Dr.Satej M. Patel
Opp.Renaissance hotel, chankyapuri road,
Nr. Kargil peyrol pump, off SG-Highway,
Ahmedabad–380060. info@satejglobalscience.com

SATEJ GLOBAL SCIENCE
Think Globally Act Locally

GSTNO: 24ARZPP5827C1ZD

GroLyfe
(Promotes Growth, Nurtures Life)

An Innovative formulation of Secondary Metabolites from Callus Extract of Aloe Vera & Tulsi plant.

Contents:

Each 1 ml. liquid contains: Aloe Barbandensis Miller Callus Extract
Ocimum Sanctum Callus Extract

Usage: 5 -10 drops once/twice daily or as directed by the Healthcare Professional

GroLyfe offers excellent results in the Prevention and treatment of following diseases:

- AIDS.
- Cancer.
- Insulin dependent Diabetes.
- Liver Cirrhosis.
- Chronic Kidney diseases.
- All Infection Diseases.

100% NATURAL

GroLyfe Liquid is an Innovative formulation of Secondary Metabolites from Callus Extract of Tulsi & Aloe-Vera Plant indigenously developed by us.

Secondary Metabolites are compounds produced in other metabolic pathways & although they may not be important for essential functioning of the plant but are responsible for defense purposes, regulate the metabolic activity within cells & oversee the overall development of the plant.

In human Life, these compounds are used as Medicines, Flavourings or Relaxing Drugs especially Essential Oils. Secondary Metabolites (around two lac compounds have been identified) are often classified in three major classes: Alkanoids, Terpenoids & Phenolics.

Some of the Prominent Examples are Alkaloids (Atropine, Morphine, Quinidine, Ephedrine, etc), Terpenes (Azadiriachtin, Artemisin), Flavonoids (Luteolin) & Tannins (Tannic Acid).

Grolyfe contains around 25-30 Secondary Metabolites which can be used in various Life-threatening conditions (AIDS, Cancer, RA & other Auto-Immune Diseases)

Grolyfe offers excellent results in Terminal illnesses & chronic disorders like AIDS, Cancer, RA, Diabetes or Dysfunctional Body Organs (Liver, Kidney, Pancreas, etc.)

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GSTNO: 24ARZPP5827C1ZD

As per some pre-clinical study based on animal model many therapeutic application is possible from Ocimum Sanctum Extract. Like

1). **Anti-Diabetic Properties**
2). **Cardio protective**
3). **Wound Healing Activity**
4). **Radio-Protective Effect**
5). **Highly Significant Recovery in Genotoxicity.**
6). **Antioxidant Properties**
7). **Hypolipidemic Activities.**
8). **Anti-microbial Properties.**
9). **inhibit the Transcriptional Expression of Genes.**
10). **Gastro protective Effect.**
11). **Immunomodulatory Effect.**
12). **Support the Central Nervous System.**
13). **Analgesic, Anthelmintic, Anti-inflammatory Activity**
14). **Anticancer Activity.**

Note:

Anti-Cancer In-vitro study & Antiviral Testing Reports attached herewith.
We can conduct more studies depending on our Target condition/Audience.
We are the first for launched this type of product. This time we have no any competitor in world.

A Report Of
In Vitro Anti-cancer activity of Ocimum Sanctum Callus Extract

:: Submitted To::
Satej Global Science
Suvas Apartment,
Behind Park Avenue Bunglow, Thaltej,
Ahmedabad-380059, Gujarat, India.

:: Submitted By ::

ACCUPREC
RESEARCH LABS PVT. LTD.
Ultimate solution...
FDCA LICENCE NO. GTL/37/31

Accuprec Research Labs Pvt. Ltd.
Opp. Zydus Pharmez, Changodar Bavla Highway,
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Ahmedabad - 382213, Gujarat, India.
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Report of "*In Vitro* Anti-cancer activity of *Ocimum Sanctum* Callus Extract"

Name of Product	*Ocimum Sanctum* Callus Extract
Method Followed	Cytotoxicity study by MTT Assay (ISO 10993-5:2009)
Testing Facility	Accuprec Research Labs Pvt. Ltd. Opp. Zydus Pharmez, Changodar- Bavla Highway, Nr. Matoda Patiya, Post- Matoda, Ahmedabad, Gujarat 382213, India.
Sponsor	Satej Global Science Suvas Apartment, Behind Park Avenue Bunglow, Thaltej, Ahmedabad-380059, Gujarat, India.
Study Period	4 Days
Turn around period	14 Days
Report Number	ARL/3994/2017
Report Date	04/11/2017

Prepared By (Sign/Date)	Reviewed By (Sign/Date)	Approved By (Sign/Date)
Ms. Kruti Upadhyay (Research Assistant)	Dr. Manish Rachchh (CEO)	Dr. Rina Gokani (CSO)
04/11/2017		04/11/2017

TABLE OF CONTENTS

I. STATEMENT OF COMPLIANCE

We, the undersigned hereby declare that the study entitled "*In Vitro* Anti-cancer activity of *Ocimum Sanctum* Callus Extract" was performed under our supervision in compliance with OECD principles of Good Laboratory Practices (OECD, 1998) & ISO17025. Characterization of the test material was performed by the sponsor. The objective laid down in the study protocol was achieved. No unforeseen circumstances were observed which might affect the quality or integrity of the study.

The report represents a true and accurate results obtained. We accept the responsibility for validity of the data, as well as the interpretation, analysis, documentation and reporting of the results.

The report comprises of total 16 pages and includes statement of compliance, quality assurance statement, study personnel detail, experimental design, results, discussion, conclusion, reference and period of archival.

Date: 04/11/2017

04/11/2017
Ms. Kruti Upadhyay
Asst. Study Director

04/11/2017
Dr. Manish Rachchh
Study Director

04/11/2017
Mr. Bhargav Gohel
DM, QA

04/11/2017
Dr. Rina Gokani
Q. A. Head

II. QUALITY ASSURANCE STATEMENT

This study report has been reviewed by the Quality Assurance Unit of Accuprec Research Labs Pvt. Ltd., for compliance with the OECD Principles of GLP & ISO 17025.

This statement confirms that the study report accurately reflects Study data. The summary of inspections performed during the course of the study is as follows:

Sr. No.	Type of Inspection	Date of Inspection	Phases of Study inspected
1	Study Based	01/11/2017	Preparation of test sample
2	Study Based	02/11/2017	Evaluation of cell Viability

Date: 04/11/2017

04/11/2017
Mr. Bhargav Gohel
DM, QA

04/11/2017
Dr. Rina Gokani
Q. A. Head

III. ABBREVIATION

GLP	- Good Laboratory Practice
Gm	- Gram
hr	- Hour
ISO	- International Organization for Standardization
Kg	- Kilogram
Mg	- Milligram
MSDS	- Material Safety Data Sheet
EDTA	- Ethylenediaminetetraacetic acid
IPA	- Isopropyl Alcohol
FBS	- Fetal Bovine Serum
MEM	- Minimum Essential Medium
NCCS	- National Centre for Cell Science

1. STUDY INFORMATION

Report No.	: ARL/3994/2017
Study Title	:"*In Vitro* Anti-cancer activity of *Ocimum Sanctum* Callus Extract".
Sponsor	: Satej Global Science Suvas Apartment, Behind Park Avenue Bunglow, Thaltej, Ahmedabad-380059, Gujarat, India.
Testing Facility	: Accuprec Research Labs Pvt. Ltd. Opp.Zydus Pharmez, Changodar- Bavla Highway, Nr. Matoda Patiya, Post- Matoda, Ahmedabad, Gujarat 382213, India
Name of Asst. Study Director	: Ms. Kruti Upadhyay
Sign of Asst. Study Director	: 04/11/2017
Name of Study Director	: Dr. Manish A. Rachchh
Sign of Study Director	: 04/11/2017
Name of DM - Q. A.	: Mr. Bhargav Gohel
Sign of DM - Q. A.	: 04/11/2017
Name of Q. A. Head	: Dr. Rina Gokani
Sign of Q. A. Head	: 04/11/2017

2. SUMMARY

The objective of the study now reported was to determine *in vitro* anti-cancer activity of "*Ocimum Sanctum* Callus Extract", by performing cytotoxicity test by MTT assay using Caco-2 cell line.

3. INTRODUCTION

3.1. OBJECTIVE

The study was conducted to establish *in vitro* anti-cancer activity of the *Ocimum Sanctum* Callus Extract using Caco-2 cell line.

3.2. TEST GUIDELINES:

Cytotoxicity test by MTT assay using Caco-2 cell line.

