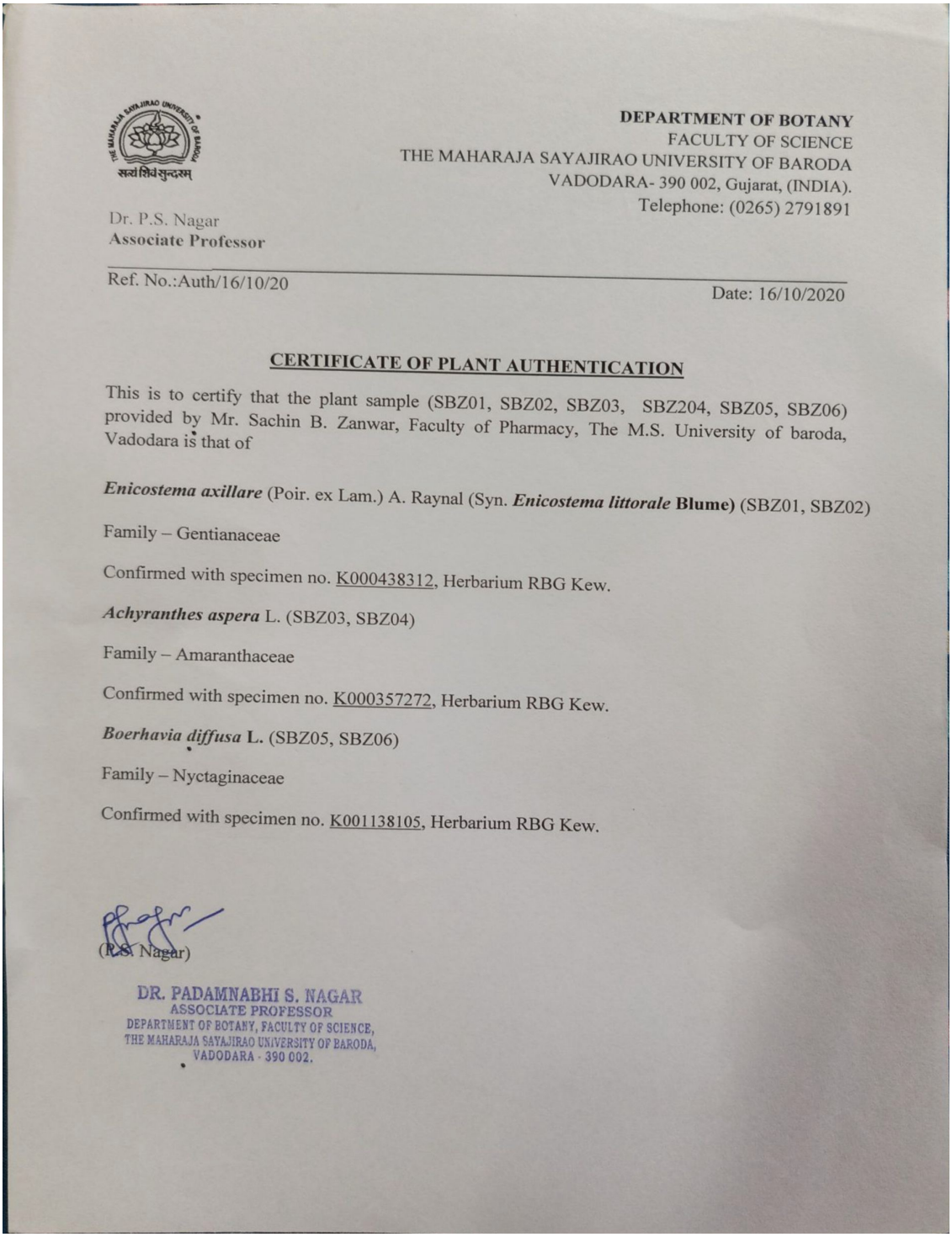
8. Annexure

8.1 Herbarium Certificate



DEPARTMENT OF BOTANY
FACULTY OF SCIENCE
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA- 390 002, Gujarat, (INDIA).
Telephone: (0265) 2791891

Dr. P.S. Nagar
Associate Professor

Ref. No.:Auth/16/10/20

Date: 16/10/2020

CERTIFICATE OF PLANT AUTHENTICATION

This is to certify that the plant sample (SBZ01, SBZ02, SBZ03, SBZ204, SBZ05, SBZ06) provided by Mr. Sachin B. Zanwar, Faculty of Pharmacy, The M.S. University of baroda, Vadodara is that of

Enicostema axillare (Poir. ex Lam.) A. Raynal (Syn. ***Enicostema littorale*** **Blume)** (SBZ01, SBZ02)

Family – Gentianaceae

Confirmed with specimen no. K000438312, Herbarium RBG Kew.

