

BIOACTIVE PHYTOCHEMICALS FROM MARIGOLD

by

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Centre for Rural Development and Technology

Submitted

in fulfilment of the requirements of the degree of Doctor of Philosophy

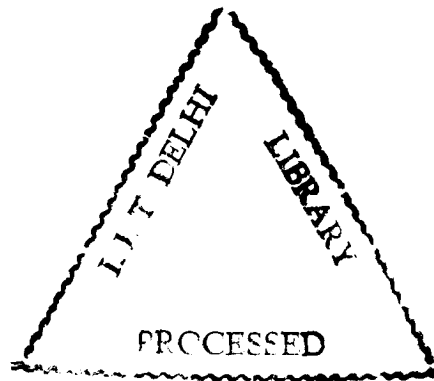
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CERTIFICATE

This is to certify that the thesis entitled, "BIOACTIVE PHYTOCHEMICALS FROM MARIGOLD" being submitted by Mr. D. Vidya Sagar Swamy to the Indian Institute of Technology, Delhi for the award of Doctor of Philosophy is a record of bonafide research work carried out by him under our guidance and supervision in conformity with the rules and regulations of Indian Institute of Technology, Delhi.

The research report and results presented in this thesis have not been submitted, in part or in full, to any other university or institute for the award of any degree or diploma.


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ACKNOWLEDGEMENT

Gratitude can seldom be expressed in words.

Beginning with the formulation of research problem, till date I have been especially privileged one to receive guidance from my supervisors Prof. Padma Vasudevan and Dr. S. N. Naik whose academic excellence and constant encouragement steered me through the work all the way and all the time. I would seize the opportunity to express my deep sense of gratitude for their motivational urge, valuable analysis, criticism and personal affection which instilled in me immense confidence to continue my research right from the beginning of my research work till the accomplishment of the goal.

I am extremely grateful to Professor Y. R. Rao, RRL Bhubaneshwer for contributing valuable suggestions and providing the GCMS facility.

With profound respect, I wish to express deepest sense of gratitude to Prof. Harro Lentz, Dr. Zimmermann, Dr. P. K Mittal, Dr. Nambiar, Dr. Sateesh kumar. Dr. C. K. Katiyaar and Dr. Mahapatra for the help they provided during the research.


I am grateful to Prof. Rajendra Prasad, Prof. R.C. Maheshwari, Prof. Santosh, Dr. V.P.S. Sorayan, Dr. V.K. Vijay, Dr. Satywati Sharma, Dr. Dilbagh Kaur, Dr. S.K. Jain, laboratory and office staff of the Center for providing lab facilities and timely requirements for my research work.

When one owes so many, it is almost impossible and even invidious to single out names. However, I am indebted to my friends Vivek, Prabhat, Anshu, Anuja, Arpita, Chitra, Padma, Munna, Srini, Vimal, Tarun and P.K. Rout for their constant encouragement and wholehearted moral support without which it would have been difficult to finish this task.

I feel pleased to acknowledge the love, affection and support received from Mr and Mrs. Venu Gopal, Mr. And Mrs. Vijay Krishana, K.R.B. Reddy, Kiran, Ranga Rao, Ravi, Rishi, Sharat, Supraj, Lekha, Mamun and Ms. Jyothi Manjari Naik.

I express my sincere thanks to Ramar, Rooplal, Lala Ram, Kuldeep Singh, Badri Prasad, Ram Kumar, Ramesh, Govind Hanuman Singh for their co-operation. Thanks are also due to my friends particularly D. K. Mishra, Aravind, Ganesan, Mohanty, Tony, Dhananjay, Mohini, Suman, Madhu and Neetu.

Lastly, I am ever indebted to my parents and all my family members especially to my sister (Madhavi), Brother-in-law (Dr. Sampath) and beloved Sharat Chandra whose blessings, love and moral support brought me here up to.


(D. Vidya Sagar Swamy)

ABSTRACT

*Floriculture is an age-old farm activity with immense potential for generating remunerative self-employment among small and marginal farmers. In Haryana, a state in North India, farmers cultivate marigold (*Tagetes erecta* and *Tagetes patula*) as a major crop in a multi crop system by rotating it with other agricultural and horticultural crops like tomatoes and other vegetables. Currently, the cropping area covers around 1600 hectares of land in the Farrukhnagar block. The flowers are generally sold in the nearby markets in Delhi and are being used for decorative, ornamental, social and religious purposes. For assessing the prevailing situation, a field survey was done in the Farrukhnagar block of district Gurgaon, Haryana. The survey indicated that during the flowering season there is a sharp day to day fluctuations in the price offered for the flowers, thus affecting the net economic returns. Due to non-uniform flowering and market fluctuations more than 30-50% of flowers are left in the field. Often, after transporting to the market the farmers are not able to sell their produce and have to throw their unmarketed flowers, resulting in losses in transportation and labor wages. Due to a sudden plunge in market price there are times when the farmers do not even get compensated for labour and transport charges and suffer great losses. In the light of this, it was felt that better returns with value addition to the farmer could be ensured if the crop is exploited for its multiple applications. All the plant parts (leaves, root, stem and unmarketed flowers) may be used for the extraction of phytochemicals. Hence, it was decided to conduct the relevant experiments with the flowers and other plant parts (leaves and herbage).*

