Journal of King Saud University – Science 32 (2020) 3273–3278

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Bioactivity of *Syzygium aromaticum* (L.) Merr. & L.M.Perry extracts as potential antimicrobial and anticancer agents

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ARTICLE INFO

Article history:
Received 2 March 2020
Revised 19 August 2020
Accepted 6 September 2020
Available online 12 September 2020

Keywords:
Syzygium aromaticum, Antibacterial
Minimum inhibitory concentration
Anti-cancer assay, GC–MS

ABSTRACT

Objectives: Cancer is the most common cause of death every year. Moreover, high incidence of bacterial resistance to the most frequently used antibiotics contributes a significant death and disability worldwide. Hence, formulation of novel antimicrobial and anticarcinogenic agents is required.
Methods: In the current study, the antibacterial efficiency of clove extracts (acetonic, dichloromethane, ethanolic, and petroleum ether) against four pathogenic bacterial strains [*Escherichia coli, Salmonella typhi, Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA)] was estimated by disc diffusion method. Furthermore, the anticancer potency of *S. aromaticum* extracts against HCT human colon carcinoma was appraised using MTT assay. The phytochemical ingredients of the most effective extract were determined using gas chromatography–mass spectrometry (GC–MS) analysis.
Results and conclusion: The dichloromethane extract presented the highest antibacterial potency against the pathogenic isolates recording minimum inhibitory concentration (MIC) of 1 mg/disc against MRSA strain and 0.5 mg/disc against both of *E. coli* and *S. typhi* strains. Furthermore, the minimum bactericidal concentration (MBC) of clove dichloromethane extract was recoded at 2 mg/disc against MRSA stain, and 1 mg/disc against *E. coli* and *S. typhi* strains. The dichloromethane extract of clove showed the lowest cytotoxic activity against HCT cell line whereas, the ethanolic extract exerted the highest efficacy with relative IC_{50} of 6.71 and 2.53 μg/ml respectively. GC–MS analysis revealed that the clove dichloromethane extract was comprised of eugenol (50.65%) and eugenyl acetate (12.54%) as major active components. In conclusion, clove extracts could be utilized as potential antibacterial and anticarcinogenic agents.

1. Introduction:

Multidrug resistant bacteria causing infectious diseases were responsible for significant mortality, particularly in developing countries (Nii-Trebi, 2017). Bacterial resistance in the U.S contributed to over 2 million disorders and 23,000 deaths per year according to statistics of the CDC center (Okwu et al., 2019). High prevalence of methicillin-resistant *Staphylococcus aureus* strains (MRSA) was recorded in both health-care and community environments (David and Daum, 2010). Epidemiological studies reported the variation in MRSA infection rates among different countries, for example: 33%–43% in Nigeria (Okwu et al., 2014); 25%–50% in India (Arunkumar et al., 2017). *Staphylococcus aureus* was responsible for approximately 1064 hospitalizations and 241,188 disorders in the United States, according to statistics of CDC center (Scallan et al., 2011). Ingestion of the *S. aureus* enterotoxin followed by a 6–10 hrs incubation period resulted in food poisoning episodes as vomiting, diarrhea, nausea, abdominal cramps, dizziness, and general weakness (Ravensbergen et al., 2017). In contrast, *Salmonella* spp. belonging to Enterobacteriaceae group were reported to be majorly accountable for foodborne outbreaks in the U.S (Control and Prevention, 2012). Reportedly, a high incidence of salmonellosis outbreaks was recorded by a total of 94,625 infections in Europe for the year 2015 (Authority, Prevention et al., 2018). Furthermore, the pathogenic strains of

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2020.09.009

E. coli were responsible for significant morbidity and mortality worldwide, for example, *E. coli* strain O157:H7 was recorded to cause 63,000 outbreaks, 2100 hospitalizations, and 20 deaths every year (Croxen et al., 2013). Cancer is considered as one of the outstanding causes of death worldwide that is characterized by uncontrolled multiplication of normal human cells (Mubeen and Kini, 2012). The main conventional therapies commonly used for cancer management were chemotherapy, radiotherapy and surgery (Ju et al., 2018). The conventional therapies target both cancer and healthy cells nonselectively so that it is necessary to elaborate new anticancer drugs using medicinal plants as a substitution of these conventional therapies avoiding incidence of cancer cell resistance and reducing the toxicity resulting from using single drug (Aumeeruddy and Mahomoodally 2019). Furthermore, medicinal plants were reported as a prospective source of antimicrobial bioactive agents because of their constituents as tannins, flavonoids, phenolic compounds and alkaloids (Djeussi et al., 2013).

