spectrophotometric and microfluidic paper-based analytical device (µPADs)) were used to estimate the antioxidant capacity as follows:

Method A: spectrophotometric detection

In order to evaluate the antioxidant activity of GK ethanolic extract, the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH assay) was performed. Briefly,

two mL of GK ethanolic extract was added to 2 mL of a 0.0094 % DPPH solution dissolved in MeOH. A synthetic reference vitamin C was used as a stable antioxidant. After mixing and allowing to stand for thirty minutes at room temperature, the optical absorbance of the reactant was read at 517 nm. The antioxidant activity was calculated applying the below equation [13]:

$$\% \textbf{ Inhibition} = \frac{\textbf{A blank} - \textbf{A sample}}{\textbf{A blank}} * \textbf{100}$$

Where A blank: is the control absorbance, and A sample: is the GK extract absorbance[9].

Method B: microfluidic paper-based analytical device (μPADs) detection:

To construct the calibration graph for DPPH using μPADs device, 5 μL of different concentrations ranging (,0.000770.0015,0.0031,0.0062 and 0.0125) g mL^{-1} of GK ethanolic extract were spotted into detection circular reaction zone individually, then 2 μL of DPPH reagent was added using a micropipette and left to dry at room temperature. A synthetic reference vitamin C was used as a stable antioxidant. Samsung note 9-camera phone was used to capture images that were analyzed using Image J software (National Institutes of Health, USA).

2.8 Estimation of the COX-2 activity in thyroid cancer patients:

The activity of the COX-2 enzyme was estimated by an assay for the peroxidase activity in the serum of thyroid cancer patients based on a colorimetric procedure[24]. This method depends on measuring the enzyme-catalyzed oxidation of tetramethyl phenylenediamine (TMPD) by hydrogen peroxide and measuring the blue color at 610 nm. One unit of activity is defined as the amount of enzyme required to convert 1μmol of hydrogen peroxide to the product under assay conditions [25].

2.9 Studying the effect of GK ethanolic extract and anti-inflammatory drugs on the activity of COX-2 in sera of thyroid cancer patients:

The effect of GK ethanolic extract and anti-inflammatory drug (Aspirin) on the activity of COX-2 is studied after preparing extract and drug at different concentrations. A stock solution and serial diluents have been prepared as follows:

1-Aspirin (0.5 g) was dissolved in 1 ml of ethanol and the volume was completed to 9 ml by D.W as a stock solution (0.05 g mL^{-1}) to prepare a serial dilutions(0.00077, 0.0015,0.0031,0.00625 and 0.0125) g mL^{-1} from the stock solution [26].

2-GK ethanolic extract (0.5g) was dissolved in 1 ml of ethanol and the volume was completed to 9ml with D.W. as a stock solution of (0.05 g mL^{-1}), then serial dilutions were prepared (0.00077, 0.0015,0.0031,0.00625 and 0.0125) g mL^{-1} in 20 ml volumetric flask from the stock solution [27]. The COX-2 activity was measured in the patient's serum as follows: 2.74 mL of tris buffer + 40 μL of each inhibitor in different concentrations was added to (100 μL) of serum (mixing 5 patients samples).The inhibition percentage was calculated by comparing the activity with and without inhibitors under the same conditions according to the equation:

$$\% \textbf{ Inhibition} = \textbf{100} - \frac{\textbf{The activity in the presence of inhibitor}}{\textbf{The activity in the absence of inhibitor}} * \textbf{100}$$

3. Results and Discussion

3.1 Antimicrobial activity

The GK three extracts ethanol, water and ethyl acetate were tested for their antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella sp.*, and *Candida albicans* (Table 1). Initial data showed that the zone of inhibition of ethylacetate GK extract were higher than that of ethnol and water. However, these results was omitted due to the high inhibition zone of ethylacetate blank solution. Therefore, ethanolic GK extract was chosen for further antimicrobial analysis using different concentrations in the rang of (0.00077, 0.0015, 0.00312,0.00625,0.0158, and 0.05) g mL^{-1}.

