EVIDENCE BASED HERBAL MEDICINE

NANOBIOTECHNOLOGY APPROACH TO CANCER RESEARCH

Part - V

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EVIDENCE BASED HERBAL MEDICINE – PART-V

(Scientific validation of Pharmacological action of AYUSH drugs)

AUTHOR

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By

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PREFACE

India is one of the richest biodiversity country in the world and its nature gifted medicinal plants which are essential for treatment of human diseases. 80% world human population depends on herbal medicine for human wellness. Natural medicinal herbs enriched with novel bioactive molecules, nutraceuticals and micronutrients are responsible for therapeutic and medicinal benefits. AYUSH is leading herbal based therapeutic system for treatment of many diseases practiced in India for more than 2000 years, Siddha and Ayurveda system of medicine is oldest practice by ancestors. Scientific validation of therapeutic actions of plants and poly herbal formulation is essential for authentication of pharmacological action and therapeutic efficacy. Evidence based herbal medicine is significant for proof of concept to verify medicinal plants therapeutic potential and medicinal values. This text book of "Evidence Based Herbal Medicine - Part V" is a collection of research articles of scientific validations of pharmacological actions of selected medicinal plants and poly herbal formulations helps to distinguish and discover the therapeutic benefits by modern scientific methods.

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EVIDENCE BASED HERBAL MEDICINE - PART - V

In vitro antioxidant and antibacterial activity of *Mucuna pruriens* – Poonaikali

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Abstract *– Mucuna pruriens is a plant species belongs to Fabaceae family and it having nutritional and pharmaceutical bioactive molecules. Many researchers reported that Mucuna as a good nutritional supplement in livestock feed and as a fodder crop. Approximately 120 species have been reported from worldwide and 15 species from India. Most of the species had been studied for its nutraceuticals potential and few reported for its pharmaceutical values. The phytochemicals from Mucuna used for the management of male infertility and nervous disorders. In the present study, in vitro antioxidant activity and antibacterial activity of Mucuna pruriens seeds extract have been studied.*

Keywords *– Mucuna pruriens, in vitro antioxidant activity, antibacterial activity*

1. INTRODUCTION

The genus *Mucuna*, belonging to the Fabaceae family, subfamily Papilionaceae (Table 1), includes approximately 151 species of annual and perennial legumes [1]. *Mucuna* is an annual, climbing shrub with long vines (Figure 1). During tender age, the plant is completely covered with fuzzy hairs, but when older, it is almost completely free of hairs. The leaves are tripinnate, ovate, reverse ovate, rhombus-shaped, or widely ovate. The husk is very hairy and carries up to seven seeds. The seeds are flattened uniform ellipsoid and thick [2]. It is considered a viable source of dietary

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proteins due to its high protein concentration. The most important phytochemical compounds present in this plants are alkaloids, flavonoids, tannins, and phenolic compounds [3]. The chemical constituents may be used for the various purposes such as activity against pathogenic bacteria [4]. *M. pruriens* is used to treat diabetes mellitus, impotence and different types of cancer whereas the seeds have various functions such as many free radical-mediated diseases control, atherosclerosis, diabetes, analgesic, arthritis, antipyretic activity, parkinson and nervous disorders [5,6].

Table 1: Scientific classification

Kingdom	Plantae
Family	Fabaceae
Genus	*Mucuna*
Species	*pruriens*

Figure 1: *Mucuna pruriens* plant pod and seeds

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2. MATERIALS & METHODS

2.1. Preparation of extract

10 grams of dried powdered *Mucuna pruriens* (Poonaikali) seed materials was boiled with 100 ml of ethanol for 48 hours in a round bottomed-flask and filter. The filtrates (PK ET) are dried under vacuum and dried extract used for an activity.

2.2. In vitro antioxidant activity

Antioxidant activity of the ethanolic extract of *Mucuna pruriens* (PK ET) was estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals [7]. 100 µL of *Mucuna pruriens* (PK ET) extracts were taken in the microtiter plate. The 100 µL of 0.1% methanolic DPPH was added to the samples and incubated in dark conditions for 30 minutes. The samples were then observed for discoloration; the plate was considered strong and weak positive from purple to yellow and pale pink respectively and read on the Elisa plate reader at 490 nm. Standard ascorbic acid was used as a reference. In triplicates, all the analysis was performed and average values were taken. The Radical scavenging activity was calculated using the below-mentioned equation:

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance ofcontrol)] x 100

2.3. Antibacterial activity

Ethanolic extract of *Mucuna pruriens* (PK ET) was screened for their antibacterial activity against the bacterial strains, *Streptococcus pneumonia* (MTCC 655) and *Klebsiella pneumoniae* (MTCC 39). To assess the antibacterial activity, a well-diffusion assay was carried out. 17 hrs of old bacterial cultures were inoculated over the agar surface of Mueller Hinton agar plates using sterile cotton swabs for the well diffusion assay. After 10 min, wells were cut using a cork borer and each well was loaded with 100 µl of compound from 10 mg/ml, 20 mg/ml and 50 mg/ml concentration

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stock (100 µg/well) along with DMSO control. At 37°C, the plates were incubated for 24 h [8]. Susceptibility was assessed on the basis of the diameter of the zone of inhibition (ZoI) against the test pathogens.

3. RESULTS & DISCUSSION

The ethanolic extracts of *M. pruriens* seeds have demonstrated antioxidant and antimicrobial activities in the presence of bioactive compounds. In the present study the ethanolic extract of *M. pruriens* seeds exhibited good *in vitro* antioxidant activity. An average inhibition percentage of 1000 mg/ml PK-ET extract is 66.6% (Table 2). The antioxidant activity results is significantly high when compared with standard ascorbic acid.

Table 2: *In vitro* antioxidant activity of *Mucuna pruriens* seed extract

Sample code	nm	COD	SOD	Inhibition (%)	Average %
PK ET	520	0.79	0.25	68.3	66.6
PK ET	520	0.79	0.32	59.4	
PK ET	520	0.79	0.28	64.5	
PK ET	520	0.79	0.26	67.0	
PK ET	520	0.79	0.25	68.3	
PK ET	520	0.79	0.22	71.1	
Ascorbic acid std.	520	0.73	0.04	94.5	93.5
Ascorbic acid std.	520	0.73	0.06	91.7	
Ascorbic acid std.	520	0.73	0.04	94.5	

Many researchers reported that ethanolic extract of *M. pruriens* seeds had antimicrobial properties against wide microbial strains. In the present study, the PK-ET extract is mainly effective against *Streptococcus pneumonia* (MTCC 655) and *Klebsiella pneumoniae* (MTCC 39). The antibacterial potency was evaluation by zone of inhibition (ZI) where *Streptococcus pneumonia* (MTCC 655) showed higher ZI (24 mm) (Figure 2) than *Klebsiella pneumoniae* (MTCC 39) ZI (22 mm) (Figure 3).

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Figure 2: Antibacterial activity of PK-ET extract against *Klebsiella pneumoniae* (MTCC 39)

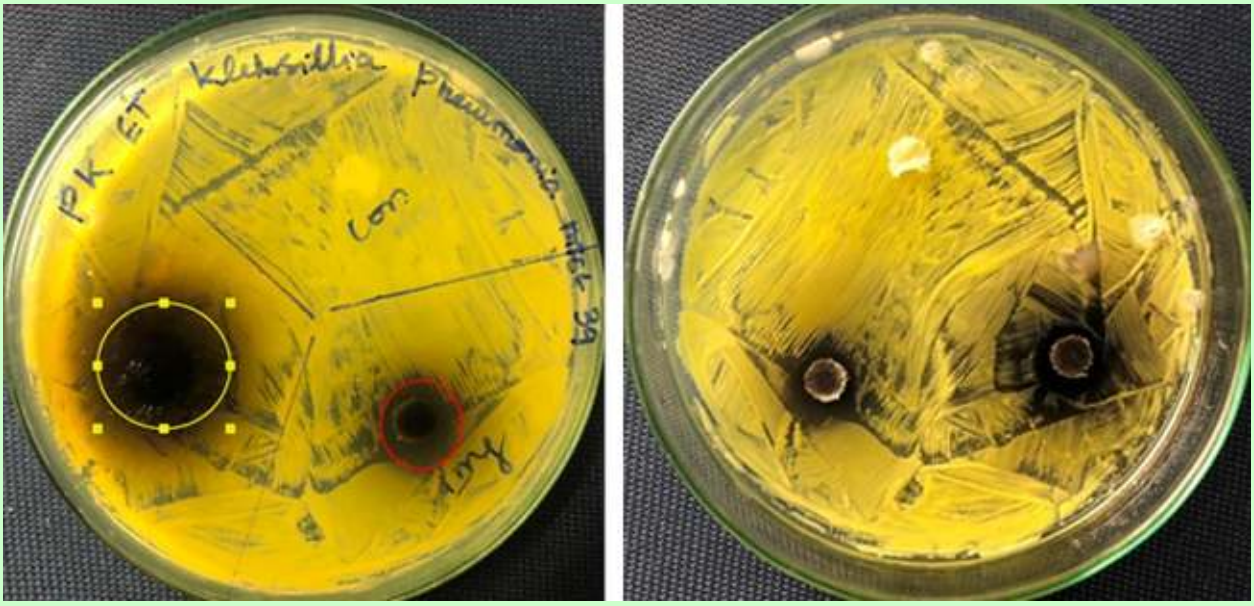
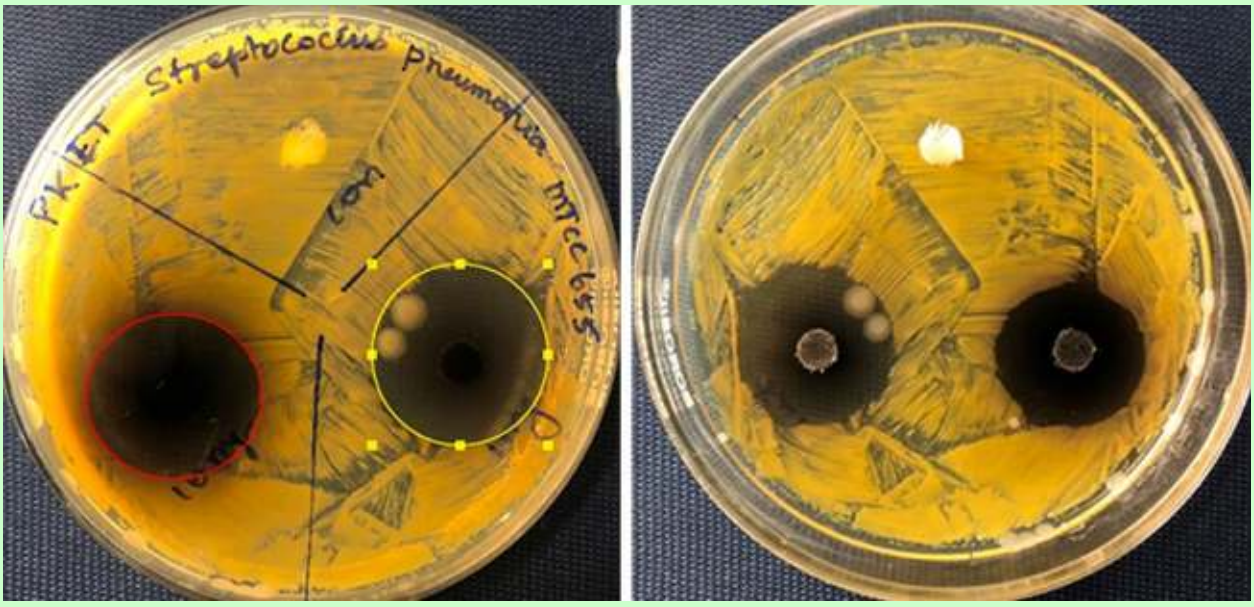


