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Phytochemical Profile, Antimicrobial, Antioxidant Activity and Cyclooxygenase 2 Inhibitory Properties of Nutmeg (Myristica Fragrans) Seeds Extract

Muthanna C. Urabee, Jwan O. Abdulsattar,* Zaizafoon N. Nasif

Chemistry Department, College of Science, Mustansiriyah University, Baghdad, Iraq

Abstract

The present study was conducted with the aim to extract phytochemical constituents of nutmeg (*Myristica fragrans*) seeds using methanol and acetone solvents, respectively by soxhlet apparatus with a yield of (1.47gm/100 gm) for methanol solvent and (2.11gm/100gm) for acetone solvent. The two extracts were tested for their antimicrobial, antioxidant and anti-inflammatory activities. Agar well diffusion method was adopted against four bacterial species including two Gram-positive bacteria (*Staphylococcus aureus and Staphylococcus epidermidis*) and two Gram-negative bacteria (*Escherichia coli, and Klebsiella sp.*) as well as one yeast (*Candida albicans)* to determine the antimicrobial activity. The present study showed that the tested microorganisms were inhibited by nutmeg acetone extract with an inhibition zone ranging between (11-15 mm), especially toward *Candida albicans* with 15 mm diameter while no inhibition zone was observed using methanol extract. Chemical constituents of nutmeg acetone extract were estimated using Atomic Absorption Spectrometry (AAS) and Gas Chromatography-Mass Spectroscopy (GC-MS) measurements revealed the presence of 150 µg/dl Cu, 280 µg/dl Zn, 6.1 µg/dl Pb, 0.08 µg/dl Cd, 0.09 µg/dl Cr, 0.0012 µg/dl Ni and 0.027 µg/dl Mn and 32 extra compounds. Other phytochemical detections including alkaloids, tannins, terpenoid, saponins, glycosides, resins, and flavonoids were investigated using standard procedures. Total phenol content and radical scavenging activity were measured quantitatively using Folin-Ciocalteu and 2,2-diphenylpicrylhydrazyl (DPPH) methods, respectively and were compared qualitatively with the microfluidic paper-based analytical device (µPADs) as a new platform. The extract contains a high concentration of phenolic compounds (0.6217 mg/ml) and the DPPH assay for acetone extract indicated a high amount of antioxidant compounds. The effect of nutmeg acetone extract on cyclooxygenase (COX-2) enzyme activity has been done and showed that the nutmeg extract can inhibit COX-2 activity better than the ability of anti-inflammatory drug (Aspirin). Our findings indicate the potential use of nutmeg acetone extract as a source of antimicrobial, antioxidant, and anti-inflammatory agent.

Keywords*: Nutmeg seed extract; microfluidic paper-based analytical device (µPADs); antimicrobial activity; antioxidant activity; Cyclooxygenase (COX-2).*

1. Introduction

Due to the tremendous bioactive compounds found in plants that can be used as antimicrobials, antioxidants, anti-inflammatory, anti-infectious, and anti-tumours, the botanic kingdom has witnessed accelerated attention in therapeutic, cosmetic, food and pharmaceutical areas as a significant natural and safe alternative to synthetic compounds[1]–[3]. Nutmeg, which is the seeds of Myristica fragrans that belongs to the Myristiceae family is a tropical, evergreen tree disturbed widely in America, Africa, and Asia continents [4][5]. It is widely known for its warm taste and pleasant fragrance, therefore used in flavouring various types of meats, vegetables, teas, baked goods and others[6][7]. Also, nutmeg is used in the manufacture of many cosmetic products such as creams, soaps, shampoos, perfumes, etc [8]. Historically, nutmeg was a key ingredient in medical preparations that were recommended to treat stomachaches, diarrhoea, mouth sores, joint pains, insomnia, lowering blood pressure, and dissolving kidney stones [2][9][10].

Standard analytical methods were used to confirm the metabolites constitute profile of nutmeg seeds that are responsible for their bioactivities. β-caryophyllene and eugenol in nutmeg extract are responsible for high antioxidant activity due to the presence of hydrogen atoms in the allylic or benzylic

*Corresponding author e-mail: jwan.abdulsattar@uomustansiriyah.edu.iq.; (Jwan O. Abdulsattar).

