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ASSESSMENT OF HEATING EFFECT ON CHEMICAL CONSTITUENTS OF MUSTARD OIL THROUGH HPTLC PROFILING

A, Singh[a]*, P. Raghaw [a], B. Dwivedi[a], R. Singh[a], P.Pant[a], M.M.Padhi[a]

[a]Central Council for Research in Ayurvedic Sciences, Janakpuri, Delhi-110058.

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***Correspondence for Author**
Dr. A, Singh
Central Council for Research in Ayurvedic Sciences, Janakpuri, Delhi-110058.

ABSTRACT

Several studies have been carried out for chemical analysis of mustard oil, even chemical composition/ fatty acid composition of mustard has been established by using chromatographic and spectroscopic methods. As being essential food commodity, in Indian kitchens it is usually heated up for preparation of a variety of food stuffs. Owing to excessive heat or prolonged continuous boiling, the chemical composition may be altered or the constituents of nutrient value may be decomposed or destroyed. In the present study the effect of heat/boiling on the chemical composition has been assessed. Authentic oil was heated up to 400^{o}C, followed by collection of sample at every 20^{o}C interval. Then the samples so collected were subjected to the HPTLC study. HPTLC profiles were compared with each other as well as with the bands observed in the unheated sample. Some of the bands which were present in the unheated sample were absent after heating at 300^{o}C and above, likewise some new bands also appeared after a certain temperature range.

KEYWORDS: Mustard oil, HPTLC plates, phosphomolybdic acid.

INTRODUCTION

Mustard oil is one of the most widely consumed oils because of its culinary, religious and medicinal properties since time immemorial [2]. Mustard oil is placed prominently in the Indian system of medicine and its medicinal properties are well documented in the classical *Avurvedic* texts [1, 3]. This oil is being used in cooking for the last several centuries where it is usually heated up to its boiling point but hardly any attempt has been made to assess the effect of temperature on the composition of oil using simple analytical tools. It's pharmacopoeial standards have been laid down in Aurvedic pharmacopoeia of India however

no HPTLC study is carried out. Most official pharmacopoeias use reverse phase HPTLC techniques which are a bit expensive for day to day industrial uses [4]. In the present study effect of heat/boiling on **chemical composition**/ FAC has been assessed. Normal phase thin layer chromatography is used to study the effect of temperature on composition of mustard oil.

MATERIALS AND METHODS

Experimental

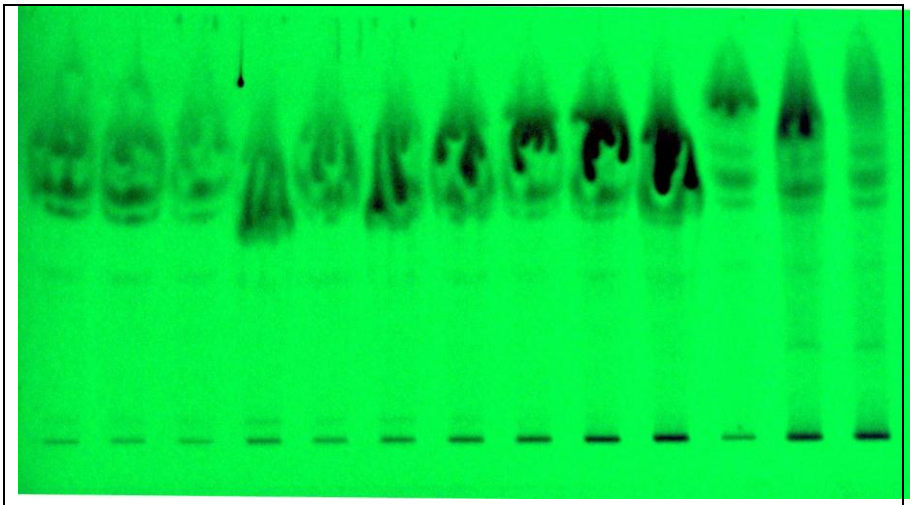
Mustard oil was procured from the local market. 200ml of this oil was heated up to 400^{o}C, using controlled heating followed by collection of samples at every 20^{o}C interval. The temperature was measured using an infrared thermometer (Mextech) at the ambient temperature of 30^{o}C. The sample collection was started from 50^{o}C. 10 ml oil was taken out in a separate vial and marked with the sample id. These samples were subjected to HPTLC study.