4. STUDY PERSONNEL

Study Director	**: Dr. Manish A. Rachchh**
Research Assistant	**: Ms. Kruti Upadhyay**
Research Assistant	**: Ms. Kanchan Khare**
Research Assistant	**: Mr. Amol Kharat**
DM - Q.A.	**: Mr. Bhargav Gohel**
Q. A. Head	**: Dr. Rina Gokani**

5. MATERIALS AND METHODS

5.1. TEST ARTICLE DETAILS

Test Article	*Ocimum Sanctum* Callus Extract
Storage Conditions	The test article was stored at room temperature.
Stability	The stability of the test article formulations, under the storage conditions used in this study, is the responsibility of the Sponsor.
Safety Precautions	Standard laboratory safety procedure was employed for handling the dose formulations. Specifically, gloves and eye protection were worn while administering doses.
Date of Initiation of Study	28/10/2017
Date of Completion of Study	02/11/2017

5.2. MATERIALS

Sr. No.	Name of material	Type	Make
A	Caco-2, Human epithelial cells	Cell line	NCCS
B	Minimum essential medium	Medium	Hi Media
C	Phosphate Buffer pH 7.4	Medium	Hi Media
D	Fetal Bovin Serum	Medium	Hi Media
E	Trypsin -EDTA	Medium	Hi Media
F	Antibiotic (Streptomycin)	Reagent	Hi Media
G	Test Sample	-	Provided by Sponsor

5.3. Instruments:

1. CO_2 Incubator
2. Water bath
3. Inverted Microscope
4. Biosafety Cabinet
5. Microplate Reader

5.4. Preparations:

Test:

The test sample was prepared with phosphate buffered saline at different concentrations such as 10µL/mL, 50µL/mL, 100µL/mL, 500µL/mL and 1000µL/mL (undiluted extract); immediately prior to use.

Positive Control:

Cyclophosphamide (2 mg/ml) was freshly prepared in complete medium (MEM medium + 10% FBS) immediately prior to use. When necessary, pH was adjusted to 7.4 using 1 N HCl or 1 N NaOH.

Blank:

Complete medium (MEM medium + 10% FBS) having 2% antibiotic in incubator at 37°C in 5% CO_2 under aseptic condition for 24 hours.

Negative Control:

Phosphate buffered saline was used as negative control.

MTT Solution:

MTT was prepared in fresh PBS at a concentration of 1 mg/ml and adjust the pH 6.0-6.5. Solution is sterilized by sterile filtration using syringe filters (pore size 0.22 µm). The solution should be used on the same day.

6. EXPERIMEMNTAL DESIGN

6.1. Sub culturing of cell line

- **Detachment of cells**

Cell culture flask was removed from deep freezer and immediately thawed by keeping the flask in water bath at 37°C. The process of thawing was performed fast so that during thawing the cells did not get damaged. After the culture was thawed , the flask was removed from water bath and sprayed with 70% IPA on outer surface so that the outer exposed surface got disinfected and then the flask was placed in laminar air flow. 3-5 ml of 0.25% trypsin-EDTA was added in T-25 flask to detach cells from the flask under sterile condition in aseptic cabinet. The flask was shaken in horizontal direction so that each and every cell got exposed to trypsin solution. Flask was then incubated for 2-3 min at 37°C, 5% CO_2. The flask was observed under microscope to see whether cells were detached. Detached cells appeared in round shape and floated in the medium. After incubation immediately 20% FBS was added to stop the detachment process.

- **Viable cell count**

Counting of cells per ml of medium was carried out using hemocytometer. 10µl of cell suspension was taken and 20µl of trypan blue dye (0.5%) was added. Mixture of cell suspension and dye was dropped on hemocytometer. The unstained (viable) and stained (non viable) cells were counted separately, % cell viability was calculated.

6.2. Splitting of culture cells

After cell calculation the cells were splitted to half in two different sterile centrifuge tubes. In each tube the volume of fresh medium was added in such a way that the final concentration of cells in the medium would be (approximately: 4.25×10^5) in T-25 flask. The culture was transferred in T-25 flask and incubated for 4 Days in humidified atmosphere in incubator maintained at 37^0C temperature and 5% CO_2.

6.3. Preparation of Test extract

The test sample was prepared with phosphate buffered saline at different concentrations such as 10µL/mL, 50µL/mL, 100µL/mL, 500µL/mL and 1000µL/mL (undiluted extract); immediately prior to use.

Preparation of Blank, Positive Control and Negative Control:

Two T 25 flasks having 2 ml cell suspension, 2 ml fresh complete medium having 2 % antibiotic were prepared. Further, 2 ml fresh complete medium having 2 % antibiotic was added in control flask. The flasks were then incubated in incubator at 37°C in 5% CO_2 for 48 h.

6.4. Evaluation of Cytotoxicity:

i) Microscopic examination:

Following incubation, the culture wells were examined microscopically to evaluate cellular characteristics and % lysis.

Each Flask was evaluated for % lysis and cell characteristics and categorized as per below:

Grade	Reactivity	Condition of cell culture
0	None	No lysis
1	Slight	Occasional lysis
2	Mild	No extensive lysis
3	Moderate	Not more than 70 % of cell layers contain rounded cells or are lysed
4	Severe	Nearly complete cell lysis

ii) MTT assay

Step I: Growing cells in 96 well micro titer plate

Cell cultures was removed from culture flasks by enzymatic digestion (trypsin/EDTA). Dispense 100 µl culture medium only (blank) into the peripheral wells of a 96-well tissue culture microtitre plate. In the remaining wells, dispense 100 µl of a cell suspension of 1×10^5 cells/ml (= 1×10^4 cells/well). Incubate cells for 24 h (5% CO_2, 37°C) so that cells form a half-confluent monolayer. This incubation period ensures cell recovery, and adherence and progression to exponential growth phase. Each plate was examined under a phase contrast microscope to ensure that cell growth was relatively even across the microtitre plate.

STEP II: Contact of cells with test extract

After 24 h incubation, culture medium was aspirated from the cells. Per well, 100 µl of treatment medium containing either the appropriate concentration of sample as well as positive control, negative control and in three well (control) where 100 µl fresh medium was added. Cells were incubated for 24 h (5% CO_2, 37°C).

Step III: Examination of Cytotoxicity

After 24 h treatment, each plate was examined under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells.

After the examination of the plates, the culture medium from the plates was carefully removed. 50 µl of the MTT solution (1 mg/ml & pH 6.0-6.5) was then added to each test well and the plates were further incubated for 4 h in the incubator at 37°C. MTT solution was descanted and 100 µl of Dimethyl sulphoxide was added in each well. Well plate was transferred to a microplate reader equipped with a 570 nm filter to read the absorbance (reference wavelength 650 nm).

Step IV: Data analysis

A decrease in number of living cells results in a decrease in the metabolic activity in the sample. % cell Viability was calculated from following equation:

$$\% \text{ cell viability} = \frac{OD_{570e} \times 100}{OD_{570b}}$$

Where, OD_{570e} is the mean value of the measured optical density of the test sample as well as positive control;

OD_{570b} is the mean value of the measured optical density of negative control .

Interpretation:

In microscopic examination, achievement of numerical grade less than 2, based on Tables 1 of.6.4, is considered a non-cytotoxic effect. The lower % cell viability value indicates the higher Cytotoxic potential of the test item. If viability is reduced to < 70 % of the negative control then it confirm that the test material possess Cytotoxic potential.

7. RESULTS

MTT ASSAY

The test sample was evaluated for its cytotoxicity against human epithelial cell line (Caco-2) using MTT assay. Different concentrations of the extract, such as; 10, 50, 100, 500 and 1000 µl/ml shows 58.50%, 66.75%, 65.49%, 82.74% and 84.63% cytotoxicity towards Caco-2 cell line, respectively.

Further, standard sample (Cyclophosphamide) showed 100 % cytotoxicity (**Annexure 1**).

8. DISCUSSION:

In the present study, in vitro cytotoxicity testing of *Ocimum Sanctum* Callus Extract was performed using human epithelial cell line (Caco-2). The test sample was subjected to cell proliferation assay by MTT. The results revealed that the extract showed significant cytotoxicity on Caco-2 cell line. Overall, the cell growth inhibition by the extract observed in this study was concentration dependant. Undiluted extract has shown 84.63% cytotoxic potential against Caco-2 cell line.

9. CONCLUSION:

It can be concluded that the test materials *Ocimum Sanctum* Callus Extract has shown cytotoxic potential under the prescribed test conditions. Maximum cytotoxicity observed was 84.63% by undiluted extract of *Ocimum sanctum.*

04/12/2017

Study Director

Dr. Manish A. Rachchh

10. REFERENCE

Roy M, Chakrabarty S, Siddiqi M, Bhattacharya RK. Induction of apoptosis in tumor cells by natural phenolic compounds. Asian Pacific J Cancer Prev2002;3:61–7.

11. LIST OF ATTACHMENT

Annexure	Content
Annexure 1	% cell viability (MTT Assay)
Annexure 2	Microscopic Examination
Annexure 3	Photographs of Caco-2 Cell Line (Blank, Positive Control, Negative Control and Test)

12. ARCHIVES

All original raw data, draft study plan and report, approved study plan and final report will be retained in the archives of Accuprec Research Labs Pvt Ltd, Ahmedabad, Gujarat, India for a period of 5 years. At the end of the archiving period, the Sponsor's instructions will be sought either to extend the archiving period or to return or dispose of the archived material.