Table 1: Mean and standard deviation (SD) values of (S1 water extract) (S2 ethanolic extract) (S3 ethylacetate extract) against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella sp.* and *Candida albicans*.

SampleNo.	Gram-positive				Gram-negative				Fungi	
	S.aureus		*S. espidermis*		*E .coil*		*Klebsiella sp.*		*C.albicans*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
S1	12.3	0.81	12.3	0.47	20 .2	1.24	16.2	0.81	16.2	1.63
S2	13.5	0.47	12.4	0.33	24.3	1.24	18.3	1.24	17.2	1.24
S3	14.2	0.81	16.2	0.47	26.2	1.31	20.2	1.24	23.3	0.81

The results of the antimicrobial investigations using different concentrations of the GK ethanolic extract were given in Table 2. It indicates that different bacterial species demonstrated different levels of sensitivities toward the tested samples of GK extract. The diameter for the zone of inhibition for GK ethanolic extract ranged from 11.3 to 13.2 mm at various concentrations used. The maximum inhibitory effect was towards *E.coil* and the minimum inhibitory effect was towards *S. epidermis*.

Table 2: Mean and standard deviation (SD) values of GK ethanolic extract against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella sp.*, and *Candida albicans* at different concentrations.

Sample No.	Gram-positive				Gram-negative				Fungi	
	S.aureus		*S. espidermis*		*E .coil*		*Klebsiella sp.*		*C.albicans*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
S1 (0.0158 gmL^{-1})	-	-	11.3	0.43	13.2	1.64	12.7	0.47	12.54	0.43
S2 (0.00625 gmL^{-1})	-	-	-	-	11.5	1.24	11.8	1.26	11.63	1.08
S3 (0.0031 gmL^{-1})	-	-	-	-	-	-	-	-	-	-
S4 (0.0015 gmL^{-1})	-	-	-	-	-	-	-	-	-	-
S5 (0.00077 gmL^{-1})	-	-	-	-	-	-	-	-	-	-

3.1 Phytochemical compounds of kohlrabi plant extract

The phytochemical screening compounds that exists in GK three extracts including ethanol, water, and ethyl acetate were examined using astandard qualitative chemical analysis. The three solvents were chosen due to their availability, high polarity, economical, and low toxicity[10]. The study included the Alkaloids, Tannins, Terpenoids and Steroids, Saponins, Glycosides, Resin, and flavonoids compounds presence.

Table 3. Phytochemical analysis for ethanol, water, and ethyl acetate GK extracts.

Phytochemicals	Reagents	Remarks	Ethanol extract	Water extract	Ethyl acetate Extract
Alkaloids	Picric acid	The appearance of yellow color	+	+	-
Tannins	Lead acetate reagent Ferric Chloride	The appearance of gelatin solution The appearance of yellowish color	++	++	--
Terpenoids and Steroids	Glacial acetic acid Sulphuric acid	The appearance of brown color The appearance of dark blue color	+	+	-
Saponins	Vigorous shaking	The appearance of white color	+	+	-
Glycosides	Benedict reagent	The appearance of brown color	+	-	-
Resin	Ethanol	Turbidity	+	+	-
Flavonoids	Ethanol KOH	The appearance of yellow color	+	-	-

The results in Table 3 showed the presence of Alkaloids, Tannins, Terpenoids and Steroids, Saponins, Glycosides, Resin, and Flavonoids compounds in ethanolic GK extract, while in GK water extract all mentioned phytochemicals were presented except for Glycoside, and Flavonoids. On the other hand, the extract of ethyl acetate did not give any of the phytochemicals substances because of the very little solubility of active compounds in ethyl acetate[5,18,30]. Some of these results agreed with other published work which stated the existence of phytochemicals such as Glucosinolate, Phenols, Anthocyanins, Carbohydrates, Saponins, Flavonoids and Terpenoids while Tannins, Alkaloids and Steroids were absent which can be attributed to the extraction method, type of solvent and GK part[11,14]. The importance of initial phytochemical screening compounds is to detect the presence of pharmaceutically active compounds that is necessary for the drug industry. Quantitative analysis of GK extract showed that the tested moisture was (2.22%) of total kohlrabi plant extract nutritional content, while ash percentage was (15.233%). This indicates a low level of moisture in the plant, while the percentage of ash indicates the carbohydrate content of the plant, which is also considered low[29]