The genus *Syzygium* has been reported as the largest genus of flowering plants that was comprised of approximately 1200–1800 species (Mahomoodally et al., 2020). *Syzygium aromaticum* belonging to Myrtaceae family was of medical importance due to its antimicrobial, antioxidant, anti-cancer, anti-inflammatory and anti-diabetic properties (Lau and Rukayadi 2015). The essential oil of clove suppressed the growth of *S. aureus* and *E. coli* strains with inhibition zone diameters of 16 and 6 mm respectively (Saikumari et al., 2016). *Syzygium aromaticum* extracts were also reported to prohibit the proliferation of HeLa (cervical cancer), Te-13 (esophageal cancer), DU-145 (prostate cancer) and MCF-7 (breast cancer) cell lines significantly (Dwivedi et al., 2011).

Excessive incidence of multidrug resistant bacterial strains necessitates the formulation of novel antimicrobial therapeutic agents. Furthermore, high death rate globally due to cancer and the side effects of commonly used chemotherapeutic agents necessitate the formulation of new anti-carcinogenic agents. Thus, the current study was conducted to detect the antibacterial efficiency of clove extracts against pathogenic bacterial strains. Moreover, the anticancer potency of *S. aromaticum* extracts was detected using the MTT assay.

2. Material and methods

2.1. Plant extracts preparation

Clove buds were collected from the local market in Riyadh, Saudi Arabia, identified by herbarium of the Botany Department, College of Science, King Saud University and deposited with voucher number of (KSU-14682). All active phytochemicals were extracted using four different organic solvents (petroleum ether, dichloromethane, acetone and ethanol) with different polarities of 0.1, 3.1, 5.1, and 5.2, respectively. The buds were washed with 0.5% sodium hypochlorite solution (NaOCl) for disinfection, washed with sterilized distilled water and dried. Grinding of the buds was performed using a mechanical mortar to attain a homogenized powder. 50 g of clove powder was drenched in 200 ml of different solvents and was incubated for 48 h at 25 °C over a magnetic stirrer. Centrifugation of different solvent extracts was carried out at a speed of 9000 rpm for 10 min, filtered using a Whatman filter paper to obtain clear filtrates and discard of the plant remainders. Eventually, the solvents were evaporated using a rotatory evaporator in order to concentrate the extracts. The extracts were preserved at 4 °C till use and the extraction yields were calculated as shown in the subsequent formula: Percentage of the extract yield = (R/S) × 100 where R referred to the extract residue weight and S referred to the weight of the raw sample.

2.2. Antimicrobial efficiency of the clove extracts

2.2.1. Preparation of bacterial inocula

Bacterial strains subjected to the study were (*S. aureus*, *Staphylococcus*-MRSA, *S. typhi* and *E. coli*) and were attained from culture collection of Botany and Microbiology Department, College of Science, King Saud University, Saudi Arabia. The microbial strains were subcultured in Mueller-Hilton agar slants and kept for 48 h at 35 °C to obtain fresh inoculums. The microbial growth was gathered using 5 ml of sterile saline water. Finally, adjustment of absorbance was done at 580 nm using a spectrophotometer to obtain a microbial count of 10^7/ml.

2.2.2. Antibacterial assay

Disk diffusion method was done to detect the antibacterial efficacy of *S. aromaticum* extracts against some Gram-positive strains as (*S. aureus* and MRSA) and other Gram-negative isolates as (*E. coli* and *S. typhi*). About fifteen ml of Mueller-Hinton agar medium was poured into sterile Petri dishes as a basal layer followed by the addition of 10 ml seeded medium that was previously inoculated with bacterial suspension (1 ml of 10^7CFU/100 ml of medium) to obtain 10^5 CFU for each ml of the medium (Yassin et al., 2020a). 8 mm sterile paper disks were impregnated with clove extracts at a concentration of 10 mg/ml and put over the seeded plates. Chloramphenicol as antibacterial agent (30 μg/discs) was utilized as positive control against different bacterial strains according to CLSI (CLSI, 2000). The seeded plates were kept in a refrigerator at 4 °C for 2 h to permit the diffusion of *S. aromaticum* extract through the medium. Incubation of plates was achieved at 35 °C for 48 h and the diameters of suppressive zones were calculated using a Vernier caliper.