Date: 04/11/2017

04/11/2017

Ms. Kruti Upadhyay
Asst. Study Director

04/11/2017

Dr. Manish Rachchh
Study Director

04/11/2017

Mr. Bhargav Gohel
DM, QA

04/11/2017

Dr. Rina Gokani
Q. A. Head

IV. CERTIFICATE

This is to certify that the test material "*Ocimum Sanctum* Callus Extract", supplied by Satej Global Science, Suvas Apartment, Behind Park Avenue Bunglow, Thaltej, Ahmedabad-380059, Gujarat, India., has been tested for *in vitro* anti-cancer activity using Caco-2 cell line.

The test material was found to be "Cytotoxic " on human epithelial cell line (Caco-2). This indicate that it possess anti-cancer property. Maximum cytotoxicity observed was 84.63% by undiluted callus extract.

Accuprec Research Labs Pvt. Ltd., is approved by the Food & Drug Administration, Gujarat State, Gandhinagar, through License No. GTL/37/31.

Date: 04/11/2017

04/11/2017

Ms. Kruti Upadhyay
Asst. Study Director

04/11/2017

Dr. Manish Rachchh
Study Director

04/11/2017

Mr. Bhargav Gohel
DM, QA

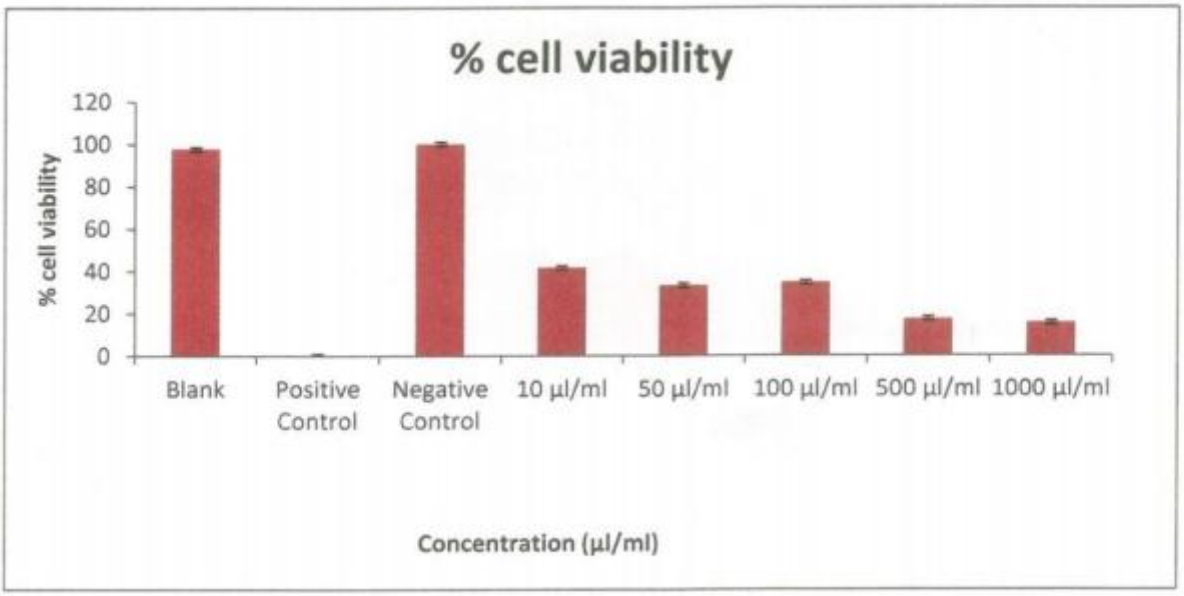
04/11/2017

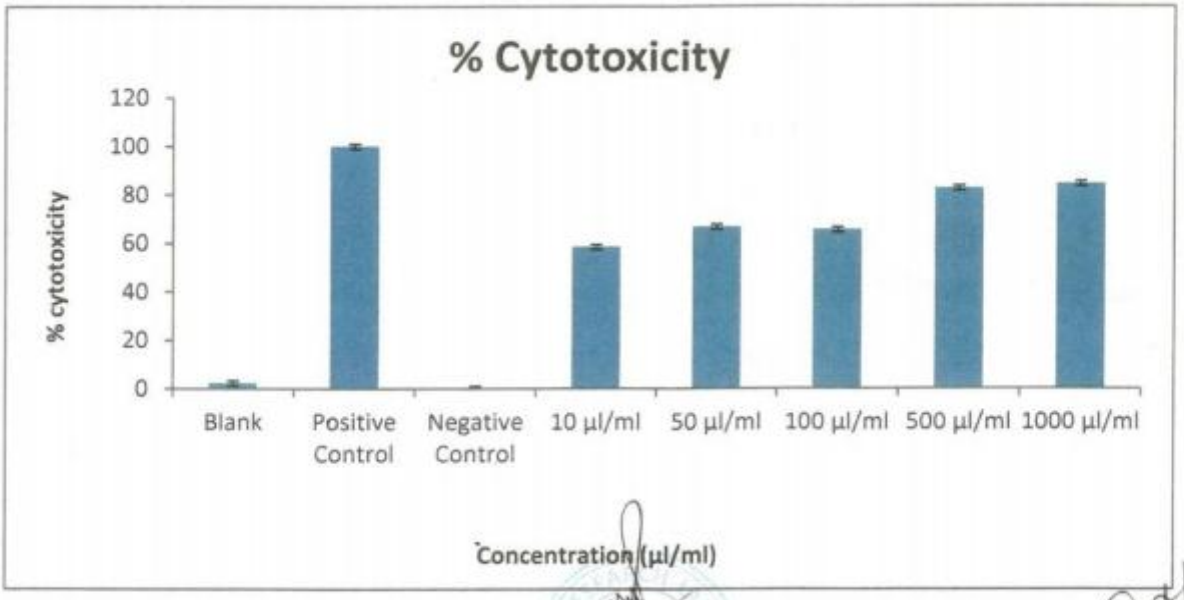
Dr. Rina Gokani
Q. A. Head

Annexure 1

Study Title: *In Vitro* Anti-cancer activity of Ocimum Sanctum Callus Extract.

Optical Density								
Sample	Blank	Positive Control	Negative Control	Test				
				10 µl/ml	50 µl/ml	100 µl/ml	500 µl/ml	1000 µl/ml (Undiluted Extract)
1	0.542	0.000	0.527	0.207	0.218	0.203	0.093	0.084
2	0.498	0.000	0.548	0.214	0.123	0.153	0.097	0.079
3	0.512	0.000	0.513	0.238	0.187	0.192	0.084	0.081
Avg.	0.517	0.000	0.529	0.220	0.176	0.183	0.091	0.081
% cell viability	97.733	0.000	100.000	41.499	33.249	34.509	17.254	15.365
% cell cytoxicity	2.267	100.000	0.000	58.501	66.751	65.491	82.746	84.635





04/11/2017

ACCUGREEN

Annexure 2

Study Title: *In Vitro* Anti-cancer activity of Ocimum Sanctum Callus Extract.

Grade								
Sample	Blank	Positive Control	Negative Control	Test				
				10 µl/ml	50 µl/ml	100 µl/ml	500 µl/ml	1000 µl/ml (Undiluted Extract)
1	0.000	4.000	0.000	2.000	2.000	3.000	3.000	3.000
2	0.000	4.000	0.000	2.000	3.000	2.000	3.000	4.000
3	0.000	4.000	0.000	2.000	2.000	2.000	3.000	4.000
Avg.	2.000	2.000	1.000	2.167	2.333	2.667	3.333	3.667

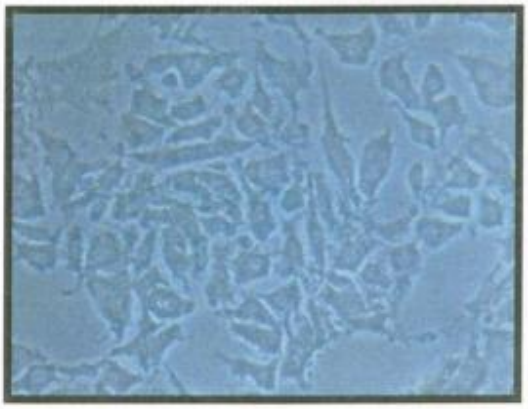
04/11/2017

Annexure 3

Microscopical examination of Anti-cancer activity of *Ocimum Sanctum* Callus Extract performed using Human epithelial cell line (Caco-2) following ISO 10993-5:2009 guideline.

