Anti-Tumor, Antioxidant and Antimicrobial and the Phenolic Constituents of Clove Flower Buds (Syzygium aromaticum)

Abd El Azim MHM, El-Mesallamy AMD, El-Gerby M, and Awad A

1Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt
2Medicinal and Aromatic Plants Department, Desert Research Center, Cairo, Egypt
3Department of Chemistry, Faculty of Science, Jazan University, Jizan 2097, Saudi Arabia

Abstract

Eleven phenolic compounds were identified from the methanolic extract of Cloves flowers buds (Syzygium aromaticum L.) by chromatographic methods. Efficiency of the methanolic extract of licorice roots as anticancer agent for breast, colon and liver was tested. The results showed that the IC₅₀ were (31 μg/mL for anti-colon cancer, 29.7 μg/mL for anti-breast cancer and 18.7 μg/mL for anti-hepatic cancer). This extract showed strong antioxidant activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH.) as compared with vitamin C. Antimicrobial activity of the methanol extract of licorice roots was studied against three bacterial and four fungal strains at concentration 0.1 ml and 0.3 ml (10 mg/1 ml). The extract showed strong inhibitory effect for most species at concentration 0.3 ml (10 mg/ml).

Keywords: Phenolic; Anti-tumor; Antioxidant; Antimicrobial

Introduction

Main objectives of this study were to evaluate antitumor, antioxidant, antimicrobial and phenolic constituents of clove flower buds. Cloves (Syzygium aromaticum L.) are the aromatic dried flower buds of a tree in the family Myrtaceae. The genus comprises about 1100 species, 62 species are found in Australia and are generally known as lilipillies, brush cherries [1].

Syzygium species have been reported to possess antibacterial [2], and anti-inflammatory activity [3]. It was reported that the buds of Syzygium aromaticum were used in folk medicine as diuretic, odontalgic, stomachic, tonicardiac, aromatic condiment properties and condiment with carminative and stimulant activity [4]. Clove exerted immunomodulatory/anti-inflammatory effects by inhibiting LPS action. A possible mechanism of action probably involved the suppression of the nuclear factor-kb pathway by eugenol, since it was the major compound found in clove extract [7]. Eugenol is the active component of clove Syzygium aromaticum and it is also present in number of other aromatic plants like basil, cinnamon and bay leaves [8].

Materials and Methods

Plant material

Clove flowers buds (Syzygium aromaticum L.) 500 gm were provided from Lotus Company (Sekem Group, Egypt) in June 2010. The taxonomic identification of plant material was confirmed by Botany Department, Faculty of Science, Zagazig University (Egypt).

Ultra-violet spectrophotometric analysis

Chromatographically, pure materials dissolved in analytically pure methanol were subjected to UV spectrophotometric investigation in 1 ml capacity quartz cells Zeiss spectrometer PMQ-II. In case of flavonoids, AlCl₃, AlCl₃/HCl, fused NaOAc/H₃BO₃, and NaOMe reagents were separately added to methanolic solution of the investigated material and UV measurements were then carried out [9].

Nuclear magnetic resonance spectroscopic analysis

Jeol ECA 500 MHz NMR Spectrometer at 500 MHz, (Institute Fur Chemie, Humboldt Universität zu Berlin, Germany). 1H chemical shifts (δ) were measured in ppm, relative to TMS and 13C NMR chemical shifts to DMSO-d₆ and converted to TMS scale by adding 39.5. Typical conditions: spectral width=8 KHz for 1H and 30 KHz for 13C, 64 K data points and a flip angle of 45°C.

Mass spectrometric analysis

The isolated pure compounds were subjected, in most cases to Fast Atom Bombardment (positive and negative) Mass Spectroscopic Analysis (FAB-MS) on MM 7070 E spectrometer (VG analytical). Some other compounds were subjected to electron spray ionization mass spectroscopic analysis (ESI-MS) a Varian Mat1 12-ET Spectrometer. All measurements were carried out at Institute Fur Chemie, Humboldt Universität zu Berlin, Germany [10].

