

ANTIOXIDANT ACTIVITY OF SYZYGIUM AROMATIUM AND CINNAMOMUM VERUM SEED EXTRACTS

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Abstract: The present study was carried out for the evaluation of antioxidant activities of clover (*Syzygium aromaticum* L.) and cinnamon (*Cinnamomum verum* L.). The research work was carried out at the Institute of Molecular Biology, University of Lahore. The samples of clovers and cinnamon were collected from Lahore and were crushed to prepared sample in n-hexan, and ethanol extracts for evaluation of antioxidant activities. The highest antioxidant activities of ethanol extract from cinnamon indicated that the cinnamon may be used as an active ingredient to control stress conditions of cells for stress avoidance. The alkaloids, coumarins, saponins, flavonoids, tannins, sterols, quinones, triterpenoids, anthocyanins, leucoanthocyanins and terpenoids were tested for both ethanol and n-hexan extracts which indicated that most of the components were found present in both of the plant species, which revealed that these herbal plant seeds may be used as potential medicinal plants.

Keywords: clovers, cinnamon, antioxidant, ethanol, n-hexan

Introduction

As shown from the chemical diversity, the saponins have a very wide range for biological activities collectively. Many saponins have antioxidant, antibacterial and antiherbivore activity so these play a vital role in plant defense mechanism (Lo *et al.*, 2004; Roby *et al.*, 2013). The use of cinnamon (*Cinnamomum verum* L.) also showed antioxidant and antimicrobial activities against different bacterial strains including *Bacteroides succinogenes*, *E. coli*, *Streptococcus bovis*, *Staphylococcus aureus*, *Ruminococcus flavefaciens* and fungal strains including *Penicillium roqueforti*, *Mucor plumbeus*, *Zygosaccharomyces rouxii*, *Candida lipolytica*, *Debaryomyces hansenii*, *Aspergillus flavus*, *Pediococcus halophilus* and *Pichia membranaefaciens*. The use of cinnamon oil was carried out for the inhibition of microbial growth under control conditions. The cinnamon (*Cinnamomum verum* L.) largely produced by Indonesia up to 43% among all of the cinnamon producing countries while China contributes up to 33% in total world production of cinnamon (Diao *et al.*, 2014; Zhang *et al.*, 2016). The clovers (*Syzygium aromaticum* L.) also an important plant which shows antioxidant and antimicrobial activities against bacterial strains like *Clostridium E. coli*, *sticklandii*, *salmonella* along with at some extant against fungal strains. The antioxidant and antimicrobial activities of clovers are due to the presence of soluble

phenolics. The leaves of clovers are usually rich in with isoflavonoids, biochanin A and formionin (Anwar *et al.*, 2009; Singh and Majumdar, 1999). The presence of phenolic compounds showed the inhibitory activities against the growth and multiplication of microbes. The phenolic compounds in clovers also caused the fermentation of amino acids. The clover leaves and shoots may be used as antioxidants and antimicrobial medicines to prevent human, food and animals from attacks of microbes (Chesson *et al.*, 1982; Dickinson *et al.*, 1988; Lee *et al.*, 2004). The chemical contents have been used for the formation of fungicidal lotions, antioxidant, pesticides and insecticides due to its insect repellent activities (Dua *et al.*, 2013; Kaithwas *et al.*, 2011; Singh *et al.*, 2005). The sterols and aglycone triterpenes themselves encompass a wide range of structure with the nature of cyclization, oxidation in structure and variation in the degree. Secondly, variation with respect to sugar molecules and their numbers and nature of glycosylation, the presence of sugar chains and types of inter sugar chain linkages. Single sugar is attached at C-3 position in mono desmosidic saponins. While at C-28 position in bidesmosidic saponins (like triterpenoid saponins), and in steroid saponins sugar are attached at C-26 position (Chesson and Forsberg 1997; Sullivan, 2009). The rumen bacterial strains caused disease in the intestine of animals which may lead towards the death of animal. These bacterial strains can be controlled or killed through the use of plant

phytochemical or phenolic compounds as antimicrobial agents which are also used as potential antioxidants (Chesson *et al.*, 1982; Stewart *et al.*, 1997).