3.2 Determination of trace metals concentration in GK extract

An atomic absorption spectroscopy instrument (flame and flameless mode) was used to determine the concentration of trace metals in GK samples[31,32]. As can be seen from Table 4, the concentration of Cu, Zn, Pb, Cr, Ni, and Mn were in concentrations of 9.34×10^{-4}, 37.5×10^{-4}, 8.3×10^{-4},$0.0811\times0^{-4,}$ 0.053×10^{-4}, 0.007×10^{-4} and 0.014×10^{-4} g mL^{-1}, respectively.

Table 4. The concentration of trace elements in GK ethanolic extract.

Trace elements	Cu	Zn	Pb	Cd	Cr	Ni	Mn
Concentration in g mL^{-1}	9.34×10^{-4}	37.5×10^{-4}	8.3×10^{-4}	0.081×10^{-4}	0.053×10^{-4}	0.007×10^{-4}	0.014×10^{-4}

The Brassicaceae family was generally rich in trace elements. Results allow pre-selection of the most

perspective tubers as possible dietary sources of essential minerals and trace elements[31].

3.4 Gas Chromatography-Mass Spectrometry (GC-MS) analysis:

To analyze the existence of phytochemical compounds in ethanolic GK extract, GC-MS was used. Table 5 displays the presence of the number of compounds with various retention times including amino acids, organic acids, carbohydrates, sugar alcohols and an amine, which were detected and normalized against the internal standard signal intensity[7]. Most of these compounds contain functional groups such as NH, COOH, OH, etc that give the plant extract importance as an anti-oxidant, and anti-inflammatory agent [32]. The GC-MS chromatogram of GK extract (figure 1) revealed the extract was rich in phenolic compounds, terpenes, alkaloids, phenolic acids, quinones, steroids, saponins, and tannins which were in agreement with Change *et al* research paper which stated the existence of 45 metabolites such as organic acids, amino acids, sugars, and an amine[33]. Also, eleven compounds including glycosylated indole alkaloid derivative and three fructose derivatives were identified by Ritu *et al* group [32]. Marios *et al* discovered twenty-eight phenolic compounds from methanol/water (60:40, v/v) extract[34] .In addition, Woo *et al* profiled the presence of glucosinolates, anthocyanins, carotenoids, and other secondary metabolites in the skin and flesh of GK and RK[12]. Due to a lack of library data and authentic samples, some GC-MS peaks remained unidentified[35].

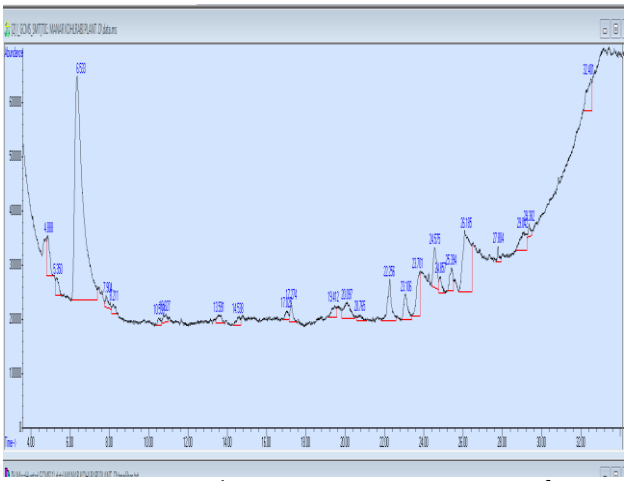


Fig. 1. GC-MS chromatogram investigation of GK extract.