Extraction and isolation

500 gm from dried clove buds exhaustively extracted under reflux over a water bath with 5 liters of a methanol/bidistilled water (3:1) mixture for 3 hours. The solvent was removed under reduced pressure at about 45°C. The residual finally yielded 30 gm of a sticky dark brown material.

Fractionation of the extract, (30 gm dissolved in 100 ml aqueous
methanol 3:1) over Sephadex LH-20 (200 gm) column (150 X 4.5 cm) and elution with methanol/bidistilled water mixtures of decreasing polarities for gradient elution led to the desorption of sex individual fractions (I-VI) which were dried, individually, in vacuum, and then subjected to rechromatography for several times to obtain a pure phenolic compounds. The structure of these compounds was confirmed by comparison of their physical and spectral data.

**SRB assay of cytotoxic activity**

Human tumor cell lines were obtained frozen in liquid nitrogen (-180°C) from the American Type Culture Collection. The tumor cell lines were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. Measurement of potential cytotoxicity activity of the methanolic extract of licorice roots against the liver carcinoma cell line (HepG2), colon carcinoma cell line (HTC116) and breast carcinoma cell line (MCF7) was tested by SRB (Sulphorhodamine-B) assay using the method of Skehan et al. [5]. This experiment was conducted in the National Cancer Institute, Cairo, Egypt.

**Di phenyl picryl hydrazide assay**

The free radical scavenging effect of plant extracts was assessed by the decolouration solution of DPPH radical according to Letelier et al. [2], in Faculty of Agriculture Research Park–Cairo University (FARP). This assay was realized essentially by the method described by Joyce et al. [6], and modified by [11-13].

**Antimicrobial activities of the methanolic extract**

Strains were obtained from the bacteria stock present at the Research Laboratory of bacteriology, Faculty of Science, Zagazig University. Gram- positive and Gram-negative bacteria species tested were *E. coli* (KQ103), *Staphylococcus aureus* (LC405) and *Salmonella typhi* (RS57) and fungi species (Laboratory collection strains) were *Fusarium oxysporum*, *Aspergillus niger*, *Penicillium* sp. and *Trichoderma* sp.

**Antibacterial activity**

*In vitro* antimicrobial assay of the methanolic extract was carried out according to pour plate technique at two concentrations 0.1 ml and 0.3 ml (10 mg/ml). Culturing and incubated of different bacteria species were carried out at 37 °C for 24 hours. After the elapse of incubation periods, the diameter of inhibition zones was measured [14].

**Antifungal activity**

Czapek Dox media used for cultivation of fungal species. The medium was seeded with different fungal species. After solidification of media on plates, make pores in agar with cup-borer (15 mm) diameter. Two concentrations 0.1 ml and 0.3 ml (10 mg/ml) of the methanolic extract were transferred into the well. Dimethyl Foramide (DMF) was used only as a control. The plates were incubated for 7 days at 30°C. The inhibition zone formed by the extract against the particular test fungal strain determined as the antifungal activities of the extract [14].

**Results and Discussion**

Structure elucidation: Investigation of the phenolic compounds was done by fractionation of the extract, over polyamide column and elution with methanol/bidistilled water, and then subjected to re-chromatography for several times led to the separation of eleven pure phenolic compounds. The structure of these compounds was confirmed by comparison of their physical and spectral data with those of reported compounds:
H-2&H-6’) also Ms (m/z): 317.0 [M- - H, 100%], 287.1 [M- - C\textsubscript{7}H\textsubscript{5}O\textsubscript{3}, 5.2%], 271.1 [M- - C\textsubscript{3}H\textsubscript{5}O\textsubscript{3}, 9.2%], 242.1 [M- - C\textsubscript{4}H\textsubscript{2}O\textsubscript{2}, 5.1%], 203.1 [M- - C\textsubscript{4}H\textsubscript{2}O\textsubscript{2}, 4.2%, ion A] and 169.1 [ion A – H\textsubscript{2}O\textsubscript{2}, 4.9%].