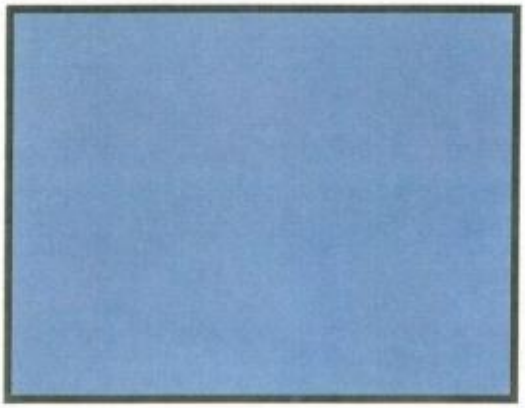
Sample Name: *Ocimum Sanctum* Callus Extract

Blank



Morophology of control cells (magnification 20 X)

Positive Control (Cyclophosphamide)



Morophology of Positive Control (magnification 20 X)

Negative Control (Phosphate Buffered Saline)

Morophology of Negative Control (magnification 20 X)

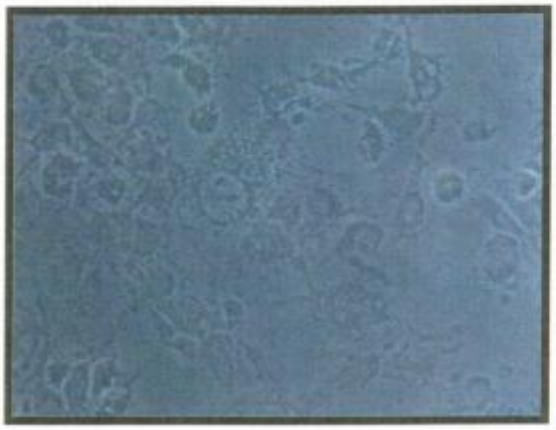
Test (10µL/mL) (*Ocimum Sanctum* Callus Extract)

Morophology of Test (*Ocimum Sanctum* Callus Extract) treated cell (magnification 20 X)

04/11/2017

Test (50µL/mL)

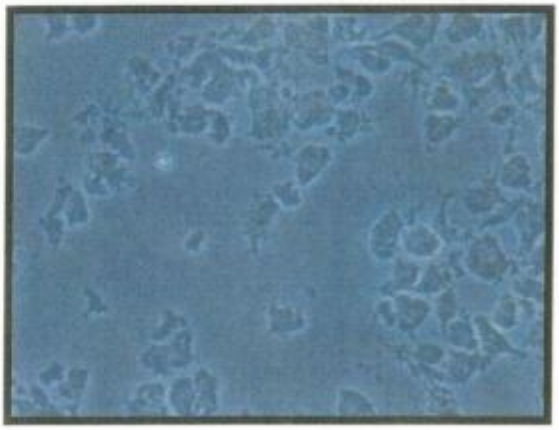
(Ocimum Sanctum Callus Extract)



Morophology of Test (*Ocimum Sanctum* Callus Extract) treated cell (magnification 20 X)

Test (100µL/mL)

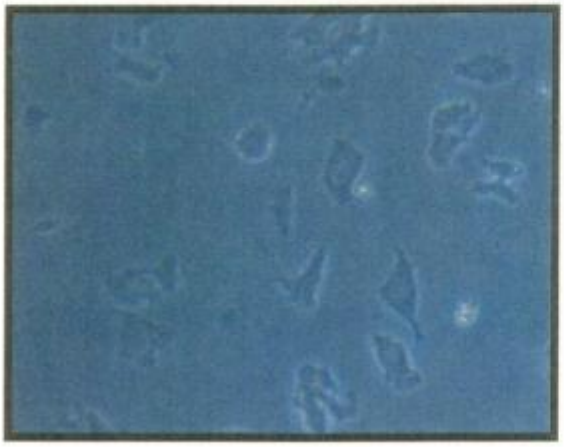
(Ocimum Sanctum Callus Extract)



Morophology of Test (*Ocimum Sanctum* Callus Extract) treated cell (magnification 20 X)

Test (500µL/mL)

(Ocimum Sanctum Callus Extract)



Morophology of Test (*Ocimum Sanctum* Callus Extract) treated cell (magnification 20 X)

Test (1000µL/mL)

(Ocimum Sanctum Callus Extract)

Morophology of Test (*Ocimum Sanctum* Callus Extract) treated cell (magnification 20 X)

04/11/2017

क्षेत्रीय जैवप्रौद्योगिकी केन्द्र
राष्ट्रीय महत्ता की संस्था, जैवप्रौद्योगिकी विभाग,
भारत सरकार द्वारा यूनेस्को के तत्वावधान में स्थापित
Regional Centre for Biotechnology
An Institution of National Importance,
Established by the Department of Biotechnology
Government of India, Under the auspices of UNESCO

United Nations
Educational, Scientific and
Cultural Organization

क्षेत्रीय जैव प्रौद्योगिकी केन्द्र
Regional Centre
for Biotechnology

SARS-CoV2 Antiviral Testing Report

(To be used only for research purpose)

Assay Method – Cytotoxicity

- The assay is done in a 96-well plate format in 3 wells for each sample.
- 1x10e4 VeroE6 cells were plated per well and incubated at 37-degree C overnight for the monolayer formation.
- Next day, cells were incubated with the test substance (TS) at the indicated concentration. The cells without test substance was the control.
- 24 and 48 hours later, cells were stained with Hoechst 33342 and Sytox orange dye.
- Images were taken at 10X, 16 images per well, which covers 90% of well area using ImageXpress Microconfocal (Molecular Devices).
- Hoechst 33342 nucleic acid stain is a popular cell-permeant nuclear counterstain that emits blue fluorescence when bound to dsDNA. It stains all the live and dead cells.
- Sytox orange dye stains nucleic acids in cells with compromised membranes. This stain is an indicator of cell death.
- First, the software will count total number of cells in the Hoechst image.
- In the Sytox image, it will count, among Hoechst positive cells, how many cells are positive for Sytox.

Assay Method – Antiviral screening

- The assay is done in a 96-well plate format in 3 wells for each sample.
- 1x10e4 VeroE6 cells were plated per well and incubated at 37-degree C overnight for the monolayer formation.
- Next day, cells were incubated with the test substance (TS) at the indicated concentration. The cells without test substance was the control. The cells were infected with SARS-CoV2 at a MOI of 0.01.
- 24 and 48 hours later, viral RNA was extracted from 100 µl culture supernatant and subjected to qRT-PCR (in duplicates) where Ct values for N and E gene sequence were determined.
- Inhibition of virus replication is determined based on the fold change in the Ct value in TS-treated cells compared to the control.
- Remdesivir was used as a positive control for viral inhibition.

Hirpendra Singh

क्षेत्रीय जैवप्रौद्योगिकी केन्द्र
राष्ट्रीय महत्ता की संस्था, जैवप्रौद्योगिकी विभाग,
भारत सरकार द्वारा यूनेस्को के तत्वावधान में स्थापित
Regional Centre for Biotechnology
An Institution of National Importance,
Established by the Department of Biotechnology
Government of India, Under the auspices of UNESCO

United Nations
Educational, Scientific and
Cultural Organization

क्षेत्रीय जैव प्रौद्योगिकी केन्द्र
Regional Centre
for Biotechnology

Results

Compound	Concentration	% Cell viability		% inhibition of virus replication			
		24 h post-treatment	48 h post-treatment	24 h post-infection		48 h post-infection	
				E	N	E	N
Remdesivir	10 µM	99.23	94.37	92.62	93.55	99.96	99.96
Grolyfe	10 µg/ml concentration	53.66	42.06	96.13	96.38	99.96	99.95
Grolyfe	1 µg/ml concentration	83.94	91.38	56.47	63.16	34.54	39.73
Grolyfe	0.1 µg/ml concentration	84.89	100.75	-	-	-	-

Nirpendra Singh
7/00/2020

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(Estd. by Dept. of Biotechnology, Govt. of India, under the auspices of UNESCO)
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GROLYFE

Contain: Ocimum Sanctum Callus Extract (Callus dehydrated powder of Shyam Tulsi)
Aloe Barbandensis Mill. Callus Extract (Callus Dehydrated Powder of Aloevera)

As per some pre-clinical study based on animal model many therapeutic application is possible from Ocimum Sanctum Extract. Like

1). **Anti-Diabetic Properties**
2). **Cardio protective**
3). **Wound Healing Activity**
4). **Radio-Protective Effect**
5). **Highly Significant Recovery in Genotoxicity.**
6). **Antioxidant Properties**
7). **Hypolipidemic Activities.**
8). **Anti-microbial Properties.**
9). **inhibit the Transcriptional Expression of Genes.**
10). **Gastro protective Effect.**
11). **Immunomodulatory Effect.**
12). **Support the Central Nervous System.**
13). **Analgesic, Anthelmintic, Anti-inflammatory Activity**
14). **Anticancer Activity.**

THERAPEUTIC APPLICATIONS

Pre-Clinical studies (Animal model)

During the last two decades, *O. sanctum* L. has demonstrated various pre-clinical activities in animal models *in vitro* testing. Some such notable findings are reported here:

(1) Antidiabetic

Extract of *O. sanctum* L. significantly decreases the blood glucose, glycosylated hemoglobin and urea with a concomitant increase in glycogen, hemoglobin and protein in streptozotocin-induced diabetic rats. This extracts also resulted in an increase in insulin and peptide levels and glucose tolerance.
The constituents of *O. sanctum* L. extracts have stimulatory effects on physiological pathways of insulin secretion, which may underlie its reported antidiabetic action.