Achyranthes aspera L. (SBZ03, SBZ04)

Family – Amaranthaceae

Confirmed with specimen no. K000357272, Herbarium RBG Kew.

Boerhavia diffusa **L.** (SBZ05, SBZ06)

Family – Nyctaginaceae

Confirmed with specimen no. K001138105, Herbarium RBG Kew.

(P.S. Nagar)

DR. PADAMNABHI S. NAGAR
ASSOCIATE PROFESSOR
DEPARTMENT OF BOTANY, FACULTY OF SCIENCE,
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA,
VADODARA - 390 002.

8.2 Certificate of analysis for alcoholic extract of *A. aspera*

AMSAR PRIVATE LIMITED

47, Laxmibai Nagar, Industrial Estate, Fort, INDORE - 452006

Certificate of Analysis

1. Sample : **ACHYRANTHES ASPERA (APAMARG) STANDARDIZED DRY EXTRACT**
2. Batch No. : 2613..
3. No.F/D : 594
4. Supplier : AMSAR
5. Category : INTERMEDIATE
6. Date of Receipt in Lab : 08/10/2021
7. Date when test completed : 14/10/2021

8.	PROTOCOLS OF TEST APPLIED	STANDARD TO WHICH COMPARED	ACTUAL RESULT
1	DESCRIPTION	BROWN POWDER WITH CHARACTERISTIC ODOUR	COMPLIES
2	ORGANOLEPTIC TEST	BITTER TASTE	COMPLIES
3	WATER SOLUBLE EXTRACTIVES BY API METHOD TAKING 1 GM SAMPLE	NOT LESS THAN 70%	86.34%
4	SAPONINS	NOT LESS THAN 7%	7.36%
5	LOSS ON DRYING	NOT MORE THAN 8%	4.24%
6	ASH	NOT MORE THAN 20%	13.88%
7	BULK DENSITY	>0.6 gm/ml	0.76 g/ml
8	SIEVE TEST POWDER PASSES THROUGH 40 MESH	NOT MORE THAN 90%	COMPLIES
9	pH	4.5 - 7.5	6:15
10	HEAVY METALS		
	a) As	NOT MORE THAN 03PPM	COMPLIES
	b) Pb	NOT MORE THAN 10PPM	COMPLIES
	c) Cd	NOT MORE THAN 0.30PPM	COMPLIES
	d) Hg	NOT MORE THAN 1PPM	COMPLIES
11	MICROBIAL PROFILE		
	a) TOTAL PLATE COUNT	NOT MORE THAN 1000 CFU/G	370 CFU/G
	b) YEAST & MOULDS	NOT MORE THAN 100 CFU/G	ABSENT
	c) E.COLI	ABSENT	ABSENT
	d) SALMONELLA	ABSENT	ABSENT

Page # 1

Opinion of Analyst: The sample Conforms to the standard compared.

Statement: MANUFACTURING DATE : OCTOBER - 2021
EXPIRY DATE : 3 YEARS FROM THE DATE OF MANUFACTURE

FOR AMSAR PRIVATE LIMITED

Sample Tested by:

Report Authorised by:

Testing Chemist

Dr. (Mrs.) C. R. Bhatt
Q. C. MANAGER
Q C Manager

8.3 Certificate of analysis for alcoholic extract of *B. diffusa*

AMSAR PRIVATE LIMITED

47, Laxmibai Nagar, Industrial Estate, Fort, INDORE - 452006

Certificate of Analysis

1. Sample : **BOERHAAVIA DIFFUSA (PUNERNAVA) STANDARDIZED DRY EXTRACT**
2. Batch No. : 2699.
3. No.F/D : 680
4. Supplier : AMSAR
5. Category : INTERMEDIATE
6. Date of Receipt in Lab : 09/11/2021
7. Date when test completed : 15/11/2021