9) Quercetin (C\textsubscript{15}H\textsubscript{10}O\textsubscript{6}, 45 mg), UV (MeOH): max=255, 268, 370 nm, (MeOH + NaOAc): max=254, 276, 375 nm, (NaOAc + H\textsubscript{2}O\textsubscript{2}): max=272, 388nm, (MeOH + AlCl\textsubscript{3}): max=270, 360, 440 nm and (AlCl\textsubscript{3} + HCl): max=258, 400 nm; \(^1\text{H}-\text{NMR (DMSO-d\textsubscript{6})}: \delta 6.19 (d, J=2.5, H-6), 6.4 (d, J=2.5, H-8), 7.64 (d, J=2.5, H-2’), 6.88 (d, J=8.5, H-5’), 7.53 (dd, J=2.5&8.5, H-6’). \(^1\text{C}-\text{NMR (DMSO-d\textsubscript{6})}: \delta 147.0(\text{C-2}), 135.8(\text{C-3}), 176.2(\text{C-4}), 160.5(\text{C-5}), 99.2(\text{C-6}), 164.0(\text{C-7}), 93.7(\text{C-8}), 156.4(\text{C-9}), 103.5(\text{C-10}), 122.2(\text{C-1‘}), 115.3(\text{C-2‘}), 145.1(\text{C-3‘}), 148.0(\text{C-4‘}), 115.6(\text{C-5‘}) and 120.2(\text{C-6‘}). \text{Ms (m/z)}: 285.1 [M- - H, 100%, ion A], 242.1 [M- - CO\textsubscript{2}, 5.3%, ion C], 135 [ion B–C\textsubscript{2}H\textsubscript{6}, 5.6%, ion C], 111 [ion C–2H\textsubscript{2}O, 12.5%].

10) Kaempherol (C\textsubscript{15}H\textsubscript{10}O\textsubscript{6}, 23 mg), Rf values (x100): 00 (H\textsubscript{2}O), 10 (HOAc-6), 85 (BAW). UV (MeOH): max=268, 369 nm, (MeOH + NaOAc): max=270, 310, 375 nm, (NaOAc + H\textsubscript{2}O\textsubscript{2}): max=270, 320, 372 nm, (MeOH + AlCl\textsubscript{3}): max=270, 305, 360, 430 nm and (AlCl\textsubscript{3} + HCl): max=278, 316, 413nm. \(^1\text{H}-\text{NMR (DMSO-d\textsubscript{6})}: \delta 6.4 (d, J=2.5, H-8), 6.8 (d, J=2.5, H-6), 8.14 (d, J=8, H-2’ and H-6’), 6.89 (d, J=8, H-3’ and H-5’). \(^1\text{C}-\text{NMR (DMSO-d\textsubscript{6})}: \delta 146.8(\text{C-2}), 135.4(\text{C-3}), 175.9(\text{C-4}), 161.0(\text{C-5}), 98.6(\text{C-6}), 162.4(\text{C-7}), 93.8(\text{C-8}), 156.4(\text{C-9}), 103.7(\text{C-10}), 121.9(\text{C-1‘}), 129.9(\text{C-2‘} and \text{C-6‘}), 115.8(\text{C-3‘} and \text{C-5‘}) and 159.5(\text{C-4‘}). \text{Ms (m/z)}: 285.1 [M- - H, 100%, ion A], 242.1 [M- - CO\textsubscript{2}, 5.3%, ion C], 203.1 [ion A–C\textsubscript{5}H\textsubscript{4}O\textsubscript{3}, 4.6%, ion B] and 169.1 [ion B–H\textsubscript{2}O\textsubscript{2}, 5.7%].