Table 5: GC–MS analysis revealed the presence of active compounds in GK extract.

peak	Retention time	Area%	Name of the compound	Formula	Mol.Wt.(g mol^{-1})
1	4.683	0.87	malonic acid	$[(CH_3)_4N][CH_3C(O-)=Cr(CO)_5]$	309.24
2	6.535	54.7	D-Limonene	$C_{10}H_{16}$	136.23
3	8.208	0.79	Pentane, 1-methoxy-	$C_6H_{14}O$	102.1748
4	13.688	0.89	Ethanol, 2-bromo-	C_2H_5BrO	124.96
5	17.281	1.75	2,15-Pentadecanetriol	$C_{15}H_{32}O_3$	260.41
6	19.482	0.88	7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin	$C_{14}H_{14}O_3$	230.26
7	20.051	1.73	Fumaric acid, 3-hexyl tridecyl ester	$C_{23}H_{42}O_4$	382.5772
8	23.118	3.29	1-Hexanamine	$CH_3(CH_2)_5NH_2$	101.19
9	23.704	4.56	8,8-Dimethyl-7,9-dioxabicyclo nonane-3-carboxylicacid, methyl ester	$C_{22}H_{30}O_3$	342.5
10	23.899	0.94	alpha.-l-Mannose semicarbazon pentaacetate	$C_{18}H_{25}N_3O_{12}$	475.404
11	24.587	4.28	Oleic Acid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	282.46
12	24.868	0.98	Oxirane-2-carboxylic acid, 2-amino carbonyl-3-methyl-3-(1-methylethyl)-, ethyl ester	$C_3H_4O_3$	88.06
13	25.454	3.29	cis-9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254.41
14	26.066	6.74	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356.5399
15	27.790	0.78	cis-Vaccenic acid	$C18H34O_2$	282.5
16	28.895	1.23	(E)-13-Docosenoic acid	$C_{22}H_{42}O_2$	338.5677
17	29.379	0.80	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.5
18	31.766	1.18	Benz[e]azulene-3,8-dione	$C_{19}H_{24}O_6$	348.4
19	32.293	2.27	10-Methyl-E-11-tridece-1-ol acetate	$C_{16}H_{30}O_2$	254.41

3.5 Total phenolic contents

Total phenolic concentrations were estimated using the gallic acid and GK ethanolic extract calibration curves as given in table (6) and figure (2), and expressed as gallic acid equivalents per mL. The total phenolic concentration in GK ethanolic extract was 0.793 g mL-1, indicating significant phenolic acid values. This has to do with the extraction of soluble phenolic acids that are both nonpolar and semipolar. The antibacterial activity is thought to be attributed to phenolic chemicals. The high antioxidant activity of the extracts could be attributed to their high phenolic content [36].

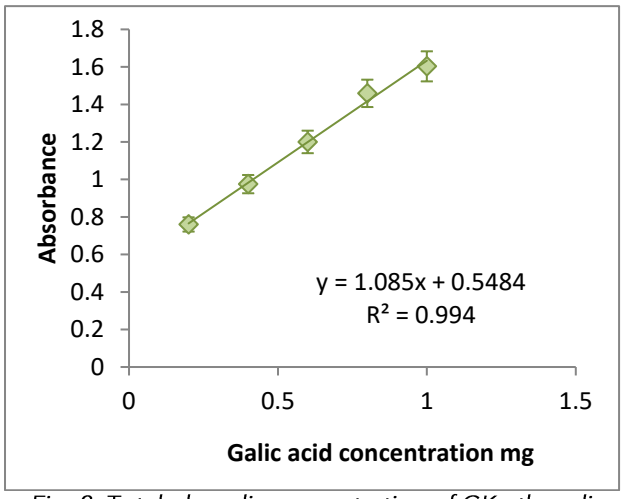


Fig. 2: Total phenolic concentration of GK ethanolic extract

Table 6: Total phenolic of GK ethanolic extract		
Concentration (gallic acid) g mL^{-1}	Absorbance	Total phenolic content in kohlrabi plant extract g mL^{-1}
0.2	0.760	-
0.4	0.975	-
0.6	1.2	-
0.8	1.459	-
1	1.603	-
Extract	1.408	0.793

3.6 Free radical scavenging activity (quantitative determination)

The DPPH radical scavenging is a sensitive antioxidant assay and is independent of substrate polarity. DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. This preliminary study indicates the interesting anti-oxidative stress activity of GK ethanolic extract suggesting its promising applications as a medicinal source for the treatment and prevention of free radicals associated diseases[33]. Firstly, qualitative determination of radical scavenging was done using a UV-VIS Spectrophotometer. From table 7 and figure 3, data showed a good percentage of antioxidants that the extract contains when compared with vitamin C, which is a strong and effective antioxidant DPPH scavenging affects 85.14%.