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positions that can be easily denoted by peroxy radicals produced under oxidative stress [8]. In addition, Lignans and their glycosides show high antioxidant properties as they simply donate phenolic hydrogen or electrons to the acceptors including lipid peroxyl groups species [8]. Carvacrol, γ-cymene, α-pinene, β-pinene, β-caryophyllene and others are identified as the nutmeg constituents responsible for the antimicrobial activity as well as anti-inflammatory and antifungal activities which can be explained due to their involvement in membrane disruption [11]–[13]. Nutmeg exhibit an anti-carcinogenic effect related to their ability to hinder the hepatic carcinogen-metabolizing enzymes, like aryl hydrocarbon hydroxylase.

Cyclooxygenase (COX), the main catalyst enzyme in the prostanoid synthesis, has two main known isoforms, COX-1 and COX-2 [14]. Cyclooxygenase 2 (COX-2) has been implicated in several physiological and pathophysiological processes including inflammation, tumour growth, and renal injuries. Catalyze the first, rate-limiting step in the formation of such prostanoids as prostaglandin and thromboxane eicosanoids from phospholipase [15]. Chuan et al reported that the pericarp of M. fragrans was evaluated for its bioactive components using in vitro antioxidant and anti-inflammatory assays. Hexane, ethyl acetate and methanolic extracts inhibited lipid peroxidation (LPO) by 82.5, 70.1 and 73.2%, and cyclooxygenase enzymes COX-1 by 44, 44 and 42% and COX-2 by 47, 41 and 36%, respectively, at 100 μg/mL. The bioassay-guided purifications of extracts yielded 20 compounds belongs to neolignans (0.13%), phenylpropanoids (0.28%), phenolic aldehyde (0.35%), triterpenoids (0.06%), triglycerides (0.20%), sugars (10.2%) and steroids (0.49%). Pure isolates 1−5 inhibited LPO by 70−99% and 3−12 inhibited COX-1 and -2 enzymes by 37−49% [16].

Conventional solvent extraction methods including ethanol, acetone, methanol, and water were used to extract phytochemical constitutes from nutmeg seeds[17]–[20]. but these extraction methods suffer from many disadvantages such as high cost, a time-consuming, final product containing solvent residual, low extract yield, large volumes of reagents and samples alongside with high amount of chemical waste and contamination that can affect the environment [6]. As a substitution approach to conventional solvent extraction; soxhlet apparatus that is considered as user-friendly, with the ability to produce a free residual solvent product, reduce chemical waste and contamination [6][21]–[23] was used to fulfils the green chemistry concept for an ideal extraction method for industrialized companies and researchers, targeting clean and safe chemical processes [6]. Hence, the present study was designed to investigate the antimicrobial effect, phytochemical constituents, antioxidant activities, and cytotoxicity of nutmeg seeds extract.

2.1 Collection and preparation of nutmeg seeds extract:

The dried seeds of nutmeg collected from a local market in Baghdad, Iraq in September 2020, were washed and the extract was prepared according to Ozaki et al procedure with modification [24]. A mass of 14.28 gram of the crude powder of nutmeg seeds was refluxed with 100 ml of methanol, and acetone (1:7) solvents, respectively in the soxhlet apparatus for 8 hours to extract the bioactive compounds within the nutmeg dried seeds. Then, the solution was filtered through a filter paper (Whatman Grade No.18) to eliminate residual solids and evaporated to dryness under vacuum at 40°C. Afterwards, the dried extract was weighted and the stock solution of the extract was prepared by weighing one gram of dried extract and diluted with 10 ml of methanol and acetone, respectively. A serials from the stock solution were prepared in the range of (0.00312, 0.00625, 0.0125, 0.025, 0.05, 0.1) mg /mL.