Unravel the possible mechanism of glucose-lowering activity of *O. sanctum* L. in male mice. The study suggested that *O. sanctum* L. decreases the serum concentration of both cortisol and glucose and also exhibited antiperoxidative effect. Therefore *O. sanctum* L. may potentially regulate corticosteroid- induced diabetic mellitus.

In another study the effect of O. sanctum L. on three important enzymes of carbohydrate metabolism [glucokinase (gk), hexokinase (hk) and phosphofructokinase (PFK) along with glycogen content of insulin-dependent (skeletal muscle and liver) and insulin-independent tissues (kidneys and brain) was studied in streptozotocin (STZ, 65 mg/kg)-induced model of diabetes for 30 days in rats.

Administration of O. sanctum L. extracts 200 mg/kg for 30 days lead to decrease in plasma glucose levels by approximately 9.06 and 24.4% on 15th and 30th day. O. sanctum L. significantly decreased renal but not liver weight (expressed as % of body weight) O. sanctum L. glycogen content in any tissue; also O. sanctum L. partially corrected the activity of glucokinase (gk), hexokinase (hk) and phosphofructokinase (PFK) distributed in the diabetic control.

Tulsi (*O. sanctum* L.) leaf powder was fed at the 1% level in normal and diabetic rats for a period of one month and the result indicated a significant reduction in fasting blood sugar urogenic acid, total amino acids level. This observation indicates the hypoglycemic effect of *O. sanctum* L. in diabetic rats. also reported that oral administration of extract of leaves of *O. sanctum* L. led to marked lowering of blood sugar level in normal, glucose-fed hyperglycemic and streptozotocin-induced diabetic rats. Furthermore, the extract potentiates the action of exogenous insulin in normal rats. The activity of the extract was
91.55 and 70.43% of that of Tolbutamide in normal and diabetic rats, respectively.

(2) Cardiac activity

Oral feeding of extract of *O. sanctum* L. (100 mg/kg) to male Wister rats subjected to chronic-resistant stress (6 h/day for 21 days) significantly prevented the chronic-resistant stress/induced rise in plasma

cAMP level, myocardial superoxide dismutase and catalase activities as well as the light microscopic changes in the myocardium.

Rats fed with fresh leaf homogenate of *O. sanctum* L. (50 and 100 mg/kg body weight) daily 30 days inhibit isoproterenol-induced changes in myocardial superoxide dismutase, glutathione peroxidase and reduced glutathione.

In another study effect of pre- and co-treatment of extract of *O. sanctum* L. at different doses (25, 50, 75, 100, 200 and 400 mg/kg) was investigated against isoproterenol (ISO, 20 mg/kg, Sc) myocardial infarction in rats. *O. sanctum* L. at the dose of 25, 50, 75 and 100 mg/kg significantly reduced glutathione (GSH), superoxide dismutase and LDH levels. In this study, it was observed that *O. sanctum*
L. at the dose of 50 mg/kg was found to demonstrate maximum cardio protective effect.

The generation of drug-induced oxygen radicals in heart cells led to cardiac lipid membrane peroxidation. Urosolic acid (UA) isolated from *O. sanctum* L. have been identified as a protector against Adriamycin (ADR)-induced lipid per oxidation. Protection with UA was 13 and 17% in liver and heart microsomes, respectively.

(3) Wound healing activity

Evaluated the wound healing effect of aqueous extract of *O. sanctum* L. in rats. Wound-breaking strength in incision wound model, epithelization period and percent wound concentration in excision wound model were studied owing to increase per cent wound contraction. *Ocimum sanctum* L. may be useful in the management of abnormal healing such as keloids and hypertropic scars. Extract of leaves of *O. sanctum* L. was investigated for normal wound healing and dexamethasone-depressed healing. The extract significantly increased the wound breaking strength, wound epithelializes fast and wound contraction was significantly increased along with increase in wet and dry granulation tissue weight and granulation tissue breaking strength. The extract also significantly decreases the anti-healing activities of dexamethasone in all wound healing models.

(4) Radio-protective effect

Radio-protective effect of aqueous extract of *O. sanctum* L. (40 mg/kg, for 15 days) in mice exposed to high doses (3.7 MBq) of oral 131 iodine was investigated by studying the organ weights, lipid peroxidation and antioxidant defense enzyme in various target organs like liver, kidney, salivary glands and stomach at 24 h after exposure.

Pretreatment with *O. sanctum* L. in radioiodine-exposed group showed significant reduction in lipid peroxidation in both kidney and salivary glands. In liver, reduced glutathione (GSH) levels showed significant reduction after radiation exposure while pretreatment with *O. sanctum* L. exhibited less depletion in GSH level even after 131 iodine exposure. However, no such changes were observed in the stomach. The results indicate the possibility of using aqueous extract of *O. sanctum* L. for ameliorating 131 iodine induced damage to the salivary glands.

Two polysaccharides isolated from *O. sanctum* L. could prevent oxidative damage to liposomal lipids and plasmid DNA induced by various oxidants such as iron, AAPH and gamma radiation. Reported that two water-soluble flavonoids, Orientin (Ot) and Vicenin (Vc), isolated from the leaves of *O. sanctum* L. provide significant protection against radiation, lethality and chromosomal aberration *in vivo*. In order to select the most effective drug concentration, fresh whole blood was exposed to 4 Gy of cobalt-60 gamma radiation with *O. sanctum* L. without a 30 min pretreatment with 6.25, 12.5, 15, 17.5 and 20 micron of Ot/Vc in micronucleus test. Radiation significantly increased the micronucleus (MN) frequently. Pretreatment with either Ot or Vc at all concentration-dependent manner, with optimum effect at 17.5 µm.

The effect of aqueous extract of leaves of *O. sanctum* L. against radiation lethality and chromosome damage was studied by radiation-induced lipid peroxidation in liver. Adult Swiss mice were injected with 10 mg/kg of gamma radiation 30 min after last injection. Glutathione (GSH) and the antioxidant enzymes glutathione transferase (GST), reductase (GSRx), peroxidase (GSPx) and superoxide dismutase (SOD) as well as lipid peroxide (LPx) activity were estimated in the liver at 15 min, 30 min, 1, 2, 4 and 8 h post- treatment. Aqueous extract itself increased the GSH and enzymes significantly above normal level, whereas radiation significantly reduced all the values and significantly increased the lipid peroxidation rate, reaching a maximum value at 2 h after exposure (3.5 times of control). Aqueous extract significantly reduced the lipid peroxidation and accelerated recovery to normal levels.

In a comparative study of radioprotection by ocimum flavonoids and synthetic aminothiol protectors in mouse showed Ocimum flavonoids as promising human radiation protectant. In this study, adult Swiss mice were injected intraperitoneally with 50 µg/kg body weight of Orientin (OT) or vicenin (Vc) 20 mg/kg body weight of 2-ercaptopropionyl glycine (MPG) 150 mg/kg body weight of WR2721 and exposed to whole body irradiation of 2 Gy gamma radiation 30 min later.

After 24 hours, chromosomal aberrations were studied in the bone marrow of the femur by routine metaphase preparation after colchicines treatment. Pretreatment with all the protective compounds resulted in a significant reduction in the percentage of aberrant metaphases. Vicenin produced the maximum reduction in per cent aberrant cells while MPG was the least effective; OT and WR-2721 showed an almost similar effect.Investigated the radio-protective effect of the leaf extract of *O. sanctum* L. (OE) in combination with WR-2721 (WR) on mouse bone marrow.

Adult Swiss mice were injected intraperitoneally with OE (10 mg/kg for five consecutive days) alone or 100-400 mg/kg WR (Single dose) *O. sanctum* L. combination of the two and whole body was exposed to 4.5Gy gamma irradiation (RT). Metaphase plates were prepared from femur bone marrow on days 1, 2, 7 and 14 post-treatment and chromosomal aberrations were scored. Pretreatment with OE or WR individually resulted in a significant decrease in aberrant cells as well as different types of aberrations. The combination

of the two further enhanced this effect; resulting in a two-fold increase in the protection factors (PF = 6.68) compared to 400 mg/kg WR alone.

(5) Genotoxicity

In vivo cytogenetic assay in Allium cepa root tip cells has been carried out to detect the modifying effect of *O. sanctum* L. aqueous leaf extract against chromium (Cr) and mercury (Hg)-induced genotoxicity. It was observed that the roo ts post-treated with the leaf extract showed highly significant recovery in mitotic index (MI) and chromosomal aberrations. When compared to pre-treated (Cr/Hg) samples, the lower doses of the leaf extract were found to be more effective than the higher doses.

Immu-21, a poly-herbal formulation containing *O. sanctum* L. and other herbal extracts when given at 100 mg per kg daily over 7 days and 300 mg/kg daily over 14 days inhibited both cyclophosphamide (40 mg/kg i.p.)-induced classical and non-classical chromosomal aberration (40–60% of control). This also reduces the increase in micronuclei in the bone marrow erythrocytes of mice treated with cyclophosphamide.