2.3. Detection of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) is specified as the least concentration of clove extract showing antibacterial potency. MIC was detected for clove dichloromethane extract as it demonstrated the highest antibacterial efficacy. Detection of MIC was achieved by disk diffusion method as mentioned above, in which 8 mm sterile filter paper disks were impregnated with clove extracts (0.25, 0.5, 1.0, 2.0, 4.0, 8.0 mg/disc). Plates were refrigerated at 4 °C for 2 h to permit the diffusion of extracts through the medium. Eventually, the plates were put in the incubator at 35 °C for 48 h and the diameters of suppressive zones were imputed using a Vernier caliper.

2.4. Determination of minimum microbicidal concentration

Minimum bactericidal concentration (MBC) was specified as the least clove concentration resulting in removal of bacterial growth. Inocula were collected from the suppressive zones of MIC concentration and the two other sequential concentrations and plated onto Mueller-Hinton agar plates. The plates were finally put in the incubator at 35 °C for 48 h.

2.5. In vitro anticancer assay

Human colon carcinoma cells (HCT) were supplemented from Zoology dept., Faculty of Science, King Saud University. Anticancer efficacy of clove (acetonic, dichloromethane, ethanolic and petroleum ether) extracts was done using MTT assay. Anti-cancer potency of *S. aromaticum* extracts against HCT cells was performed as described by (Yassin et al., 2020b).

2.6. GC–MS analysis of clove extracts

Chemical investigation of clove dichloromethane extract was performed as it exhibited the highest antibacterial potency. The chemical analysis was determined using the GC–MS (Trace 1300/Tsq 8000 Triple Quadrupole) with a TG 5MS column (30 m × 0.25 mm, 0.25 μm film thickness). Conditions of GC/MS analysis were optimized as: The carrier gas was Helium with a flow rate of 1 ml/min, 250 °C was the temperature of injector and detector, 1:50 was the split ratio, oven temperature was 280 °C and it was programmed to be increased at a rate of 5 °C/min, and sample injection volume was 1.0 μl. Conditions of mass spectrometry were optimized as follows: 70 eV was the ionization potential; mass range from *m*/*z*, 40–400 amu; 2000 V was the electron multiplier energy. The phytochemicals of clove dichloromethane extract were detected by comparing the GC–MS results with reference spectral mass data and retention times of the NIST database (Yassin et al., 2020c).

3. Results

3.1. Extracts yield

The highest extraction yield (7.21%) was attained using dichloromethane as a solvent followed by ethanol (5.21%), petroleum ether (4.21%), and acetone (2.96%).

3.2. Antibacterial assay

All *S. aromaticum* extracts exhibited antimicrobial potency against the pathogenic bacterial strains with different sensitivity patterns as demonstrated in Fig. 1. The dichloromethane extract of clove showed the highest antimicrobial efficacy against the concerned bacterial isolates (*S. aureus*, MRSA, *S. typhi,* and *E. coli*) with inhibition zone diameters of 18.20, 17.25, 21.15, and 24.2 mm, respectively as listed in Table 1. The Gram-negative strains (*E. coli* and *S. typhi*) were more susceptible to different clove extracts than the Gram-positive strains (*S. aureus*, MRSA).

3.3. Detection of minimum inhibitory concentration

Minimum inhibitory concentration (MIC) for the dichloromethane extract of clove was detected as that extract revealed the highest efficiency against the concerned pathogenic bacterial strains, for example, MIC for *S. aureus* and MRSA was 1 mg/disc and exhibited suppressive zones of 9.63 and 8.67 mm, respectively. In contrast, MIC of dichloromethane extract against Gram-negative strains (*E. coli* and *S. typhi*) was 0.5 mg/disc with corresponding suppressive zones of 9.23 and 10.64 mm, respectively as listed in Table 2. MIC data confirmed that the Gram-negative isolates were more sensitive to clove extracts than the Gram-positive ones.