Sample Preparation and HPTLC Analysis

15 milligram of each sample was dissolved in 2 ml of hexane separately in eppendorf tube, 8 µl of each sample was applied on Merck Aluminum plate pre-coated with silica gel 60F_{254} of 0.2mm thickness by linomat IV applicator. The plate was developed in solvent system of Toluene: Ethyl acetate:: 90:10. The plate then air dried and visualized under UV 254 & 366nm. The plate was derivatized in phosphomolybdic acid (in alcohol) reagent and heated at 105^{o}C till the colour of the spot appeared on visualization under white light. In HPTLC profile, Rf values of heated sample's bands were compared with that of unheated sample.

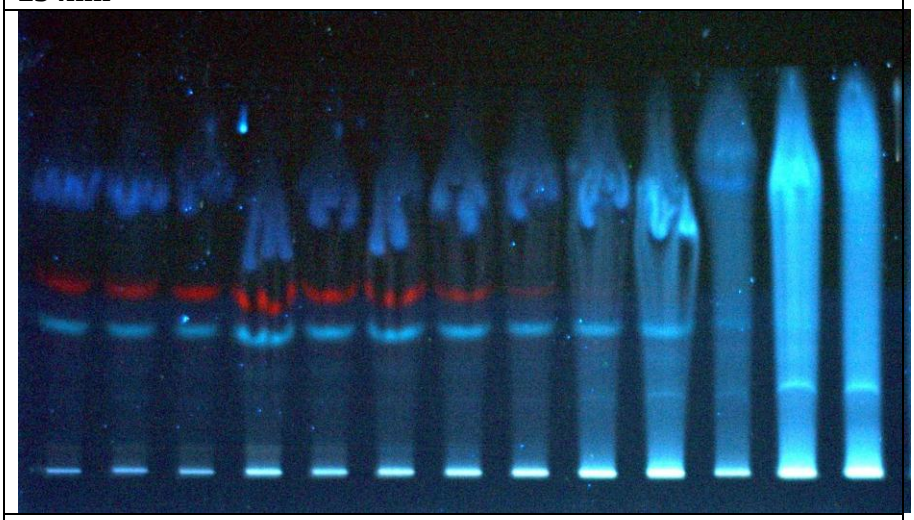
RESULT AND DISCUSSION

The study shows that the HPTLC pattern of mustard oil has been developed on normal phase silica plates. The plate was observed in UV light at 254, 366nm and after derivatization which yielded significant information. In HPTLC profile a number of bands appeared at different R_f under UV 254, 366 & after derivatization. At 254nm two bands, at 366nm, two different bands and three bands in white light after derivatization were observed in majority of heated samples. The details of band's colour & R_f values are given in **Figure.1a, 1b &1c and Table.1** respectively. Inferences are also summarized in the **Table.2.**