Figure 3: Antibacterial activity of PK-ET extract against *Streptococcus pneumonia* (MTCC 655)



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EVIDENCE BASED HERBAL MEDICINE - PART - V

In vitro antioxidant and cytotoxic activity of Stress Nil

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[2]*Department of Pharmaceutical Biotechnology, JSSAHR Academy of Research and Education College of Pharmacy, Ooty – 643004*

Abstract *– Stress Nil capsule is an authentic herbal supplement designed to provide instant and adequate relief from stress and anxiety. This product by Pure Nutrition is enriched with the goodness of Withania somnifera (ashwagandha) that has proven beneficial to reduce stress levels and anxiety and provides sound sleep. It's an all-natural product composed of a blend of ingredients that allows the body to relax, reduces stress, boost cognitive health and ensures a stress-free life. Also, its regular intake can enhance the mood while dealing with mood swings effectively.*

Keywords *– Stress, in vitro antioxidant activity, cytotoxic activity*

1. INTRODUCTION

Withania somnifera, also known as ashwagandha, Indian ginseng, and winter cherry, it has been an important herb in the Ayurvedic and indigenous medical systems for over 2000 years [1]. The roots of the plant are categorized as rasayanas, which are reputed to promote health and longevity by augmenting defense against disease, arresting the ageing process, revitalizing the body in debilitated conditions, increasing the capability of the individual to resist adverse stress and by creating a sense of mental wellbeing [2]. *Mentha piperita* L. is an important medicinal herb [3]. Herbalists consider peppermint as an astringent, antiseptic, antipruritic, antiemetic, carminative,

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vermifuge, diaphoretic, analgesic. *Mentha piperita* leaf is used as a remedy for common cold, inflammation of the mouth, pharynx, liver, as well as disorders in the gastrointestinal tract such as nausea, vomiting, diarrhea, cramps, flatulence and dyspepsia [4]. Long pepper (*Piper longum*), sometimes called Javanese, Indian, or Indonesian long pepper, is a flowering vine in the family Piperaceae cultivated for its fruit, which is usually dried and used as a spice [5]. Long pepper is a close relative of *P. nigrum*, which gives black, green, and white pepper and has a similar but generally hotter flavor. This plant is inexpensive, readily available, and effective for many diseases, including cancer, inflammation, stress, depression, diabetes, obesity, and hepatotoxicity [6]. Leaves of *Melissa officinalis* are used for their digestive, carminative, antispasmodic, sedative, analgesic, tonic, and diuretic properties, as well as for functional gastrointestinal disorders [7]. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. When combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity. The polyherbal formulation "Stress Nil" containing ingredients such as *Withania somnifera, Mentha piperita, Piper longum* and *Melissa officinalis*. This formulation is known to against stress. Stress Nil capsule is an authentic herbal supplement designed to provide instant and adequate relief from stress and anxiety. This product by Pure Nutrition is enriched with the goodness of *Withania somnifera* (ashwagandha) that has proven beneficial to reduce stress levels and anxiety and provides sound sleep. It's an all-natural product composed of a blend of ingredients that allows the body to relax, reduces stress, boost cognitive health and ensures a stress-free life. Also, its regular intake can enhance the mood while dealing with mood swings effectively.

Table 1: Polyherbal composition of "Stress Nil"

Plant name	Family
Withania somnifera	Solanaceae
Mentha piperita	Lamiaceae

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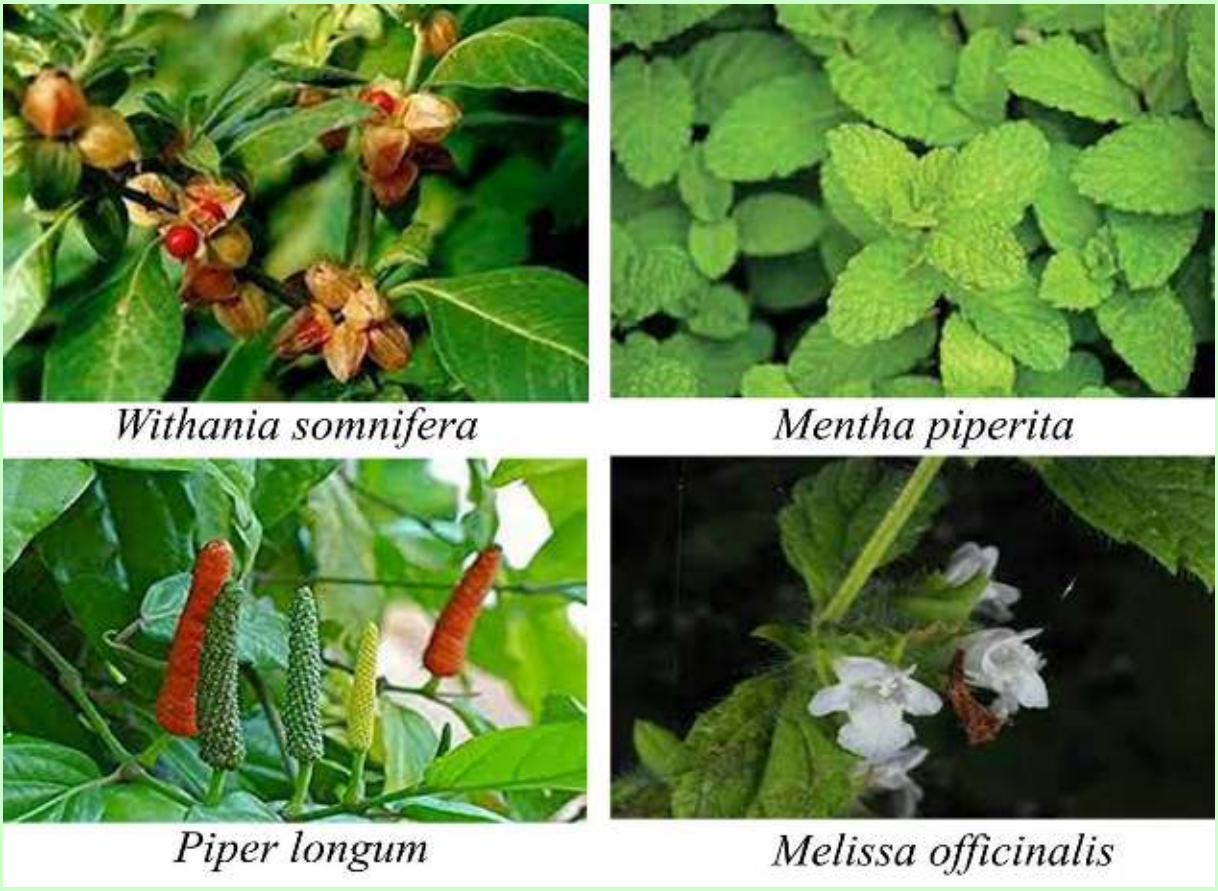
Piper longum	Piperaceae
Melissa officinalis	Lamiaceae

Figure 1: Photographs of polyherbs used for Stress Nil preparation



2. MATERIALS & METHODS

2.1. Preparation of extract

10 grams of dried powdered poly herbal materials were boiled with 100 ml of ethanol for 48 hours in a round bottomed-flask and filter. The filtrates (SK) are dried under vacuum and dried extract used for an activity.

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2.2. In vitro antioxidant activity

Antioxidant activity of the ethanolic extract of *Mucuna pruriens* (PK ET) was estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals [8]. 100 µL of *Mucuna pruriens* (PK ET) extracts were taken in the microtiter plate. The 100 µL of 0.1% methanolic DPPH was added to the samples and incubated in dark conditions for 30 minutes. The samples were then observed for discoloration; the plate was considered strong and weak positive from purple to yellow and pale pink respectively and read on the Elisa plate reader at 490 nm. Standard ascorbic acid was used as a reference. In triplicates, all the analysis was performed and average values were taken. The Radical scavenging activity was calculated using the below-mentioned equation:

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance ofcontrol)] x 100

2.3. Anticancer activity

The minimum essential medium (Eagle) with 2 mM I glutamine and Earle's BSS adjusted to contain 1.5 g/L Na bicarbonate, 0.1 mM non-essential amino acids and 1.0 mM Na pyruvate 90%; fetal bovine serum, 10%. The human brain neuroblastoma cell lines were incubated in Growth Conditions: 37°C temperature at 5% Carbon-dioxide atmosphere. Remove growth medium from flask. Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the cell sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cells come off substratum. Aspirate with fresh medium and dispense cell suspension into new flasks. Stress Nil were studied for anticancer activity against human brain neuroblastoma cell lines and vero cells through MTT assay [9]. The parameters such as inhibitory concentration (IC50-concentration required to inhibit the growth of 50% cancer cells) and cytotoxic concentrations were measured for the predictions of anticancer activity and the cytotoxicity potential of prepared extracts.

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3. RESULTS & DISCUSSION

Stress nil investigated for *in vitro* antioxidant activity and anticancer activity to explore possible therapeutic potentials. Stress nil had significant antioxidant activity of more 90 percent inhibition (Tables 1-3) of free radicals when compared with standard ascorbic acid of 97 percent under similar conditions free radicals is responsible for extensive damages of neurocells due to oxidative stress leads to neuritis.