3.4. Detection of minimum bactericidal concentration

The microbicidal activity of the effective extract was evaluated to detect the lowest concentration presenting cidal activity. Minimum bactericidal concentration (MBC) of clove dichloromethane extract was 2 mg/disc against *S. aureus* and *MRSA* strains, whereas it was 1 mg/disc against *E. coli* and *S. typhi* isolates.

3.5. Cytotoxicity assay

The ethanolic extract of clove exerted the maximum anticancer potency against HCT cell line with IC_{50} of 2.53 μg/ml. In contrast, dichloromethane extract of clove demonstrated the least efficacy against HCT cells with relative IC_{50} of 6.71 μg/ml. Moreover, the petroleum ether and acetonic clove extracts showed a moderate cytotoxic efficiency against HCT cells recording IC_{50} of 6.48 and 2.91 μg/ml respectively as demonstrated in Fig. 2.

3.6. GC–MS analysis of clove extracts

Chemical investigation of clove dichloromethane extract was performed as it exhibited the highest antibacterial potency. The dominant constituent of clove dichloromethane extract was found to be eugenol (50.65%), followed by eugenyl acetate (12.54%), allopurinol, dimethyl (10.98%), and isoquinoline,1-[3-methoxy-5-hydroxybenzyl]-1,2,3,4,5,8-hexahydro-6-methoxy (9.86%) (Table 3).

4. Discussion

High incidence of multidrug resistant pathogens worldwide contributes to a significant mortality rate every year (Chan et al., 2014). The current study demonstrated that the *S. aromaticum* (acetonic, dichloromethane, ethanolic, petroleum ether) extracts possessed a potential anti-MRSA activity with suppressive zones of 14.85, 17.255, 16.55, and 16.65 mm, respectively. These findings were in accordance with those of Mandal et al. (2011) who confirmed the antibacterial efficacy of *S. aromaticum* extract against MRSA isolates with inhibitory zones ranging from 19 to 23 mm. Pandey and Singh (2011) stated that clove methanolic extract exerted antibacterial potency against *S. aureus* and *E. coli* strains recording MIC values of 0.385 and 2.31 mg/ml respectively.

Bacterial food spoilage constitutes the most common cause of food poisoning especially by Gram-negative (*E. coli* and *S. typhi*) and Gram-positive (*S. aureus* and MRSA) bacterial strains (Solomakos et al., 2008). The potent antibacterial activity of

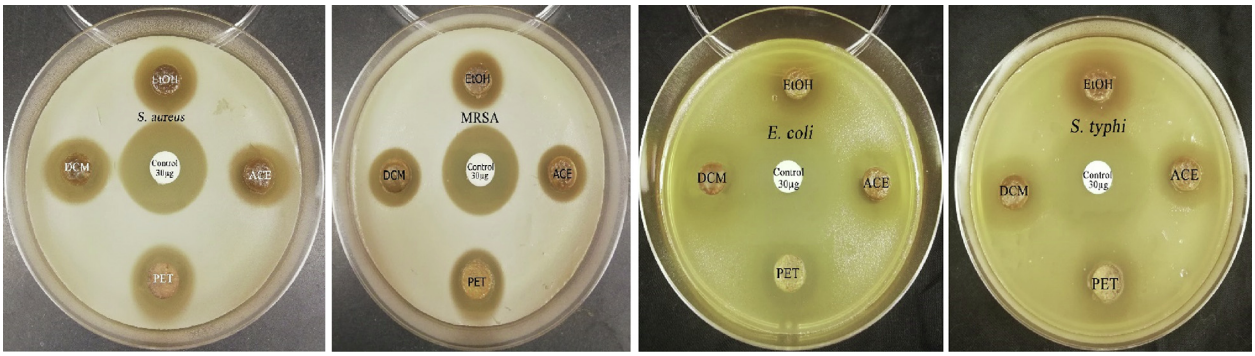

Fig. 1. Antibacterial activity of clove extracts (10 mg/disc) against different bacterial strains. **EtOH:** ethanolic extract, **ACE**: acetonic extract, **PET:** petroleum ether extract, **DCM:** dichloromethane extract. **Control:** Chloramphenicol antibacterial agent (30 μg/disc).

Table 1
Antimicrobial activity of clove extracts against different pathogenic bacterial strains.