A distinguishing feature of T. patula flower and leaf is the presence of conspicuous brown-black essential oil glands, which on steam/hydro distillation yield essential oil. The presence of fragrance in the essential oil makes the flowers useful for commercial applications. Scented flowers are the natural sources for extraction of perfumes and flavors. These extracts may be in the form of concrete, absolute, attar, essential oil etc. Experiments were hence carried out to assess the quality and quantity of essential oil from the fresh, shade dried and sun dried flowers and leaves; from fresh herbage (end crop); and of concrete and carotenoid pigments from the fresh and dried flower petals.

Hydro-distillation was done with plant materials dried to different extent. Fresh leaves have a higher amount of essential oil content (0.24%) in comparison to flowers (0.05%). One ton of fresh herbage (end crop) was also extracted in a large-scale (1 ton capacity) steam distillation unit. A yield of 1.5 litre per ton was obtained.

Solvent extraction of fresh flowers was carried out in 25-litre volume glass jar with n-hexane by cold percolation to determine the percent of crude extract. Batch and column studies with dried plant material of different particle sizes (100-300 μ and 500 μ) using n-hexane were conducted to determine the oleoresin content. Cold percolation of fresh flowers yielded 0.3 % concrete. Soxhlet and column extraction of dried crushed petals yielded in the range of 7.9-9% and 8.0-8.8%, respectively.

The essential oil was identified by GC/MS. The oil components are a mix of several mono and sesqui-terpenes. Out of 68, 74, and 69 components from fresh, shade and sun dried flower 45, 43 and 42 compounds were identified, respectively. Similarly, in the fresh, shade and sun dried leaf, out of 64, 48 and 71 components 44, 33 and 42 were identified. Thus the identified of components accounts in the range of 85 to 95 %. The terpenoid profile of oil from leaf and flower were similar. The major components in flower essential oil were Piperitone (34.6%), limonene (10.9%), Terpinolene (7.7%), E -caryophyllene (5.4%) and Z and E B-Ocimene (4.2% and 4.9%). In the leaf essential oil the major components were Piperitone (41.6%), limonene (12.6%) Terpinolene (15.5%), E -caryophyllene (1.96%) and Z and E β -Ocimene (2.3% and 3.5%). Concrete was analyzed by HPLC, HPTLC, NMR, IR, UV and Mass spectral analysis. Carotenoid pigments were identified as lutein and xanthophyll. Lutein and xanthophyll are found in the form of its fatty ester. The fatty acids were further characterized by gas chromatography and found to be palmitate and steric.

"In vitro" biocidal studies with essential oil obtained from herbage were conducted against various plants and human pathogenic microorganisms by "Zone of inhibition method". Bacteriostatic and fungistatic experiments against plant and human pathogens with different concentration (1:1000, 1:100, 1:10, 2:10, 3:10, 4:10, 5:10, 6:10, 7:10, 8:10, 9:10) essential oil: ethanol and also with 40, 50 and 60 μ l essential oil showed a dose dependent decrease with all the microorganisms. E. coli and B. megatarium were inhibited even at dilutions as low as 1:100 while for the other bacterial strains 1:10 was the minimum dilution at which the growth was inhibited. The fungal strains P. funiculosum and F. solani were inhibited at

1:1000 dilutions and T. viridie was completely inhibited at 60 µl of essential oil. Also, larvicidal studies against fourth instar larvae of Aedes aegypti, Anopheles stephensi, and Culex quinquefasciatus showed the lethal concentration for 50% mortality (LC₅₀) after 24 hours was 12.0, 13.6 and 22.8 ppm, respectively.

Cost benefit analysis, based on prevailing costs, shows that small-scale steam and solvent extraction units, which could be run on a cooperative basis at the village level, are technoeconomically viable. By introducing such units, all the plant parts and un-marketed flowers can be utilized to ensure value addition to the farmer by fully exploiting this crop for its multiple applications. Based on the survey and experimental findings, an economically remunerative technology package has been proposed for small and marginal farmers.

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Appendix

Biodata