Annexure IX

UNIVERSITY GRANT COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI-110002

Final Report of the work done on the, Minor Research Project

1. TITLE OF THE RESEARCH PROJECT: 'Pharmacognostic and phytochemical standardization and anticancer studies on the leaves of *Aegle marmelos* corr.'

2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR: Dr. Rajbir Bhatti, Assistant Professor, Dept of Pharmaceutical Sciences, Guru Nanak Dev University , Amritsar

3. NAME AND ADDRESS OF THE INSTITUTION: Dept of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar.

4. UGC APPROVAL LETTER NO AND DATE : F.No. 41-1370/2012 (SR), 30 Jul, 2012

5. DATE OF IMPLEMENTATION: 1-08-12

6. TENURE OF THE PROJECT: 01-08-2012 to 01-08-2014

7. GRANT ALLOCATED: Rs. 1,55,000/- (Rupees One Lakh fifty five thousand only)

8. TOTAL GRANT RECEIVED: Rs. 1,02,500/-

9. FINAL EXPENDITURE: Rs. 92755/- (Ninety two thousand seven hundred fifty five only)

10. TITLE OF THE PROJECT: 'Pharmacognostic and phytochemical standardization and anticancer studies on the leaves of *Aegle marmelos* corr.'

11. OBJECTIVE OF THE PROJECT : The current study was designed the objectives of morphological and histological study of the leaves of *A. marmelos*, preliminary phytochemical studies of the extracts, evaluation of the effect of leaf extracts of *Aegle marmelos* for anticancer activity, bioactivity derived fractionation of the most active extract of *Aegle marmelos,* HPTLC studies on the most active extract, attempt at PASS assisted design and synthesis of analogues of active phytoconstituents.

12. WHETHER OBJECTIVES WERE ACHIEVED: Yes (Details attached in summary)

13. ACHEIVEMENTS FROM THE PROJECT: The project was helpful in establishing the pharmacognostic parameters of *Aegle marmelos* leaves. The grant helped in completing the Ph.D. of the PI. One research paper (attached). Bhatti R, Singh J, Saxena AK, Suri N, Ishar MPS. (2013). Pharmacognostic standardization and antiproliferative activity of Aeglemarmelos (L.) Correa leaves in various human cancer cell lines. **Indian Journal of Pharmaceutical Sciences** 75(6):628-634 [**Impact factor: 0.762]** has been published. Additionally, one presentation made in 100th Indian Science Congress at University of Calcutta, Kolkatta from Jan 3-7, 2013.

14. SUMMARY OF THE PROJECT: Attached herewith

15. CONTRIBUTION TO THE SOCIETY: The project was helpful in establishing the pharmacognostic parameters of *Aegle marmelos* leaves. *Aegle marmelos* is a well documented medicinal plant and also used in Ayurvedic formulations such as chavanparash. The project was undertaken to establish the pharmacognostic characteristics of the leaves of the plant and carrying out anti-proliferative studies on its extract. The ethanol extract and chloroform extract of the plant were found to have appreciable anti-cancer activity when tested on cancer cell lines. The results of the plant may be useful in carrying out further studies. One paper was published in the **Indian Journal of Pharmaceutical Sciences**.

16. WHETHER ANY PH.D ENROLLED/PRODUCED OUT OF THE PROJECT: Research proposal helped in completing the Ph.D. of the PI and Ph.D. was awarded by Guru Nanak Dev University, Amritsar in May 2015.

17. NO. OF PUBLICATIONS OUT OF THE PROJECT: **01** (attached)
Poster presentation: **01** (National, Indian Science Congress)

Signature of the PI

Registrar
Guru Nanak Dev University

UGC MINOR PROJECT REPORT

SUMMARY

Title: Pharmacognostic and phytochemical standardization and anticancer studies on the leaves of *Aegle marmelos* corr.

Principal Investigator: Rajbir Bhatti, Assistant Professor, Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar

Introduction:

Aegle marmelos (L.) Correa (Rutaceae) is a medicinal plant indigenous to Asia and is widely grown in the Indian sub-continent. The plant has been explored for a range of therapeutic activities including anti-hyperglycemic, lipid lowering, radioprotective and antimicrobial action. Cancer is prevalent in epidemic proportions world wide. The clinical management of cancer has seen a revolutionary change in the past decades and this has led to drastic improvement in the prognosis of cancer. However, the side effects of the currently available chemotherapeutic agents have led to a renewed interest in alternative medicines so as to provide an integrative strategy in cancer management. The hypothesis of synergy has been proposed to make the plant derived medicines as adjuvant or alternatives in managing chronic ailments such as cancer as the wide array of naturally occurring combinations of phytoconstituents on one hand kills the cancer cells and on the other hand provides nourishment to the surviving cells thereby reducing cancer cachexia. Furthermore, the availability of highly advanced techniques available for characterization and isolation of phytocontituents have made them appealing sources of new drugs. (Jesse *et al*., 2009). Taking into consideration, the huge potential of plant medicines, the World Health organization has outlined guidelines for the standardization of plant medicines. Literature reveals that useful criteria for identifying and authenticating a plant drug may be established by pharmacognostic standardization. Also, these parameters are helpful in the quality control of plant drugs (Bhaskar & Balakrishnan, 2010; Reddy et al., 1999; Srivastava et al., 2004, 2006).