2.2 Antibacterial Activity comparative studies

The antimicrobial activity of nutmeg extract was done using the agar well diffusion method which was adopted from K. Ibrahim et al [2] using two Gram-positive bacteria (Staphylococcus aureus and Staphylococcus epidermidis) and two Gram-negative bacteria (Escherichia coli, and Klebsiella sp.) as well as Candida albicans (yeast) which was kindly supplied by Department of Biology, Mustansiriyah University. Each bacteria was grown in Muller Hinton agar. After 18 hours at 37˚C, a colony was picked with a sterile loop and added to 5 ml of fresh Muller Hinton broth. The mixture for each isolate was adjusted to reach 0.5 Mcfarland by using saline solution. By using a sterile swab, each of the test bacteria was seeded evenly on a fresh Muller Hinton agar. The agar was left to dry and a 4 mm in diameter well was cut using a sterile cork borer. The agar discs were removed and filled with 0.1 ml of different concentrations of the nutmeg extract and allowed to diffuse at room temperature for 2 hours. The plates

were then incubated at 37 ºC for 24 hours. Replicates were carried out for the extract against each of the tested organism. Simultaneous addition of the respective solvents instead of extracts was carried out as controls. After incubation, the diameters of the inhibition zones (mm) were measured [25].

2.3 Qualitative and quantitative analysis of chemical plant extract components

2.3.1 Qualitative nutmeg extract phytochemical analysis

phytochemicals qualitative analysis were carried out for the existence of alkaloids, tannins, terpenoids and steroids, saponins, carbohydrates, resins, and flavonoids using standards procedure [26].

2.3.2 Ash and moisture determination

For the ash determination; the S. S. Nielsen procedure was followed [27]. On the other hand, the moisture procedure was determined by the B. C. O'Kelly procedure [28].

2.3.3 Atomic absorption spectroscopy (AAS) analysis and Gas chromatography-mass analysis (GC-MS)

AAS was used to determine the concentration of trace elements of plant extract using acid digestion procedure [29]. While GC-MS was used to determine the chemical consistent of the plant extract [30].

2.3.4 Determination of total phenolic compounds

A volume of 100 µl aliquot of the extract was transferred to a volumetric flask, containing 46 ml distilled water (H2O) and 1 ml of Folin–Ciocalteu reagent. After 3 minutes, 3 ml of 2% Na2CO3 was added and the mixture was incubated for 2 hours at 25°C. The absorbance was measured at 760 nm. Gallic acid (Sigma-Aldrich, 0.2–1 mg/mL gallic acid) was used as the standard for the calibration curve, and the total phenolic contents were expressed as mg gallic acid equivalents per mL[31]. Qualitative determination of phenol content was done using a new technology of microfluidic paper-based analytical device (µPADs) designed according to Carrilho et al [32] by the addition of 5 µL of nutmeg extract and 5 µL of Folin–Ciocalteu reagent to the detection circular reaction zone using a micropipette. After 3 minutes, 5 µL of 2 % Na2CO3 was added to the detection circular reaction zone and left to dry. Images were taken using a Samsung note 9-camera phone and analyzed via image J freeware (National Institutes of Health, USA).

2.3.5 Determination of free radical scavenging activity

Quantitative determination of antioxidant was done using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Two ml of different dilutions of the extract in acetone were added to 2 ml of a 0.0094 % methanol solution of DPPH (Sigma-Aldrich). Vitamin C (1 mM) (Sigma-Aldrich) a stable antioxidant, was used as a synthetic reference. After 30 minutes incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I %) was calculated in the following way:

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

Where A blank: is the absorbance of the control reaction (containing all reagents except the test compound), and A sample: is the absorbance of the tested compound. Tests were carried out in triplicate[33]. Qualitative determination of antioxidant was determined using the microfluidic paper-based analytical device (µPADs) by spotting of 5 µL of nutmeg extract and 5 µL of DPPH reagent into detection circular reaction zone using a micropipette and left to dry. Images were taken using a Samsung note 9-camera phone and analyzed via image J freeware (National Institutes of Health, USA).

2.4 Estimation of the COX-2 activity in ovarian cancer patients

The activity of the COX-2 enzyme was estimated by an assay for the peroxidase activity in serum of ovarian cancer patients based on a colorimetric procedure [34]. 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 condition [35].