Clove extracts (10 mg/disc)	Inhibition zone diameter (mm) of bacterial strains			
	Gram + ve bacteria		Gram −ve bacteria	
	S. aureus	MRSA	*S. Typhi*	*E. coli*
Acetonic extract	16.05 ± 0.09	14.85 ± 0.26	19.60 ± 0.28	17.80 ± 0.35
Dichloromethane extract	18.20 ± 0.63	17.25 ± 0.09	21.15 ± 0.43	24.20 ± 0.06
Ethanolic extract	18.10 ± 0.34	16.55 ± 0.12	19.45 ± 0.49	19.12 ± 0.23
Petroleum ether extract	15.90 ± 0.46	16.65 ± 0.14	18.95 ± 0.25	18.55 ± 0.14
Chloramphenicol (30 μg/disk)	26.55 ± 0.16	25.30 ± 0.29	28.05 ± 0.89	31.80 ± 0.34

Table 2
MIC's of clove dichloromethane extract against pathogenic bacterial strains.

Concentration of clove extract (mg/disc)	Inhibition zone diameter (mm) of bacterial strains			
	Gram + ve bacteria		Gram − ve bacteria	
	S. aureus	MRSA	*S. Typhi*	*E. coli*
0.25	0.00 ± 0.0	0.00 ± 0.00	0.00 ± 0.0	0.00 ± 0.0
0.50	0.00 ± 0.0	0.00 ± 0.00	9.23 ± 0.34	10.64 ± 0.12
1.00	9.63 ± 0.18	8.67 ± 0.26	12.34 ± 0.41	13.65 ± 0.56
2.00	12.35 ± 0.25	11.18 ± 0.31	15.18 ± 0.12	16.23 ± 0.17
4.00	14.23 ± 0.19	13.21 ± 0.47	17.33 ± 0.24	19.23 ± 0.32
8.00	16.45 ± 0.38	15.29 ± 0.08	19.14 ± 0.15	21.18 ± 0.29

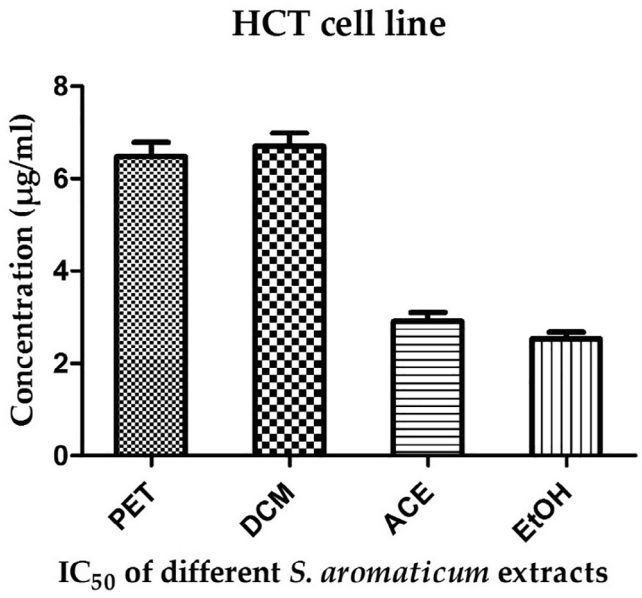

Fig. 2. Anticancer activity of clove extracts against HCT human colon carcinoma cell line. **EtOH:** ethanolic extract, **ACE**: acetonic extract, **PET:** petroleum ether extract, **DCM:** dichloromethane extract.

S. aromaticum extracts against food spoilage bacteria supports the utilizing of these extracts in the formulation of natural food preservatives avoiding antimicrobial resistance to chemical preservatives and the deleterious impact of these preservatives to the human health (Bialonska et al., 2010).