Table 1: *In vitro* anti-oxidant activity of Stress Nil concentration 1 microg/ml

S. No.	Wavelength (nm)	COD	SOD	Percent inhibition (%)
1	520	0.67	0.06	91
2	520	0.68	0.05	92.6
3	520	0.69	0.01	98.5
4	520	0.69	0.02	97.1
5	520	0.68	0.04	94.1
6	520	0.69	0.05	93
Average				94.3

Table 2: *In vitro* anti-oxidant activity of standard Vitamin C concentration 1 microg/ml

S. No.	Wavelength (nm)	COD	SOD	Percent inhibition (%)
1	520	0.70	0.03	95.7
2	520	0.69	0.01	98.5
3	520	0.69	0.02	97.1
Average				97.1

Table 3: *In vitro* anti-oxidant activity of Stress Nil concentration 1 microg/ml

S. No.	Wavelength (nm)	COD	SOD	Percent inhibition (%)
1	520	1.43	0.14	90.2
2	520	1.46	0.13	91.0
3	520	1.41	0.16	88.6
4	520	1.41	0.16	88.6
5	520	1.40	0.11	92.1
6	520	1.44	0.12	91.2

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Average	90.3

Table 4: *In vitro* anticancer activity of Stress Nil against neuroblastoma cell line

Avg.	Conc.	% Viability
0.03	250	13.63636
0.0543	125	24.68182
0.111	62.5	50.45455
0.169	31.25	76.81818

Figure 2: Graph showing percentage of neuroblastoma cell viability

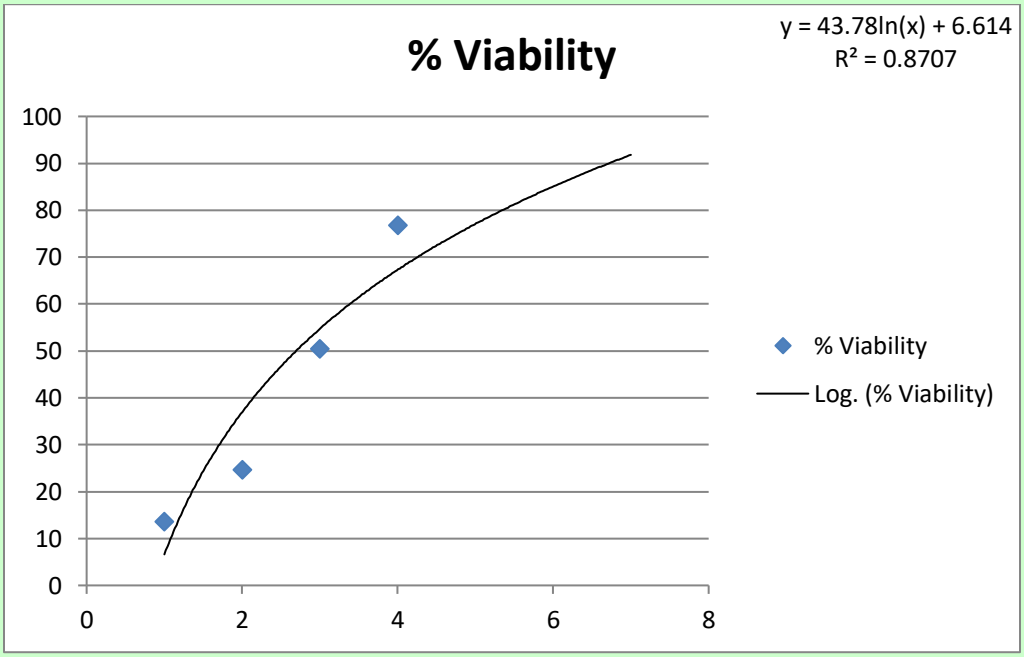

Table 5: *In vitro* anticancer activity of Stress Nil against vero cell line

	IC_{50}	67.04801
Avg	Conc	% Viability
0.11	250	50
0.1209	125	54.95455
0.1299	62.5	59.04545
0.1394	31.25	63.36364
Vero	**IC50**	**259.1222**

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Figure 3: Graph showing percentage of vero cell viability

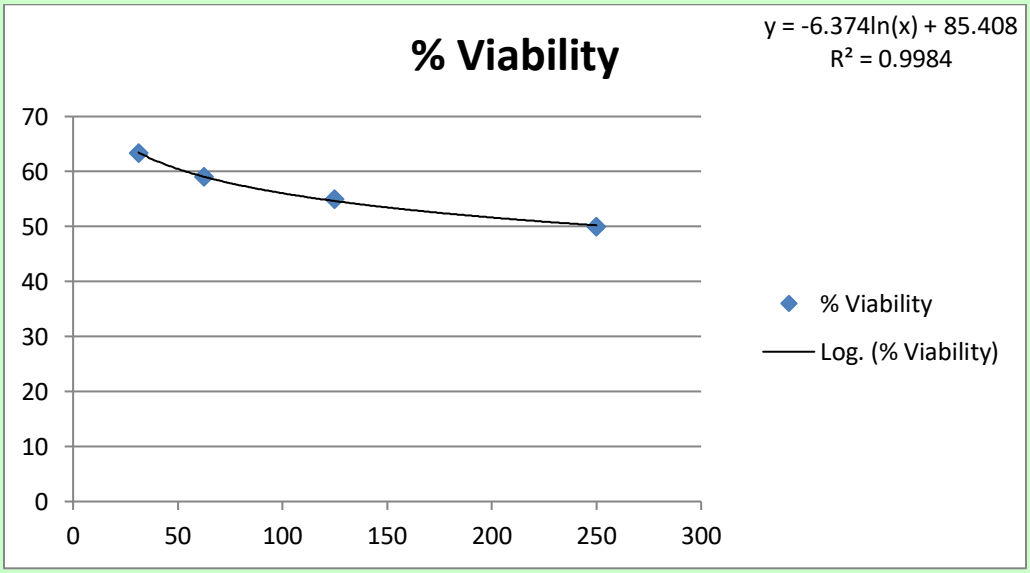

Figure 4: Photograph showing cell viability

Stress nil also test for *in vitro* anticancer activity against neuroblastoma cells and cytotoxicity against normal Vero cell to understand anticancer potential against neuroblastoma cells. From the study stress nil extract inhibits neuroblastoma cells at 76 microg/ml and non toxic to normal Vero cells at 259 microg/ml .this study demonstrate that stress nil had significant anticancer and antioxidant potentials (Tables 4 & 5 and Figures 2, 3 & 4).

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4. CONCLUSION

Polyherbal formulation with potential activity is essential for therapeutic potentials and present study documented for stress nil poly herbal formulation had significant scavenger of free radicals and also had good anticancer activity against neuroblastoma cells. This is first kind of report in the world of literature.

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In vitro antioxidant and antimicrobial activity of *Eclipta alba*

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Abstract *– Eclipta alba (Family: Asteraceae) is a natural gifted plant, very good source of a variety of bioactive molecules and also documented for a board-spectrum of pharmacological actions. The present work is in preparation of aqueous extract from Eclipta alba by hot continues the extraction process to achieve the complete extraction process. Prepared EA-ME (methanolic extract) was screened for their antibacterial activity. It was found EA-ME extract significantly in vitro antioxidant activity by DPPH assay with 79.05% at 1 mg/ml.*

Keywords *– Eclipta alba; antibacterial activity; antioxidant activity*

1. INTRODUCTION

Eclipta alba is a common folk medicinal plant which is generously known as bhringraj in Indian traditional medicine and as false daisy in English [1]. It belongs to the Asteraceae family, found in almost all over the globe. *Eclipta alba* is a small annual herb whose stem is usually erect, flat or round, blackish green, profusely branched and pubescent (Figure 1) [2]. Leaves are opposite, 3 to 5 cm long and blackish green in colour. The inflorescence is a head with 6 to 8 mm diameter. The flower is solitary, white, achene, compressed, and narrowly winged. Many blackish seeds are present in fruit [3]. This plant is known to have various pharmacological properties and is traditionally used in treatment of epilepsy [4]. Phytochemical studies on *Eclipta alba* discovered

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the presence of alkaloids like nicotine and ecliptine and bio-active steroidal alkaloids like verazine, dehydroverazine, ecliptalbine [5]. The plant is known to have some important pharmacological activities such as antimicrobial, antinociceptive, analgesic, antiinflammatory, antiviral, hepatoprotective, immunomodulatory activity, etc [6].

Table 1: Botanical classification

Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae
Genus	*Eclipta*
Species	*alba*

Figure 1: Photographs of *Eclipta alba* plant

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2. MATERIALS & METHODS

2.1. Preparation of extract

Eclipta alba powder was subjected to hot continuous extraction in soxhlet apparatus to using methanol (EA-ME) and ethanol (EA-ET) extracts to get different crude extracts. The extract were concentrated under vacuum and dried extracts EA ME used for in vitro antioxidant activity and antibacterial activity.

2.2. In vitro antioxidant activity

Antioxidant activity of the methanolic extract of *Eclipta alba* (EA-ME) was estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals [7, 8]. 100 µL of *Eclipta alba* (EA-ME) extract was taken in the microtiter plate. The 100 µL of 0.1% methanolic DPPH was added to the samples and incubated in dark conditions for 30 minutes. The samples were then observed for discoloration; the plate was considered strong and weak positive from purple to yellow and pale pink respectively and read on the Elisa plate reader at 490 nm. Standard ascorbic acid was used as a reference. In triplicates, all the analysis was performed and average values were taken. The Radical scavenging activity was calculated using the below-mentioned equation:

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance ofcontrol)] x 100

2.3. Antimicrobial activity

Methanolic extract of *Eclipta alba* (EA-ME) were screened for their antimicrobial activity against bacteria *Staphylococcus aureus*, *Streptococcus pyogens*, *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa* [9, 10]. To assess the antimicrobial activity, a well-diffusion assay was carried out. 17 hrs., of old bacterial cultures were inoculated over the agar surface of Mueller Hinton agar plates using sterile cotton swabs for the well diffusion assay. After 10 min, wells were cut using a cork borer and each well was loaded with 100 µl of compound from 10 mg/ml, 20

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mg/ml and 50 mg/ml concentration stock (100 µg/well) along with DMSO control. At 37°C, the plates were incubated for 24 h. Susceptibility was assessed on the basis of the diameter of the zone of inhibition (ZoI) against the test pathogens and the results are tabulated. The antibacterial activity of methanolic extract of *Eclipta alba* (EA-ME) were tested against human pathogens of *Staphylococcus aureus*, *Streptococcus pyogens*, *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa*.

3. RESULTS & DISCUSSION

Extracts of *Eclipta alba* (EA-ME / EA-ET) investigated for in vitro antioxidant activity and antibacterial activity to explore possible therapeutic potentials. *Eclipta alba* had significant antioxidant activity of more 79.05 percent inhibition (Tables 1 & 2) of free radicals when compared with standard ascorbic acid of 97 percent under similar conditions free radicals is responsible for antibacterial activity.

Table 1: *In vitro* anti-oxidant activity of *Eclipta alba* (EA-ET) 1 microg/ml

S. No.	Concentration	Wavelength (nm)	COD	SOD	% of inhibition
1	0.1	520	0.89	0.22	75.2
2		520	0.85	0.25	70.5
3		520	0.90	0.24	73.3
4		520	0.84	0.23	72.6
5		520	0.89	0.22	75.2
6		520	0.89	0.20	77.5
7	0.2	520	0.89	0.37	58.4
8		520	0.89	0.30	61.6
9		520	0.89	0.37	60.4
10	0.3	520	0.89	0.41	53.9
11		520	0.80	0.42	42.6
12		520	0.84	0.39	53.5
13	0.4	520	0.89	0.58	34.8
14		520	0.89	0.54	39.3
15		520	0.89	0.52	40.2
16	Ascorbic acid	520	0.89	0.14	84.2
17		520	0.89	0.12	83.1

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Table 2: *In vitro* anti-oxidant activity of *Eclipta alba* (EA-ME) 1 microg/ml					
S. No.	**Concentration**	**Wavelength (nm)**	**COD**	**SOD**	**% of inhibition**
1	0.1	520	0.98	0.17	82.65
2		520	0.97	0.19	80.41
3		520	0.95	0.19	80.00
4		520	0.91	0.21	76.92
5		520	0.90	0.18	80.00
6		520	0.91	0.20	78.02
7	Ascorbic acid	520	0.89	0.14	84.2
8		520	0.89	0.12	83.1

Extracts of *Eclipta alba* (EA-ET / EA-ME) exhibited antibacterial activity against *Streptococcus pyogens*, *Staphylococcus aureus*, *Klebsiella Pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*. *Staphylococcus aureus* showed maximum zone of inhibition in 100 µl, moderately sensitive in 20 µl. *Klebsiella pneumoniae* showed maximum zone of inhibition in 100 µl, moderately sensitive in 20, 40 and 80 µl. In positive control no zone of inhibition this showed that *Klebsiella pneumoniae* was resistant to Gentamycin. *Escherichia coli* showed maximum zone of inhibition in 100 µl, moderately sensitive in 20, 40 and 80 µl. *Pseudomonas aeruginosa* showed maximum zone of inhibition in 100 µl, moderately sensitive in 20, 40 and 80 µl. In all four bacteria 100 µl showed maximum concentration, so the concentration of the sample was 100 µl (Tables 3& 4 and Figures 2 & 3).