Objectives:

The current study was designed the objectives of morphological and histological study of the leaves of *A. marmelos*, preliminary phytochemical studies of the extracts, evaluation of the effect of leaf extracts of *Aegle marmelos* for anticancer activity, bioactivity derived fractionation of the most active extract of *Aegle marmelos,* HPTLC studies on the most active extract, attempt at PASS assisted design and synthesis of analogues of active phytoconstituents.

Materials and methods:

Soxhlet extraction of the leaves was carried out using hexane, petroleum ether, chloroform and ethanol extracts of the shade dried leaves. The extracts were subjected to tests fo phytochemical characterization. Evaluation of anti-cancer activity was carried out using six human cancer cell lines including colon (CoLo-05), ovary (IGR-OV-1), lung (A-549), leukemia (THP-1), prostrate (PC3), and breast (MCF-7) cancer cell lines. Column chromatography of the most active extract was carried out for the purpose of bioactivity derived fractionation.

PASS assisted design: Two analogues of imperatorin were synthesized. 9-(4-Bromo-phenyl)-4-methyl-furo[2,3-*h*]chromen-2-one was synthesized by the following procedure. Acetophenone (0.05 M) dissolved in glacial acetic acid (20 ml) was taken and bromine (0.05 M) in acetic acid was slowly added with constant stirring in cool condition. The reaction mixture was then heated on water bath. When the colored lightened to straw yellow, the reaction was brought to room temperature and then added to ice cold water. The precipitate was obtained. It was filtered and recrystallized from ethanol. 7-Hydroxy-4-methyl coumarin was synthesized by dissolving resorcinol (0.18M) in ethyl acetoacetate (0.18 M) at 15˚C. conc. H_2SO_4 was added dropwise over 30 min with constant stirring. The precipitate so obtained was filtered and recrystrallized from methanol as cream colored needles. However, the analogues so synthesized did not show anticancer activity in cell lines.

Results and discussion:

The pharmacognostic standardisatrion was carried out and the morphological and microscopic features of the leaves of *Aegle marmelos* were undertaken. Preliminary phytochemical analysis revealed the presence of terpenoids, coumarins, alkaloids and anthraquinones in the ethanol and chloroform extract. The hexane and petroleum ether extract was found to contain tannins, terpenoids and reducing sugars. Maximal anti-proliferative activity was evident in the ethanol extract (AME) which showed maximum inhibition of 69% in colon and breast carcinoma cell lines at a dose of 100 μg mL^{-1}. The AME yielded five major fractions in column chromatography of which only three were found to be bioactive. The IC_{50} of AME-2, AME-4 and AME-5 leukemia cell line was found to be 12.5, 86.2 and > 100 μg mL^{-1} respectively. The high pressure thin layer chromatography of the most active fraction revealed the presence of furanocoumarin imperatorin.

Conclusions:

The study established the histological features of the leaves of *A. marmelos.* Phytochemical analysis led to the presence of alkaloids, anthraquinones, coumarins, glycosides, tannins, triterpenoids etc. in the most active i.e. ethanol extract of the leaves. Column chromatography was carried out to obtain five fractions (AME-1, AME-2, AME-3, AME-4 and AME-5) out of which only three were found to be bioactive. Maximum activity was evident against leukemia cell line and IC_{50} values of 12.5, 86.2 and > 100 μM respectively were obtained for AME-2, AME-4 and AME-5. The furanocoumarin imperatorin was identified in the HPTLC of AME-2.

References:

Bhaskar VH, Balakrishnan N. (2010). Pharmacognostic studies on *Pergularia daemia* roots. *Pharm Biol*, 48, 427- 432.

Bhatti R, Singh J, Saxena AK, Suri N, Ishar MPS. (2013). Pharmacognostic standardization and antiproliferative activity of *Aegle marmelos* (L.) Correa leaves in various human cancer cell lines.(2013). Indian J of Pharm Sci 75(6):628-634

Jesse W, Li H, Vaderas JC. (2009). Drug discovery from natural products: end of an era or an endless frontier. Science, 325, 161-165.

Reddy YSR, Venkatesh S, Ravichandran T, Suburatju T, Surseh B. (1999). Pharmacognostic studies of *Wrightia tinctoria* bark. *Pharm Biol*, 37, 291-295.

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