(6) Antioxidant

The antioxidant capacity of essential oils obtained by steam hydrodistillation from *O. sanctum* L. was evaluated using a high-performance liquid chromatography (HPLC) based hypoxanthine xanthine oxidize and OPPH assays. In hypoxanthine xanthine oxidase assay, strong antioxidant capacity was evident from *O. sanctum* L. (IC50 = 0.46 µL/ml).

In another study the aqueous extract of *O. sanctum* L. significantly increases the activity of anti-oxidant enzymes such as superoxide dismutase, catalase level in extract-treated group compared to control.

Aqueous extract of *O. sanctum* L. inhibit the hypercholesterolemia-induced erythrocyte lipid per oxidation activity in a dose-dependent manner in male albino rabbits. Oral feeding also provides significant leaver and aortic tissue protection from hypercholesterolemia-induced per oxidative damage.

The effect of extract of *O. sanctum* L. leaves in cerebral reperfusion injury as well as long-term hypo perfusion was studied by Yanpallewar *et al*. *Osimum sanctum* L. pretreatment (200 mg/kg/day for 7 days) prevented reperfusion-induced rise in lipid peroxidation and superoxide dismutase. *Osimum sanctum* L. pretreatment also stabilized the levels of tissue total sulfhydryl group during reperfusion.

(7) Hypolipidemic

Administration of *O. sanctum* L. (0.8 gm/kg body weight/day) for four weeks, in cholesterol-fed (100 mg/kg body weight/day) rabbits significantly decreases serum cholesterol, triacylglycerol and LDL + VLDL cholesterol as compared to untreated cholesterol-fed group suggesting the hypo-cholesterolemic activity of *O. sanctum* L.

Fresh leaves of *O. sanctum* L. mixed OS 1 and 2 g in 100 gm of diet given for four weeks brought about significant changes in the lipid of normal albino rabbits. This resulted in significant lowering in serum total cholesterol, triglyceride, phospholipids and LDL-cholesterol level and significant increase in the HDL-cholesterol and total fecal sterol contents.

(8) Antimicrobial

Study suggested that higher content of linoleic acid in *O. sanctum* L. fixed oil could contribute towards its antibacterial activity. The oil show good antibacterial activity against *Staphylococcus aureus, Bacillus pumius* and *Pseudomonas aeruginosa*, where *S. aureus* was the most sensitive organism.

studied that the aqueous extract of *O. sanctum* L. (60 mg/kg) show wide zones of inhibition compared to alcoholic extract against *Klebsiella, E. coli, Proteus, S. aureus* and *Candida albicans* when studied by agar diffusion method. Alcoholic extract showed wider zone for *Vibrio cholerae*.

(9) Effect on gene transcription

The genes that have direct role in artherogenesis include LDRL, LxRalpha, PPARs, CD-36 because these genes control lipid metabolism, cytotoxin production and cellular activity within the arterial wall. To know whether or not the polyphenols extracted from *O. sanctum* L. have any effect on the transcription of these genes, cultured human mononuclear cells in the presence of polyphenols extracted from *O. sanctum* L. Transcriptional expression of these genes was measured by using RT-PCR and SCION IMAGE analysis software. These polyphenolic extracts were found to have the inherent capacity to inhibit the transcriptional expression of these genes.

(10) Gastroprotective

The standardized extract of leaves of *O. sanctum* L. (OSE) given in doses of 50–200 mg/kg orally twice daily for five days showed dose-dependent ulcer protective effect against cold-restraint stress-induced gastric ulcers.

Optimal effective dose (100 mg/kg) of OSE showed significant ulcer protection against ethanol and pyloric ligation induced gastric ulcer but was ineffective against aspirin-induced ulcer. OSE (100 mg/kg) also inhibits the offensive acid pepsin secretion and lipid per oxidation and increases the gastric defensive factors like mucin secretion, cellular mucus and lifespan of mucosal cells.

evaluated the anti-ulcerogenic activity in cold-restraint (CRU), aspirin (ASP), alcohol (Al), pyloric ligation (PL) induced gastric ulcer models in rats, histamine-induced (HST) duodenal ulcer in guinea pigs and ulcer healing activity in acetic acid induced (AC) chronic ulcer model. *Osimum sanctum* L. at a dose of 100 mg/kg was found to be effective in CRU (65.07%), ASP (63.49%), Al (53.87%), PL (62.06%) and HST (61.76%) induced ulcer models and significantly reduced free, total acidity and peptic activity by 72.58, 58.63 and 57.6%, respectively, and increased mucin secretion by 34.61% conclusively *Osimum sanctum* L. could act as a potent therapeutic agent against peptic ulcer disease.

The antiulcerogenic property of *O. sanctum* L. was studied in pyloric-ligated and aspirin-treated rats. The extract of reduced ulcer index, free and total acidity on acute and chronic administration seven days pretreatment increased the mucus secretion also. So it may be concluded that *O. sanctum* L. extract has anti-ulcerogenic property against experimental ulcers and it is due to its ability to reduce acid secretion and increase mucus secretion.

(11) Immunomodulatory effect

Immunotherapeutic potential of aqueous extract of O. sanctum L. leaf in bovine sub-clinical mastitis (SCM) was investigated after intramammary infusion of aqueous extract. The results revealed that the aqueous extract of *O. sanctum* L. treatment reduced the total bacterial count and increased neutrophil and lymphocyte counts with enhanced phagocytic activity and phagocytic index.

In another study, the immunomodulatory effect of *O. sanctum* L. (OSSO) was evaluated in both non-stressed and stressed animals. *Osimum* sanctum L. (3 ml/kg, Ip) produced a significant increase in anti- Sheep red blood cells (SRBC) antibody titer and a decrease in percentage histamine release from peritoneal mast cell of sensitized rats (humoral immune responses) and decrease in food pad thickness and percentage leucocyte migration inhibition (cell-mediated immune responses). Co-administration of diazepam (1 mg/kg, Sc), a benzodiazepine (BZD) with OSSO (1 mg/kg, IP) enhanced the effect of OSSO on resistant stress induced

changes in both humoral and cell-mediated immune responses. Further, flumazenil (5 mg/kg, IP) a central BZD receptor antagonist inhibited the immunomodulatory action of OSSO on resistant stress induced immune responsiveness. Thus, OSSO apparatus to modulate both humoral and cell-mediated immune responsiveness and these immunomodulatory effects may be mediated by GABAnergic pathway.

investigated the immunoregulatory profile of extract and an aqueous suspension of *O. sanctum* L. leaves to antigenic challenge of *Salmonella typhosa* and sheep erythrocytes by quantifying agglutinating antibodies employing the Widal agglutination and sheep erythrocyte agglutination tests and E-rosette formation in albino rats. The data of the study indicate an immunostimulation of humoral immunogenic response as represented by an increase in antibody titer in both the Widal and sheep erythrocyte agglutination tests as well as by cellular immunologic response represented by E-rosette formation and lymphocytosis.

(12) Sexually transmitted disease

Extract of *O. sanctum* L. caused inhibition of *Neisseria gonorrhoeae* clinical isolates and WHO organization strains. The activity is comparable to penicillin and ciprofloxacin.

(13) Effect on central nervous system (CNS)

Different extracts of stem, leaf and stem callus (induced on slightly modified Murashige and Skoog's medium and supplemented with 2,4-dichlorophenonyacetic acid and kinetin) were tested for anticonvulsant activity by maximal electroshock model using Phenytoin as standard. It was observed that ethanol and chloroform extractives of stem, leaf and stem calli were effective in preventing tonic convulsions induced by transcorneal electroshock.

extract of leaves of *O. sanctum* L. prolonged the time of lost reflex in mice due to pentobarbital, decreased the recovery time and severity of electroshock and pentylenetetrazole-induced convulsions and decreased apomorphine-induced fighting time and ambulation in 'open field' studies. In the forced swimming behavioral despair model, the extract lowered immobility in a manner comparable to Imipramine. This action was blocked by haloperidol and sulpiride, indicating a possible action involving dopaminergic neurons. In similar studies, there was a synergistic action when the extract was combined with bromocriptine, a potent D2-receptor agonist.

Nootropic agents are a new class of drugs used in situations where there is organic disorder in learning abilities. Joshi and Parle assessed the potential of *O. sanctum* L. extract as a nootropic and anti-amensic agent in mice. Aqueous extract of derived whole plant of *O. sanctum* L. ameliorated the amensic effect of scopolamine (0.04 mg/kg), diazepam (1 mg/kg) and aging-induced memory deficits in mice. Elevated plus maze and passive avoidance paradigm served as the exteroceptive behavioral models. *O. sanctum* L. extract decreased transfer latency and increased step-down latency, when compared to control (piracetam-treated), scopolamine and aged groups of mice significantly. So *O. sanctum* L. preparation could be beneficial in the treatment of cognitive disorders such as dementia and Alzheimer's disease.

extract of *O. sanctum* L. root extract at a dose of 400 mg/kg (ip increases the swimming time of mouse in a despair swim test model, suggesting a central nervous system stimulant and/or anti-stress activity of *O. sanctum* L.

