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In vitro anti-hepatitis B virus Activity and Chemical Profile Of Bioactive Compounds From *Syzygium Aromaticum* and *Olea Europaea*

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Abstract

The hepatitis B virus (HBV) is the major cause of liver disease, and it develops into cirrhosis, chronic hepatitis, and hepatocellular carcinoma. Medicinal plants are traditional medicine and are used to cure several diseases. In this study, antioxidant assay by DPPH, total phenolic and flavonoid contents of *Syzygium aromaticum* and *Olea europaea* extracts, and their anti-viral effect against HBV. The chemical profile of the most active extracts was analyzed by LC/MS/MS, investigating the anti-HBV activity of methanol extracts for reducing the relative HBV replication in infected HepG2 cells with archived serum samples compared with other tested extracts of the two plants. The results proved that *S. aromaticum* methanol extract has higher phenolic and flavonoid content (150.71±1.24 mgGAE/g extract, 310.61±0.67 mg Rutin/g extract, respectively) than *O. europaea* methanol extract (108.77±1.17 mg GAE/g extract, 93.14 ± 2.11 mg Rutin/g extract, respectively). *S. aromaticum* has higher antioxidant activity than *O. europaea* (16.85 ± 0.81 and 36.62 ± 1.17 µg/ml). Nevertheless, the most potent anti-HBV extract in both plants was methanol. Hence, LC/MS/MS Fingerprint exhibited the presence of six compounds in *O. europaea* methanol extract including oleuropein, oleuropein glucoside, Rutin, and p-Coumaric acid while, *S. aromaticum* methanol extract exhibited ten compounds include caffeic acid, ellagic acid, galloyl glucose, and isobiflorin. So, both plants demonstrated promising anti-HBV activity. These findings suggest that both *S. aromaticum* and *O. europaea* hold promise as sources of anti-HBV agents, deserving further exploration for developing novel therapeutics against HBV infection.

Keywords: *Olea europaea* - LC/MS/MS- *Syzygium aromaticum* - phenolic content- flavonoid content

Introduction

Hepatitis B virus (HBV) infection is a major global health concern that affects about 257 million people with chronic infection and is estimated to cause approximately 887,000 deaths globally each year; of these, 990,000 (66%) occurred in the World Health Organisation (WHO) African Region. The virus causes both acute and chronic hepatitis, which can result in liver cirrhosis and hepatocellular carcinoma. Although there are effective vaccines and antiviral therapies available, HBV infection continues to be a major public health concern, especially in developing nations with limited access to healthcare ^[1]. Many natural products have been investigated in an effort to find more side-effect-free, efficient therapies for HBV infection. Growing interest has been seen in the use of natural materials as possible sources of anti-HBV medicines in recent years ^[2].

Several chemical components and biological processes that are intrinsic to these plant entities have been identified by phytochemistry, emphasising the myriad secondary metabolites that they generate ^[2]. Alkaloids, flavonoids, terpenoids, and phenolic compounds are some examples of these compounds. They frequently play important roles in a plant's defensive mechanisms and ability to adapt to its surroundings. Understanding plant biology, ecology, and evolutionary adaptation is greatly enhanced by the analysis of these chemical constituents. In addition, it is a fundamental component of many other domains, including industry, agriculture, nutrition, and medicine, where the therapeutic, dietary, and economic benefits of substances produced from plants are extensively studied in relation to human health. Therefore, the study of phytochemistry emphasises how closely plants and human civilisation are linked ^[3].

Two plants that have historically been investigated for medical purposes are clove (*Syzygium aromaticum*) and olive (*Olea europaea*) ^[4]. Cloves are members of the Myrtaceae family. Clove's bioactive ingredients, which include flavonoids, gallic acid, eugenol acetate, phenolic acids, and terpenoids, are used in cosmetics and medicinal spices ^[5]. Antibacterial, antinociceptive, antimicrobial, anti-diabetic, anti-thrombotic, antifungal, and anticancer properties are all present in its essential oil. Clove has anti-inflammatory compounds that help reduce pain and inflammation ^[6-8].

Tropical and warm regions of the world are home to olives (*Olea europaea*), which are members of the Oleaceae family ^[4]. One of the main crops grown worldwide for edible oil and olive fruit production is the olive ^[9, 10]. Olive leaves are thought to be an inexpensive raw material that can be a good source of goods with high added value (phenolic chemicals). In addition to more complex elements like secoiridoids, it contains a number of bioactive chemicals, including oleuropein, vanillic, gallic, coumaric, and caffeic acids, tyrosol, and hydroxytyrosol ^[11,12]. It has been discovered that the olive leaf extract possesses antibacterial, anti-inflammatory, and antioxidative properties against bacteria and fungus ^[12-14]. This is the first comparison of total phenolic and flavonoid levels, DPPH radical scavenging activity, and anti-HBV activity amongst the plants, given the significance of each plant.

This work aims to investigate the efficacy of bioactive compounds in *S. aromaticum* and *O. europaea* on the anti-HBV activity and to provide a comprehensive analysis of the chemical characterization of these compounds.