2.4.1 Studying the effect of nutmeg and anti-inflammatory drugs on the activity of COX-2 in sera of ovarian cancer patients

The effect of nutmeg 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 (0.5gm) was dissolved in 1 ml of ethanol and volume completed to 4 ml by Deionized D.W as a stock solution 0.1 g/ mL to prepare a serial

dilutions (0.05, 0.025, 0.0125, 0.00625, and 0.00312) g/ mL from the stock solution[36].

2. Nutmeg extract (0.5 gm) was dissolved in 1 ml of acetone and volume was completed to 4 ml with Deionized D.W as a stock solution 0.1 g/ mL then serial dilutions was prepared (0.05, 0.025, 0.0125, 0.00625, 0.00312) g/ mL in 20 ml volumetric flask from the stock solution [37]. 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) then the method was followed as described in section (2.4). The inhibition percentage was calculated by comparing the activity between with and without inhibitors under the same conditions according to the equation:

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

2.5 Statistical analysis

All the experimental data are presented as mean ± SD of three individual samples. Antimicrobial effect was measured in terms of zone of inhibition and the effect was calculated as a mean of triplicate tests. Data are presented as percentage of free radical scavenging and excel analysis was used to calculate the percentage of COX-2 inhibition at different concentrations of the extract. The total phenolic content was calculated from the standard curve.

3. Results and Discussion

3.1 Antimicrobial activity

Acetone and methanol nutmeg seeds extracts were tested for their antimicrobial activity. Preliminary screening against Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella sp. and Candida albicans was performed. Acetone extract at the concentration of 0.1 gm/ml (S7) showed an effect against all tested microorganisms. Overall, Candida albicans, was the most affected microorganism as listed in table (1) while the methanol extract showed no effect against tested microorganisms as shown in table (2).

There are different inhibition mechanisms against microorganisms such as cell membrane damage, inhibition of protein synthesis and disruption of cell biological functions and cell membranes by specific enzymes. The nature of bacterial cell wall is influenced by the presence of compound like α-Pinene and β-pinene (pinene-type monoterpene hydrocarbons) which is involved in the membrane cell wall disruption by the lipophilic compounds [38]. In addition, the nature of gram-negative bacteria cell wall with high lipid content (up to 20 %) compared with 0-2% for gram-positive is responsible for the resistance of microorganisms such as Escherichia coli [2]. While gram-positive bacteria show more sensitivity to the antimicrobial compounds found in nutmeg [33]. It is concluded that nutmeg acetone extract has antimicrobial activity against gram-positive bacteria in contrast to gram-negative bacteria which are resistant to both acetone and methanol extracts [2]. While Candida albicans showed a higher sensitivity to acetone extract ranging from 11 to 15 mm diameter. Our results agree with a recent study in 2021 which reported that nutmeg seed essential oil showed high antifungal activity compared with their antibacterial activity against tested microorganisms[39].

3.2 Phytochemical compounds of nutmeg seeds extract

To examine the presence of phytochemical compounds in nutmeg seeds acetone extract; a standard qualitative chemical analysis was done (as listed in table 3) to build a good platform for a better view of the nutmeg extract compounds.

As can be seen from the table (3), nutmeg seeds acetone extract contains alkaloids, terpenoids, steroids, saponins, glycosides, resins, flavonoids and tannins. The present study results agree with a similar study conducted [11]. An alkaloid found in the extract has an important biological property such as cytotoxicity and it is used in allopathic systems [26]. Steroidal compounds are of great importance and interest in pharmacy due to their relationship with compounds such as sex hormones mostly used in the development of female contraceptive pills [31]. Also, glycosides are useful in lowering blood pressure and are used in the treatment of congestive heart failure and cardiac arrhythmia [26]. Terpenoids present in the extract are used in the treatment of cough, asthma and hay fever. Traditionally, saponins have been extensively used as detergents and pesticides, also known for their protection against hypercholesterolemia and have antibiotics properties. In addition to their industrial applications as foaming and surface-active agents and also beneficial health effects [40]. The growth of many fungi, yeast, bacteria and viruses are inhibited by tannins, which act as antioxidant similar to phenols [26]. Quantitative analysis of nutmeg extract showed that the tested moisture was (13.56 %) of total nutmeg extract nutritional content, while ash percentage was (0.176 %).

