British Journal of Pharmacy

https://doi.org/10.5920/bjpharm.1391

British Journal of Pharmacy

www.bjpharm.hud.ac.uk

Proceedings of the 14th APS International PharmSci 2023

Development, characterisation of menthol based non-alcoholic propolis extracts and its effect on flavonoid composition of propolis analysed using RP-HPLC

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ARTICLE INFO

Received: 31/07/2023
Revised: 15/08/2023
Accepted: 18/08/2023
Published: 30/12/2023

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KEYWORDS: Propolis; non-alcoholic extract; RP-HPLC ; flavonoids;

SUMMARY

Propolis had been used as anti-inflammatory, immunomodulatory, antibacterial and antiviral agent since ages and proven to cure wounds, sore throats and colds. Propolis show poor solubility, hence several methods have been applied to improve its solubility in water, oil and waxy mixtures, as ethanolic extracts have its limitation to be applied in the pharmaceutical and cosmetic industry. To address this issue menthol based propolis extract in combination with lactic acid and salicylic acid was developed and its chemical composition was analysed. HPLC of menthol based non -alcoholic extracts indicated the presence of active ingredients similar to alcoholic extract. These solvents find their application in the cosmetic and pharmaceutical industry as topical formulations, as menthol enhances the permeability of the active ingredients.

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INTRODUCTION

This study aims to develop menthol based non-alcoholic extract of propolis in combination with salicylic acid, lactic acid and propylene glycol .The chemical make-up and biological characteristics of propolis have received a lot of attention from researchers all over the world in recent decades. Propolis has a highly intricate chemical profile that differs with respect to many of variables like plant source, season of harvest, location, kind of bee flora, climate variations, and honey bee species at the time of harvest.(A. and F. N. 2007)

Propolis is most frequently used as an immune stimulant, as a tool to fight off colds due to its antibacterial and antiviral action, as a natural treatment for skin issues due to its calming and healing effects, as well as in the oral cavity to treat small ulcers and canker sores, to relieve redness and itching of the urinary tract, and finally to restore the balance of the gastric mucosa(Hossain et al. 2022).The elements flavonoids, phenolic acids, and their esters are in charge of this activity.(Silva* and 2019).

MATERIALS AND METHODS

All standards and chemicals such as menthol, salicylic acid, lactic acid, propylene glycol and chemical reagents for HPLC were from Merck Pvt Ltd. Propolis raw samples were provided by Natures Laboratory Ltd.

CHROMATOGRAPHIC CONDITIONS

The HPLC method used here has already been reported by (D. G. Watson 2006). The HPLC Analysis was carried out using a Chrom-master (Hitachi) HPLC system The system was fitted with an ACE 5 C_{18} column (250 × 4.6 mm i.d., 5 µm) and a security guard cartridge. The following linear

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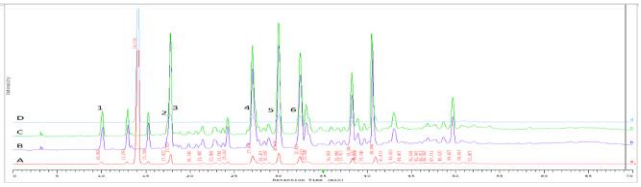
gradients of mobile phase methanol (solvent A) and 0.1% formic acid (solvent B) at a flow rate of 1 mL/min and 10 min equilibration time was used between runs. The chromatogram was monitored at 290 nm. (Soumaya Touzani et al. 2021)

RESULTS AND DISCUSSION

Non – alcoholic extracts were prepared by combining 1 mole menthol with 1 mole lactic acid or salicylic acid along with 0.5 mole propylene glycol (B&D) and then propolis was added and heated at 50°C about 20mins until a clear solution is formed. Later the sample was filtered and injected into HPLC system for analysis.

Alcoholic extracts of propolis samples showed the presence of Ferulic acid, Quercetin, Cinnamic acid, Naringenin, Chrysin, Galangin, Pinocembrin while other biomarkers such as Gallic acid, Rutin, CAPE, p-coumaric acid, chlorogenic acid were not found in these samples.



1-Ferulic acid, 2- cinnamic acid, 3- Naringenin, 4- Pinocembrin, 5-Chrysin, 6- Galangin.

Fig. 1. The relative chromatograms of non- alcoholic extracts a)MSD b)MLA c)MLA- PG d)MSD-PG

Sample A has missing Ferulic acid whereas sample B and C had missing cinnamic acid, and all samples had missing Quercetin

Sample	Ferulic acid (µg/g)	Quercetin (µg/g)	Cinnamic acid (µg/g)	Naringenin (µg/g)	Pinocembrin (µg/g)	Chrysin (µg/g)	Galangin (µg/g)
A	0	0	3103.16	3931.4	44095.5	5396.9	8151.81
B	593.9	0	0	2428.5	21983.6	2835.83	4560.17
C	583.74	0	0	1981.23	23025.14	2882.97	4501.12
D	1033.43	0	2206.14	3380.32	43227.26	5090.88	8077.23
Control	6141.82	4595.8	16430.32	11465.11	167665.6	28668.35	48663.74

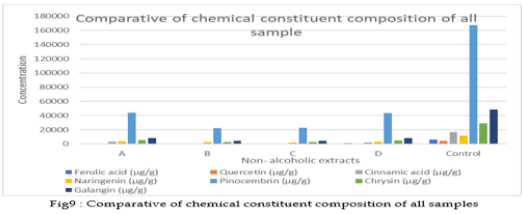


Fig9 : Comparative of chemical constituent composition of all samples

A-msd4, B- mla4 C – mla5, D- msd5, Control- alcoholic extract

Fig 2: Quantification of chemical constituents present in control and non alcoholic extracts.

Ferulic and cinnamic acid are agents that cause skin inflammation and irritation, itching, of skin which are among the reported side effects of propolis. As these chemical constituents are missing the extracts these show potential application in cosmetic industry.

CONCLUSIONS

The non-alcoholic extract of propolis depicted the presence of active ingredients similar to alcoholic extract. The menthol base non -alcoholic solvents can be applied in pharmaceutical and cosmetic industry as they are proven to enhance permeability and are not harmful as ethanolic extracts.

ACKNOWLEDGEMENTS

I would like to acknowledge University of Bradford, for providing this opportunity and support for the smooth conduct of research and also extend my gratitude to Nature's laboratory Ltd for providing the resources needed and their supervision and contribution.

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