Materials and methods

The research was conducted in Microbiology Lab at the Institute of Molecular Biology and Biotechnology, The University of Lahore. The research work was done in the time period from November 2019 to March 2020. The seed samples were included *Syzygium aromaticum* and *Cinnamomum verum* (*Trifolium pretense* L.) which was collected from market of Lahore, Pakistan.

Preparation of plant extracts

The dried samples were brought to the laboratory and crushed into small pieces by using laboratory grinder. After grinding for clovers (*Syzygium aromaticum* and *Cinnamomum verum* (*Trifolium pretense* L.) were dipped in two different solvents ethanol and n-hexan ether depending on the amount required and kept for 2 weeks with vigorous shaking but the amount of samples was kept the different. All samples were taken in grams (g) and solvents per milliliters (ml). After keeping the samples in the solvents for 2 weeks and samples were filtered using a whatman filter paper into a new bottle and then placed in the rotary evaporator. After the rotary process, samples were collected in falcon tubes and labeled accordingly and then placed in Petri plate for at least 1-2 days to evaporate completely. Dried extracts were collected and stored at refrigerator 4°C for further used detail shown in table below.

Antioxidant activity

Antioxidants are capable to inhibit or either delayed oxidation processes, which occurred under the effects of the atmospheric O₂ or ROS (reactive oxygen species). They were used to stabilize polymeric products of foodstuffs, pharmaceuticals, petrochemicals and cosmetics. Antioxidants are involved in defense mechanism or immune system of organism against varieties of pathologies which are usually associated with the attacked of free oxygen radicals or ROS. To check the antioxidant properties of clovers (*Syzygium aromaticum* L.) and cinnamon (*Cinnamomum verum* L.) plant DPPH assay was applied:

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay

DPPH, known formally as 2, 2-diphenyl-1-picrylhydrazyl, was a cell-permeable, stable free radical that was commonly used to evaluate the ability of compounds to acted as free radical scavengers or hydrogen donors and to measure the antioxidant activity of flower, leaves, stem and roots extracts. The reaction of DPPH with an antioxidant or reducing compound produced the corresponding

hydrazine DPPH, which could be followed by color changed from purple (absorbance at 515-528nm) to yellow (Adedayo *et al.*, 2004). The antioxidant activity of the extracts was determined using the DPPH free radical scavenging assay described with some modifications. Briefly, the universal bottle was contained 501.1L of clovers (*Trifolium pretense* L.), Alsi (*Linum usitatissimum* L.), fennel or sonf (*Foeniculum vulgare* L.), cinnamon (*Cinnamomum verum* L.) extracts in concentrations from 1 to 5 mg/ml, 0.004% (w/v) solution of DPPH was added. The obtained mixture was vortexed, incubated for 30 min in room temperature in a relatively dark place and then was read using spectrophotometer at 517 nm. The blank was 80% (v/v) methanol. Ascorbic acid (vitamin C) was used for comparison. Measurements were taken in triplicate. DPPH scavenging effect was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{(A_0 - A_1)/A_0 \times 100}{100}$$

Where A₀ was the absorbance of negative control (0.004% DPPH solution) and A was the absorbance in presence of extract. The results were reported as IC₅₀ values and ascorbic acid equivalents (AAE, mg/g) of clovers (*Syzygium aromaticum* L.) and cinnamon (*Cinnamomum verum* L.) extracts.

Results and discussions

The ethanol and n-hexan extracts from clover and cinnamon were used to find out the antioxidant activities present in the spices plants. The highest antioxidant activity (2.678) through ethanol plant extract at 50 µg/ml concentration was reported by cinnamon sample while lowest (0.267) was recorded for clover. The highest antioxidant activity (0.568) through ethanol plant extract at 100 µg/ml concentration was reported by cinnamon sample while lowest (0.557) was recorded for clover. The highest antioxidant activity (2.80, 1.173, 300) through ethanol plant extract at 200 µg/ml, 150 µg/ml and 250 µg/ml concentrations was reported by Cinnamon sample while lowest (0.228, 0.153, 0.238) was recorded for clover respectively (Table 1). The lowest antioxidant activity (0.231) through n-hexan plant extract at 50 µg/ml concentration was reported by clover sample while higher (0.275) was recorded for Cinnamon. The highest antioxidant activity (0.714) through n-hexan plant extract at 100 µg/ml concentration was reported by cinnamon sample while lowest (0.411) was recorded for clover. The highest antioxidant activity (1.241) through n-hexan plant extract at 150 µg/ml concentration was reported by cinnamon sample while lowest (0.171) was recorded for clover. The highest antioxidant activity (1.752) through n-hexan plant extract at 250 µg/ml

