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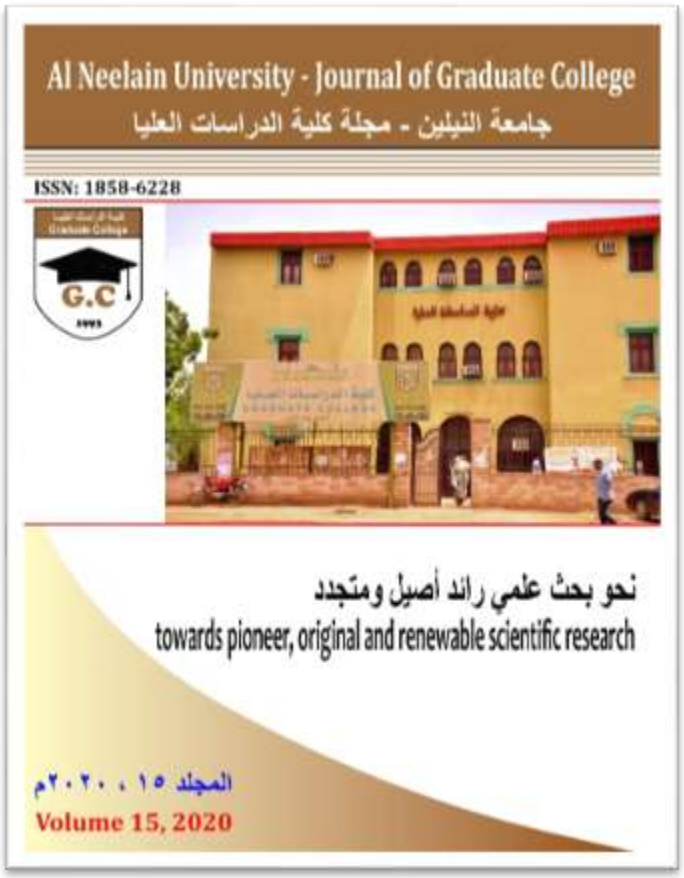

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GC-MS analysis, Anti-oxidant and Anti-inflammatory Properties of Lemon Essential Oil

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Abstract

Introduction: Medicinal plants play a vital role in drug discovery, and there is a worldwide interest in searching for new safe photochemical compound drugs. As the long-term uses of NSAIDs cause adverse side effects and damage human biological system such as liver, gastrointestinal tract etc.

Objective: The goals of this research is to analyze the chemical compounds of lemon peel essential oil using GC-MS and to investigate its in-vitro capabilities to inhibit protein denaturation as an anti-inflammatory and its antioxidant properties.

Methodology: Essential oil was prepared by using hydrodistillation technique. Ten milliliters of the essential oil of lemon were analyzed using Gas Chromatography-mass spectroscopy (GC/MS) at the Department of Biochemistry, the University of Science and Technology. The anti-inflammatory activity was carried out according to modification of the in-vitro protein denaturation bioassay methods of Jagtap et al (2011) and Shallangwa et al (2013). Ten milliliters of oil were mixed with DMSO and diluted with PBS (0.2M, pH7.4). The test mixtures (5 ml each) made up of 0.2 ml of egg albumin, 2.8 ml of PBS (pH7.4) and 2ml of varying concentrations of the extract. Test solution was incubated at 37oC in Corsair Heating and Catering limited incubator for 15 min. Denaturation was induced at 60oC in water-bath for 10min after cooling the turbidity was measured at 660nm. Diclofenac sodium was used as reference drug. Each experiment was done in triplicate and average was taken. The percentage of inhibition of denaturation was calculated. The antioxidant activity of the oil was assessed against the standard (Ascorbic acid), based on radical scavenging effect of the stable 1, 1-DPPH-free radical activity. The diluted working solutions of the test extracts were prepared in methanol, where oil was prepared in DMSO. A solution of 0.004% of DPPH was prepared in methanol and only 1 ml of it was mixed with 1 ml of sample solution or standard solution. These mixtures were kept in dark for 30 min. The optical density was measured at 517 nm and percentage of inhibition was calculated.

