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Evaluation of essential oils from *Boswellia sacra* and *Teucrium mascatense* against acetyl cholinesterase enzyme and urease enzyme

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Abstract

Enzyme inhibition is one of the most important areas of pharmaceutical research which resulted in the discoveries of several useful drugs. The prime aim of this study is to identify effective natural inhibitors against pharmacologically important enzymes such as acetylcholinesterase enzyme and urease enzyme. In present study, we evaluated the essential oils extracted from medicinally important plants including *Boswellia sacra* Flückiger (frankincense) and *Teucrium mascatense* Boiss. Two major constituents of frankincense essential oil including (+)- -pinene and (R)-+-limonene were also investigated against these enzymes. The essential oils were extracted from *Boswellia sacra* and *Teucrium mascatense* which are native plants to the southern and northern areas of Oman, respectively. In this study, the essential oil of frankincense exhibited significant inhibition with IC_{50} value of 0.043±0.02 mg/mL, against acetylcholinesterase enzyme while against urease enzyme it has shown good inhibition with IC_{50} value of 0.17 ± 0.05 mg/mL. The essential oil obtained from the *Teucrium mascatense* was found to be in active against both the enzymes. On acetylcholinesterase enzyme inhibition assay, the (+)- - pinene exhibited significant inhibition (IC_{50} = 0.094±0.01 mg/mL) while (R)-+- limonene was found to be inactive on this assay. Against urease enzyme (+)- - pinene and (R)-+- limonene showed moderate activity 40.06±1.03 % and 19.5±1.00 %, respectively. Interestingly, the mixture of equal concentration of (+)- - pinene and (R)-+- limonene exhibited 70% inhibition with IC_{50} value of (0.195 ± 0.10 mg/mL) which shows the synergistic relationship between them. Promising inhibitory potential of frankincense essential oil and (+)- -pinene, against acetylcholinesterase enzyme and urease enzyme indicated their potential therapeutic role to manage Alzheimer's disease and stomach ulcers, respectively.

Key Word: *Boswellia sacra, Teucrium mascatense*, essential oil, acetylcholinesterase enzyme, urease enzyme, (+)- - pinene

Introduction

The plant *Boswellia sacra* Fluckiger belongs to the family Burseraceae. *B. sacra* trees are commonly found in the Dhofar region of the Sultanate of Oman and southern Yemen in arid forest, on the steep and slopes in the mountains [1,2]. Frankincense is an aromatic resin acquired from trees of the genus Boswellia. Frankincense is particularly vital as its aroma has significance for religious practices since the time of ancient Egyptians and these resins have also shown therapeutic properties [3,4]. It has great medicinal value and commonly used to treat various problems related to digestion, respiration, stomach, dental infections, fever, cough and muscle pain [5-7].

Chemical constituents of essential oils obtained from Boswellia sp. differ considerably due to change in conditions such as harvesting time, storage, geographical sources of resins, climates and techniques of sample preparations [8]. The essential oil from *Boswellia sacra* is effective to control cell viability and induce apoptosis to the human pancreatic cancer cell lines which might be an alternative therapeutic agent for treating patients with pancreatic adenocarcinoma [4]. The plant *Teucrium mascatensis* Boiss. belongs to the family Lamiaceae. It usually grows on rocky hills and mountains in northern Oman [9]. *T. mascatensis* is traditionally used to reduce fever. The essential oil of *T. mascatensis* is useful against Gram positive cocci and yeasts [10]

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In modern age research, enzymes are the excellent targets for pharmacological intervention due to their important roles in life processes. Although enzymes are vital for life, dysegulated enzyme activity can create disease states. One effective approach of chemotherapeutic intervention is the selective inhibition of critical enzymes of infectious organisms to control infectious diseases. Since small molecules have shown great potential to inhibit enzyme activity, therefore various enzymes are the common targets of pharmaceutical companies for new drug discovery [11].