concentration was reported by cinnamon sample while lowest (0.464) was recorded for clover. The highest antioxidant activity (0.565) through n-hexan plant extract at 200 µg/ml concentration was reported by cinnamon sample while lowest (0.232) was recorded for clover (Table 2). The alkaloids, coumarins, saponins, flavonoids, tannins, sterols, quinons, triterpenoids, anthocyanins, leucoanthocyanins and terpenoids were tested (Tables 3) for both ethanol and n-hexan extracts of clover and cinnamon which indicated that most of the components were found present in both of the species, which revealed that these herbal plants may be used as potential medicinal plants. The ethanol and n-hexan extracts from clovers and cinnamon were used to find out the

antioxidant activities present in the spices plants. The highest antioxidant activities of ethanol extract from Cinnamon and clover indicated that the cinnamon and clover may be used as an active ingredient to control stress conditions of cells for stress avoidance. The highest antioxidant activities of ethanol extract from Cinnamon indicated that the cinnamon may be used as an active ingredient to control stress conditions of cells for stress avoidance. The highest antioxidant activities of ethanol extract from Cinnamon and clover indicated that the cinnamon and clover may be used as an active ingredient to control stress conditions of cells for stress avoidance (Anwar *et al.*, 2009; Heng *et al.*, 2006; Lee *et al.*, 2004; Matan *et al.*, 2006).

Table 1. Antioxidant activities of spices by ethanol extract

Doses	clover (<i>Syzygium aromaticum</i> L.)	Cinnamon (<i>Cinnamomum verum</i> L.)
50 µg/ml	0.267	2.678
100 µg/ml	0.557	0.568
150 µg/ml	0.228	2.80
200 µg/ml	0.153	1.173
250 µg/ml	0.238	3.00

Table 2. Antioxidant activities of spices by n-hexan extract

Doses	clover (<i>Syzygium aromaticum</i> L.)	Cinnamon (<i>Cinnamomum verum</i> L.)
50 µg/ml	0.231	0.262
100 µg/ml	0.411	0.714
150 µg/ml	0.171	1.241
200 µg/ml	0.232	0.565
250 µg/ml	0.464	1.752

Table 3. Phytochemical composition of clover (*Syzygium aromaticum* L.) and Cinnamon (*Cinnamomum verum* L.) seed extract

Phytochemical	clover (<i>Syzygium aromaticum</i> L.)		Cinnamon (<i>Cinnamomum verum</i> L.)	
	Ethanol extract	n-hexan extract	Ethanol extract	n-hexan extract
Alkaloids	+	+	+	+
Coumarins	+	+	+	+
Saponins	-	+	+	+
Flavonoids	+	+	-	+
Tannins	+	-	-	-
Sterols	+	+	+	+
Quinons	+	+	+	+
Triterpenoids	-	-	+	-
Anthocyanins	-	-	+	-
Leucoanthocyanins	+	+	-	+
Terpenoids	+	+	+	+

Conclusions

The alkaloids, coumarins, saponins, flavonoids, tannins, sterols, quinons, triterpenoids, anthocyanins, leucoanthocyanins and terpenoids were tested for both ethanol and n-hexan extracts of clover (*Syzygium aromaticum* L.) and Cinnamon (*Cinnamomum verum* L.) which indicated that most of the components were found present in both of the species, which revealed that these herbal plants may be used as potential medicinal plants. It was concluded that the Cinnamon (*Cinnamomum verum* L.) and clover (*Syzygium*

aromaticum L.) may be used as potential antibiotics and antioxidants.

Conflict of interest

The authors declared absence of conflict of interest.

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