The clove dichloromethane extract exerted the highest antimicrobial efficacy against the pathogenic bacterial strains, which may be assigned to the high percentage of eugenol and eugenyl acetate compounds of 50.65 and 12.54% respectively as presented in GC–MS results. GC–MS results were in accordance with that of Tischer et al. (2019), who reported the effectiveness of eugenyl acetate compound against *S. aureus* and *E. coli* strains recording inhibitory zones of 33 and 37.55 mm, respectively. Moreover, the efficiency of eugenol as antimicrobial agent was confirmed by Qiu et al. (2010) who reported the antibacterial efficacy of eugenol against *S. aureus* with MIC value of 256 μg/ml. Nazzaro et al. (2013) attributed the efficacy of eugenol as antibacterial agent to the free hydroxyl group in eugenol molecule. The free hydroxyl groups of eugenol block the enzymatic activity of microbial cells through binding to the bacterial proteins. The antimicrobial potency of *S. aromaticum* extracts may be ascribed to its eugenol content which affects the permeability of cytoplasmic membrane negatively resulting in disturbance of ions, ATP transport and initiation of cell death (Devi et al., 2013). The high phenolic content of clove oil causes microbicidal action against different bacterial pathogens through disruption of the active transport, electron flow and proton motive force resulting in coagulation of bacterial cell contents (Elhoussine et al., 2010). The mode of eugenol action against *S. aureus* strains was confirmed by Das et al. (2016) who explained that eugenol compound induces bacterial cell toxicity through production of reactive oxygen species (ROS) resulting in disturbance of

Table 3
Phytochemical components of clove dichloromethane extract.

Compounds	Chemical formula	Mol. weight	RT	% of Total
3-[(Z)2-Phenylethenyl]cholestan-2-one	$C_{35}H_{52}O$	488.80	9.271	4.17
2H-Pyrazino[1,2-b]isoquinoline-1,3(4H,6H)-dione,2-(4-fluorophenyl)-11,11a-dihydro-8,9-dimethoxy	$C_{20}H_{19}FN_2O_4$	370.40	9.668	4.60
Eugenol	$C_{10}H_{12}O_2$	164.20	10.773	50.65
Acetyl eugenol	$C_{12}H_{14}O_3$	206.24	11.310	12.54
Allopurinol, dimethyl	$C_7H_8N_4O$	164.16	11.531	10.98
Allocryptopine	$C_{21}H_{23}NO_5$	369.41	11.676	7.19
Isoquinoline,1-[3-methoxy-5-hydroxybenzyl]-1,2,3,4,5,8-hexahydro-6-methoxy	$C_{18}H_{23}NO_3$	301.40	11.787	9.86

cell membrane permeability, growth inhibition of microbial cells, DNA damage and finally bacterial cell death.

Other researchers attributed the antibacterial efficacy of clove extracts to the destructive action of clove oil that acts on different bacterial cell components due to its hydrophobic feature resulting in partitioning lipids of mitochondria and cell membrane (Xu et al., 2016). Also, clove oil targets the proteins of the electrons transport system in bacterial cell membrane causing inhibition of microbial growth (Wongsawan et al., 2020).

On the other hand, medicinal plant extracts provide a powerful tool in controlling malignant nature of cancer cells avoiding the side effects of chemotherapeutic agents (Mbaveng et al., 2011). *Syzygium aromaticum* extracts are rich in tannins which were reported to possess potential anticancer activity (Batiha et al., 2020). The cytotoxic efficiency of *S. aromaticum* extracts was confirmed by Kumar et al. (2014), who demonstrated the cytotoxic impact of clove oil to the MCF-7 breast cancer cell line. The potent anticancer activity of *S. aromaticum* extracts may be attributed to their phytoactive constituents of eugenol which was proved to perform antiproliferative action against malignant melanoma cells, oral squamous carcinoma and androgen-insensitive prostate cancer cell lines (Carrasco et al., 2008).

5. Conclusion

The current study confirmed the potent antimicrobial potency of *S. aromaticum* extracts against different pathogenic bacterial strains. Clove dichloromethane extract exhibited the highest antimicrobial activity against bacterial strains. The prospective antimicrobial efficacy of clove extracts authenticated the ability of utilizing these extracts in the production of novel antimicrobial agents. Results on the antibacterial activity of clove extracts against food poisoning bacterial strains emphasize the application of these extracts in formulation of natural food preservatives. Strong anticancer activity of clove extracts against HCT cancer cell line supports the ability of utilizing these extracts in formulation of natural anticarcinogenic agents.

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.

Acknowledgment

Author M.A.El-Sheikh extends his appreciation to the Researchers Supporting Project Number (RSP-2020/182), King Saud University, Riyadh, Saudi Arabia.

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