Acetylcholinesterase (EC 3.1.1.7, AChE), also known as red blood cell cholinesterase, is found in the blood and neural synapses. Acetylcholinesterase (AChE) is the most important enzyme which regulates the level of acetylcholine in the brain [12,13]. Alzheimer's disease (AD) is a common type of dementia in the aging population due to acetylcholine deficiency. Enhancement of acetylcholine levels in the brain is one of the approaches to treat Alzheimer's disease [14]. The inhibitors of acetyl cholinesterase enzyme actually block the cholinesterase enzyme to break down acetylcholine. In this way both the level and duration of the neurotransmitter action is increased in the body. The general mechanism of action is shown in Figure.1 [15].

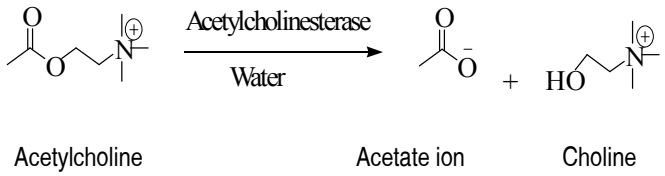

Figure.1. Mechanism of action of acetyl cholinesterase enzyme.

The inhibition of AChE is the most efficient therapeutic approach to increase the level of acetylcholine and thus to treat the Alzheimer's disease (AD) [16,17]. In these circumstances the evaluation and identification of new potent inhibitors against acetyl cholinesterase enzyme is highly desirable. [18-22]

Urease enzyme is the most prominent protein component of bacteria called *Helicobacter pylori* (H. pylori). Urease (EC 3.5.1.5) is a nickel dependent metalloenzyme which catalyzes to produce ammonia and carbamate from the hydrolysis of urea. In next step, carbamate spontaneously hydrolyzes to form carbonic acid and another molecule of ammonia [23]. The overall reaction is shown below in Figure.2;

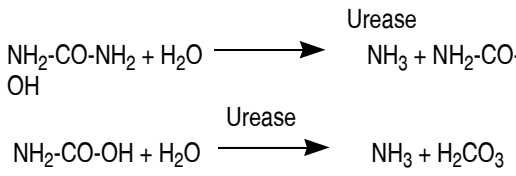

Figure 2. Mechanism of action of urease enzyme.

The net effect of these reactions is an increase in pH which neutralizes acids of stomach and thus facilitates survival and colonization of the dangerous pathogen. Most of the urease is found in bacterial cytoplasm which causes the pH change and acid resistance [24]. Urease enzyme is involved in stomach ulcer and infections of urinary and gastrointestinal tracts. Killing *H. pylori* with antibiotics can be useful to cure duodenal ulcer however, the antibiotic resistance causes various complications. Therefore, the urease inhibitors have attracted the attention as much safer potential anti-ulcer drugs [24, 25].

Previously, our research group UoN, Chair of Oman's Medicinal Plants and Marine Natural Products, at the University of Nizwa, Oman, has been extensively involved in the identification of medicinally important plants of Oman, extraction and analysis of essential oils, isolation of secondary metabolites from different medicinal plants including *Boswellia sacra* Flückiger (frankincense) and their charachetrization [1,7].

Material And Methods

Chemicals

Required chemicals such as acetylcholinesterase (AChE) type VI-S, from electric eel, acetylthiocholine iodide (ATCI), 5,5 -dithiobis[2-nitrobenzoic acid] (DTNB), urease (EC 3.5.1.5) from Jack bean, sodium nitroprusside,sodium hydroxide, NaOCl, K_2HPO_4, KH_2PO_4, Ethylenediamine tetraacetic acid (EDTA), LiCl , phenol and thiourea, dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich. Moreover, essential oils, (+)- - Pinene and (R)-+- Limonene were obtained from our research group.