Table 7: Determination of DPPH free radical for GK extract.			
the concentration of the extract (µg mL^{-1})	DPPH scavenging effect (%)	Mean of DPPH scavenging effect (%)	Standard Deviation of DPPH scavenging effect (%)
Vitamin C	93.31	93.88	0.07
0.00077	22.9	22.33	0.05
0.0015	23.63	24.8	0.09
0.0031	37.41	37.78	0.04
0.0062	55.15	55.45	0.06
0.0125	85.14	86.21	0.12

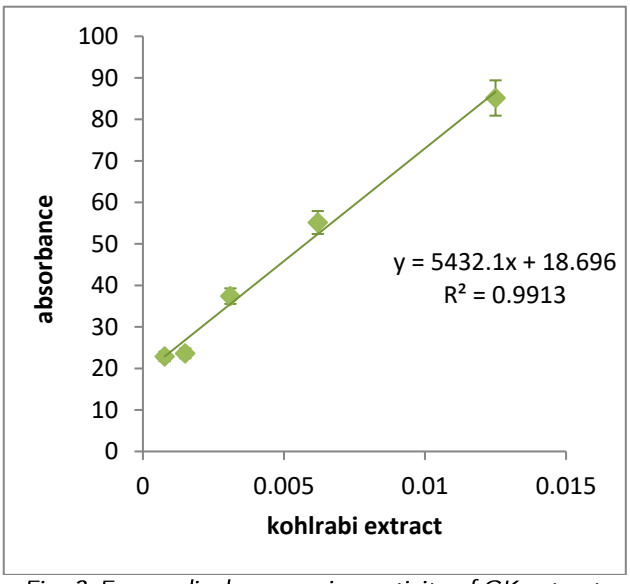
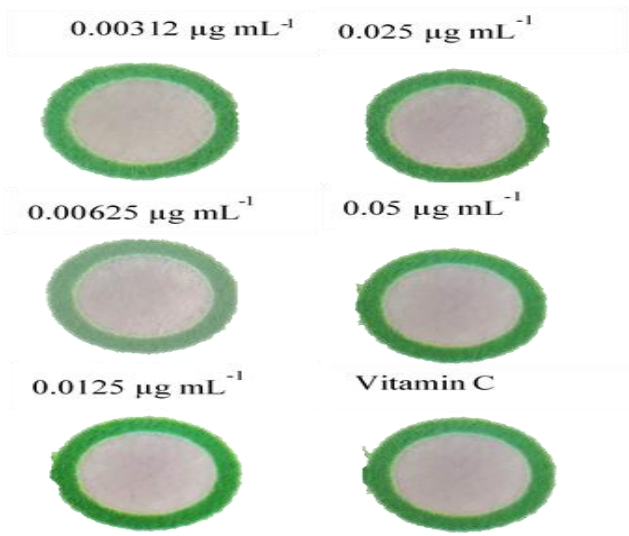


Fig. 3: Free radical scavenging activity of GK extract.



Following the spectrophotometric determination of DPPH free radical for GK extract, a new technique called microfluidic paper-based analytical device (µPADs) was used for quantitative analysis of GK extract in the range of (0.00312-0.05) µg mL^{-1}. A fairly weak color intensity after the addition of the extract to the DPPH reagent was observed and the absorbance tend to vary, thus this range is not suitable for quantitative analysis rather than semi-quantitative analysis. As can be seen in figure 4.