Materials and Methods

Plant Material

Syzygium aromaticum cloves, or flower pods, weighed 1 kg, and *Olea europaea* leaves, weighed one kilogramme, were gathered at Orman Garden. Dr. Rim Samir Hamdi, a professor of plant taxonomy at Cairo University's Faculty of Science, recognised the plants. These plants were powdered after being dried. Theodor Bilharz Research Institute's Department of Medicinal Chemistry maintained the voucher specimen (No. C15/4/19) of the plants for research purposes.

Plant Extraction

1 kg of powdered *S. aromaticum* cloves and *O. europaea* leaves were extracted four times over the course of two weeks using 85% aqueous methanol to yield a methanolic extract of each plant. This extract was then defatted with petroleum ether (PET) and fractionated using n-butanol (n-BuOH). Using a rotatory evaporator, the aqueous residue was also dried until it was completely evaporated (BUCHI). For the purposes of this investigation, moisture was maintained away from the methanolic extract and other fractions of each plant.

Determination of total phenolic content

Using a colorimetric assay based on methods described by Singleton et al. ^[15] with minor changes, the phenolic content of the two examined methanolic extracts was determined using Folin-Ciocalteu reagent; gallic acid was employed as standard. Using this approach, 500 µl of the Folin–Ciocalteu reagent, 1.5 ml of sodium carbonate (20%), and 100 µl of each extract at a concentration of 100 µl/ml were combined. After shaking the mixture and adding distilled water to make it equal to 10 ml, it was let to stand for two hours. At 765 nm, the absorbance was measured in relation to a blank that contained gallic acid and distilled water as a standard. Three separate measurements of the phenolic content were made. The gallic acid equivalent (mg GAE/g extract) was used to express the total phenolic content in milligrammes per gramme of dry weight extract.

Determination of total flavonoid content

Using rutin as a standard, the total flavonoid content of the two tested methanolic extracts was calculated using the methods outlined by Kumaran and Karunakaran ^[16]. Following a mixture of 100 µl of plant extract in methanol (100 µg/ml) and 100 µl of aluminium trichloride in methanol (20 mg/ml), the mixture was diluted to 500 µl with methanol. Each test's absorbance was measured at 415 nm in comparison to the blank after 40 minutes. Every determination was made three times. Each plant extract's total flavonoid content was calculated as milligrammes of rutin equivalents per gramme of extract (mg RE /g extract).

Determination of DPPH radical scavenging activity

Using the Akroum et al. ^[17] technique, each methanolic extract was evaluated for its ability to scavenge DPPH radicals. One millilitre of DPPH (0.1 mM 1,1-diphenyl-2-picrylhydrazyl) in methanol

was well mixed with three millilitres (100 µg/ml) of each plant extract. The mixture was thoroughly shaken and then allowed to sit at room temperature in the dark for half an hour. A UV/VIS spectrophotometer was used to quantify the absorbance at 517 nm in relation to methanol, which served as the blank. Ascorbic acid served as the reference standard. The only ingredients in the controls are methanol and DPPH; no extract is present. Each experiment was conducted three times. Each extract's capacity to scavenge free radicals is expressed as a decrease in the percentage of DPPH. The IC₅₀ is the concentration of each extract required, relative to ascorbic acid, to scavenge 50% of DPPH radicals. A lower IC₅₀ value indicates a higher level of scavenging or antioxidant activity in the plant extracts.

Antiviral Assay

Cell cultures and inoculum preparations

HepG2 cells were cultured in DMEM that was enhanced with 10% foetal bovine serum and a 70% sheet of 1x antibiotic antimycotic mix. In a 24-well plate, 105 cells were planted per well with 4 millilitres of full culture media. The serum of chronic hepatitis B (106 IU/ml) was used to precipitate and centrifuge 20% PEG-8000/2.5 M NaCl to extract HBV particles (DNA of HBV) ^[18].

No of copies = $x \text{ ng} * 6.0221 * 10^{23} \text{ molecules per mole} / N * 660 \text{ (g/mole)} * 1 * 10^9 \text{ (ng/g)}$

Where; X: the amount of amplicon per ng, N: length of dsDNA (HBV= 3.2 kb), 660 (g/mole): average mass of 1 bp dsDNA, 1 IU/ml of HBV= 5.6 copies/ml= 50 mg/ml.

Transfection protocol

The transfection reagent ESCORT (1 mg/ml Sigma Aldrich) was utilised, and the ratio of DNA/liposome was 3 µg/6 µg. The DNA was suspended in a serum-free, antibiotic-free medium at a concentration of 3 µg/100 µl. Following its procedure, the transfection reagent was dissolved in a serum-free, antibiotic-free media. After gently tipping the finger to combine the DNA and transfection reaction, the mixture was incubated for 45 minutes at room temperature. 24 well-plates containing cells were twice rinsed with 500 µl of serum-free antibiotic-free solution. Next, 400 µl of 2% serum-containing media were added to each well, and 100 µl of DNA/liposome complex was added dropwise. In a CO₂ incubator, cells were cultured for 24 hours at 37°C. Before testing the samples, the cells were rinsed twice with 500 µl of serum-free, antibiotic-free media. In accordance with our earlier research on the cytotoxicity of HepG2, the tested concentration for each fraction was found ^[8]. Only the transfected cells were present in the negative samples. The quantities under test were diluted in 500µl of full medium containing 5% serum. The cells were cultured in a CO₂ incubator for twenty-four hours at 37 oC. After collecting the supernatant, cells were resuspended in lysis solution in order to extract DNA.