Methods

Essential oil isolation using NEOS apparatus

Essentail oil extraction was carried out by using the procedure reported by Hussain J. et al. 2013. Fresh samples 100 g each powder form were heated using a fix power of 400 W for 30 mints at 100 C in water (1 L). The extraction was continued at 100 C with continuous stirring until no more essential oils were obtained. The essential oils were collected, dried over anhydrous Na_2SO_4 and stored at 4 C until further use. Extractions were performed at least three times and the mean values were reported. NEOS (new essential oil system) was carried out with a Milestone technologies (Model: IEC 60825-1; 2007) microwave apparatus with a maximum delivered power of 900 W variable in 10 W increments and 650 nm wave length. During experiment, time, pressure and power were controlled with the "easy-WAVE" software [26-28].

Assay Protocol for Acetyl cholinesterase Enzyme

Inhibition of acetylcholinesterase enzyme was determined using Ellman's colorimetric method as modified by Eldeen et al., 2005. In this assay 96-well plate was used and each reaction well contained 25 µL of 15 mM ATCI dissolved in water, 125 µL of 3 mM DTNB in Buffer (50 mM Tris–HCl, pH 8), and 25 µL of plant extract of concentration 0.5 mg/mL dissolved in DMSO. In blank sample 25 µL plant extract was replaced by 25 µL of Tris-HCl buffer. In control sample also 25 µL of Tris-HCl buffer was used in place of 25 µL of plant extract. 96-well plate was then incubated at 25 °C for 15 minutes. Thereafter, 25 µL AChE (0.2 U/mL) was added to the wells and the absorbance measured five times consecutively every 45 s. In blank sample 25 µL of AChE were replaced by 25 µL of Tris-HCl buffer. The reaction was monitored for 5 min at 405 nm [14,29,30]. The percentage inhibition was calculated using the equation:

Inhibition % = 1 (blank sample/control) 100

Assay Protocol for Urease Enzyme

Urease enzyme inhibition activity was determined by following the assay procedure described by Serwar M et al, with slight modification [31]. The sample solution containing 25 µL of Jack bean Urease, 55 µL of buffer and 100 mM urea were incubated with 5 µL (0.5 mg/mL.) of the test compounds at 30 C for 15 min in 96-well plate. The ammonia produced during this reaction was measured by indophenol method and used to determine the urease inhibitory activity. The phenol reagent (45 µL, 1% w/v phenol and 0.005% w/v sodium nitroprusside) and alkali reagent (70 µL, 0.5% w/v sodium hydroxide and 0.1% NaOCl) were added to each well and the increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). The change in absorbance was analysed. The assays were performed at pH 8.2 (0.01 M $K_2HPO_4.3H_2O$, 1.0 mM EDTA and 0.01 M $LiCl_2$). Thiourea was used as the standard inhibitor.

Statistical Analysis

The results were expressed as mean ± SEM while the EZ-fit software (Perrella Scientific Inc., Amherst, U.S.A.) was used to calculate the IC_{50} values (µg/mL). IC_{50} values were measured by using different concentrations of the active samples.

Results and Discussion

The plant *Boswellia sacra* is commonly known for the production of fragrant gums and resins since ancient times. *B. sacra* is mainly restricted to the southwest of Oman and some regions of Yemen and Somalia. The frankincense is prepared from pieces of yellowish brown gum excreted by the trunk of the *B. sacra* trees. Both Oman and Yemen have been involved in the production and export of the frankincense to other areas of the world since prehistoric time [2, 32].

Generally, chemical constituents of essential oils obtained from various Boswellia differ significantly due to change in physical conditions however; alpha-pinine is the main component of essential oils obtained from various Boswellia sp. even at different temperatures. Previously, frankincense essential oil has shown activity against proliferation and induced cytoxocity to various pancreatic cancer cells *in vitro*. It was also effective to induce tumor cell death, suppress and reverse tumor growth in mouse model [3,4].