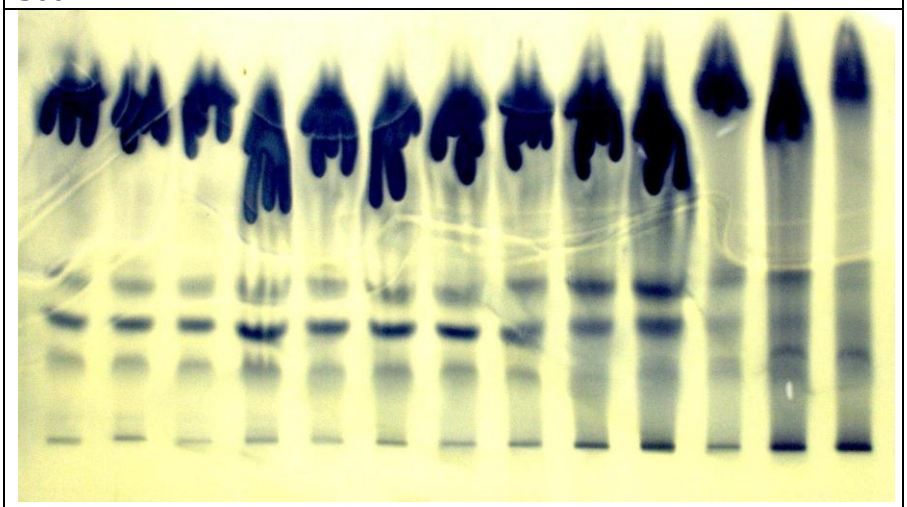
1 2 3 4 5 6 7 8 9 10 11 12 13

Figure 1a: HPTLC fingerprint of profile of mustard oil at 254nm



1 2 3 4 5 6 7 8 9 10 11 12 13

Figure 1b: HPTLC fingerprint of profile of mustard oil at 366nm



1 2 3 4 5 6 7 8 9 10 11 12 13

Figure 1c: HPTLC fingerprint of profile of mustard oil after derivatization

Sample, mustard oil in hexane (8µl); Toluene: Ethyl acetate:: 90:10		
Sample id: heating temperature	**Sample id: heating temperature**	**Sample id: heating temperature**
1. Normal 2. 150°C 3. 200°C 4. 220°C 5. 240°C	6. 260°C 7. 280°C 8. 300°C 9. 320°C 10. 340°C	11. 360°C 12. 380°C 13. 390°C

Table 1: R_f Values of mustard oil in hexane.

Wave Length(nm)	Temperature(°C)												
	Normal	150	200	220	240	260	280	300	320	340	360	380	390
254	0.04, 0.36	0.04, 0.36	0.04, 0.36	0.04, 0.36	0.04, 0.36	0.04, 0.36	0.04, 0.36	-	-	-		0.22, 0.36	0.22, 0.36
366	0.32, 0.42	0.32, 0.42	0.32, 0.42	0.32, 0.42	0.32, 0.42	0.32, 0.42	0.32, 0.42	0.32, 0.42	0.20, 0.32	0.20, 0.32	0.20, 0.32	0.20	0.20
Derivatized in white light	0.17, 0.27, 0.38	0.17, 0.27, 0.38	0.17, 0.27, 0.38	0.17, 0.27, 0.38	0.17, 0.27, 0.38	0.17, 0.27, 0.38	0.17, 0.27, 0.38	0.17, 0.27, 0.38	0.17, 0.27, 0.38	0.17, 0.27, 0.38	0.17, 0.27, 0.38	0.23, 0.27, 0.38	0.23

Table 2: Inference.

Wavelength	Band Position(Rf)	Inference
254nm	Band at R_f 0.04 which was present in the normal oil, started to disappear after 300°C Band at R_f 0.36 which was present in the normal oil, started to disappear after 380°C	Some of the heat labile components at R_f 0.04 and 0.36 started to degrade after being heated at mentioned temperature
366 nm	Band at R_f 0.32 which was present in the normal oil, started to disappear after 340°C Band at R_f 0.42 which was present in the normal oil, started to disappear after 320°C	Some of the temperature labile components at R_f 0.32 and 0.42 started to degrade after heated at mentioned temperature
	Band at R_f 0.20 started to appear after 300°C which was not present in the normal oil	Some new degradation product started to appear at R_f 0.2 after heated at mentioned temperature
Derivatized in white light	Band pattern started to fade away after 340°C	

Effect of temperature on HPTLC profile is clearly visible as some bands have started to disappear after 300°C, it seems either these were volatile compounds which volatilized during the course of heating or were heat sensitive compounds which degraded as the temperature was raised.

CONCLUSION

It is clear that the HPTLC pattern can be developed on normal phase silica plates. It is also lucidly conclusive that excessive heating degrades some of the phytochemical constituents of oil. Further study may be taken up to ascertain the nature of compounds which are heat labile.

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REFERENCES

1. Ayurvedic Pharmacopoeia of India, Dept. of AYUSH, Ministry of Health and Family Welfare, Government of India, Part-I, Volume-VI, First Edition, 2008; 220-221.
2. Anthelmintic activity of castor oil and mustard oil, Journal of Phytology, 2011; 3(3):12-14.
3. Mustard and its uses in Ayurveda, Ram Manohar P, Reshmi Pushpan & Rohini S, Indian Journal of Traditional Knowledge, 2009; 8(3):400-404.
4. European Pharmacopoeia 6.0, Council of Europe, Strasbourg, 2008; 106.