8.	PROTOCOLS OF TEST APPLIED	STANDARD TO WHICH COMPARED	ACTUAL RESULT
1	DESCRIPTION	BROWN POWDER WITH CHARACTERISTIC ODOUR	COMPLIES
2	ORGANOLEPTIC TEST	BITTER TASTE	COMPLIES
3	WATER SOLUBLE EXTRACTIVES BY API METHOD TAKING 1 GM SAMPLE	NOT LESS THAN 70%	82.72%
4	ALKALOIDS	NOT LESS THAN 0.1%	0.17%
5	HEAVY METALS		
	a) As	NOT MORE THAN 03PPM	COMPLIES
	b) Pb	NOT MORE THAN 10PPM	COMPLIES
	c) Cd	NOT MORE THAN 0.30PPM	COMPLIES
	d) Hg	NOT MORE THAN 1PPM	COMPLIES
6	MICROBIAL PROFILE		
	a) TOTAL PLATE COUNT	NOT MORE THAN 1000 CFU/G	340 CFU/G
	b) YEAST & MOULDS	NOT MORE THAN 100 CFU/G	ABSENT
	c) E.COLI	ABSENT	ABSENT
	d) SALMONELLA	ABSENT	ABSENT

Opinion of Analyst: The sample Conforms to the standard compared.

Statement: MANUFACTURING DATE : NOVEMBER - 2021
EXPIRY DATE : 3 YEARS FROM THE DATE OF MANUFACTURE

Sample Tested by:
Testing Chemist

Report Authorised by:

FOR AMSAR PRIVATE LIMITED
Dr. (Mrs.) G. R. Bhatt
Q. C. MANAGER
Q C Manager

Page # 1

8.4 Certificate of analysis for alcoholic extract of *E. littorale*

AMSAR PRIVATE LIMITED

47, Laxmibai Nagar, Industrial Estate, Fort, INDORE - 452006

Certificate of Analysis

1. Sample : ENICOSTEMMA LITTORALE (MAMEJAVA) STANDARDIZED DRY EXTRACT
2. Batch No. : 2473.
3. No.F/D : 453
4. Supplier : AMSAR
5. Category : INTERMEDIATE
6. Date of Receipt in Lab : 17/08/2021
7. Date when test completed : 23/08/2021

8.	PROTOCOLS OF TEST APPLIED	STANDARD TO WHICH COMPARED	ACTUAL RESULT
1	DESCRIPTION	BROWN POWDER WITH CHARACTERISTIC ODOUR	COMPLIES
2	ORGANOLEPTIC TEST	BITTER TASTE	COMPLIES
3	ALCOHOL SOLUBLE EXTRACTIVES IN 90% ALCOHOL BY API METHOD TAKING 1 GM SAMPLE	NOT LESS THAN 50%	52.64%
4	BITTERS	NOT LESS THAN 4%	4.72%
5	HEAVY METALS		
	a) As	NOT MORE THAN 03PPM	COMPLIES
	b) Pb	NOT MORE THAN 10PPM	COMPLIES
	c) Cd	NOT MORE THAN 0.30PPM	COMPLIES
	d) Hg	NOT MORE THAN 1PPM	COMPLIES
6	MICROBIAL PROFILE		
	a) TOTAL PLATE COUNT	NOT MORE THAN 1000 CFU/G	360 CFU/G
	b) YEAST & MOULDS	NOT MORE THAN 100 CFU/G	ABSENT
	c) E.COLI	ABSENT	ABSENT
	d) SALMONELLA	ABSENT	ABSENT

Opinion of Analyst: The sample Conforms to the standard compared.

Statement: MANUFACTURING DATE : AUGUST - 2021
EXPIRY DATE : 3 YEARS FROM THE DATE OF MANUFACTURE

Sample Tested by:

Report Authorised by:

Testing Chemist

FOR AMSAR PRIVATE LIMITED

Dr. (Mrs.) C. R. Bhatt
Q. C. MANAGER

Q C Manager

Page # 1

8.5 IAEC approval

IOM

13th September, 2021

Institutional Animal Ethics Committee
(I A E C)-SPARC Ltd.