DNA extraction using the phenol-chloroform method

The DNA was isolated using Butler's ^[19] technique. 500 µl of lysis buffer (100 mM tris pH 8.5, 4.7 M guanidinium thiocyanate) is added to the collected cells. To lyse the cells and break down the proteins, 20 µl of proteinase K was added, and the mixture was then incubated at 65°C for one to two hours. After that, 5 µl of 10 mM RNase A was added, and the mixture was left to breakdown RNA for 15 to 30

minutes at room temperature. DNA was precipitated with isopropanol after extractions employing phenol: chloroform: isoamyl alcohol and chloroform:isoamyl alcohol were carried out. After centrifuging the DNA to create a pellet, 70% ethanol was used to wash it, and any leftover ethanol was either air-dried or quickly centrifuged. The DNA pellet was then completely dissolved in TE buffer in preparation for further use or storage. Using NanoDrop 2000, the concentration and purity of extracted DNA were assessed.

Conventional PCR

To optimize the annealing temperature for further quantitative PCR analysis, conventional PCR was performed using internal control β -actin and HBV gene-specific primers (**Table 1**). 200 ng of extracted DNA, 200 μ M dNTPs, 1 μ M of each forward and reverse primer, and 25 μ l of 2x Taq ready-to-use master mix were combined to create a total volume of 50 μ l. A range of annealing temperatures (52–60 oC) was examined ^[20].

Table 1: Primers sequence for gene expression analysis using qPCR

Primer	Sequence	Reference
β -actin Forward	GGACTT CGAGCAAGAGATG	Samir <i>et al.</i> ⁽²²⁾
β -actin Reverse	CCTTCTGCATCCTGTC	
HBV Forward	GATGTGTCTGCGGCGTTTAA	(Liu <i>et al.</i> , 2017)
HBV Reverse	GCAACATACCTTGATAGTCCAGAAGAA	

Agarose gel electrophoresis

Agarose (Molecular Biology Grade) was prepared in 1x TAE buffer at a percentage of 2%. The DNA samples containing 1/10th volume of sample loading buffer were loaded electrophoresed in 1x electrode buffer at 120 volts. The fractionated DNA fragments were stained in 0.5 μ g/ml ethidium bromide for 3-5 min, then visualized under UV light (302 nm wavelengths) and photographed ^[21].

Quantitative PCR

A total of 20 μ l of materials were used, including 2 μ l of 200ng DNA, 5 pmoles of forward and reverse primers, and 10 μ l of 2x SYBR Green/ROX PCR Master Mix (Biovision Co.). Initial denaturation at 95°C for 15 minutes was part of the qPCR procedures. Next, the PCR products were subjected to 50 cycles of denaturation (95°C for 30 s), annealing (55°C for 20 s), extension (72°C for 25 s), and melting curve analysis (from 60 to 95°C). DNA was absent from the negative controls, which were nuclease-free water. To guarantee high-quality DNA extraction and target normalisation using a $2^{-\Delta\Delta C_t}$ technique, the reference gene used was β -actin ^[22].

LC/MS/MS

Using LC/MS/MS studies performed using a Sciex 4000 QTRAP® system LC system paired with a hybrid triple quadrupole LIT (linear ion trap), comprising Harvard apparatus 11 plus, the phenolic compounds in the methanolic extracts of *O. europaea* L and *S. Aromaticum* were identified. Pump: A SUPELCO Discovery HS-F5 column (3 µm, 3×150 mm; Sigma-Aldrich Co., Steinheim, Germany) was used for the isolation. the isocratic elution using methanol, water, and formic acid as the mobile phase (50:50:0.1, v/v/v). Both the run time and the flow rate were 15 minutes. The analytical column's temperature was adjusted to 30°C. There was a 5 µL injection volume. Analyst® 1.6.3 with Hotfix is the software.

Statistical analysis

With GraphPad Prism 8 (San Diego, California, USA), it was documented. To evaluate the importance of distinctions between cancerous and normal cells, a T-test was utilised. Within a group of fractions, an ANOVA was run. A difference deemed statistically significant was defined as having a p value less than 0.05.

Results and Discussion

Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of each plant's methanolic extract were ascertained in this study and are listed in (**Table 2** and **Fig 1**). Because of methanol's polarity and capacity to solubilize and recover optimal levels of phenolic from plants, it is commonly cited as an effective solvent for the extraction of antioxidant chemicals in previously published research [23, 24]. Additionally, the kind of plant material may have an impact on variations in the recovery of phenolic and flavonoids from natural products [5]. When compared to other previous reports [25, 26, 14], our results showed a high amount of phenolic (150.71 ± 1.24 and 108.77 ± 1.17 mg GAE/g extract) and flavonoid contents (310.61 ± 0.67 and 93.14 ± 2.11 mg Rutin/g extract) for both *S. aromaticum* and *O. europaea* leaves, respectively. It was evident that the extract from *S. aromaticum* had more flavonoids than phenolics. This indicates that flavonoids outweigh phenols in the plant. This is entirely consistent with reports from Shabir *et al.* [27] and Sajid *et al.* [28] on *S. aromaticum*.