Table 3: *In vitro* antibacterial activity of *Eclipta alba* (EA-ET)						
S. No.	**Name of the bacteria (ATCC strain)**	**Zone formation in mm**				**Control**
		20µl	**40 µl**	**80 µl**	**100 µl**	**Gentamycin**
1	*Staphylococcus aureus*	14 mm	13 mm	13 mm	18 mm	20 mm
2	*Klebsiella pneumoniae*	12 mm	12 mm	12 mm	17 mm	No zone
3	*Escherichia coli*	12 mm	12 mm	13 mm	18 mm	20 mm
4	*Pseudomonas aeruginosa*	15 mm	13 mm	14 mm	15 mm	22 mm

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Table 4: *In vitro* antibacterial activity of *Eclipta alba* (EA-ME)						
S. No.	Name of the bacteria (ATCC strain)	Zone formation in mm				Control
		20µl	40 µl	80 µl	100 µl	Gentamycin
1	*Staphylococcus aureus*	11 mm	12 mm	14 mm	15 mm	10 mm
2	*Klebsiella pneumoniae*	18 mm	18 mm	16 mm	19 mm	5 mm
3	*Escherichia coli*	12 mm	12 mm	12 mm	5 mm	17 mm
4	*Pseudomonas aeruginosa*	5 mm	11 mm	14 mm	19 mm	18 mm

Figure 2: Antibacterial activity of *Eclipta alba* extract (EA-ET)

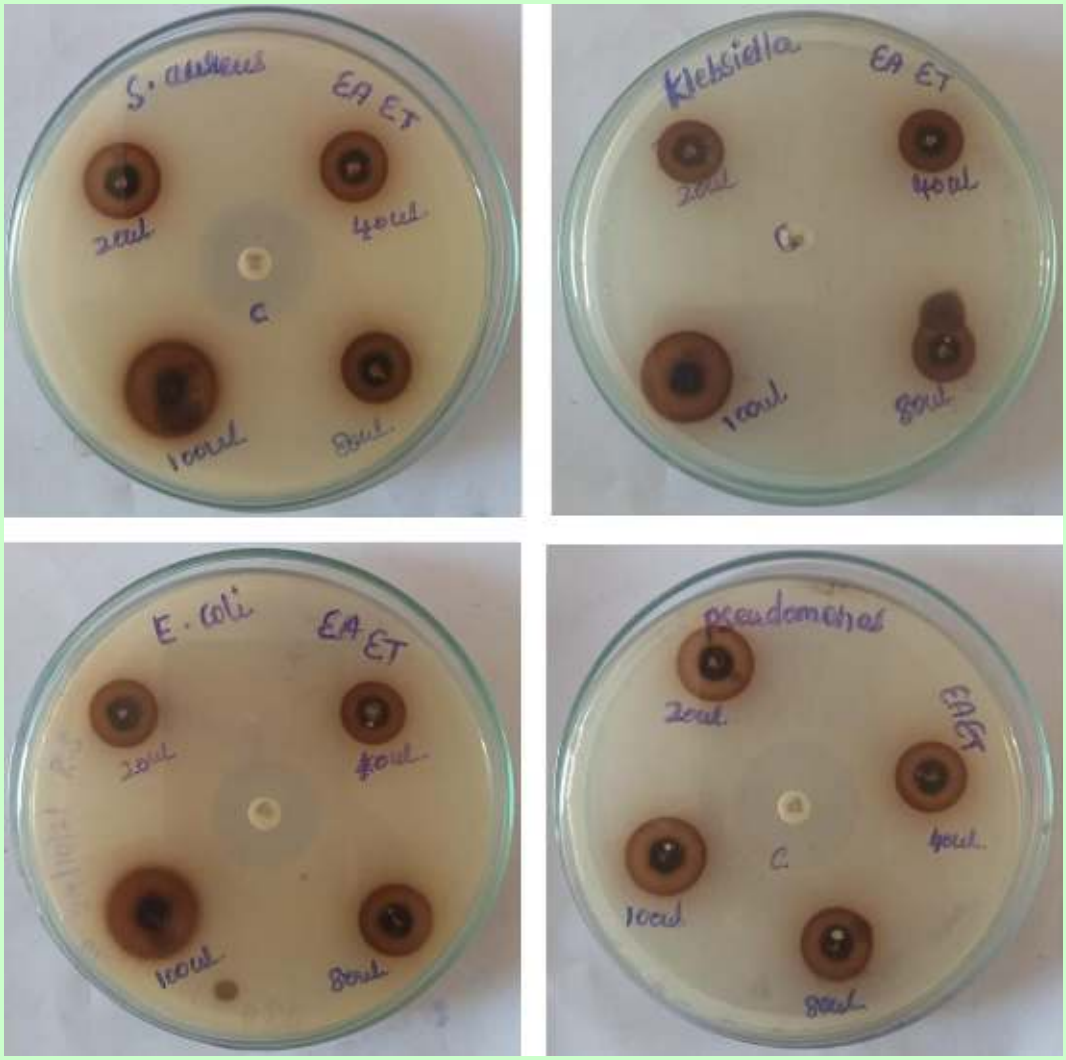

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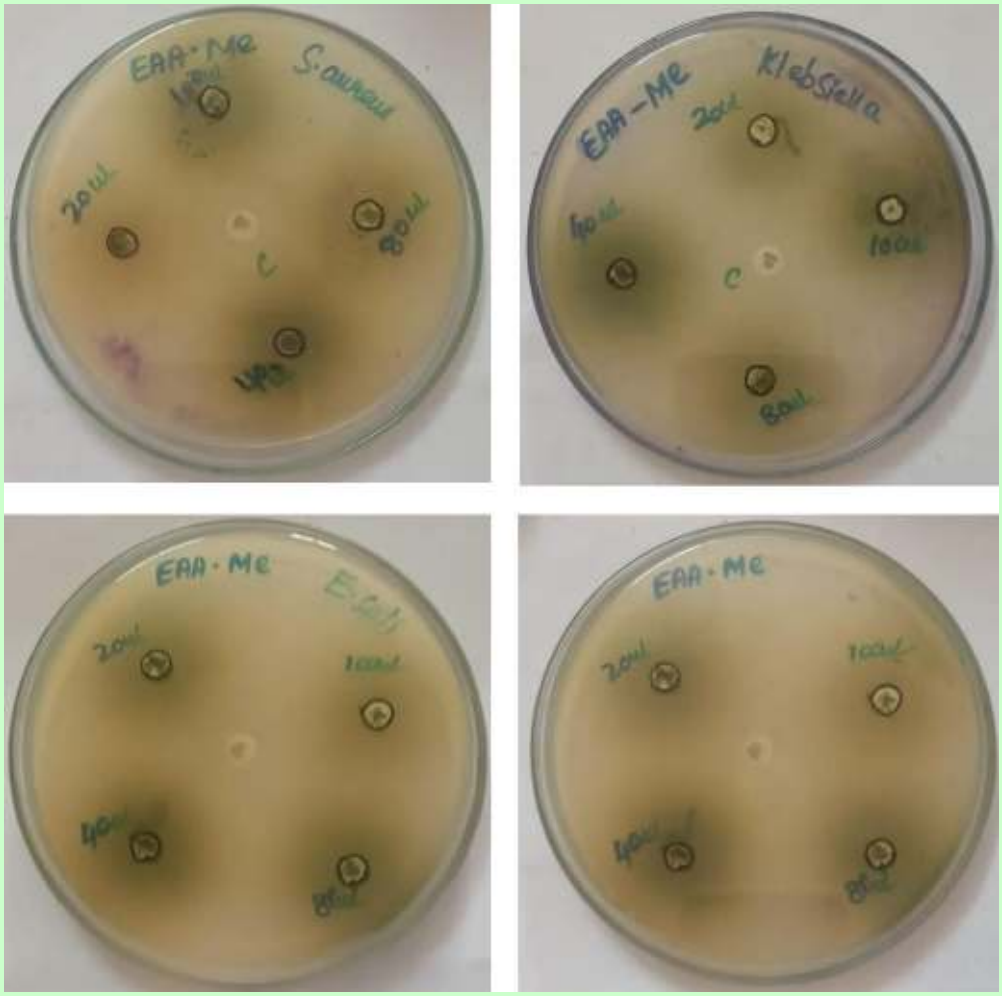
Figure 3: Antibacterial activity of *Eclipta alba* extract (EA-ME)



4. CONCLUSION

Methanolic extract of *Eclipta alba* (EA-ME) with potential activity is essential for therapeutic potentials and present study documented for its significant scavenger of free radicals and also had good antibacterial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*. Further investigation needed to study more pharmacological potential.

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EVIDENCE BASED HERBAL MEDICINE - PART - V

In vitro antioxidant and antimicrobial activity of Cansure capsules

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Abstract *– Cansure capsule is an authentic herbal supplement designed to provide an adequate relief from cancer and stress. It is a polyherbal product made up of Withania somnifera (Nattu amukkara), Ocimum sanctum (Thulasi), Centella asiatica (Vallarai), Cynodan dactylon (Aruganpul), Spheorabthus amarabthoicdes (Sivakaranthai), Bacopa monniera (Neer Brahami) and Lactose IP (Paal sarkkarai). Cansure capsule has proven beneficial to reduce stress levels and malignant cells and provides cure for cancer. It is a polyherbal product composed of a blend of ingredients that allows the body to fight against advanced cancer of the ovaries, breast, non-small cell lung cancer, and Kaposi sarcoma. Moreover this capsule may interferes with the growth of cancer cells, which are eventually destroyed. In the present study in vitro antioxidant and antibacterial properties and effect of different enzyme hydrolysis,(a-chymotrypsin, Trypsin, Papain, and Pepsin) were analysis in Cansure capsule by plate methods.*

Keywords *– Cansure capsule, in vitro antioxidant activity, antibacterial activity, cancer treatment*

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1. INTRODUCTION

The concept of polyherbalism to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. When combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity [1]. Cansure capsule is an authentic herbal supplement designed to provide an adequate relief from cancer and stress. It is a polyherbal product made up of *Withania somnifera* (Nattu amukkara), *Ocimum sanctum* (Thulasi), *Centella asiatica* (Vallarai), *Cynodan dactylon* (Aruganpul), *Spheorabthus amarabthoicdes* (Sivakaranthai), *Bacoba muneri* (Nithyakalyani) and Lactose IP (Paal sarkkarai) (Figure 1 and Table 1).

Withania somnifera, also known as ashwagandha, Indian ginseng, and winter cherry, it has been an important herb in the indigenous medical systems for over 2500 years [2]. The roots of the plant are categorised as rasayanas, which are reputed to promote health and longevity by augmenting defence against disease, arresting the ageing process, revitalizing the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and by creating a sense of mental wellbeing [3]. Many studies indicate ashwagandha possesses antioxidant, anticancer, anxiolytic, adaptogen, memory enhancing, antiparkinsonian, antivenom, antiinflammatory, antitumor properties [4].

