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In vitro plasma absorption studies of essential oils from *Blumea eriantha* (Indian origin) using HSGC and GCMS

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Introduction

Traditionally, Indians are well known for using herbal extracts and essential oils for medicinal purposes. Now a days, some of these medicines have gained attention of people worldwide due to their proven fact of no side effects.

This poster gives glimpse of how chromatography can be useful to study behavior of these oils in blood plasma. *Blumea eriantha*, a wild herb (shown in Fig. 1), commonly known as '*Nimurdi*' in Inida, is known for its medicinal use for skin and kidney ailments. This essential oil has shown great antimicrobial activity against *Propionibacterium acnes, Staphylococcus epidermidis, Staphylococcus aureus* and *Streptococcus pyogenes*.[1] The activity of this essential oil in plasma is studied using Shimadzu GCMS-QP2010 Ultra along with Headspace (HT3) from Teledyne Tekmar (shown in Fig. 2). It is observed that certain components from *Blumea eriantha* oil show greater rate of absorption in plasma.

Experimentation

Extraction of essential oil

The entire upper portion including aerial part, stem and leaves of *Blumea eriantha* were collected from Seawoods, Navi Mumbai, Maharashtra, India during the March 2010. The authentication of plant was carried out at Agharkar Research Institute, Pune, Maharashtra, India. This plant material were chopped into small pieces and 750g was subjected to hydro distillation using clevenger type apparatus of capacity 5 L.

Fig. 1 Plant *Blumea eriantha*

Table 1 MIC and MBC of *Blumea eriantha* oil

	MIC (%v/v)	MBC (%v/v)
Propionibacterium acnes	0.39%	1.56%
Staphylococcus epidermidis	1.56%	6.25%
Staphylococcus aureus	0.19%	0.78%
Streptococcus pyogenes	0.09%	0.39%

Sample Preparation

Stock solution (15% v/v) of essential oil from *Blumea eriantha* was prepared in DMSO. For blank plasma sample, 1000µL of rabbit plasma was taken in headspace GC vial and was analyzed immediately on headspace GCMS. In two separate headspace vials, 1000 µL of blank plasma was spiked with 500 µL of above stock solution and the vials were sealed immediately. Out of the two vials one vial was analyzed other vial was kept at room temperature for 24Hrs and then analyzed. The conditions used for headspace and GCMS are given in Table 2. For antimicrobial activity study, MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concentration) values were measured using 'Well diffusion' method (shown in Table 1).

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Fig. 2 Head Space (HT3) and GCMS-QP2010 Ultra

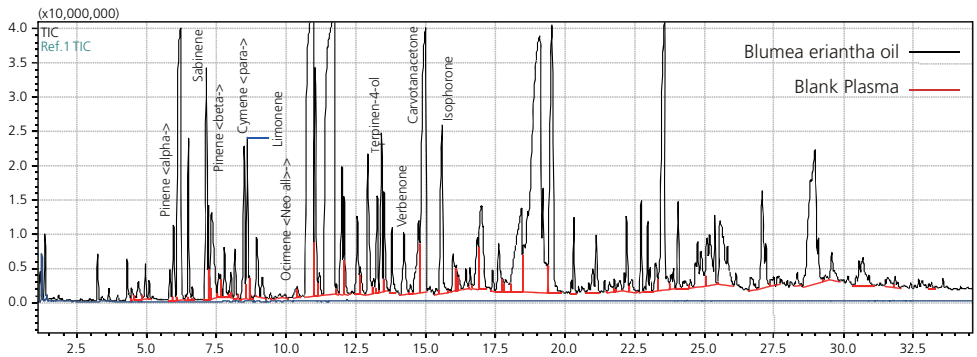

Fig. 3 *Blumea eriantha* essential oil zoom chromatogram overlaid with blank rat plasma

Instrumental Parameters

Table 2 Head Space (HT3) and GCMS conditions

Head Space Conditions	
Valve Oven Temp.	95°C
Transfer Line Temp.	110°C
Platen/Sample Temp.	80°C
Sample Equil.Time.	25.00 min
Pressurize	8 PSIG
Pressurize Time	2.00 min
Pressurize Equil.Time	0.20 min
Loop Fill Pressure	6 PSIG
Loop Fill Time	2.00 min
Injection Time	1.00 min

GC Conditions			MS Conditions	
Injection Temp.	266.00°C		Ion Source Temp.	200.00°C
Injection Mode	Split		Interface Temp.	260.00°C
Column Flow	0.93 mL/min		Solvent Cut Time	1.00 min
Split Ratio	40.00		Detector Gain	0.84
Oven T emp Program			Start *m/z*	40.00
Rate	Temperature (°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		

Results and Discussion

Plasma samples are non volatile in nature and most of the essential oils are volatile in nature. Headspace technique was found to be faster and better technique, than any other extraction method of analysis, for *in vitro* studies of such mixtures. *Blumea eriantha* oil was found to have more than 100 components (shown in Fig. 3), out of which, ten major components have shown greater rate of absorption in plasma over 24Hrs study. Following Table 3 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 *Blumea eriantha*.[2] The *in vitro* absorption of essential oil analysis in rabbit plasma has revealed the selective absorption of some of the components. This can be further confirmed by *in vivo* studies of these constituents using Headspace GCMS.

Table 3 Component area reduction after 24Hrs treatment of oil in rat plasma

No.	Name of Compound	RT in min	Area at 0Hr	Area at 24Hrs	Reduction in Area	%Reduction in Area
1	PINENE <ALPHA-> DB5-319	5.85	3270401	3025100	245301	7.50%
2	SABINENE DB5-379	7.14	37952345	33973478	3978867	10.48%
3	PINENE <BETA-> DB5-386	7.22	8679755	8201496	478259	5.51%
4	CYMENE <PARA-> DB5-471	8.49	28317366	24669575	3647791	12.88%
5	LIMONENE DB5-481	8.61	21025197	16947795	4077402	19.39%
6	OCIMENE <NEO-ALLO-> DB5-731	9.60	1022982	784380	238602	23.32%
7	TERPIN-4-OL DB5-820	12.92	1803519	1543957	259562	14.39%
8	VERBENONE DB5-894	13.80	432819	371237	61582	14.23%
9	CARVOTANACETONE DB5-994	14.83	2156584	1488121	668463	31.00%
10	ISOPHORONE DB5-675	15.51	2805367	2357188	448179	15.98%

Conclusion

Headspace coupled with GCMS is fast and reliable tool for analysis of volatiles in complex matrices. During *in vitro* study of essential oil in rat plasma, extraction of oil is difficult, time consuming and process losses may lead to wrong interpretation, which are minimised using headspace GCMS. Present study indicates that, among hundreds of components present in essential oil, only few components show higher activity in plasma. This technique can be further used to study dynamics of individual components in plasma and their selectivity against various microbes.

References

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