Results and discussion: Analysis of the essential oil by GC-MS has shown that lemon essential oil is rich with D-Limonene and Geraniol. Inhibition of protein albumin denaturation by essential oil was increased in a dose-dependent manner at concentration of 400 to 2000µg/ml (123% and 138.5%, respectively). However, essential oil exhibited moderate antioxidant activity; the maximum activity was determined at 1000µg/ml. (60.84+0.25%). IC_{50} value for essential oil was amounted to 912.74µg. The presence of these bioactive components (Limonene and Geraniol) may be behind the anti-inflammatory and antioxidant activities of lemon essential oil.

Conclusion and recommendation: The essential oil of lemon was capable of limiting the process of protein denaturation, and possess antioxidant activity; these activities are related to the major terpenoids and other lemon constituents detected by GC-MS. Therefore, we recommend further investigation for designing potent anti-inflammatory and antioxidant drugs.

Keywords: Lemon; antioxidant; anti-inflammatory, citrus

Introduction

A medicinal plant is a plant that has similar properties as conventional pharmaceutical drugs. Humans have used them throughout history to either cure or lessen symptoms from an illness. A pharmaceutical drug is a drug that is produced in a laboratory to cure or help an illness. Typically, pharmaceutical drugs are modeled after compounds found in medicinal plants (WHO, 1994). Medicinal plants typically have essential oils in their tissues or seeds that prevent bacteria, molds, or other microbes from growing, which confers antimicrobial properties. Common herbs like peppermint, basil, oregano, thyme, and rosemary have essential oils that prevent microbial growth (Ali *et al.*, 2002).

Inflammation is complex process which is frequently associated with pain involve: increase of vascular permeability, increase of protein denaturation and membrane alteration. Common use alternative drugs such as substance product from medicinal plant (Leelaprakash *et al.*, 2010).

Antioxidant is molecule that inhibits the oxidation of other molecules. oxidation is a chemical reaction that transfer electron or hydrogen from substance to an oxidizing agent. Oxidation reaction can produce free radical, in turn, these radicals can start chain reactions, when the chain reaction occurs in a cell it can cause damage or death to the cell, antioxidant terminate this chain reaction by removing free radical (Moharram *et al.*, 2014).

Ancient Chinese medicine, still practiced today, utilizes everything from plant leaves to bark when treating illness. Chinese medicine has medicinal combinations to prevent cancer, lessen the effects of menopause, increase fertility, reduce blood pressure, and even make people more alert to help them study for tests or work. These same compounds have allowed humans to survive throughout the ages, and now modern medicine is learning about these compounds to further medicine today (Eltohami, 1997). Despite tremendous advances in modern medicine, plants continue to make important contributions to health care as witnessed by the increasing interest in alternative therapies (Rates, 2001).

Herbs are used in many domains, including medicine, nutrition, flavorings, beverages, dyeing, repellents, fragrances and cosmetics (Djeridane *et al.*, 2006). Traditional herbal medicine as a major African socio-cultural heritage, obviously in existence for several hundreds of years, however, today it has been brought into focus for meeting the goals of a wider coverage of primary health care delivery, not only in Africa but also to various extents in all countries of the world (Elujoba *et al*., 2005) because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last few years have seen a major increase in their use in the developed world (Kamboj, 2000). The world Health Organization (WHO) has for several decades, supported, promoted and assisted the development of traditional medicine in the bid to move the African health agenda forward, particularly for the less-developed countries (Elujoba *et al.,* 2005). In certain African countries, up to 90% of the population still relies exclusively on plants as a source of medicines (Hostettmann *et al.*, 2000). There has been a growing interest in the alternative therapies in recent years, especially those from plants. The WHO estimates that about 65-80% of the world's population living in developing countries depends essentially on medicinal plants (herbs) for primary health care (Chmielewski *et al*., 2005). Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, hypolipidemic, antimutagenic effects and anti-carcinogenic potential (Cai *et al.*, 2004).