Ocimum sanctum belongs to family Labiateae and OS is very important for their therapeutic potentials. The secondary metabolites present in *Ocimum* have been reported to have multiple biological effects, including antioxidant activity and anticancer activity [5]. Potential sources of antioxidants have been found in leaves, oilseeds, barks and roots. Natural antioxidants from plant sources are potent and safe due to their harmless nature. *O. sanctum* extracts are used as remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning and malaria [6].

Centella asiatica, a clonal, perennial herbaceous creeper belonging to the family Umbellifere (Apiceae) is found throughout India growing in moist places. Its chemical constituents have wide therapeutic applications in areas of antimicrobial, anti-inflammatory, anticancer,

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neuroprotective, antioxidant, and wound healing activities [7]. *C. asiatica* extract significantly increase the anti-oxidant enzymes, like superoxide dismutase (SOD), catalase and glutathione peroxidase (GSHPx) and decrease the anti-oxidants like glutathione (GSH) and ascorbic acid [8].

Cynodon dactylon is commonly known as "Aruvaum pullu" and belongs to family of Poaceae [9]. The plant is traditionally used as an agent to control diabetes in India. *C. dactylon* keeps several biological activities such as antibacterial, antimicrobial, anticancer, antiviral and wound healing properties [10]. Furthermore, it has been extensively used in traditional medicines to treat varied ailments such as cough, headache, diarrhea, cramps, epilepsy, dropsy, dysentery, hemorrhage, hypertension, hysteria, measles, snakebite, sores, stones urogenital disorders, tumors and warts [11].

Sphaeranthus amaranthoides Linn is a small procumbent herb found in semi-aquatic environment. The leaves are palmately 3-foliolate with stems r ooting and pubescent with appressed hairs. It belongs to the family Asteracea [12]. The plant is used in the treatment of eczema, blood disorders, stomach worms, filarial and fever. Several researchers reported that anti-bacterial activity of the ethanolic extract of the plant by disc diffusion method showed that the extract was active against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Enterococcus faecalis* and *Escherichia coli* [13]. The reported activities were wound healing effect, anti-diarrhoeal effect, antimicrobial effect, anticancer, analgesic and anti-inflammatory activities, hepatoprotective effect, antidiabetic activity, antimutagenic activity and antioxidant effect [14].

The plant *Bacopa monnieri* (water hyssop, Brahmi, thymeleaved Gratiola, herb of grace, and Indian pennywort) is a perennial, creeping herb native to the wet lands of India [15]. *Bacopa monniera* is characterized by its typical chemical composition which predominantly includes compounds like dammarane-type triterpenoid saponins called as bacosides, with jujubogenin or pseudo-jujubogenin moieties as their aglycone units. The various chemical constiuents exhibited antibacterial, antimicrobial, anticancer, and antiviral and wound healing properties [16].

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In view of the above literature the present study is focused to identify the antioxidant and antimicrobial activities of the polyherbal capsule "Cansure" and prove its efficacy against fighting against cancer cells and other tumor cells.

Figure 1: Photograph of Cansure product & polyherbs

Table 1: Polyherbal composition of "Cansure"

Botanical name	Vernacular name	Part used
Withania somnifera	Nattu amukkara	Root
Ocimum sanctum	Thulasi	Leaf
Centella asiatica	Vallarai	Leaf
Cynodan dactylon	Aruganpul	Leaf
Spheorabthus amarabthoicdes	Sivakaranthai	Leaf
Bacopa monniera	Neer brahami	Flower
Lactose IP	Paal sarkkarai	Additives

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2. MATERIALS & METHODS

2.1. Preparation of extract

Cansure capsule powder was subjected to hot continuous extraction in soxhlet apparatus to using ethanol to get crude extract. The extract was concentrated under vacuum and dried extract was used for *in vitro* antioxidant activity and antibacterial activity.

2.2. In vitro antioxidant activity

Antioxidant activity of the ethanolic extract of Cansure capsule powder was estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals [17, 18]. 100 µL of Cansure capsule powder extract was taken in the microtiter plate. The 100 µL of 0.1% methanolic DPPH was added to the samples and incubated in dark conditions for 30 minutes. The samples were then observed for discoloration; the plate was considered strong and weak positive from purple to yellow and pale pink respectively and read on the Elisa plate reader at 490 nm. Standard ascorbic acid was used as a reference. In triplicates, all the analysis was performed and average values were taken. The Radical scavenging activity was calculated using the below-mentioned equation:

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance ofcontrol)] x 100

2.3. Antimicrobial activity

Ethanolic extract of Cansure capsule powder was screened for their antimicrobial activity against bacteria species such as *Escherichia coli* (MTCC 1652), *Pseudomonas aeroginosa* (MTCC424), *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 96) [19, 20]. To assess the antimicrobial activity, a well-diffusion assay was carried out. 17 hrs, of old bacterial cultures were inoculated over the agar surface of Mueller Hinton agar plates using sterile cotton swabs for the well diffusion assay. After 10 min, wells were cut using a cork borer and each well was loaded with 1 mg/ml of compound from stock (1 mg/ml/well) along with DMSO control. At 37°C, the plates were incubated for 24 h. Susceptibility was assessed on the basis of the diameter of the zone

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of inhibition (ZoI) against the test pathogens and the results are tabulated. In this experiment distilled water was used as control.

2.4. Anticancer activity

The minimum essential medium (Eagle) with 2 mM I glutamine and Earle's BSS adjusted to contain 1.5 g/L Na bicarbonate, 0.1 mM non-essential amino acids and 1.0 mM Na pyruvate 90%; fetal bovine serum, 10%. The human brain neuroblastoma cell lines were incubated in Growth Conditions: 37°C temperature at 5% Carbon-dioxide atmosphere. Remove growth medium from flask. Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the cell sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cells come off substratum. Aspirate with fresh medium and dispense cell suspension into new flasks. Cansure studied for anticancer activity against human brain neuroblastoma cell lines and vero cells through MTT assay [21]. The parameters such as inhibitory concentration (IC50-concentration required to inhibit the growth of 50% cancer cells) and cytotoxic concentrations were measured for the predictions of anticancer activity and the cytotoxicity potential of prepared extracts.

3. RESULTS & DISCUSSION

Extracts of Cansure capsule powder investigated for *in vitro* antioxidant activity and antibacterial activity to explore possible therapeutic potentials. Cansure capsule powder had significant antioxidant activity of 76.7 percent inhibition (Table 2) of free radicals when compared with standard ascorbic acid of 85.1 percent under similar conditions.

Enzyme hydrolysis

Chymotrypsin cleaves peptide bonds by attacking the unreactive carbonyl group with a powerful nucleophile, the serine 195 residue located in the active site of the enzyme which briefly becomes covalently bonded to the substrate, forming an enzyme-substrate intermiediate. Selectively cleaves

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(cuts) off pieces of amino acids from the protein chain. Cansure samples was specifically chymotrypsin cleaves phenylalanine, tyrosine, and tryptophan bonds, or in other words the aromatic amino acids. Cansure samples have more efficient to cleave these amino acids starting from the C-terminus of the protein including Trypsin, Papain, and Pepsin inhibitor were compare to other compounds (Figure 2) (V. Vikram *et al*., 2021).

Figure 2: Effect of Cansure samples on different enzyme activity *in vitro* assay

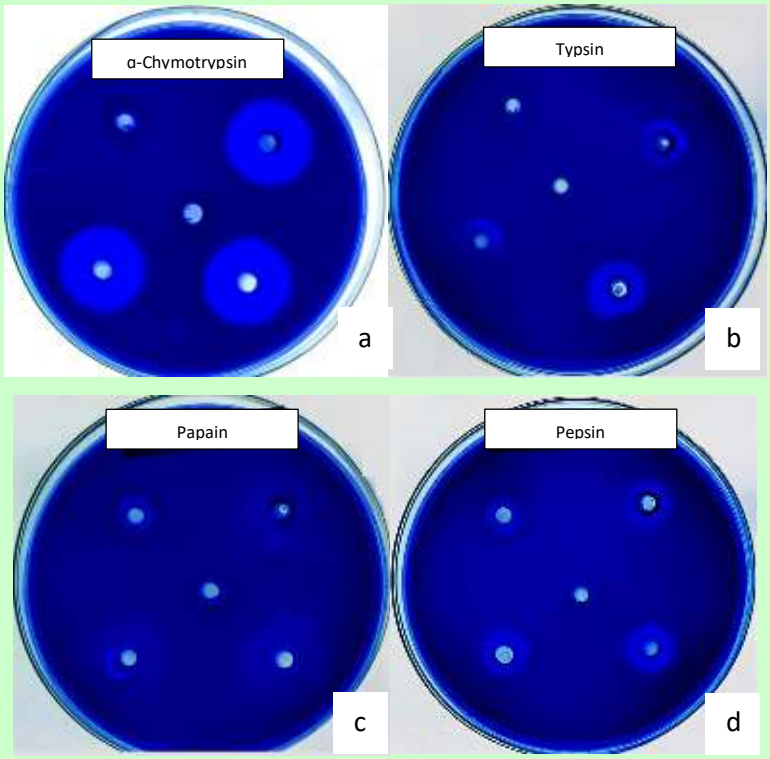

Table 2: *In vitro* anti-oxidant activity of Cansure capsule powder 0.1 microg/ml

S. No.	Concentration	Wavelength (nm)	COD	SOD	% of inhibition	Average
1	0.1	520	0.79	0.22	72.1	76.7%
2		520	0.81	0.17	78.0	
3		520	0.80	0.18	77.5	
4		520	0.79	0.21	73.4	
5		520	0.79	0.18	77.2	

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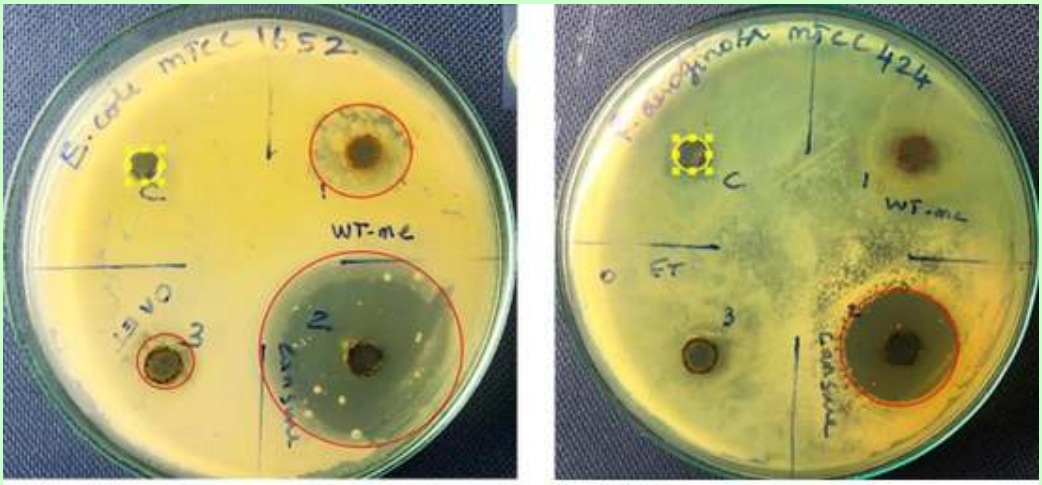
6		520	0.79	0.14	82.2	
7	Ascorbic acid	520	0.79	0.14	83.2	85.1%
8		520	0.79	0.10	87.3	
9		520	0.79	0.11	86.0	

Extracts of Cansure capsule powder exhibited maximum antibacterial activity against *Escherichia coli* (MTCC 1652) and *Bacillus subtilis* (MTCC 441) showed maximum zone of inhibition in 1 mg / ml concentration whereas, *Pseudomonas aeroginosa* (MTCC424) and *Staphylococcus aureus* (MTCC 96) were exhibited moderately sensitive against Cansure powder extract. In this experiment there was no zone identified in control (Table 3 and Figure 3).

