ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 6, Suppl 3, 2013

ISSN - 0974-2441

Research Article

DEVELOPMENT OF ANALYTICAL METHOD FOR IDENTIFICATION OF ESSENTIAL OIL FROM *BLUMEA ERIANTHA* DC IN PLASMA.

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Received: 24 May 2013, Revised and Accepted: 16 July 2013

ABSTRACT

Objective
The present study was designed to develop a head space gas chromatography method using mass spectrometric detection for identification of essential oil constituents from *Blumea eriantha* DC in plasma.
Methods
The essential oil was extracted by hydrodistillation using Clevenger type apparatus. The rabbit plasma samples were spiked with essential oil and were further analyzed using dynamic head-space sampling with gas chromatography-mass spectrometry (GC-MS).
Results
The analysis of plasma samples spiked with essential oil has revealed the selective absorption of some of the essential oil components.
Conclusion
Analytical method has been developed for identification of essential oil constituents in plasma which will be helpful in further *in vivo* pharmacokinetic studies of the essential oil.

Keywords Blumea eriantha DC, Dynamic Headspace Sampling, GC-MS, Plasma.

INTRODUCTION

Almost all studies on essential oils research focus on their extraction, chemical composition, and wide application in the food and cosmetics industries and traditional medicine [1]. Therefore it is often believed that essential oils are completely safe, as they are natural in origin. Essential oils are rich blend of highly functional molecules. They are highly concentrated, volatile and fat-soluble in nature. Therefore mainly differ from the water-soluble whole herb extracts used in herbal medicine [2]. The toxicity of essential oils can also be entirely different to that of the herb as they are lipophilic in nature and hence can pass across the membranes very efficiently [2]. As these properties are beneficial for their medicinal effects, this may also lead to their toxicity. Some of the major areas of concern about essential oil hazards include allergic contact dermatitis, photosensitization, neurotoxicity, carcinogenicity [3]. Hence it is very important to study interaction of essential oils and their constituents *in vivo* to know their efficacy as well as toxicity. As the fragrances of essential oils are complicated and difficult to characterize, there are very few reports available about their in vivo interactions in body fluids [4, 5 and 6]. It is therefore important to develop reliable analytical methodology for identification of essential oils or their constituents in body fluids.

Blumea eriantha DC (*Asteraceae*) (*B. eriantha*) is an Indian medicinal plant, mainly distributed in Karnataka, Maharashtra, Uttar Pradesh, Madhya Pradesh, Bihar and Orissa in India [7]. The essential oil obtained from *B. eriantha* possesses significant antibacterial and antifungal properties [8] and may be incorporated in dermatological medicaments [9]. The antibacterial potential of essential oil from *B. eriantha* was confirmed by *in vitro* antimicrobial testing against skin pathogens mainly acne inducing bacteria by means of broth microdilution method and time kill analysis [10]. The essential oil showed strong antibacterial activity against *Propionibacterium acnes* (MIC 0.39%), *Staphylococcus epidermidis* (MIC 1.56%), *Staphylococcus aureus* (MIC 0.19%) and *Streptococcus pyogenes* (MIC 0.09%). Further the essential oil was also studied for its chemical composition by Gas Chromatography Mass Spectrometry and antioxidant potential [11]. Total 72 components were identified in the essential oil out of which Ocim-(4E, 6Z)-ene <allo->, Caryophyllene <(E)->, Caryophyllene oxide, Carvotanacetone, Pinene <alpha->, Eudesmol <7-epi-alpha-> are major constituents. The oil showed activity as a radical scavenger at 437.92 ± 4.22 µg/ml.

The antimicrobial efficacy of essential oil from *B. eriantha* indicates its potential as a natural antibiotic in the treatment of skin infections. The in vitro efficacy in antimicrobial studies also indicate potential of *B. eriantha* essential oil for evaluation in *in vivo* efficacy models to establish their clinical benefits as an effective therapy against acne vulgaris and other skin infections. Therefore this study was undertaken to develop a reliable analytical method to analyze plasma samples from *in vivo* study. In this study we have used dynamic head-space sampling with gas chromatography-mass spectrometry (GC-MS) for extraction and identification of volatiles of *B. eriantha* essential oil from rabbit plasma samples.

MATERIALS AND METHODS

Plant Material

The entire upper portion including aerial part, Stem and leaves of *B. eriantha* were collected from Seawoods, Navi Mumbai, Maharashtra, India between the months of December to March. The identification of the plant was done at the Blatter Herbarium, St. Xavier's College, Mumbai, India, by Dr. Rajendra Shinde. The plant specimen matches with the Blatter Herbarium specimen number PD 496 of P. Divakar.

Extraction of essential oil

The fresh plant material including aerial part, stem and leaves of *B. eriantha* were chopped into small pieces. 750g of fresh plant material was subjected to hydrodistillation using Clevenger type apparatus of capacity 5 Liters. 3 liters of water was added to the plant material. The mixture was heated on heating mantle at 85^{0}C. The distillation was continued for three hours. The essential oil obtained was dried over anhydrous sodium sulphate and stored at 4^{0}C in sealed vials until analysis.