Determination of DPPH radical scavenging activity

A common metric for assessing the nutritional value of foods, plants, and their physiologically active ingredients is antioxidant content. Since synthetic antioxidants are known to cause adverse consequences including cancer, their use has been limited [29]. Discovering the most promising natural antioxidant to take the place of synthetics has gained significant attention lately. According to our research, both plants' methanolic extracts have antiradical action, as seen in (**Fig. 2**). Compared to *O. europaea*, *S. aromaticum* exhibits more antioxidant activity; its IC₅₀ was (16.85 ± 0.81 µg/ml), whereas *O. europaea*'s is (36.62 ± 1.17 µg/ml). In the model of stable lipophilic radicals known as DPPH, lipid oxidation sets off a series of lipophilic radical reactions. Antioxidants can react with DPPH By lowering the quantity of DPPH radicals to the quantity of their accessible hydroxyl groups [30]. Our findings demonstrated a clear positive correlation between the plant extracts' antioxidant activity and their total phenolic and flavonoid content.

This brings us back to *S. aromaticum*'s increased phenolic and flavonoid levels as well as its enhanced capacity to scavenge radicals. Our results concur with those of other earlier studies ^[1, 31].

Cell culture

According to our previous studies, we used nontoxic concentrations (500 and 250 µg/ml) on transfected HepG2. As shown in (**Fig. 3**), microscopic examination exhibited viable and bright cells in both untreated and treated HBV-infected cells. These results confirmed the safety of the extracts used before starting the antiviral assay. The human hepatoma cell line HepG2, which is known to enable HBV replication after transfection by cloned HBV DNA, has the biosynthetic capacities of typical liver parenchymal cells ^[32].

Agarose gel electrophoresis of conventional PCR

Confirmation of optimum annealing was done using 2% agarose gel. As shown in **Fig. 4**, different isolated DNA samples from the treated HepG2 infected cells gave an obvious band using HBV primers at an annealing temperature of 55 °C.

Table 2: Total phenolic and flavonoid content of methanolic extract of *S. aromaticum* and *O. europaea*.

Tested Plant	Total Phenolic (mg GAE/g extract)	Total flavonoid (mg Rutin/g extract).
<i>S. aromaticum</i>	150.71 ± 1.24	310.61 ± 0.67
<i>O. europaea</i>	108.77 ± 1.17	93.14 ± 2.11

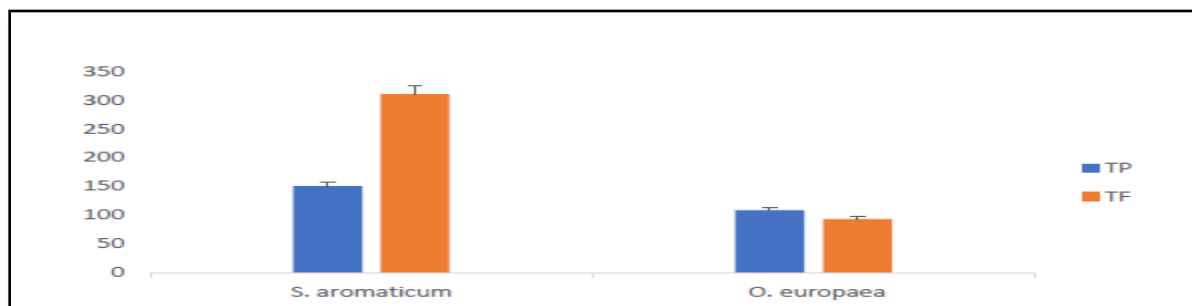


Fig (1): Total phenolic and flavonoid content of methanolic extract of *S. aromaticum* and *O. europaea*.

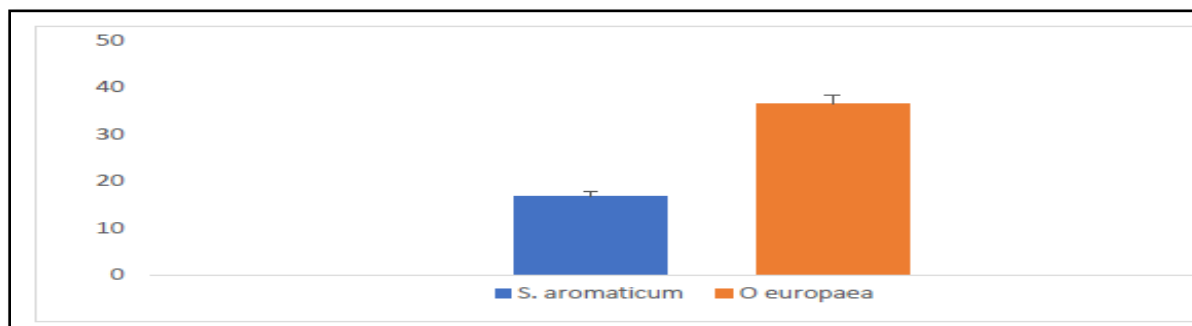


Fig (2): Scavenging activity of methanolic extract of *S. aromaticum* and *O. europaea* against DPPH. Antiviral assay.