Table 3: *In vitro* antibacterial activity of Cansure capsule powder

S. No.	Name of the bacteria (MTCC strain)	Zone formation in mm 1 mg/ml	Control (Water)
1	*E. coli* (MTCC 1652)	906 mm	No zone
2	*P. aeroginosa* (MTCC424)	866 mm	
3	*B. subtilis* (MTCC 441)	874 mm	
4	*S. aureus* (MTCC 96)	725 mm	

Figure 3: Antibacterial activity of Cansure capsule powder



Escherichia coli (MTCC 1652) *Pseudomonas aeroginosa* (MTCC424)

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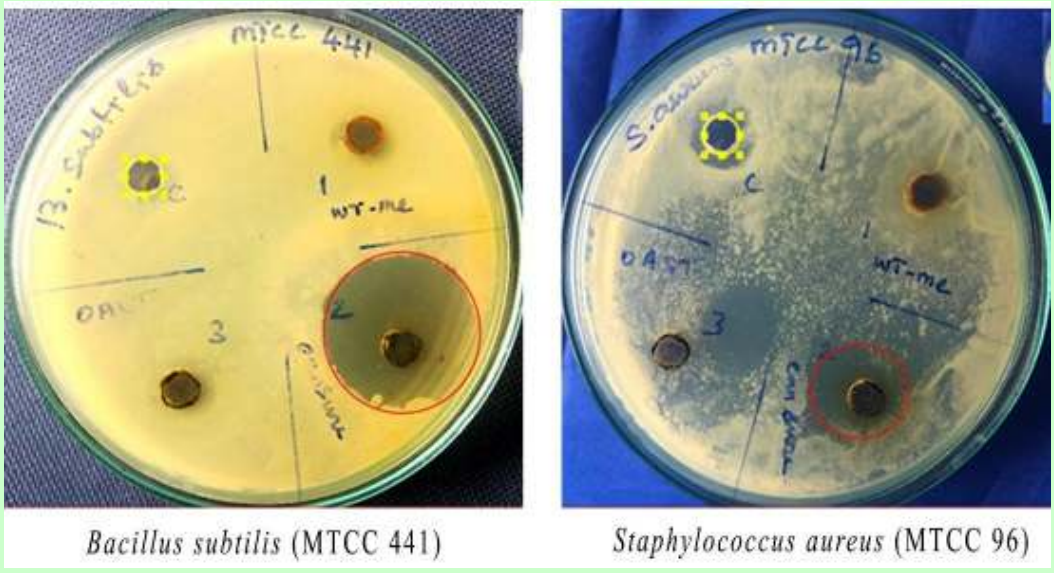

Bacillus subtilis (MTCC 441) *Staphylococcus aureus* (MTCC 96)

Cansure also test for *in vitro* anticancer activity against neuroblastoma cells and cytotoxicity against normal Vero cell to understand anticancer potential against neuroblastoma cells. From the study Cansure extract inhibits neuroblastoma cells at 36.7 microg/ml and non-toxic to normal Vero cells at 144.5 microg/ml .this study demonstrate that Cansure had significant anticancer and antioxidant potentials (Tables 4 & 5 and Figures 4 & 5).

Table 4: *In vitro* anti-cancer activity of Cansure capsule powder against neuroblastoma cells

	SK-N-SH		
Cansure	**Avg**	**Conc**	**% Viability**
	0.1296	250	30.85714
	0.1489	125	35.45238
	0.1873	62.5	44.59524
	0.2189	31.25	52.11905
	S1	**IC50**	**36.73067**

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Figure 4: *In vitro* anti-cancer activity of Cansure capsule powder against neuroblastoma cells

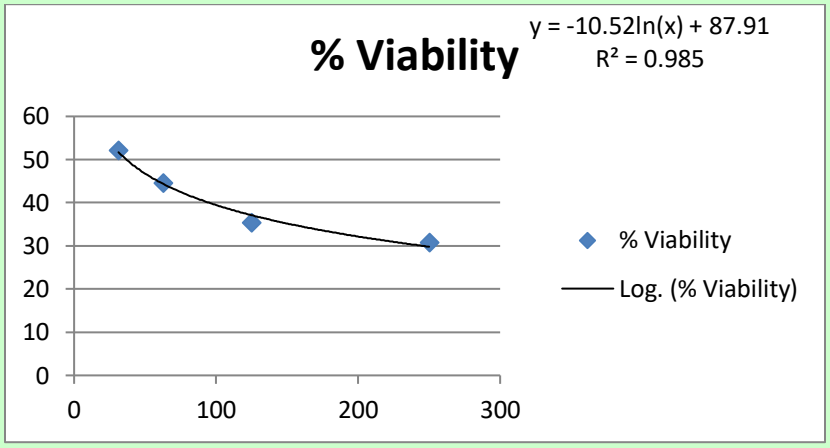
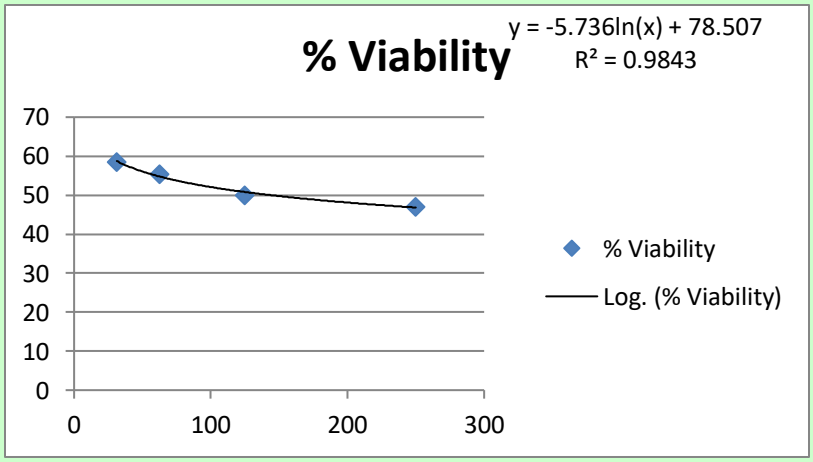


Table 4: *In vitro* anti-cancer activity of Cansure capsule powder against vero cells			
Vero	**Avg**	**Conc**	**% Viability**
Cansure	0.198	250	47.14286
	0.21	125	50
	0.233	62.5	55.47619
	0.246	31.25	58.57143
	S2	**IC50**	**144.5784**

Figure 5: *In vitro* anti-cancer activity of Cansure capsule powder against vero cells



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4. CONCLUSION

Ethanolic extract of Cansure capsule powder with potential activity is essential for therapeutic potentials and present study documented for its significant scavenger of free radicals and also had good antibacterial activity against *Escherichia coli* (MTCC 1652), *Pseudomonas aeroginosa* (MTCC424), *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 96). Cansure is effective against Further investigation needed to study more pharmacological potential.

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EVIDENCE BASED HERBAL MEDICINE - PART - V

Investigation of pharmacological actions of *Vitex negundo* and *Smilax china*

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Abstract *– Phytochemicals are ecologically derived plant secondary metabolites produced by the plants to protect them against environmental stress and invasions against pathogenic microbes. These phytochemical compounds are known to elicit beneficial as well as pharmacological actions against human ailments. It is an established fact that, the active principles present in the medicinal plants act synergistically to alleviate the primary and secondary complications of several diseases. Vitex negundo and Smilax china are widely used as a therapeutic agent as well as dietary supplement for the treatment of novel Corona virus (COVID-19). In the present study, above plants were investigated for inhibition of SARS CoV-2 spike protein (RBD) with Human ACE-2 receptor binding The results obtained evidenced that, the aqueous extract possesses significant inhibition of COVID 19 spike protein with Human ACE-2. The data presented provide scientific evidence for the antiviral activity with inhibition of viral entry and therapeutic efficacy, which in turn may be due to the presence of biologically active molecules present in the medicinal plants Vitex negundo and Smilax china.*

Keywords *–Vitex negundo; Smilax china; COVID-19; Spike protein; Human ACE-2*

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1. INTRODUCTION

Vitex negundo and *Smilax china* are the versatile medicinal plants and it comprises of several value added bioactive compounds [1,2]. It is widely used as a therapeutic agent as well as prophylactic supplement in COVID 19/SARSCoV-2 and other viral diseases. Excellent pharmacological and therapeutic benefits of above medicinal plant is due their enriched phytochemical constituents and micronutrients. The proposed plants *Vitex negundo* and *Smilax china* reported for broad spectrum of pharmacological activity due their chemical compounds and combination of medicinal plants of high therapeutic values [3-5]. The selected two medicinal plants were recommended for treatment of COVID - 19 as immunomodulator due to their therapeutic benefits [6-8]. In the present study, the ability of the aqueous extract is investigated for inhibition of SARS COV-2 spike protein. Poly pharmacy essential for radical cure of COVID-19 infections is urgent and need for the hour. Investigated medicinal plant contains variety of chemical constituents which helps to treat the SARS CoV-2.

Figure 1: Photograph of medicinal plants

Vitex negundo *Smilax china*

2. MATERIALS & METHODS

2.1. Preparation of extract

All ten medicinal plants about 100 gm taken in equal ratio of *Vitex negundo* and *Smilax china* in one liter round bottomed flask and add 1000 ml of distilled water, boiled for 5 hours, cool the

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extract, and filtered. The filtrate was evaporated under vacuum, to get dried extract. This dried extract was used for the investigation of pharmacological activity. The extract was concentrated under vacuum and dried extract was used for *in vitro* antioxidant activity and antibacterial activity.

2.2. Inhibition of COVID 2019 Spike protein RBD binding with Human ACE 2

The extracts were used for the management of COVID -19/SARS CoV-2 recommended by Ministry of AYUSH. In this present study the extracts were investigated for inhibition of COVID-19 Spike protein Receptor Binding Domain (RBD) with Human Angiotensin Converting Enzyme (H-ACE-2) to understand the mechanism of action –explore the inhibition of viral entry [9].

3. RESULTS & DISCUSSION

Plant extracts of *Vitex negundo* and *Smilax china* investigated for inhibition of COVID 2019 Spike protein Receptor binding domain (RBD) with Human Angiotensin converting enzyme (H ACE 2). All the extracts inhibits COVID 2019/SARS CoV 2 in the range of 63-89 % inhibition at 1 mg/ml (Table 1). The compounds Quercetin, Rutin and Curcumin were found to be 92.41, 49.21 and 63.48% respectively. Extract VNA and SCA had significant inhibition of 90.67 and 83% respectively at 1mg/ml. From this studies indicate extracts inhibits the viral entry by blocking the RBD with HACE 2 receptor binding and suitable for further studies.