Our present study is an effort to identify effective natural inhibitors against acetyl cholinesterase enzyme and urease enzyme. In this study, we evaluated the essential oil of frankincense obtained from Boswellia sacra, *Teucrium mascatensis* essential oil, (+)- - pinene and (R)-+- limonene against these enzymes.

Initially, 0.5mg/mL concentration of each sample was used to investigate anti-cholinesterase activity. At this concentration frankincense essential oil and alpha-pinene (1) exhibited significant inhibition 96±1.50%) and 84±2.00, respectively while R+-limonene (2) was found to be inactive (5±1.00%). Moreover, mixture of equal concentration (50:50) of alpha-pinene (1) and R+-limonene (2) showed 80±2.00 % inhibition as shown in Figure.3.

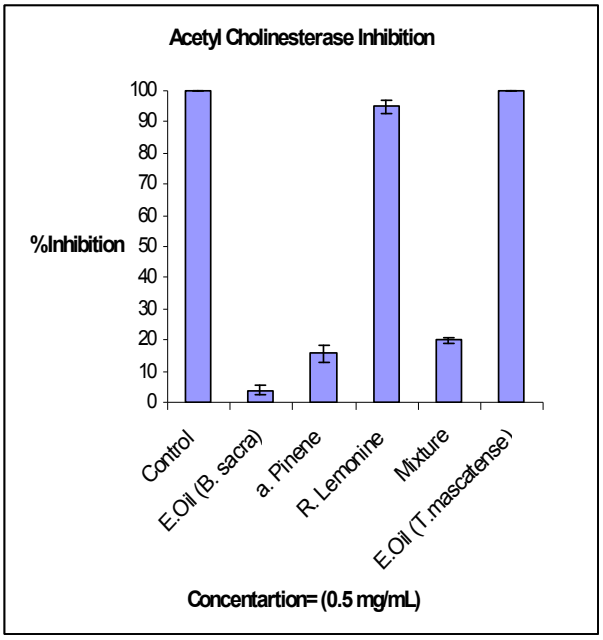

Figure.3. Acetylcholinesterase enzyme inhibition

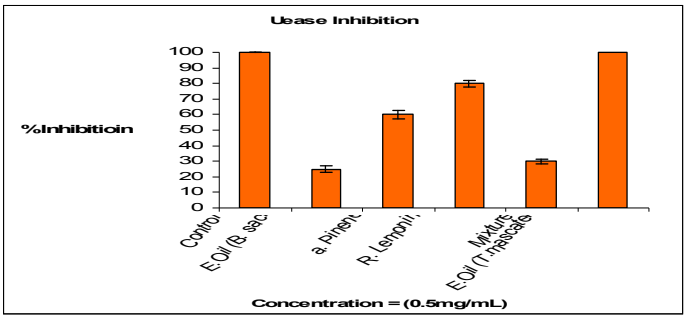

Figure.4. Urease enzyme inhibition

Similarly, against urease enzyme, frankincense essential oil exhibited good inhibition (75 ±2.00 %) while alpha-pinene (1) and R+-limonene (2) showed moderate and low activity with 40.01 ±1.50 and 19.5±1.00 % inhibition, respectively Figure.4. Interestingly, mixture of equal concentration (50:50) of alpha-pinene (1) and R+-limonene (2) showed 70±1.50 % inhibition as shown in Table-1. Galantamine was used as standard inhibitor against acetylcholinesterase enzyme while urea was used as standard inhibitor against urease enzyme with 98±1.00 and 96±2.00 % inhibition, respectively.