To,
Mr. Sachin Zanwar,
Manager - II,
Pharmacology Dept., SPARCL,
Tandalja, Vadodara - 390020

Subject: Approval of Research Proposal

Dear Mr. Sachin,

The following Research Proposal of yours has been approved in the 72nd IAEC Meeting held on 21st August, 2021.

Sr. No.	IAEC Project Title	IAEC Project Code Allotted
1	Pharmacodynamic Evaluation of Test Items/Reference Items/ Formulations in Different Rodent Models of Cancer	908

Best Regards,

13.09.21

Dr. Satish Panchal
Member Secretary - I A E C
SPARC Ltd, Vadodara.

8.6 Published paper (JNR)

JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/36504
RESEARCH ARTICLE

In Vitro and *In Vivo* Anticancer Activity of *E. littorale* Extract on Hepatocellular Carcinoma

Sachin B. Zanwar[1,2], Kirti V. Patel[2*] and Sanjay N. Mandhane[3]

[1]Preclinical Pharmacology, Sun Pharma Advanced Research Company Limited (SPARCL), Savli GIDC Estate, Manjusar, Vadodara - 391775, Gujarat, India
[2]Department of Pharmacology, Faculty of Pharmacy, Kalabhavan Campus, The Maharaja Sayajirao University of Baroda, Vadodara - 390001, Gujarat, India; kirti.patel-pharmacy@msubaroda.ac.in
[3]Rare Spring Therapeutics, Vadodara - 390012, Gujarat, India

Abstract

Enicostemma littorale Blume is a perennial herb of the Gentianaceae family. It has been noted that different parts of the plant have hepatoprotective, anti-ulcer, antioxidant, antibacterial, and hypoglycemic properties. The aim of the current study was to assess the anticancer potential of the plant *in vitro* as well as *in vivo* animal studies. First, we evaluated the cytotoxic effect of the extracts in HepG2 cells. The alcoholic extract exhibited concentration-dependent cytotoxicity. In HepG2 cells, the extract's anticancer activity was observed with an IC_{50} value of 373 ± 3.0 µg/mL. Following *in vitro* studies, the *in vivo* anticancer effectiveness of the extract was evaluated using a xenograft model. Vehicle-treated mice showed a time-dependent increase in tumor volume. Mice treated with the extract showed a decrease in tumor growth as compared to vehicle treated group, indicating the anticancer activity of the extract in tumor xenografts study. On day 21, a marked reduction in tumor volume was noted. The findings of the study suggest that the alcoholic extract of *E. littorale* is effective against hepatocellular carcinoma. To fully understand the anticancer potential of *E. littorale*, further research with specific phytoconstituents is required.

Keywords: Anticancer, Cytotoxicity, *E. littorale*, Hepatocellular Carcinoma

1. Introduction

Globally, Hepatocellular Carcinoma (HCC) is the primary liver cancer and has the third highest cancer-related death rate[1]. HCC is the seventh most frequently occurring cancer in the world[2]. Metastasis, recurrence, and the development of a new primary tumor with a poor prognosis are the three main causes of death for individuals with HCC[3]. The only proven possibly curative treatments for this cancer are percutaneous ablation, surgical resection, and liver transplantation in the early stages of the disease[4].

Targeted therapy is now restricted to sorafenib, lenvatinib, regorafenib, ramucirumab, and cabozantinib. Though these drugs have potential to improve patient survival, they face challenges of drug resistance and severe side effects. Among these, sorafenib is the first medication to receive FDA approval and is a vital treatment option for patients with advanced HCC[1,5]. However, hepatotoxicity with aberrant increase of aspartate transaminase and alanine transaminase has been recorded in 22–34 % of sorafenib-treated patients, which can result in medication discontinuation and treatment failure. Alternative and complementary medicine offer strategies to lessen side effects of the sorafenib[6]. So, there is a need to have an anticancer agent with hepatoprotective activity as an adjunct to enhance efficacy of sorafenib.

Enicostemma littorale belonging to Gentianaceae family has been utilized in traditional medicine to treat a number of diseases. In India, since many years it is been widely used to treat variety of diseases including filariasis, rheumatism, dropsy, swellings, and itching[7]. The plant is commonly called *Nagajihva* in *Ayurveda* and it has a strong bitter taste[8]. The tribal population of Gujarat uses the hot aqueous extract of *E. littorale*

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