Compounds stock: 2mg/ml		**mg/ml**	**Percent Inhibition**				
	Working: 1mg/ml	**1**	49.21	92.41	90.67	63.48	83.00
		0.5	29.16	84.40	80.00	43.03	62.88
RBD-Baculo: added 1mg/ml	RBD-working-0.5 mg/ml	0.25	11.65	78.40	66.46	21.19	43.06
		0.125	7.92	72.06	50.85	9.07	22.33
		0.0625	4.20	55.88	33.99	1.07	18.01
		0.03125	1.11	29.02	22.06	0.82	5.94
		0.015625	0.24	13.18	10.13	0.58	6.84

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		0.007813	0	0	0	0	0
			Rutin	Quercetin	VNA	Curcumin	SCA

4. CONCLUSION

Aqueous plant extracts inhibit the COVID 2019 Spike protein RBD binding with Human ACE 2 is essential for therapeutic potentials against SARS Covid. Further investigation needed to study more pharmacological potential of the plant species studied.

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EVIDENCE BASED HERBAL MEDICINE - PART - V

In vitro antibacterial activity of the phytochemical extracts from *Morinda citrifolia L* and *Vitex trifolia L*

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Abstract *– Phytochemicals are ecologically derived plant secondary metabolites produced by the plants to protect them against environmental stress and invasions against pathogenic microbes. These phytochemical compounds are known to elicit beneficial as well as pharmacological actions against human ailments. It is an established fact that, the active principles present in the medicinal plants act synergistically to alleviate the primary and secondary complications of several diseases. Noni the versatile plant (Morinda citrifoila) is known for its various therapeutic applications in East-Asian countries. Ethanolic extracts of Morinda citrifoila L Noni and seed Vitex trifolia Linn. powder were prepared by hot continuous extraction method and dried under vacuum. Dried extracts were tested against Staphylococcus aureus, Bacillus subtilis, Lactobacillus acidophilus, Escherichia coli, Pseudomonas aeruginosa and Proteus vulgaris.*

Keywords *– Morinda citrifoila; Vitex trifolia; Antimicrobial activity*

1. INTRODUCTION

Morinda citrifolia L Noni (Family: Rubiaceae) is a versatile medicinal plant with excellent pharmacological and medicinal value due to the presence of enriched bioactive molecules and

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nutraceuticals. The phytochemical content of Noni is responsible for antimicrobial activity has many therapeutic and medicinal values [1]. Noni reduces the risk of cancer [2], acts as a hepatoprotective [3], and xanthine oxidase inhibitory activity [4]. The biological potential of bioactive molecules of noni is responsible for the treatment of many diseases including cancer [5]. *Vitex trifolia* Linn. is the family Verbenaceae [6] (Figure 1). This plant is found in the tropical and subtropical regions around the world including India, Sri Lanka, china and Indonesia etc [7]. Leaves of vitex are commonly used as poultice for rheumatic pains, antimicrobial activity, inflammations, sprains [8]. It is used in curing fever, improves memory, favours hair growth, improves vision and treats leucoderma. Roots of vitex are used in treatment of painful inflammations, cough and fever [9]. Flowers are found useful in treating fever and fruits in treating amenorrhoea [10]. Present work was envisaged to study the in vitro antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus acidophilus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.

Figure 1: Photograph of medicinal plants

Morinda citrifolia *Vitex trifolia*

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2. MATERIALS & METHODS

2.1. Preparation of extract

10 gm dry powder of noni seeds were boiled with 100 ml of solvents (acetone, petroleum ether, chloroform, and ethanol) in a reflux condenser for 3 hours and filtered. The clear filtrate is dried under vacuum and dried extract ANS, PNS, CNS, and ENS is studied for biological activity.

2.2. Anti-bacterial activity

Test organisms: *Staphylococcus aureus*, *Bacillus subtilis* and *Lactobacillus acidophilus* are the gram positive bacteria and *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* are the Gram negative bacteria used as test organisms.

Procedure: Agar cup-plate method was used to assess the antibacterial activity of successive extracts.0.1 mL of Gram positive/negative bacterial test organisms was seeded into 20 ml of the sterile nutrient agar medium and poured into sterile Petri-dishes (pour-plate technique) and allowed to solidify. The six mm cups were made on the medium and dried extracts were dissolved in 10% dimethyl sulfoxide (DMSO) to obtain a concentration of 500 μg mL^{-1} and sterilized by filtration, through a Whatman filter paper No. 1 and 0.1 mL of different concentrations of extract were added to the respective cups. 0.1 mL of streptomycin at a concentration of 50 μg mL^{-1} was taken as standard reference (PC-positive control). The Petri-dishes with different concentrations of extract were kept in refrigerator at 4°C for 1 h for diffusion. After the process of diffusion, the Petri-dishes were incubated at 37°C for 24 h and the zones of inhibition were measured for the assessment of antibacterial activity using DMSO as the control (N) [11].

3. RESULTS & DISCUSSION

Antibacterial activity of the plant extracts, tested against selected microorganisms were recorded (Table 1). In the present study total of 5 successive plant extracts were selected and tested for their bioactivity. Antimicrobial activity of extracts was determined using cup plate diffusion method.

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Zone of Inhibition sizes were used to determine the susceptibility to extracts (Figure 2). The results showed that, MCF-ET 1 showed potential antibacterial activity against *Staphylococcus aureus*, *Escherichia coli, Lactobacillus acidophilus, Pseudomonas aeruginosa, Proteus vulgaris*. MCF-ET 2 showed potential antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Lactobacillus acidophilus*. KVNA 1 showed potential antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris*. KVNA 2 showed potential antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris*.

Table 1: Antibacterial activity

Strains	Extracts/Zone of inhibition (mm)				
	MCF-ET1	MCF-ET2	KVNA1	KVNA2	PC (Streptomycin)
S. aureus	11.1±0.15	11.2±0.20	10.77±0.25	10.43±0.15	22.26±0.05
B. subtilis	-	-	-	-	21.17±0.31
L.acidophilus	10.80±0.21	10.34±0.51	-	-	22.43±0.05
E. coli	11.80±0.25	11.22±0.39	-	-	21.10±0.21
P. aeruginosa	14.26±0.39	14.47±0.51	12.93±0.40	12.80±0.10	22.29±0.10
P. vulgaris	12.19±0.10	12.37±0.40	11.43±0.21	11.20±0.20	22.23±0.40

Values are expressed as mean ± SD (n = 3)

MCF-ET1 & 2 and KVNA1 & 2 extracts showed significant antibacterial potential against test organisms. The most susceptible organism in the investigation was Pseudomonas aeruginosa against which, extracts showed good inhibition zones. Maximum antibacterial activities were recorded for MCF-ET 1 & 2 extracts against Pseudomonas aeruginosa. It is well documented that the Gram-negative bacteria has difference in cell wall composition. In the present study it was observed that there is susceptibility difference between Gram-positive and Gram-negative bacteria

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indicating that the extract may have anti-bacterial activity due to cell wall inhibition as one of the mechanisms.

Figure 2: Antibacterial assay

Zone of inhibition shown by extracts for *Staphylococcus aureus*(A), *Bacillus subtilis*(B), *Lactobacillus acidophilus*(C), *Escherichia coli*(D), *Pseudomonas aeruginosa*(E), *Proteus vulgaris*(F) and Positive control (G)

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4. CONCLUSION

In conclusion, the plant extracts has the potential and broad spectrum anti-bacterial activity and may help to discover new chemical classes of antibiotic substances that could serve as selective chemotherapeutic agents for infectious diseases and their control.

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EVIDENCE BASED HERBAL MEDICINE - PART - V

Investigation of pharmacological actions of Herbex polyherbal formulations

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Abstract *– Herbex is poly herbal formulation Solanum xanthocarpum, Ocimum sanctum, Calotropis gigantea, Piper longum, Adhatoda vasica, Indigofera tinctoria, Leucas aspera, Piper nigrum and Curcuma augustifolia used traditionally for the treatment of respiratory and related disorder. In the present study we have investigated for the antioxidant activity and Interleukin 6 (IL-6) of Herbex syrup and MIC (minimum inhibitory concentration) of aqueous extract against Mycobacterium tuberculosis (H37Rv). Antioxidant activity by DPPH method was compared with standard ascorbic acid under similar conditions. Further, Interleukin 6 (IL-6) experiment was used to validate the pharmacological action of Herbex formulation. Also, antimicrobial activity of Herbex against multidrug resistant bacteria Mycobacterium tuberculosis (H37Rv) was also examined to understand microbial potential. Herbex contains medicinal plants with antimicrobial activity, antioxidant and anti-inflammatory that is essential for therapeutic efficacy. Phytoconstituents of herbex is responsible for the antioxidant, antibacterial and anti-inflammatory activities that can evaluated with suitable in vivo studies.*

Keywords *– Herbex, Interleukin 6 (IL-6), M. tuberculosis (H37Rv), antioxidant*

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1. INTRODUCTION

Herbex syrup contains combination of a versatile medicinal plants such as *Solanum xanthocarpum* [1], *Ocimum sanctum* [2], *Calotropis gigantea* [3], *Piper longum* [4], *Adhatoda vasica* [5], *Indigofera tinctoria* [6], *Leucas aspera* [7], *Piper nigrum* [8] and *Curcuma augustifolia* [9] with high therapeutic values and pharmacological actions due to their enriched phytochemical constituents, which is used for the treatment of respiratory diseases. Poly herbal formulation with antioxidant, anti-inflammatory, anti-bacterial activity essential requirement for the treatment of respiratory tract infections [10]. Current study involves the preparation of an aqueous extract from Herbex poly herbal formulation by using hot continues extraction method. Present work involves the study of the antioxidant activity and Interleukin 6 (IL-6 – pro-inflammatory cytokinin) of Herbex syrup and MIC (minimum inhibitory concentration) of aqeous extract against Mycobacterium tuberculosis (H37Rv). Antioxidant activity by DPPH method was compared with standard ascorbic acid under similar conditions.

COMPOSITION OF HERBAX SYRUP

Composition of Herbex is listed in the below mentioned table.

Composition of Herbax syrup	mg
Solanum zantho conpus	205.622
Ocimum sanctum	37.324
Calotropis gigantea	37.324
Piper longum	18.662
Adhatoda vasica	37.324
Indigofera tinctoria	18.662
Leucas aspera	37.324
Piper nigrum	18.662
Glycyrrhiza glabra	37.324
Curcuma augustifelia	200
Sugar	559.2

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2. MATERIALS & METHODS

2.1. Preparation of extract

All ten medicinal plants about 100 gm taken in equal ratio in one liter round bottomed flask and add 1000 ml of distilled water, boiled for 5 hours, cool the extract, and filtered. The filtrate was evaporated under vacuum, to get dried extract. This dried extract was used for the investigation of pharmacological activity. The extract was concentrated under vacuum and dried extract was used for MABA assay for determine the MIC against *Mycobacterium tuberculosis* (H37Rv). Herbex syrup was subjected to antioxidant and Interleukin 6 (IL-6) activity assays.

2.2. Determination of DPPH radical scavenging activity

Antioxidant activity in the sample AL-A were estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals (Odabasoglu et al., 2005) [11]. 100µL of AL-A extract was taken in the micro-titer plate. 100µL of 0.1% DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively and read the plate on Elisa plate reader at 490 nm. Standard ascorbic acid was used as reference. All the analysis was performed in triplicates and the average values were taken.