11) Apigenin (C\textsubscript{15}H\textsubscript{10}O\textsubscript{5}, 25 mg), Rf values (x100): 00 (H\textsubscript{2}O), 11 (HOAc-6), 88 (BAW). UV (MeOH): max=266, 335 nm, (MeOH + NaOAc): max=270, 300, 373 nm, (NaOAc + H\textsubscript{3}BO\textsubscript{3}): max=270, 300, 340 nm and (MeOH + AlCl\textsubscript{3}): max=277, 302, 348, 384 nm. \(^1\text{H}-\text{NMR (DMSO-d\textsubscript{6})}: \delta 6.18 (d, J=2.5Hz, H-6), 6.47(d, J=2.5Hz, H-8), 6.77(s, H-3), 6.92(d, J=8Hz, H-3’ and H-5’), 7.93(d, J=8Hz, H-2’ and H-6’). \(^1\text{C}-\text{NMR (DMSO-d\textsubscript{6})}: \delta 163.8(\text{C-2}), 102.8(\text{C-3}), 181.5(\text{C-4}), 161.3(\text{C-5}), 98.7(\text{C-6}), 163.6(\text{C-7}), 93.9(\text{C-8}), 157.2(\text{C-9}), 103.6(\text{C-10}), 121.1(\text{C-1‘}), 128.3(\text{C-2‘} and \text{C-6‘}), 115.8(\text{C-3‘} and \text{C-5‘}) and 161.4(\text{C-4‘}). \text{Ms (m/z)}: 270.2 [M+, 100%], 254 [M+ - O, 5.1%], 242 [M+ - CO\textsubscript{2}, 18.7%], 226[M+ - CO\textsubscript{2}, 4.8%], 213 [M+ - C\textsubscript{6}H\textsubscript{5}O\textsubscript{3}, 3.9%, ion A], 177 [ion A–C\textsubscript{6}H\textsubscript{5}O\textsubscript{3}, 5.4%, ion B], 167 [ion A–C\textsubscript{6}H\textsubscript{5}O\textsubscript{3}, 4.9%], 147 [ion B–HCHO, 52.5%, ion C], 135 [ion B–C\textsubscript{2}H\textsubscript{4}O\textsubscript{2}, 5.6%] and 111 [ion B–C\textsubscript{2}H\textsubscript{5}O\textsubscript{2}, 12.5%].

Anti-tumor activity

The potential cytotoxicity activity of the methanolic extract of clove buds was tested against three human cell lines [HEPG2 (liver carcinoma cell line), MCF7 (breast carcinoma cell line) and HCT116 (colon carcinoma cell line)] by SRB (Sulphorhodamine-B) assay. The results showed that the extract has strong activity against all cell lines tested. The antitumor activity of the tested extract is summarized in Figure 1. The IC\textsubscript{50} values (the concentrations of thymoquinone required to produce 50% inhibition of cell growth) of the extract against each cell line were 31 µg/ml, 29.7 µg/ml and 18.7 µg/ml for HCT116, MCF7 and HEPG2, respectively.

Antioxidant activity

The DPPH scavenging activity of the methanolic extract of clove buds is summarized in Figure 2. It was observed that the scavenging activity of the extract at all concentrations (25, 50 and 100 µl) is rather strong (42.27-80.07%) as compared with vitamin C. The remarkable antioxidant activity of methanolic extract of licorice roots might be due to the higher concentration of phenolic compounds. IC\textsubscript{50} value for the methanolic extract=44 µg/ml, while for vitamin C=17 µg/ml.

Antibacterial activity

The clove methanolic extract was showed high inhibitory effect against \(E.\) \textit{coli}, at the two concentration (0.1 and 0.3 ml), while the same extract showed high inhibitory effect against \(Salmonella\) \textit{typhi} at concentration 0.3 ml and no effect at concentration (0.1 ml). On the other hand, \(Staphylococcus\) \textit{aureus} showed the highest resistance to the extract at concentration 0.3 and 0.1ml as shown in Figure 3.
Antifungal activity

The results showed that, Clove methanolic extract has strong antifungal activity only against *Trichoderma* sp. at 0.3 ml concentration and moderated activities against *Fusarium*, *Aspergillus* sp. and *Penicillium* sp. While at 0.1 ml concentration most fungal strains showed no inhibitory activity as shown in Figure 4.

Conclusions

The overall results of this study indicate that the methanolic extract of clove buds represent a potential source of plant drugs. So, we can deduce that the methanolic extract of licorice appeared to be promising choice to be considered as antioxidant and anti-tumor medicines.

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