In the Sudan, as in many developing countries, medicinal plants have played an important role in the treatment of diseases especially in rural areas (El Ghazali *et al.,* 1994) and represent an important component of traditional medicine, and the flora of Sudan is relatively rich in medicinal plants corresponding to the wide range of ecological habitats and vegetation zones (Khalid *et al.*, 1986). However, herbal drugs were documented during comprehensive ethno-botanical investigations of El Kamali *et al*. (1996), El Ghazali *et al.* (1994, 1997) and El Kamali *et al*. (1999). Several broad-based screenings of many Sudanese medicinal plants were conducted for their antibacterial, antifungal, antiviral, anti-malarial and anthelmintic properties (Khalid *et al.,* 1986; El Tahir *et al.,* 1999a, b; Hussein *et al.,* 1999; Koko *et al.,* 2005; Elegami *et al,* 2001).

The Sudan has a unique geographical position. The climate ranges from completely arid to tropical zones with a wide range of bioclimatic regions, from the almost barren deserts in the north to the tropical rain forests in the extreme south of the country (Elthohami, 1997). Thus, the flora of the Sudan consists of 3137 species of flowering plants belonging to 170 families and 1280 genera. Of these, 278 species, 210 genera and 72 families have already been identified as medicinal, culinary and aromatic (MCA) plants (El-Amin, 1990; El Ghazali *et al.,* 1994, 1997).

Rationale

There is a worldwide interest in searching for the safe new photochemical compound drugs. Some of the medicinal plants have been experimentally validated. Anti-inflammatory drugs like NSAIDs used to reduce the swelling and pain of

inflammation. Long-term uses of NSAID cause adverse side effects and damage human biological system such as liver, gastrointestinal tract etc. Many medicinal plants are reported to have potential anti-inflammation activity.

Objective

The major goal of the current research is to analyze the chemical compounds in the lemon-peel essential oil by GC-MS and to study its *in-vitro* capabilities as an anti-inflammatory and antioxidant agent.

Materials and Methods

Preparation of the Essential oil

By using hydro –distillation technique amount of water was added to the outer shell of lemon then the mixture was heated until the oil was separated from sample.

In vitro anti- inflammatory activity:

The screening for anti-inflammatory activity was carried according to modification of the in- vitro protein denaturation bioassay methods of Jagtap, *et al* (2011) and shallangwa, *et al* (2013). Ten milliliters of lemon essential oil were mixed with minimum quantity of dimethylsulphoxide (DMSO) and diluted with phosphate buffer solution (0.2M, pH7.4), final concentration of DMSO in all solution was less than 2.5%. The test mixtures (5 ml each) made up of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, PH7.4) and 2ml of varying concentration of extract (400, 800, 1200, 1600, 2000 mg\ml). Respective test solution was incubated at 37^{o}C in Corsair Heating and Catering limited incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60^{o}C in water-bath for 10min after cooling the turbidity was measured at 660nm (UV-Visible U2800, Spectrophotometer, Hiatachi). Diclofenac sodium at the same concentration s of extracts was used as reference drug and treated similarly for determination of absorbance. Percentage of inhibition of denaturation was calculated from control where, used as no drug was added. each experiment was done in triplicate and average taken. The percentage of inhibition of denaturation was calculated by using following formula

$$\%\text{inhibition} = 100*(vt/vc-1)$$

Where; vt = mean absorbance of test sample, **vc =** mean absorbance of control

The Antioxidant Activity

The antioxidant activity of the essential oil and the standard (Ascorbic acid) were assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method of Braca *et al.* (2002). The diluted working solutions of the test extracts were prepared in methanol, where oil of lemon was prepared in dimethyl sulphoxide (DMSO). Ascorbic acid was used as standard. A solution of 0.004% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately.

These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Cecil-Elect Spectrophotometer. Methanol (1ml) with DPPH solution (1ml) was used as blank. The optical density was recorded and % of inhibition was calculated using the formula given below.

$$\%\text{Inhibition of DPPH activity} = (A-B)/A \ *100$$

Where,

A = Absorbance of the blank solution, **B** = Absorbance of the test solution.

Gas Chromatography-Mass Spectroscopy Analysis

Ten milliliters of the essential oil of lemon were analyzed using Gas Chromatography-mass spectroscopy (GC/MS) at the Department of Biochemistry, the University of Science and Technology.

Results and Discussion

The present study was conducted in the laboratories of Department of Biology and Biotechnology, Faculty of Science and Technology, Alneelain University.