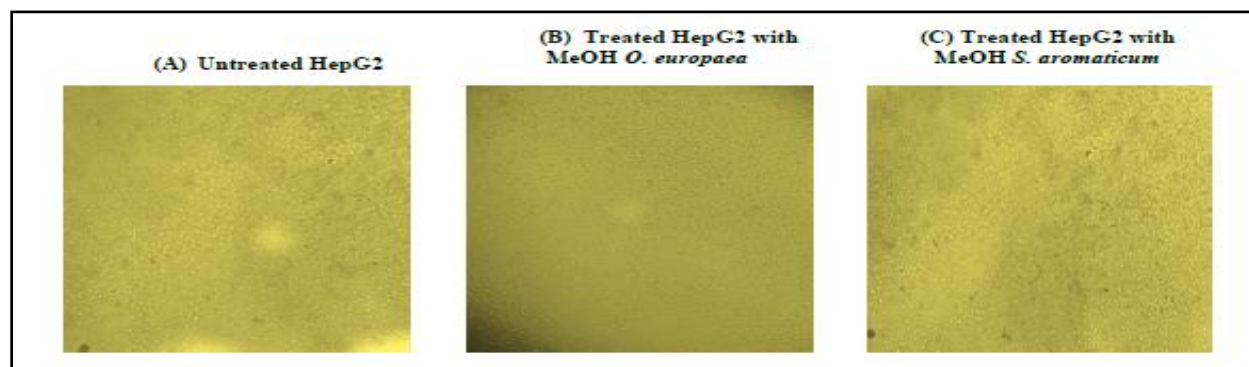


Fig (3): Microscopic examination of (A) untreated HBV-infected HepG2 cells (B) Treated cells with *O. europaea* (C) *S. aromaticum* methanol extracts.

Quantitative PCR

Results showed that *O. europaea* and *S. aromaticum* extracts had variable anti-HBV effects as shown in (Table 3 and Fig. 5) compared to untreated HBV-infected HepG2 cells. The most potent anti-HBV extract in both plants was methanol. Previously, the presence of a variety of polyphenolic chemicals, such as oleuropein, oleuropein aglycone, elenolic acid, and hydroxytyrosol in olive leaf extract demonstrates antiviral activity as the human immunodeficiency virus type 1 (HIV-1) and viral hemorrhagic septicemia rhabdovirus (VHSV). Oleuropein has been shown to have antiviral effects against the hepatitis B virus, the human immunodeficiency virus, and hemorrhagic septicemia rhabdovirus (VHSV) [33]. *S. aromaticum* extract, oil, or specific active components (such as eugenol) have historically been used for their antithrombotic, immunostimulatory, and antibacterial properties, which may also be helpful in the case of severe viral infections. Also, clove plays a broad-spectrum antiviral effect against newly emerging and re-emerging RNA and DNA viruses [34, 35].

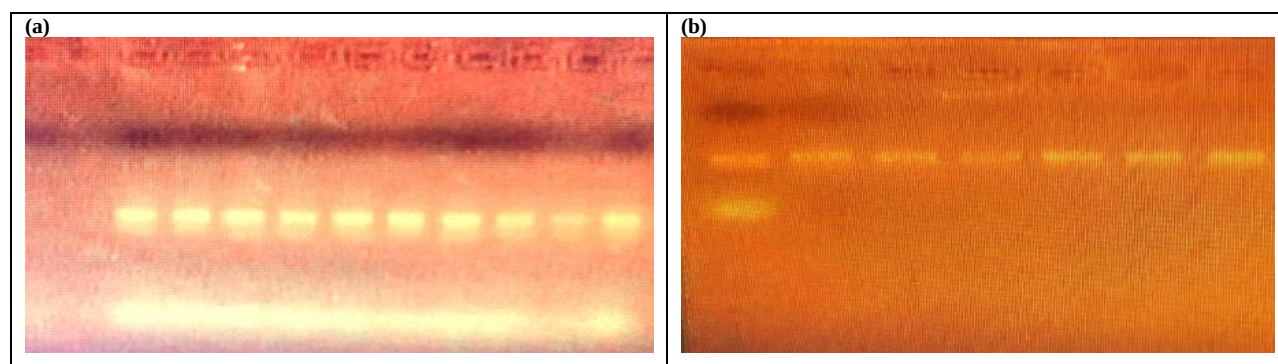


Fig. (4): 2% Agarose gel electrophoresis on PCR products showing (a) PCR on different samples using HBV primers; and (b) PCR on different samples using β -actin at 55 °C annealing temperature.

Table 3: Relative quantification of HBV in different treated HBV infected cells in compared to untreated transfected HepG2 cells for *O. europaea* and *S. Aromaticum*.