3.3 Content of trace elements in nutmeg extract

The trace elements that exist in nutmeg extract is displayed in the table (4), which specify the presence of Cu, Zn, Pb, Cr, Ni, and Mn in the concentration of 150, 280, 6.1, 0.08, 0.09, 0.0012, and 0.027 µg/dl, respectively. These elements are considered essential to human health.

3.4 GC-Mass analysis

The analysis of nutmeg seeds acetone extract using GC-MS instrument has shown various phytocompounds. As can be seen from figure (1), The GC-MS chromatogram confirmed the existence of 32 compounds with various retention times The major compounds identified in the nutmeg seeds extract are shown in figure (2) and listed in table (5), that indicate the presence of monoterpenes such as pinene, camphene, α-pinene, sabinene, myrcene, α-phellandrene, α-terpinene, limonene, 1, 8-cineole, g-terpinene, linalool, terpinene-4-ol, safrole, methyl eugenol and myristicin, as their active compounds. Several GC-MS peaks were unidentified due to the lack of library data of corresponding compounds.

Table 1: Mean and standard deviation (SD) values of acetone extract from seeds of nutmeg against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella sp.* and *Candida albicans*.

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	-	-	-	-	-	-	-	-	-	-
S2	-	-	-	-	-	-	-	-	10.33	1.24
S3	7.33	0.47	-	-	-	-	-	-	-	-
S4	8.66	1.24	-	-	-	-	-	-	11.66	1.24
S5	-	-	-	-	-	-	-	-	11	0.81
S6	-	-	-	-	-	-	-	-	12.33	0.47
S7	11.66	0.47	12	0.81	14	0.81	11.33	1.24	13	1.63

Table 2: Mean and standard deviation (SD) values of methanol extract from seeds of nutmeg against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella sp.* and *Candida albicans*.

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	-	-	-	-	-	-	-	-	-	-
S2	-	-	-	-	-	-	-	-		
S3	-	-	-	-	-	-	-	-	-	-
S4	-	-	-	-	-	-	-	-	-	-
S5	-	-	-	-	-	-	-	-	-	-
S6	-	-	-	-	-	-	-	-	-	-
S7	-	-	-	-	-	-	-	-	-	-

Table 3: Qualitative phytochemical analysis by standard procedures for nutmeg seeds acetone extract.

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

Table 4*:* Concentration of trace elements in nutmeg extracts

Trace elements	Cu	Zn	Pb	Cd	Cr	Ni	Mn
Concentration in µg/dl	150	280	6.1	0.08	0.09	0.0012	0.027

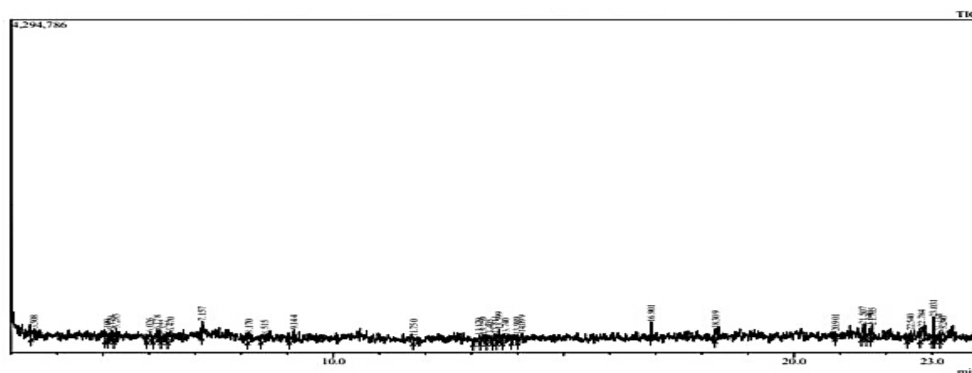

Figure 1: GC-MS chromatogram investigation of nutmeg extract.