Preparation of Rabbit Plasma Samples

The stock solution (15% v/v) of essential oil from *B. eriantha* was prepared in DMSO (Molecular Biology Grade, Himedia, India). In headspace GC vial**,** 1000µl of blank rabbit plasma was taken and was analyzed immediately on Headspace GCMS. For in vitro plasma

analysis 1000 µl of blank plasma was spiked with 500 µl of 15% essential oil stock solution. This spiked plasma samples were prepared in duplicate. Out of the two spiked plasma samples one sample was analyzed immediately on headspace GCMS as zero hour sample. The other sample was kept at room temperature for 24 hours and then analyzed.

Head Space Sampling and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The plasma samples were analyzed by gas chromatography- mass spectrometry (GC-MS) using Shimadzu GCMS QP-2010 Ultra system and Teledyne Tekmar HT3 Headspace analyzer. The system was equipped with fused silica Rtx-5 Sil MS silarylene capillary column with dimensions 30m X 0.25mm X 0.25µm. Helium was used as a carrier gas. The conditions used for HS and GCMS are given in **Table 1 and 2** respectively. Individual constituents were identified by referring to compounds known in the literature data and also by comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST 05), Flavor and Fragrance Natural and Synthetic Compounds mass spectral library database.

Table 1. Teledyne Tekmar HT3 Headspace Conditions

Head Space Conditions	
Valve Oven Temperature	95^{0}C
Transfer Line Temperature	110^{0}C
Platen/Sample Temperature	80^{0}C
Sample Equilibration Time	25.00 min
Pressurize	8 PSIG
Pressurize Time	2.00 min
Pressurize Equilibration Time	0.20 min
Loop Fill Pressure	6 PSIG
Loop Fill Time	2.00 min
Injection Time	1.00 min

Table 2. Gas Chromatography Mass Spectrometry Conditions

GC Conditions		MS Conditions	
Injection Temperature	266.00^{0}C	Ion Source Temperature	200.00^{0}C
Injection Mode	Split	Interface Temperature	260.00^{0}C
Column Flow	0.93 ml/min	Solvent Cut Time	1.00 min
Split Ratio	40.00	Detector Gain	0.84
Oven Temperature Program		Start m/z	40.00
Rate (^{0}C)	**Temperature (^{0}C)** / **Hold Time (min)**	End m/z	500.00
--	50.0 / 1.00	ACQ Mode	Scan
5.00	220.0 / 1.00		
20.00	260.0 / 15.00		

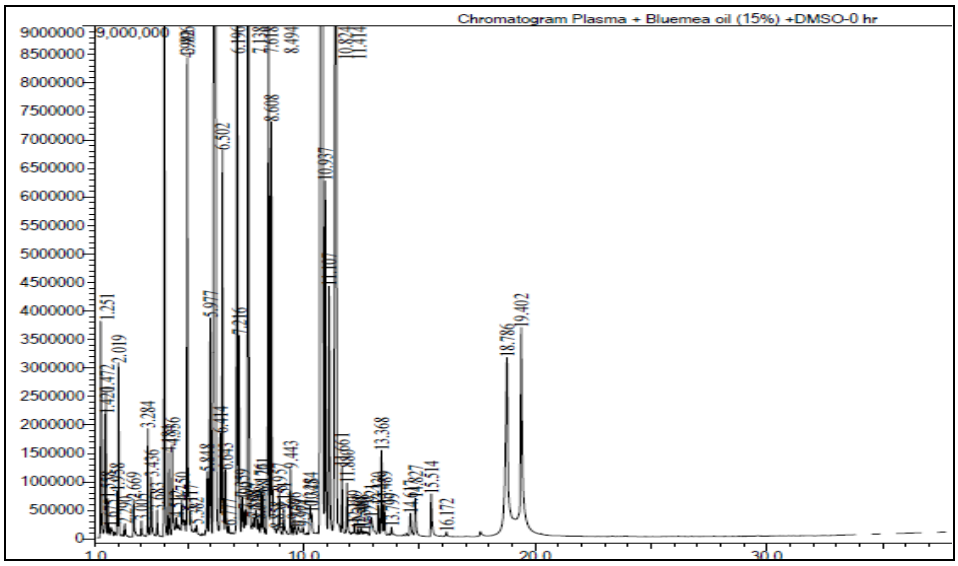

Figure 1: Total Ion Chromatogram for Zero Hour Plasma Sample

RESULTS

The use of Head space GCMS resulted in good separation of essential oil constituents. The total ion chromatograms of plasma samples from in vitro study at zero and 24 hour are shown in **Figure 1 and 2**. Approximately 70 components were identified at zero hour (**Figure 1)** out of which ten major components has shown greater rate of absorption in plasma over 24 hours. The compounds showing reduction in area and their % reduction at 24 hour with respect to zero hour area are tabulated in **Table 3.** It indicates that out of ten compounds Carvotanacetone showed maximum reduction in area (31.00%), followed by Ocimene (23.32%), Limonene (19.39%) and Isophorone (15.98%). Carvotanacetone is a terpenoid ketone commonly found in essential oil of *B. eriantha.* The in vitro absorption of essential oil analysis in rabbit plasma has revealed the selective absorption of some of the essential oil components.