Table-1: Anti-cholinesterase and anti-urease activity of various samples

S. No	Sample	Acetyl cholinesterase % Inhibition (IC_{50}= mg/mL)	Urease % Inhibition (IC_{50}= mg/mL)
1.	Essential oil (*Boswellia sacra)* **(E. Oil)b**	96±1.50 (IC_{50} = 0.043±0.02)	75±2.00 (IC_{50} = 0.17 ± 0.05)
2.	(+)- - Pinene **(1)**	84±2.00 (IC_{50} = 0.094±0.01)	40±1.50
3.	(R)-+- Limonene **(2)**	5±1.00	19.5±1.00
4.	Mixture of (+)- - Pinene and (R)-+- Limonene **(Mix)**	80±2.00 (IC_{50} = 0.102±0.015)	70±1.50 (IC_{50} = 0.195 ± 0.1)
5.	Essential oil (*Teucrium mascatensis)***(E. Oil)t**	NA*	NA
6.	Galantamine (standard) **(3)**	98±1.00 (IC_{50} = 1.00±0.21 µM)	-
7.	Urea (standard) **(4)**		96±2.00 (IC_{50} = 21.02±51µM)

The structures of alpha-pinene (1) and R+-limonene (2) and standard inhibitors are shown in Figure.5.

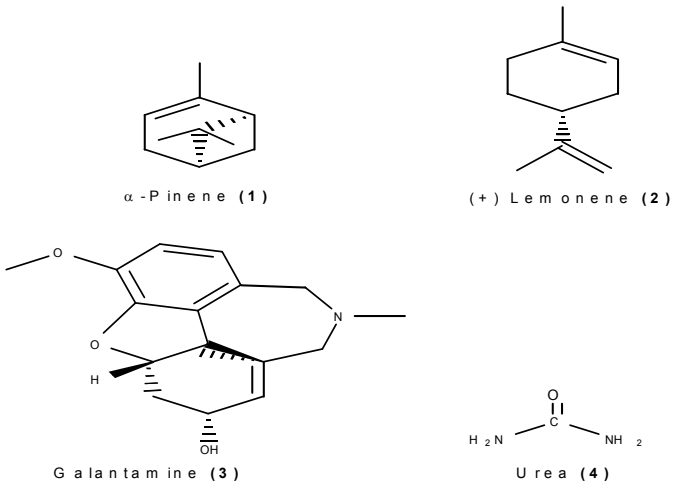

Figure 5. Structures of acetycholiesterase enzyme and urease enzyme inhibitors.

Different concentrations of the most active samples were used to calculate the IC_{50} values. On anti-cholinesterase assay, the essential oil exhibited significant inhibition with IC_{50} value of 0.043±0.02 mg/mL while against urease enzyme it has shown good inhibition with IC_{50} value of 0.17 ± 0.05 mg/mL. On acetyl cholinesterase enzyme inhibition assay, the (+)- - pinene exhibited potent activity (IC_{50} = 0.094±0.01 mg/mL) while (R)-+- limonene was found to be inactive on this assay. However, the mixture of both (+)- - pinene and (R)-+- limonene exhibited slightly less inhibition (IC_{50} = 0.102±0.015 mg/mL) as compared to (+)- - pinene alone. Against urease enzyme (+)- - Pinene and (R)-+- Limonene showed moderate activity with less than 50% inhibition (40.06±1.03 % and 20±1.00 %), respectively. Interestingly, the mixture of (+)- - pinene and (R)-+- limonene exhibited good inhibition with IC_{50} value of 0.19.05 ± 0.10 mg/mL. Galantamine (3) and urea (4) were used as standard inhibitors with IC_{50} values of 1.00±0.21 and 21.02±51µM, respectively.

Conclusion

In conclusion, these results illustrate that essential oil obtained from resins of *Boswellia sacra* has significant inhibition against

acetylcholinesterase and urease enzymes. Hence, it might have great potential to cure Alzheimer disease as well as stomach ulcers. Similarly, the (+)- - Pinene a major active constituent of frankincense essential oil has shown promising acticty against both enzymes which might have the potential as drug candidate in future. Furthermore, the present study opens the possibility of finding safe and effective natural products against these biological disorders.

Conflicts of interests

The authors declare no conflicts of interests.

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