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

Peak	Retention Time	Area%	Name of the compound	Formula	Mol. Wt.(g/mole)
1	3.508	4.39	2-[3-Chloro-4-methylphenyl]succinic acid	C11H11ClO4	242
2	5.100	2.93	4-(7-Methoxy-2H-1,3-benzodioxol-5-yl)-5-(4-me	C10H8Cl6	340
3	5.179	3.08	4-[6-(2,5-Dimethoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]pyridine	C16H13N5O2S	339
12	9.144	3.77	3-Chloro-2,2-bis(chloromethyl) propanoic acid	C5H7Cl3O2	204
13	11.750	2.58	Diethylamine, 2,2'-dichloro	C4H9Cl2N	
20	13.989	3.71	4-Acetamido-N-tert-butyl-3-nitrobenzamide	C7H15NO2	279
31	23.154	3.61	1,3,2-Dioxaphosphorinan-2-amine, N,N-dimethy	C17H20NO3P	317
32	23.240	3.19	5'-O-[N,NDimethylsulfamoyl] adenosine	C12H18N6O6S	374

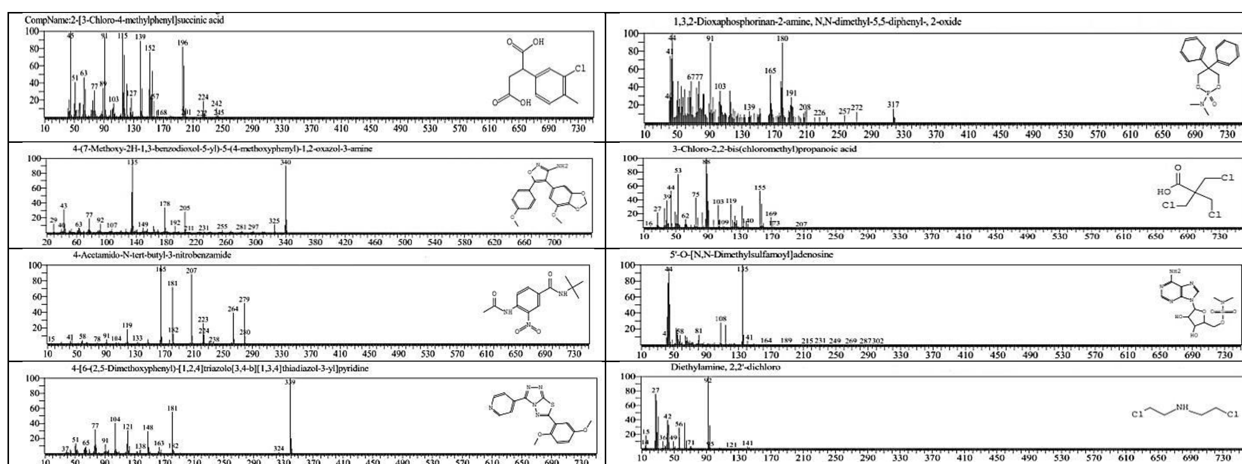

Figure 2: GC-Mass Spectrum of the main compounds identified in nutmeg seeds extract.

3.5 Total phenolic contents

Phenolic compounds are very important plant constituents because they exhibit antioxidant activity by inactivating lipid free radicals or preventing the decomposition of hydroperoxides into free radicals. The Folin-Ciocalteu method is a rapid and widely-used assay, to investigate the total phenolic content but it is known that different phenolic compounds have different responses in the Folin-Ciocalteu method [41].