Plant name	Extract	HBV RQ
Untreated cells		87.2±6.5
<i>O. europaea</i>	Pet (500)	4.8±2.5
	Pet (250)	13.9±2.7
	BuOH (500)	18.7±1.9
	BuOH (250)	47.5±2.66
	MeOH (500)	1.8±1.1
	MeOH (250)	6.6±1.8
	Aqua (500)	24±5
	Aqua (250)	63.4±7
<i>S. aromaticum</i>	MeOH (500)	2.6±6.6
	MeOH (250)	6.8±0.9
	Aqua (500)	6.4±1.85
	Aqua (250)	15.6±2.9

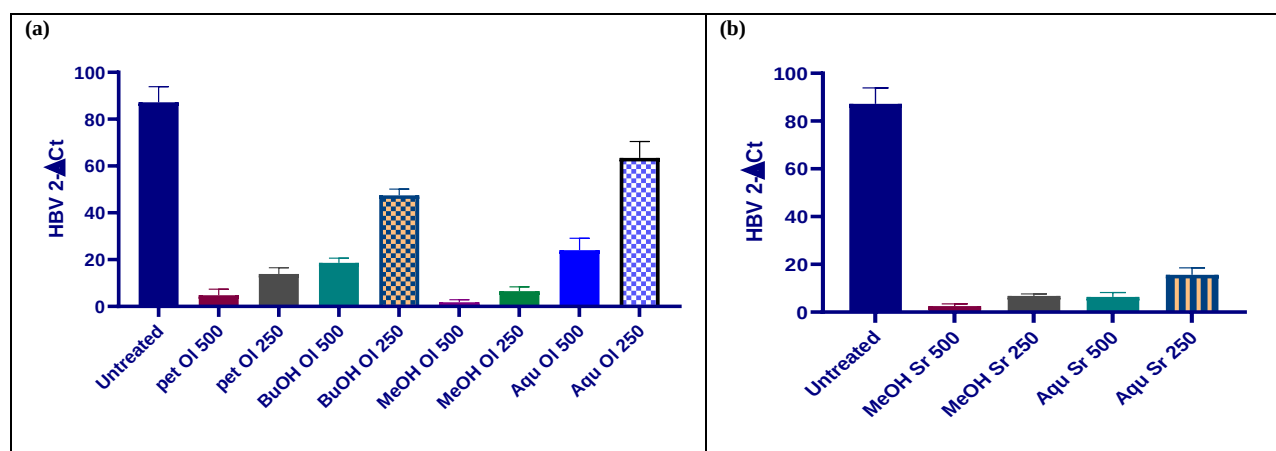


Fig. 5: Relative quantitation of HBV replication in infected HepG2 cells; (a) compared to treated cells using *O. europaea* fractions ; (b) compared to treated cells using *S. aromaticum* fractions.

LC/MS/MS

The phenolic compounds in *O. europaea* and *S. aromaticum* methanolic extracts were determined using QTRAP LC/MS/MS analysis. The phytochemicals of each plant were characterized and tentatively identified by comparing the constituent mass spectra with the previous literature. *O. europaea* L and *S. Aromaticum* methanolic extracts. Analysis of *O. europaea* methanolic extract in the Negative-ion Mode has identified six phenolic compounds but *S. aromaticum* has identified ten phenolic compounds as shown in **Fig. 6& 7**.

Analysis of O. europaea methanolic extract in the Negative-ion Mode:

Compound (1) might be 3,4-Dihydroxyphenylacetic acid. Its MS/MS yields a fragment ion at m/z 123, which corresponds to the loss of a CO_2 group from the carboxylic acid moiety. The deprotonated molecular ion $[\text{M}-\text{H}]^-$ at m/z 167 [36]. It was discovered in the water waste and olive roots [37, 38]. Tyrosol was identified as Compounds (2) because they had a pseudo molecular ion peak $[\text{M}-\text{H}]^-$ at m/z 137, followed by a peak at m/z 119 corresponding to $[\text{M}-\text{H}-\text{OH}]^-$, and m/z 105 due to $[\text{M}-\text{H}-\text{CH}_2\text{OH}]^-$ [39]. Tyrosol, which Olive has, has a variety of biological functions [40]. Compounds (3) had two peaks: m/z 117 $[\text{M}-\text{H}-28]$ owing to the loss of a CO_2 group and a pseudo molecular ion peak $[\text{M}-\text{H}]^-$ at m/z 163, followed by a peak at m/z 145 equivalent to $[\text{M}-\text{H}-18]$ owing to the loss of H_2O . As a result, this substance was identified as *p*-Coumaric acid, which was present in *O. europaea* preparations [41]. Compounds (4) displayed fragments at m/z 447 $[\text{M}-\text{H}-162]$ and a pseudo molecular ion peak $[\text{M}-\text{H}]^-$ at m/z 609.- showing the release of fragments at m/z 179 and 301 and the hexose unit, which suggested that this chemical is quercetin-O-rutinoside (Rutin) [42]. Rutin can be found in olive leaf extract [43]. The compounds (5) displayed a pseudo molecular ion peak $[\text{M}-\text{H}]^-$ at m/z 539, another fragment at m/z 377 $[\text{M}-\text{H}-162]$ -signalling glucosyl loss, and at m/z 307 $[\text{M}-\text{H}-\text{glucoside}-\text{C}_4\text{H}_6\text{O}]$. As a result, oleuropein was given to this molecule [44]. *O. europaea* has oleuropein as a major phenolic compound [43]. One excess hexose moiety was present in compounds (6), which displayed a pseudo molecular ion peak $[\text{M}-\text{H}]^-$ at m/z 701 that was 162 Da higher than oleuropein. The lack of a hexose moiety-producing daughter ion in comparison to oleuropein and its fragments at (m/z 539, 377, 307, 275) resulted in a distinctive peak. Thus, oleuropein glucoside was ascribed to this molecule [45]. Olive leaf extract is a valuable source of phenolic chemicals, which are mostly in charge of the extracts' biological activities, which include antibacterial, anticancer, and antioxidant capabilities [46, 43].