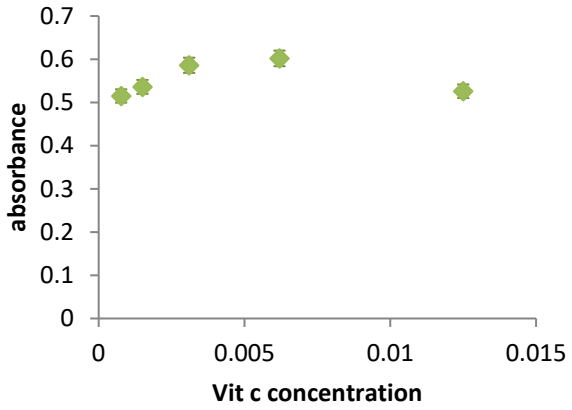


Figure 4: Free radical scavenging activity calibration graph of GK extract using μPADs.

.3.7.1 The inhibition percentage of the nutmeg extract and anti-Inflammatory drug in the activity of the COX-2 enzyme

Preparing different concentrations of drug and using it in the enzymatic reaction studied the inhibition effect of anti-inflammatory drug is Aspirin and GK ethanolic extract on the COX-2. The result of the study showed that drug exhibits a different inhibitory percentage on the activity of COX-2 as shown in table 8 and figure 5.

Table 8: Effect of GK extract and Aspirin concentrations on COX-2 activity in thyroid cancer patient.

	Concentration	Activity	%Inhibition
Aspirin	0.0158	3	42.75
	0.0062	2.9	44.66
	0.0031	2.4	54.2
	0.0015	1.99	62.1
	0.00077	1.9	63.75
Extraction			
	0.0158	0.5	90.46
	0.0062	1.8	65.65
	0.0031	1.58	69.85
	0.0015	0.4	92.37
	0.00077	0.24	95.42

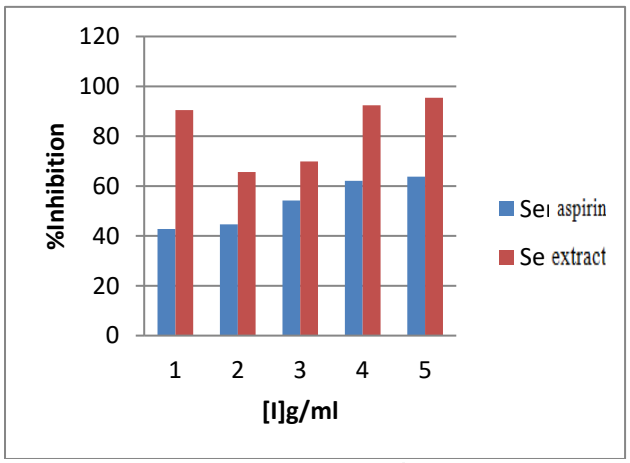


Fig 5: The inhibition percentage of the GK extract and aspirin drugs on the activity of the COX-2 in sera of thyroid cancer patients

The results study shows that the GK ethanolic extract can inhibit COX-2 activity better than the anti-inflammatory ability of the drug. The mechanism by which oil inhibits the activity of the COX-2 enzyme is not clear but the presence of fatty acids in this oil may be a cause of this inhibition where the study of Yuan *et al* [26] shows that fatty acids bind within the COX-2 site of one monomer of the dimer and inhibit the binding and oxygenation of the substrate in the COX site of the other monomer. Aspirin is an irreversible inhibitor of the COX-2 that acts as an acetylating agent where an acetyl group is covalently attached to a serine residue in the active site of the COX-2 [37].

Conclusion

The results presented in this study have cleary shown GK ethanolic extract could be considered a rich source of phytochemicals compounds. In addition to bioactive compounds that are related to antimicrobial and antioxidant activites. The anti-oxidant activity was measured using two different methods , namely spectrophotometric and μPADs and the former showed more sensitivity to calculate the antioxidative activity . Moreover, the GK extract showed an anti-inflammatory effect as it gave a better inhibition action for the human COX-2 activity rather than anti-inflammatory drug is Aspirin in sera of thyroid cancer patients. Thus, the GK plant is a potent material to treat some pathogens and can help the human antioxidant defense system.

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