Table 6: Total phenolic of nutmeg seeds extracts

Concentration (gallic acid) mg/mL	Absorbance	Total phenolic content in nutmeg extract mg/mL
0.2	0.019	-
0.4	0.050	-
0.6	0.063	-
0.8	0.075	-
1	0.084	-
Extract	0.060	0.6217

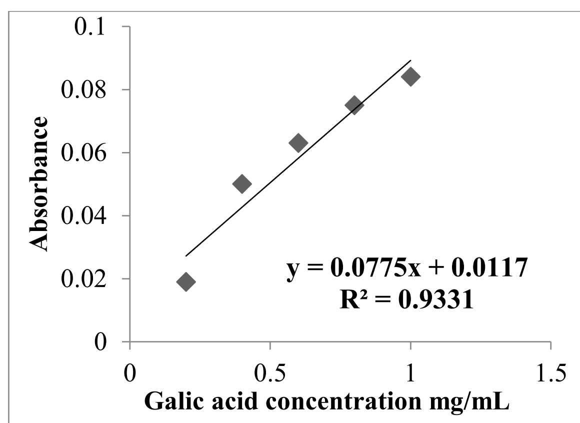

Figure 3: Total phenolic concentration of nutmeg extracts

Total phenolic contents were calculated using the calibration curve of gallic acid and nutmeg extract as shown in table (6) and figure (3) and expressed as gallic acid equivalents per mL. The total phenolic concentration was equal to 0.6217 mg/mL which, indicate the high phenolic acid levels in acetonic extracts. This can be related to the extraction of both nonpolar and semipolar soluble phenolic acids. It is suggested that the phenolics compounds, which are antioxidants, are responsible for the antibacterial activity.

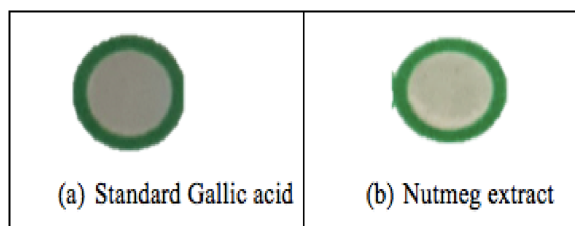

Figure 4: µPADs images for (a) standard Gallic acid, and (b) Nutmeg extract

Qualitative determination of radical scavenging was done using a new and cheap technology called microfluidic paper-based analytical device (µPADs). Experimentally, brownish colour was formed after the extract addition to Folin–Ciocalteu reagent and 2% Na2CO3, as can be seen in figure (4) indicating a good phenolic content compared with standard gallic acid

3.6 Free radical scavenging activity (qualitative and 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. The high antioxidant activity of the extracts could be attributed to their high phenolic content. This preliminary study indicates the interesting anti-oxidative stress activity of nutmeg extract suggesting its promising applications as a medicinal source for the treatment and prevention of free radicals associated diseases [42]. From table (7), data showed a good percentage of antioxidants that the extract contains when compared with vitamin C, which is a strong and effective antioxidant.

Table 7: Determination of DPPH free radical.

The concentration of the extract (µg/mL)	DPPH scavenging effect (%)	Mean of DPPH scavenging effect (%)	Standard Deviation of DPPH scavenging effect (%)
Vitamin C	92.99	92.88	0.08
0.00312	11.13	11.11	0.01
0.00625	57.63	57.53	0.06
0.0125	74.59	74.43	0.11
0.025	77.91	77.82	0.07
0.05	78.27	78.31	0.03
0.1	79.08	79.07	0.02

Qualitative determination of radical scavenging was done using the microfluidic paper-based analytical device (µPADs). The brownish colour was observed after the addition of the extract to the DPPH reagent as can be seen in figure (5) indicating a good activity of the extract against the DPPH compound.

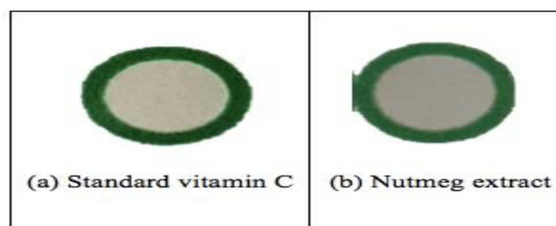

Figure 5: µPADs images for (a) standard vitamin C, and (b) Nutmeg extract

3.7 The effect of nutmeg and anti-inflammatory drugs on the activity of COX-2 in sera of ovarian cancer patients

The result of the study shows that the extracted oil has a negative impact on the activity of the COX-2 enzyme in sera of ovarian cancer patients as shown in figure (6).