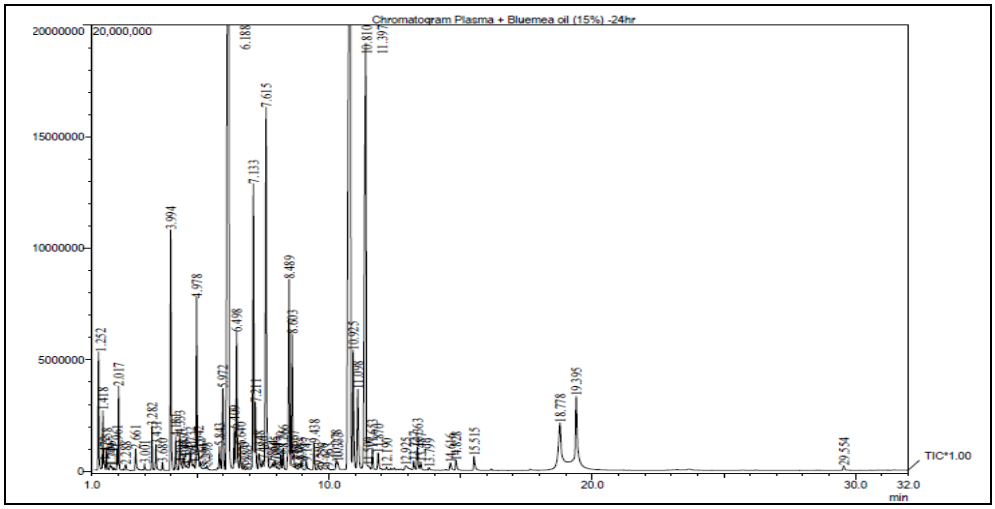

Figure 2: Total Ion Chromatogram For 24 Hour Plasma Sample

Table 3. Essential Oil Compounds with Reduced Area at 24 Hour

No.	Name of Compound	RT	Area at Zero Hour	Area at 24 Hour	Reduction In Area	% Reduction in Area
1	Pinene <Alpha->	5.85	3270401	3025100	245301	7.50
2	Sabinene	7.14	37952345	33973478	3978867	10.48
3	Pinene <Beta->	7.22	8679755	8201496	478259	5.51
4	Cymene <Para->	8.49	28317366	24669575	3647791	12.88
5	Limonene	8.61	21025197	16947795	4077402	19.39
6	Ocimene	9.60	1022982	784380	238602	23.32
7	Terpin-4-Ol	12.92	1803519	1543957	259562	14.39
8	Verbenone	13.80	432819	371237	61582	14.23
9	Carvotanacetone	14.83	2156584	1488121	668463	31.00
10	Isophorone	15.51	2805367	2357188	448179	15.98

DISCUSSION

Before beginning with *in vivo* pharmacokinetic studies, it is very important to develop an analytical method for analysis of phytoconstituents in biological samples such as blood plasma, urine etc. Usually most extensive binding of phytoconstituents occurs in the blood. Hence for more sensitive analysis it is very important to separate these phytoconstituents from interfering components of sample matrix. Therefore sample pretreatment is an essential part in analysis of such type of mixtures. This Sample pretreatment is carried out mainly for isolation of phytoconstituents from interfering matrix substances, liberation of the phytoconstituents from the protein binding sites and concentration of the phytoconstituents.

As essential oils are volatile in nature, GCMS is the chromatographic method of choice. Though essential oils are volatile in nature the plasma samples are not. Hence these samples cannot be directly injected in to the GCMS system due to their non volatile nature and for interference from plasma constituents. In such case solvent extraction, solid phase extraction methods can be employed for isolation of phytoconstituents from interfering matrix components. But chances of loss of volatile constituents during the extraction procedure are more in these extraction methods. As essential oils are highly concentrated many of them need to be applied in diluted form, hence the concentration of their constituents in body fluids is already very low. Therefore further loss of volatiles during extraction procedure can give erroneous results. Headspace analysis avoids the sample extraction procedure by directly sampling the volatile headspace from the container in which the sample is placed. Hence headspace technique was found to be fast and better technique than any other extraction methods and was employed to inject the volatile constituents directly from plasma samples. This method can also be applied for in vitro analysis of such mixtures.

CONCLUSION

Analytical methodology has been developed for the identification of constituents of essential oil of *Blumea eriantha* DC from rabbit blood plasma. This methodology includes Head space GC-MS for the determination of volatiles. The specificity of mass spectrometry has been used for identification of essential oil constituents while headspace was used for direct injection of volatile constituents directly from the plasma. The in vitro absorption of essential oil in rabbit plasma has revealed the selective absorption of some of the essential oil components.

ACKNOWLEDGMENT

Authors are grateful to Guru Nanak Institute for research and Development, Guru Nanak Khalsa College and Shimadzu Analytical India Pvt. Ltd, Mumbai for providing the excellent research facilities. We are also thankful to University Grant Commission, Ganeshkhind, Pune for providing funds for this research project.

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