(14) Antinociceptive (Analgesic)

The analgesic activity of leaf extract of *O. sanctum* L. (50, 100 mg/kg, ip; 50, 100, 200 mg/kg, po) was tested in mice using glacial acetic acid induced writhing test. *O. sanctum* L. reduced the number of writhes. *Osimum sanctum* L. (50, 100 mg/kg ip) also increased the tail withdrawal latency in mice.

(15) Anthelmintic activity

The anthelmintic activity of the essential oil from *O. sanctum* L. was evaluated by Caenorhabditis elegance model. Eugenol exhibited an ED^{50} of 62.1 µg/ml and being the predominant component of the essential oil, it was suggested as the putative anthelmintic principle.

(16) Antiinflammatory

Compounds isolated from *O. sanctum* L. extract, Civsilineol, Civsimavatine , Isothymonin, Apigenin, Rosavinic acid and Eugenol were observed for their anti-inflammatory activity or cyclooxygenase inhibitory activity. Eugenol demonstrated 97% cyclooxygenase-1 inhibitory activity when assayed at 1000 µM concentration (pn). Civsilineol, Civsimavitin, Isothymonin, Apigenin and Rosavinic acid displayed 37, 50, 37, 65 and 58% cyclooxygenase-1 inhibitory activity, respectively, when assayed at 1000 µM concentrations. The activities of these compounds were comparable to Ibuprofen, Naproxen and aspirin at 10, 10 and 1000 µM concentrations.

Study reported that linoleic acid present in different amount in the fixed oil of different species of *O. sanctum* L. has the capacity to block both the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism and could be responsible for the anti-inflammatory activity.

A extract and an aqueous suspension of *O. sanctum* L. (500 mg/kg) inhibited acute as well as chronic inflammation in rats as tested by carrageenin-induced pedal edema and cratonoil -induced granuloma and exudates, respectively, and the response was comparable to the response observed with 300 mg/kg of sodium salicylate. Both the extract and suspension showed analgesic activity in mouse hot plate procedure, and the methanol extract caused an increase in tail withdrawal reaction time of a sub- analgesic dose of morphine. Both preparations reduced typhoid–paratyphoid A–B vaccine-induced pyrexia. The antipyretic action of methanol extract and aqueous suspension was weak and of shorter duration than that of 300 mg/kg sodium salicylate.

(17) Anticancer

Fresh leaf paste (topically) aqueous and ethanolic extract (orally) for their chemopreventive activity against 7,12-dimethylbenzaanthracene (DMBA) induced (0.5%) hamster buccal pouch carcinogenesis. Incidence of papillomas and squamous cell carcinomas were significantly reduced and increased the survival rate in the topically applied leaf paste and orally administered extracts to animals.
Histopathological observation made on the mucosa confirmed the profound effect of the orally administered aqueous extract than other.

Study reported that *O. sanctum* L. leaf extract blocks or suppresses the events associated with chemical carcinogenesis by inhibiting metabolic activation of the carcinogen. In this study, primary cultures of rat hepatocytes were treated with 0–500 µg of *O. sanctum* L. extract for 24 h and then with 7,12-dimethaylbenz[a] anthracene (DMBA, 10 or 50 µg) for 18 h. Cells were then harvested and their DNA was isolated and analyzed by 32p post-labeling.

A significant reduction in the levels of DMBA/DNA adducts was observed in all cultures pretreated with *O. sanctum* L. extract. Hepatocytes that were treated with the highest dose of extract (500 µg) showed a maximum reduction of 93% in the mean values of DMBA/DNA adducts. This suggests the inhibition of metabolic activation of carcinogen.

The chemo preventive activity of *O. sanctum* L. was evaluated against subsequently injected 20-methyl cholanthrene-induced fibrosarcoma tumors in the thigh region of Swiss albino mice. Supplementation of maximal-tolerated dose (100 µl/kg body wt.) of the oil significantly reduced 20-methaylcholathrene-

induced tumor incidence and tumor volume.

The enhanced survival rate and delay in tumor incidence was observed in seed oil supplemented mice. Liver enzymatic, non-enzymatic antioxidants and lipid peroxidation end product, malondialdehyde level were significantly modulated with oil treatment as compared to untreated 20-methylcholathrene injected mice. The chemopreventive efficacy of 100 µl/kg seed oil was comparable to that of 80 mg/kg vita-E.

(18) Miscellaneous activity

Found aqueous extract of *O. sanctum* L. as the most effective aldose reductase (AR) inhibitor with a significant inhibition of 38.05% considering the AR activity of normal rat lenses as 100%. The IC50 value was found to be 20 µg/ml.

Ocimum sanctum L. extract (10 mg/kg body wt., PO) before and after mercury (HgCl 2) intoxication (5 mg/kg body wt.) showed a significant decrease in lipid per oxidation.

Serum glutamate pyruvate transaminase (SGPT) activities compared to HgCl2-induced values suggests that *O. sanctum* L. extract provides protection against HgCl2-induced toxicity in mice.

Ocimum sanctum L. fixed oil increases blood clotting time and percentage increase was comparable to aspirin and could be due to inhibition of platelet aggregation. The oil also increased pentobarbitone- induced sleeping time in rats indicating probable inhibitory effect of oil towards cytochromic enzyme responsible for hepatic metabolism of pentobarbitone.

Noise stress causes leucopenia, increased corticosterone level and enhances the neutrophil functions as indicated by increase in the candida phagocytosis and nitro blue tetrazolium reduction.

Pre-treatment with the *O. sanctum* L. extract brought back the stress altered values to normal levels indicating the stress alleviating effect of *O. sanctum* L.

Contain: Aloe Barbandensis Mill. Callus Extract (callus dehydrated powder of Aloevera)

Some novel Properties and Activities of alovera are as under below which are seen in research paper.

1). Antitumor Activity
2). Anti-Inflammatory Action
3). Wound Healing
4). Moisturizing and Anti-Aging Agent
5). Antimicrobial Activities LIKE Antiviral, Antibacterial, Antifungal Activity.
6). Anticancer Properties
7). Reduce Oxidative stress
8). Anti-Diabetic
9). Cosmetic & Skin Protection Application
10). Antiseptic

1). **Antitumor Activity**

A number of glycoproteins present in Aloe vera have been reported to have antitumor and antiulcer effects and to increase proliferation of normal human dermal cells. However, statistically significant clinical studies on the efficacy of Aloe vera on human health are very limited and often inconclusive. In recent studies, a polysaccharide fraction has shown to inhibit the binding of benzopyrene to primary rat

hepatocytes, thereby preventing the formation of potentially cancer-initiating benzopyrene-DNA adducts. An induction of glutathione S-transferase and an inhibition of the tumor-promoting effects of phorbol myristic acetate has also been reported which suggest a possible benefit of using aloe in cancer chemoprevention.

2). Anti-Inflammatory Action

The anti-inflammatory activity of Aloe vera has been revealed by a number of in vitro and in vivo studies through bradykinase activity. The peptidase bradykinase was isolated from aloe and shown to break down the bradykinin, an inflammatory substance that induces pain. A novel anti-inflammatory compound, C-glucosyl chromone, was isolated from gel extracts. Aloe vera inhibits the cyclo-oxygenase pathway and reduces prostaglandin E2 production from arachidonic acid. Fresh Aloe vera significantly reduced acute inflammation in rats (carrageenin-induced paw oedema), but not in chronic inflammation. In croton oil-induced oedema in mice, three Aloe vera sterols were able to reduce inflammation by up to 37%. Lupeol, the most active antiinflamatory sterol, reduced inflammation in a dose dependent manner. The data suggest that specific plant sterols may also contribute to the anti- inflammatory activity of. The aloe sterol includes campesterol, β-sitosterol, lupeol, and cholesterol which are anti-inflammatory in nature, helps in reducing the inflammation pain and act as a natural analgesic. Other aspirin-like compound present in Aloe is responsible for anti-inflammatory and antimicrobial properties. Even, Aloe vera extract (5.0% leaf homogenate) decreased inflammation by 48% in a rat adjuvant-induced arthritic inflammatory model.

3). Wound Healing

Wound healing is a dynamic process, occurring in 3 phases. The first phase is inflammation, hyperaemia and leukocyte infiltration. The second phase consists of removal of dead tissue. The third phase of proliferation consisting of epithelial regeneration and formation of fibrous tissue. A more recent review concludes that the cumulative evidence supports the use of Aloe vera for the healing of first to second degree burns. The wound healing property of Aloe vera gel has been attributed to Mannose-6-phosphate. Actually, glucomannan and plant growth harmone gibberellins interacts with growth factor receptors of fibrobroblast and stimulate its activity and proliferation for increases collagen synthesis in topical and oral administration of Aloe according to Hayes.