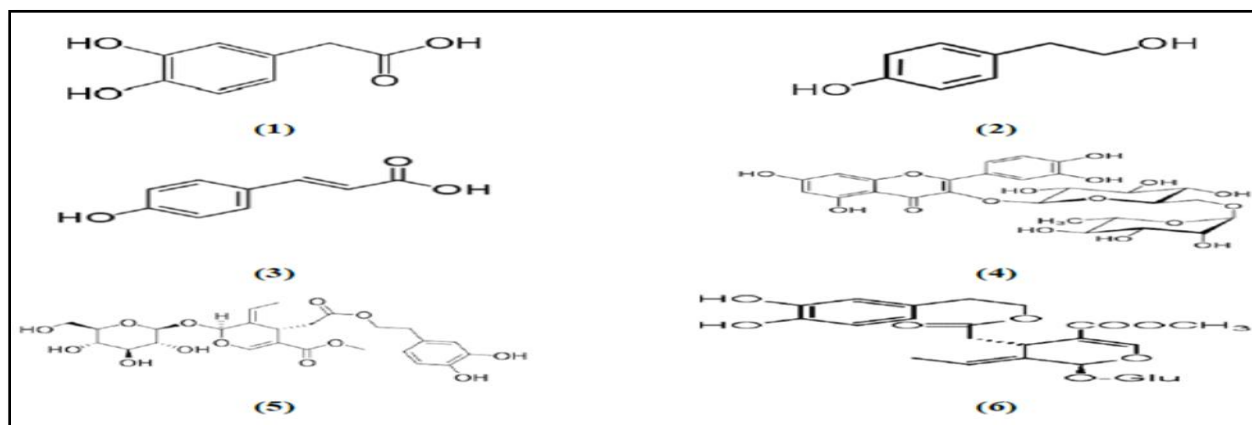


Fig. 6: Analysis of *O. europaea* methanolic extract in the Negative-ion Mode.

Analysis of S. aromaticum methanolic extract in the Negative-ion Mode:

Compounds (1) showed a pseudo molecular ion peak $[\text{M}-\text{H}]^-$ at m/z 191 and 173 $[\text{quinic acid}-\text{H}_2\text{O}]^-$. Therefore, this compound was quinic acid and it was found in clove [47, 48]. Compound (2) produced fragment ion m/z 179 $[\text{M}-\text{H}]^-$ and m/z 135 $[\text{M}-\text{H}-\text{CO}_2]^-$. It was identified as caffeic acid as a phenolic compound from clove [49, 47]. Compounds (3) showed a pseudo molecular ion peak $[\text{M}-\text{H}]^-$ at m/z 169. Produced ions at m/z 125 $[\text{M}-\text{H}-\text{COO}]^-$ and 97 $[\text{M}-\text{H}-\text{CO}_2-\text{CO}]^-$. So, this compound was assigned as gallic acid [50, 51]. Compound (4) produced a pseudo molecular ion peak $[\text{M}-\text{H}]^-$ at m/z 301, m/z 284

$[M - OH - H]^-$, m/z 257 $[M - CO_2 - H]^-$, m/z 245 $[M - 2CO - H]^-$, m/z 229 $[M - CO_2 - CO - H]^-$, m/z 89 $[M - 151 - H_2O - CO_2]$, m/z 133 $[M - 151 - H_2O]^-$, m/z 89 $[M - 151 - H_2O - CO_2]$. it was tentatively identified as ellagic acid ^[52, 47].