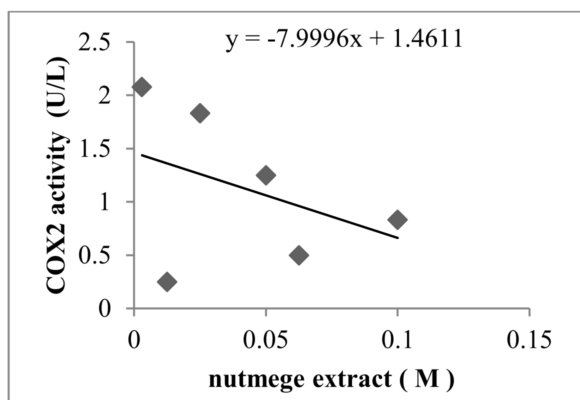

Figure 6: The Effect of nutmeg extract on the activity of COX-2 enzyme in sera of ovarian cancer patients.

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

Preparing different concentrations of each drug and using it in the enzymatic reaction studied the inhibition effect of anti-inflammatory drugs such as Aspirin and nutmeg extract on the COX-2. The result of the study showed that each drug exhibits a different inhibitory percentage on the activity of COX-2 as shown in table (8), and figure (7).

Table 8: The inhibition percentage of the nutmeg extract and some anti-inflammatory drugs on the activity of the COX-2 in sera of ovarian cancer patients.

The concentration of the extract (µg/mL)	DPPH scavenging effect (%)	Mean of DPPH scavenging effect (%)	Standard Deviation of DPPH scavenging effect (%)
Vitamin C	92.99	92.88	0.08
0.00312	11.13	11.11	0.01
0.00625	57.63	57.53	0.06
0.0125	74.59	74.43	0.11
0.025	77.91	77.82	0.07
0.05	78.27	78.31	0.03
0.1	79.08	79.07	0.02

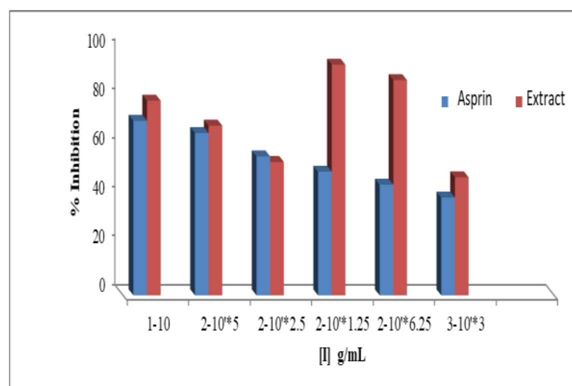

Figure 7: The inhibition percentage of the nutmeg extract and aspirin drugs on the activity of the COX-2 in sera of ovarian cancer patients

The current study shows that the nutmeg extract can inhibit COX-2 activity better than the anti-inflammatory ability of the drug as shown in Table 6. 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 [36] 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. Thus, the effect is likely to negative allosteric effect. 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 [43].

4. Conclusion

Plants contain a wide variety of antioxidants phytochemicals or bioactive molecules, which can neutralize the free radicals and thus retard the progress of many chronic diseases associated with oxidative stress and reactive oxygen species (ROS). The intake of natural antioxidants has been associated with a reduced risk of cancer, cardiovascular disease, diabetes and diseases associated with ageing. It can be concluded from the current results that acetone nutmeg extract exhibited antimicrobial activity and Candida albicans was the most affected microorganism. The extract provided better antioxidative activity by using classic methods and using a new technology called µPADs. As compared with synthetic antioxidants, which was rich with phenolic, terpenes, flavonoids, and several trace elements that were analyzed using standard methods, AAS, and GC-MS. The extract displayed an excellent inhibition action towards the human COX-2 activity and thus appeared anti-inflammatory effect. Hence,

the nutmeg extract may be exploited as a natural antioxidant and health-promoting agent that can conveniently find its appropriate therapeutic applications.

5. Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

6. Funding source

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8. References

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