The Aloe administration influence collagen composition (more type III) and increased collagen cross linking for wound contraction and improving breaking strength. It also increases synthesis of hyaluronic acid and dermatan sulfate in the granulation tissue of a healing wound. Acemannan is considered the main functional component of Aloe vera, is composed of a long chain of acetylated mannose. This complex carbohydrate accelerates wound healing and reduces radiation induced skin reactions. Macrophage-activating potential acermannan may stimulate the release of fibrogenic cytokines. Direct binding of acemannan to growth factors and their stabilization may lead to promotion of prolong stimulation of granulation tissue.

The Aloe gel has been used for the treatment of radiation burns and radiation ulcers, and complete healing has been observed in two radiation burns patients. The fresh gel was more effective than the cream as Aloe gel-treated lesions healed faster (11.8 days) compared to burns treated with petroleum jelly gauze (18.2 days) by Fulton. The 27 patients with partial thickness burns have been treated with Aloe in a placebo-controlled study.

4). Moisturizing and Anti-Aging Agent

Muco-polysaccharides help in binding moisture into the skin. The amino acids also soften hardened skin cells and zinc acts as an astringent to tighten pores. Its moisturizing effects have also been studied in treatment of dry skin associated with occupational exposure where Aloe vera gloves improved the skin integrity, decrease appearance of acne wrinkle and decrease erythema. The Aloe gel gives cooling effect and also acts as a moisturizing agent. It also has role in gerontology and rejuvenation of aging skin. This property of Aloe is because it's biogenic material. Aloe vera is used as skin tonic in cosmetic industry.

5). Antimicrobial Activities LIKE Antiviral, Antibacterial, Antifungal Activity.

5.1. Antibacterial Activity

Aloe vera was bactericidal against Pseudomonas aeruginosa and acemannan prevented it from adhering to human lung epithelial cells in a monolayer culture. A processed Aloe vera preparation inhibited the growth of fungus Candida albicans. The gel contains 99.3% of water, the remaining 0.7% is made up of solids with carbohydrates constituting for a large components. Concentrated extracts of Aloe leaves are used as laxative and as a haemorrhoid treatment. Aloe gel can help to stimulate the body's immune system. Glucomannan and acemannan have been proved to accelerate wound healing, activating macrophages, stimulating immune system as well antibacterial and antiviral effects. Streptoccocus pyogenes and Streptococcus faecalis are two microorganisms that have been inhibited by Aloe vera. Using a rat model, it was suggested that the antibacterial effect of the Aloe vera gel in vivo could enhance the wound healing process by eliminating the bacteria that contributed to inflammation. The aloe extract was potent against three strains of Mycobacterium (M. fortuitum, M. smegmatis and M. kansasii) and a strong anti-mycobacterial activity against M. tuberculosis well as antibacterial activity against P. aeruginosa, E. coli, S. aureus and S. typhi. The preliminary phytochemistry revealed presence of terpenoids, flavonoids and tannins.

5.2. Antiviral Activity

Several ingredients in Aloe vera have been shown to be effective antiviral agent. Acemannan reduced herpes simplex infection in two cultured target cell lines. Lectins, fractions of Aloe vera gel, directly inhibited the cytomegalovirus proliferation in cell culture, perhaps by interfering with protein synthesis. A purified sample of aloe emodin was effective against infectivity of herpes simplex virus Type I and Type II and it was capable of inactivating all of the viruses, including varicellazoster virus, influenza virus, and pseudorabies virus. Electron micrograph examination of anthroquinone treated herpes simplex virus demonstrated that the envelopes were partially disrupted. Such results indicate that anthraquinones extract from variety of plants are directly virucidal to enveloped viruses. These actions may be due to indirect effect due to stimulation of the immune system. The anthraquinone aloin also inactivates various enveloped viruses such as herpes simplex, varicella zoster and influenza.

5.3. Antifungal Activity

Aloe vera was evaluated on the mycellium development of Rhizoctonia solani, Fusarium oxysporum, and Colletotrichum coccodes, that showed an inhibitory effect of the pulp of A. vera on F. oxysporum at 104 µl L-1 and the liquid fraction reduced the rate of colony growth at a concentration of 105 µl L-1 in R. solani, F. oxysporum, and C. coccodes. For bacteria, inner-leaf gel from Aloe vera was shown to inhibit growth of Streptococcus and Shigella species in vitro. Agarry et al., reported that the Aloe gel inhibited the growth of

Trichophyton mentagrophytes (20.0 mm), while the leaf possesses inhibitory effects on both Pseudomonas aeruginosa and Candida albicans. In contrast, Aloe vera extracts failed to show antibiotic properties against Xanthomonas species.

Other uses for extracts of Aloe vera include the dilution of semen for the artificial fertilization of sheep, used as fresh food preservative and used in water conservation in small farms. Another constituent of Aloe vera includes saponins. These are soapy substances from the gel that are capable of cleansing and having antiseptic properties. The saponins perform strongly as anti-microbial against bacteria,viruses,fungi and yeasts.

6). Anticancer Properties

The role of Aloe in carcinogenicity has not been evaluated well. Aloe vera juice enables the body to heal itself from cancer and also from the damage caused by radio and chemotherapy that destroys healthy immune cells crucial for the recovery.

Aloe vera emodin, an anthraquinone, has the ability to suppress or inhibit the growth of malignant cancer cells making it to have antineoplastic properties.

7).Reduce Oxidative stress

Aloe is helpful in smooth functioning of the body machinery. It reduces cell-damaging process during stress condition and minimizes biochemical and physiological changes in the body. Oxidative stress refers to chemical reactions in which compounds have their oxidative state changed.

Some antioxidants are part of the body's natural regulating machinery while other dietary antioxidants are derived from diet sources. Aloevera is an excellent example of a functional food that plays a significant role in protection from oxidative stress.

8).Anti-Diabetic

The five phytosterols of A. vera, lophenol, 24-methyllophenol, 24-ethyl-lophenol, cycloartanol and 24-methylenecycloartanol showed anti-diabetic effects in type-2 diabetic mice. Aloe vera contains polysaccharides which increase the insulin level and show hypoglycemic properties.

Reviewed the beneficial effects of selective medicinal plant species such as Allium cepa, Allium sativum, Aloe vera, Azadirachta indica, Gymnema sylvestre, Syzygium cumini and Pterocarpus marsupium, and emphasize on the role of active biomolecules which possess anti-diabetic activity.

The treatment of diabetes mellitus has been attempted with various indigenous plants and polyherbal formulations. Encouraging results have been obtained from plant extracts with respect to antidiabetic activity, but still only a meager percentage of the plant world has been explored. Medicinal plants like Trigonella foenum graecum, Allium sativum, Gymnema slyvestre, Syzigium cumini and Aloe vera have been studied for treatment of diabetes mellitus.

Extracts of Aloe gum increases glucose tolerance in both normal and diabetic rats and Aloe vera sap taken for 4 - 14 weeks has shown a significant hypoglycaemic effect both clinically and experimentally. Aloe vera gel is used in reducing sugar in diabetes. The five phytosterols of A. vera, lophenol, 24- methyl- lophenol, 24-ethyl-lophenol, cycloartanol and 24- methylenecycloartanol showed anti-diabetic effects in type-2 diabetic mice.

9). Cosmetic & Skin Protection Application

Aloin and its gel are used as skin tonic against pimples. Aloe vera is also used for soothing the skin, and keeping the skin moist to help avoid flaky scalp and skin in harsh and dry weather. The Aloe sugars are also used in moisturizing preparations. Mixed with selected essential oils, it makes an excellent skin smoothening moisturizer, sun block lotion plus a whole range of beauty products.

Due to its soothing and cooling qualities, Maharishi Ayurveda recommends Aloe vera for a number of skinproblems. Aloe vera extracts have antibacterial and antifungal activities, which may help in the treatment of minor skin infections, such as boils and benign skin cysts and have been shown to inhibit the growth of fungi that cause tinea. Currently, the plant is widely used in skin care, cosmetics and nutraceuticals. Aloe vera gel has been reported to have a protective effect against radiation damage to the skin. Exact role is not known, but following the administration of Aloe vera gel, an antioxidant protein, metallothionein, is generated in the skin, which scavenges hydroxyl radicals and prevents suppression of superoxide dismutase and glutathione peroxidase in the skin.

It reduces the production and release of skin keratinocyte derived immunosuppressive cytokines such as interleukin-10 (IL-10) and hence prevents UV-induced suppression of delayed type hypersensitivity. Skin burns effect is reported and radiation dermatitis. Some researcher has been reported the contact dermatitis and burning skin sensations following topical applications of Aloe vera gel to dermabraded skin. These reactions appeared to be associated with anthraquinone contaminants in this preparation.

10). Antiseptic

The antiseptic property of Aloe vera is due to presence of six antiseptic agents namely lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols and sulphur. These compounds have inhibitory action on fungi, bacteria and viruses. Though most of these uses are interesting controlled trials are essential to determine its effectiveness in all diseases.