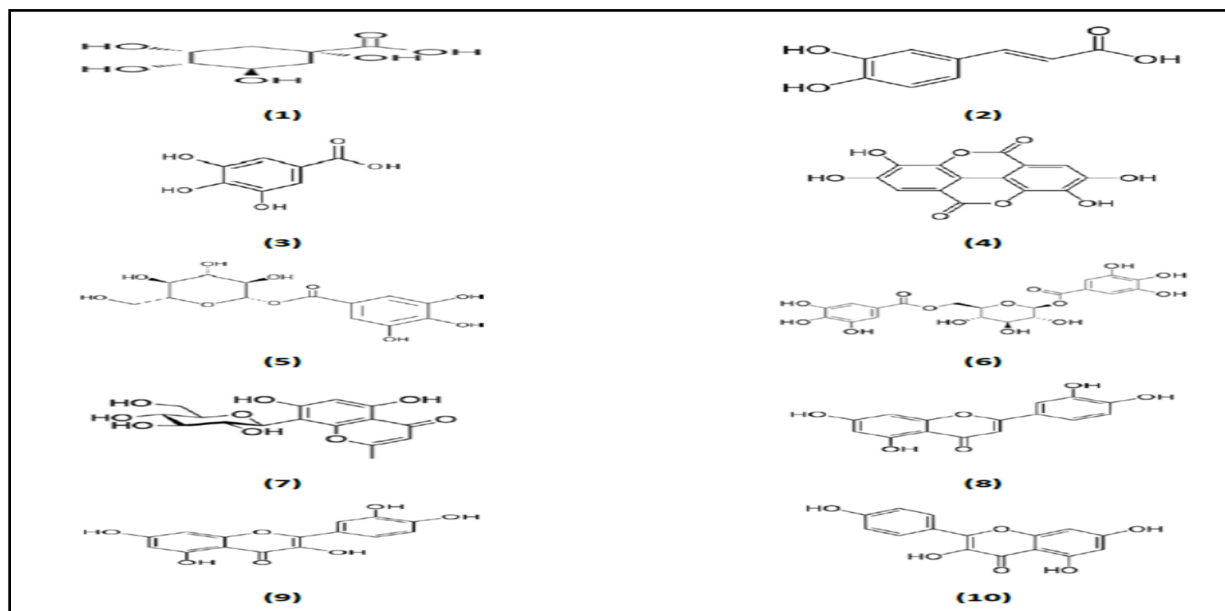


Fig. 7: Analysis of *S. aromaticum* methanolic extract in the Negative-ion Mode.

Compounds (5) showed a pseudo molecular ion peak $[M-H]^-$ at m/z 331 and the loss of C_2H_2O , CH_2O , and CH_2O from the ion at m/z 313 led to the product ions at m/z 271, 241 and 211 originated from cross-ring fragmentation of a glucose molecule. While fragment ion at m/z 125 was formed by loss of CO_2 from the galloyl group. So, this compound was assigned as galloyl glucose ^[53]. This compound would be extracted from *S. aromaticum* by supercritical CO_2 extraction ^[50].

Compounds (6) showed a pseudo molecular ion peak $[M-H]^-$ at m/z 483 and the loss of galloyl $[M - H - 152]$ – to give galloyl glucose (m/z 331) and the fragment ions m/z 313, 271, 211, and 169; it was tentatively identified as digalloyl glucose and found in clove ^[52, 47]. Compound (7) showed a pseudo molecular ion peak $[M-H]^-$ at m/z 353, m/z 233 $[M - 120 - H]^-$ due to loss of $C_4H_8O_4$, m/z 205 $[M - 148 - H - CO]^-$, m/z 191 $[M - 162]^-$ by loss glucosyl and $[m/z$ 191 – $CO]^-$; it was tentatively identified as isobiflorin that extract from flower buds ^[54, 55]. Compound (8) produced fragment ion m/z 285 $[M - H]^-$, m/z 241 $[M - 44 - H]^-$, m/z 151 $[M - 134 - H]^-$, m/z 133 $[M - 152 - H]^-$, m/z 107 $[M - 178 - H]^-$. it was tentatively identified as Luteolin ^[56]. Compound (9) produced fragment ion m/z 301 $[M - H]^-$, m/z 284 $[M - H - H_2O]^-$, m/z 273 $[M - CO - H]^-$, m/z 179 $[M - 122 - H]^-$, m/z 135 $[M - 122 - CO_2 - H]^-$, m/z 107 $[M - 122 - Da - CO_2 - CO - H]^-$, m/z 93 [phenol moiety] $^-$ and m/z 65. It was identified as quercetin ^[57]. Compound (10) produced fragment ion m/z 285 $[M - H]^-$, m/z 268 $[M - OH - H]^-$, m/z 257 $[M - CO - H]^-$, m/z 243 $[M - C_2H_2O - H]^-$, m/z 227 $[m/z$ 257 – $CHOH - H]^-$, m/z 211 $[M - 74 - H]^-$, m/z 117 $[M - 168 - H]^-$, m/z 93 [phenol moiety] $^-$, m/z 83, and m/z 65. It was identified as kaempferol ^[57]. *S. aromaticum* is rich in flavonoids that give it its activity ^[58]. These biological activities of *S. aromaticum*

are related to its compounds which include antioxidant, antibacterial, anti-inflammatory, and anticancer properties ^[58].

Conclusion

Our study compares *Syzygium aromaticum* and *Olea europaea* methanol extract and showed that have promising antioxidant activity as a scavenging DPPH free radical. This may be due to the high total phenolic and flavonoid content of both plants. Furthermore, plant extracts can reduce HBV replication when compared to control untreated cells. LC/MS/MS analysis for methanol extract of both plants exhibited the presence of phenolic and flavonoid compounds that may be the main reason for the biological activity of plants.

Authorship contribution statement

Heba Abdel-Hady: Conceptualization, Visualization, Validation, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. Eman Abdallah Morsi: Characterization of compounds conducted on previous research, Visualization, Validation, Data curation, Writing - original draft, Writing - review & editing. Hend Okasha Ahmed: Investigation of the antiviral assay, Data curation, Writing - original draft, Writing – review & editing – statistical analysis. Eman Ahmed El-Wakil: Supervision, Validation, Investigation. Mohamed Abbas Shemis: Conceptualization, Visualization, Validation, Supervision, Review & editing. All authors read and approved the manuscript.

Declaration of Competing 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.

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