***In vitro* Anti-inflammatory Activity**:

The essential oil of *Citrus lemon* was tested to detect their anti-inflammatory activity by inhibition protein denaturation. Inhibition of protein albumin denaturation by essential oil of lemon was increased with increasing of concentration used

beginning from 400 to 2000µg/ml. The highest inhibition of albumin denaturation was recorded by 2000 µg (138.5%), while the lowest inhibition of albumin denaturation was recorded by 800µg or less (123%) (Table 1 and Fig 2).

Table 1: The anti-inflammatory activity of lemon oil

Conc. (µg)	Blank	400	800	1200	2000
Absorbance	0.013	0.029	0.029	0.03	0.031
Inhibition %	100	123.1	123.1	130.8	138.5

Antioxidant activity

Table 2. Show the antioxidant activity of essential oil of lemon by inhibition free radical DPPH in vitro. Essential oil of lemon exhibited moderate antioxidant activity; the maximum activity was determined by highest concentration 1000µg. (60.84±0.25%). IC_{50} value for essential oil was amounted to 912.74µg.

Table 2: Antioxidant activity of essential oil of lemon

CONCENTRATION µg	DPPH INHIBITION %
1000	60.84±0.25
800	13.04± 0.26
600	8.08±1.03
400	6.79±0.26
200	4.22±0.26

Gas Chromatography MS Analysis

Analysis of lemon peel essential oil has shown that D-Limonene was the highest concentration followed by Geraniol. Many other components were determined but with lower concentrations.

Table 3: Chemical Compounds of Lemon peel analyzed by GC-MS:

Name	Area %	R time	Formula	M.W	Class
D-Limonene	11.37	6.732	C_{10} H_{16}	136	Mono terpene
Geraniol	9.01	11.495	C_{10} $H1_8$ O	154	Oxygenated mono terpene
2.6Octadien 1-ol,3.7 Dimethyl cis Geraniol	8.67	10.954	C_{10} H_{18} O	154	Oxygenated mono terpene
Citral	8.30	11.834	C_{10} H_{16} O	152	Oxygenated mono terpene
2.6 Octadien 3-7Dimethyl beta citral	6.98	11.219	C_{10} H_{16} O	152	Oxygenated mono terpene
Alpha- terpinol	4.95	10.190	C_{10} H_{18} O	154	Oxygenated mono terpene
Terpinen-4-ol	3.44	9.895	C_{10} H_{18} O	154	Oxygenated mono terpene

Discussion

In this study, the evaluation of anti-inflammatory effects was undertaken using the effect of essential oil of lemon on inhibition of egg albumin protein denaturation. Denaturation of proteins is well documented and is caused by inflammation process (Chandra *et al*; 2012). The ability of lemon to inhibit protein denaturation may contribute to their anti-inflammatory properties. The results of this study have shown a concentration-dependent inhibition of protein (albumin) denaturation by *oil* within the concentration ranges of 400 to 2000µg/mL studied. (Table 1, Figure 2). Denaturation of proteins is a well-documented during inflammation in conditions like rheumatism and arthritis (Umapathy *et al;* 2010). Therefore, any substance that can prevent or inhibit protein denaturation will be a good anti-inflammatory agent. The concentration (2000 µg) has the highest activity, when the concentration was able to reveal highest percent of protein denaturation inhibition (Figure 2). Essential oil of lemon showed moderate antioxidant activity by

DPPH inhibition assay. The anti-inflammatory and antioxidant activities of essential oil may be due to the presence of some bioactive components which were detected as major compounds by GC/MS analysis (D-Limonene and Geraniol) (Table). A previous study by Majnooni *et al.,* (2012) has shown that lemon has an antioxidant activity which is agreed with the present study.

Conclusion and recommendations

From the current results, we concluded that the essential oil of lemon was capable of limiting the process of protein denaturation, and to show an antioxidant activity, therefore, these activities are related to the major terpenoids and others lemon components which were detected by GC-MS. Therefore, we recommend further investigation for designing potent anti-inflammatory and antioxidant drugs which can be used for treatment of various chronic diseases such as cancer, neurological disorder, aging and inflammation.

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