The Radical scavenging activity was calculated by using the below mentioned equation:

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] x 100

2.3. Determination of IL-6 (Sub acute toxicity studies)

The serum levels of IL-6 was determined with a commercially ELISA kit following the manufacturer's instructions. The OD was measured using a micro-plate reader at 405 nm with a wavelength correction set to 650 nm. In plates of 6 wells macrophages J774A.1 were seeded at a density of 1 × 106/well and treated with CESC (25 µg/mL) or IND at a concentration of 17 µg/mL (50 µM) and incubated for 2 h. After this time, 5 µg/mL of LPS was added and the cells were

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incubated for 24 h, the supernatants were collected and stored at −80˚C until they were analyzed. The levels of IL-6 in the supernatants of the cultures of macrophages were determined using a commercial immune-enzymatic kit (PeproTech) [12].

2.4. MABA assay for determine the MIC

The inoculum was prepared from fresh LJ medium re-suspended in 7H9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase [OADC]), adjusted to an OD590 1.0, and diluted 1:20; 100 µl was used as inoculum. Each drug stock solution was thawed and diluted in 7H9-S at four-fold the final highest concentration tested. Serial two-fold dilutions of each drug were prepared directly in a sterile 96-well microtiter plate using 100 µl 7H9-S. A growth control containing no antibiotic and a sterile control were also prepared on each plate. Sterile water was added to all perimeter wells to avoid evaporation during the incubation. The plate was covered, sealed in plastic bags and incubated at 37°C in normal atmosphere. After 7 days incubation, 30 µl of alamar blue solution was added to each well, and the plate was re-incubated overnight. A change in colour from blue (oxidized state) to pink (reduced) indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in colour [13, 14].

3. RESULTS & DISCUSSION

Herbex investigated for *in vitro* antioxidant activity by DPPH assay and compared with standard ascorbic acid. Herbex syrup had significant anti-oxidant activity (87.6%) (Tables 1-3) when compared with standard ascorbic acid (1 mg/ml) by dose dependent manner. The present results showed that the serum level of IL-6 in rats, treated with the Herbex syrup (for 30 days) was at a value of 18.9 pg/ml at 200 mg/kg and 17.2 pg/ml at 400mg/kg when compared with that of 14.4 pg/ml of control group (Table 4). MABA assay to determine the MIC (minimum inhibitory concentration) of herbex syrup against *Mycobacterium tuberculosis* H37Rv exhibited significant result (MIC 25 µg/mL) (Table 5).

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Table 1: *In vitro* antioxidant activity of different doses of Herbex syrup

S. No.	NM	C.OD	S.OD	Value
0.1				
1	520	0.99	0.23	**76.6%**
2	520	0.99	0.20	**79.7%**
3	520	0.97	0.19	**80.4%**
				78.9%
0.2				
1	520	0.98	0.18	**81.6%**
2	520	0.99	0.15	**84.8%**
3	520	0.98	0.13	**86.7%**
				84.4%
0.3				
1	520	0.98	0.13	**86.7%**
2	520	0.98	0.14	**85.7%**
3	520	0.90	0.11	**88.8%**
				87.1%
0.4				
1	520	0.98	0.12	**87.7%**
2	520	0.97	0.11	**88.6%**
3	520	0.98	0.12	**87.7%**
				88.0%
0.5				
1	520	0.99	0.32	**67.6%**
2	520	0.98	0.12	**87.7%**
3	520	0.96	0.09	**90.6%**
				82.0%
Vitamin C				
1	520	0.98	0.05	**94.8%**
2	520	0.99	0.08	**91.95%**
3	520	0.99	0.11	**88.8%**
				91.8%

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Table 2: *In vitro* antioxidant activity of different doses of Herbex syrup

S. No.	NM	C.OD	S.OD	Value
0.1				
1	520	0.92	0.13	**85.8%**
2	520	0.93	0.11	**88.1%**
3	520	0.91	0.12	**86.8%**
				86.9%
0.2				
1	520	0.91	0.09	**90.1%**
2	520	0.91	0.10	**89.0%**
3	520	0.89	0.14	**84.2%**
				87.7
0.3				
1	520	0.89	0.43	**51.6%**
2	520	0.89	0.09	**89.8%**
3	520	0.89	0.07	**92.1%**
				77.8%
0.4				
1	520	0.91	0.53	**41.7%**
2	520	0.91	0.07	**92.3%**
3	520	0.89	0.10	**88.7%**
				74.2%
0.5				
1	520	0.91	0.11	**87.9%**
2	520	0.90	0.13	**85.5%**
3	520	0.91	0.11	**87.9%**
				87.1%
Vitamin C				
1	520	0.92	0.03	**96.7%**
2	520	0.93	0.04	**95.6%**
3	520	0.90	0.05	**94.4%**
				95.6%

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Table 3: *In vitro* antioxidant activity of different doses of Herbex syrup

S. No.	NM	C.OD	S.OD	Value
0.1				
1	520	1.78	0.35	**80.3%**
2	520	1.78	0.34	**80.8%**
3	520	1.79	0.25	**85.9%**
4	520	1.79	0.22	**87.6%**
5	520	1.79	0.33	**81.4%**
6	520	1.79	0.28	**84.1%**
Vitamin C				
1	520	1.44	0.06	**95.8%**
2	520	1.41	0.09	**93.6%**
3	520	1.79	0.08	**95.5%**
4	520	1.96	0.03	**97.4%**
5	520	1.71	0.08	**95.3%**
6	520	1.79	0.11	**93.8%**
				95.2%

Table 4: Sub-acute toxicity studies of Herbex cough syrup (IL-6 Level Measurement)

S. No.	Groups	Concentration of IL-6 (pg/ml)
1	Control	**14.4 ± 0.18**
2	200 mg/kg	**18.9 ± 0.31**
3	400 mg/kg	**17.2 ± 0.11**

Results were expressed as the mean ± S.E.M. of 5 rats

Table 5: MABA assay to determine the MIC

S. No.	Compound code	MIC (µg/mL)
1	Herbex	**25 (µg/mL)**
2	Isoniazid	**0.05 (µg/mL)**
3	Rifampicin	**0.1 (µg/mL)**
4	Ethambutol	**1.56 (µg/mL)**

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4. CONCLUSION

Aqueous plant extracts and herbex syrup had significant antioxidant activity in dose dependent manner. Moreover herbex syrup inhibit the secretion of pro-inflammatory cytokinin (IL-6) and arrest the growth of *Mycobacterium tuberculosis* H37Rv. Further investigation needed to study more pharmacological potential of the plant species studied.

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Investigation of pharmacological actions of Dermo herb: A polyherbal formulation

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Abstract *– Dermo herb is poly herbal formulation. Mercury (II) sulfide, Zinc oxide, Copper sulphate, Lead (II) oxide, Chaulmoogra seeds, Psoralea corylifolia, Papaver somniferum, Sesbania grandiflora, Ruellia patula, Cocos nucifera and Bee's wax-yellow used to heal skin disorders and venereal diseases. This ointment is made of effective herbs which has more medicinal properties for speedy recovery from skin disorders. In the present study we have investigated for the antioxidant activity Dermo herb. Phytoconstituents of Dermo herb is responsible for the antioxidant activities that can evaluated with suitable in vivo studies.*

Keywords *– Dermo herb, antioxidant activity, skin disorders, venereal diseases*

1. INTRODUCTION

Dermo herb ointment is a siddha proprietary medicine, made of pure Indian herbs helps to heal skin disorders and venereal diseases. This ointment is made of effective herbs which has more medicinal properties for speedy recovery from skin disorders. Dermo herb ointment contains combination of a versatile medicinal plants such as Mercury (II) sulfide, Zinc oxide, Copper sulphate, Lead (II) oxide, *Chaulmoogra* seeds, *Psoralea corylifolia*, *Papaver somniferum*, *Sesbania grandiflora*, *Ruellia patula*, *Cocos nucifera* and Bee's wax-yellow (Figure 1) with high therapeutic values and pharmacological actions due to their enriched phytochemical constituents,

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which is used for the treatment of skin and venereal diseases [1-15]. Poly herbal formulation with antioxidant, anti-inflammatory, anti-bacterial activity essential requirement for the treatment of skin and venereal diseases [13-16]. Current study involves the preparation of an aqueous extract from Dermo herb poly herbal formulation by using hot continues extraction method. Present work involves the study of the antioxidant activity of aqueous extract. Antioxidant activity by DPPH method was compared with standard ascorbic acid under similar conditions. Antibacterial activity of the extract was assessed against *Streptococcus epidermidis* (MTCC 435) and *Pseudomonas aeroginosa* (MTCC424).

Figure 1: Constituents of Dermo herb ointment

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COMPOSITION OF DERMO HERB OINTMENT

Composition of Dermo herb ointment is listed in the below mentioned table [1-15].

Table 1: Composition of Dermo herb

S. No.	Tamil name	Technical name	Part used	Form used	Quantity
1	Lingam	Mercury (II) sulfide	Mineral	Pwd.	<0.0007 mgs.
2	Pall Thuththam	Zinc oxide	Mineral	Pwd.	<0.0007 mgs.
3	Mayil Thuththam	Copper sulphate	Mineral	Pwd.	<0.0007 mgs.
4	Mirudarsirungi	Lead (II) oxide	Mineral	Pwd.	<0.0007 mgs.
5	Neeradi muthu	Chaulmoogra seeds	(Dr.Sd.)	Dc.	<0.0007 mgs.
6	karpoga arisi	Psoralea corylifolia	(Dr.Sd.)	Dc.	<0.0007 mgs.
7	Kashakasha	Papaver somniferum	(Dr.Sd.)	Dc.	<0.0007 mgs.
8	Agathi	Sesbania grandiflora	(Lf.)	Dc.	0.7194 mgs.
9	Kiranthi Nayakam	Ruellia patula	(Pl.)	Dc.	0.7194 mgs.
10	Coconut oil	Cocos nucifera	(Dr.Sd.)	Oil.	7.1942 mgs.
11	Theen melugu	Bee's wax-yellow	Animal origin	Wax.	0.8633 mgs.

Mercury sulfide can exist in several crystal structures,1 of which mercury sulfide red occurs in hexagonal structure. It can be precipitated from hydrogen sulfide and mercury salt solution [1]. Zinc oxide is an inorganic compound with the formula ZnO. It is a white powder that is insoluble in water. ZnO is present in the Earth's crust as the mineral zincite. That being said, most ZnO used commercially is synthetic. Zinc oxide is commonly found in medical ointments where it used to treat skin irritiations [2]. Copper (II) sulfate, also known as copper sulphate, are the inorganic compounds with the chemical formula $CuSO_4(H_2O)x$, where x can range from 0 to 5. The pentahydrate (x = 5) is the most common form. Copper sulphate is widely used as a fungicide, either alone or in association with other fungicides (maneb, zineb), for the treatment of

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orchards and vineyards. Lead(II) oxide, also called lead monoxide, is the inorganic compound with the molecular formula PbO. PbO occurs in two polymorphs: litharge having a tetragonal crystal structure, and massicot having an orthorhombic crystal structure. Modern applications for PbO are mostly in lead-based industrial glass and industrial ceramics, including computer components. It is an amphoteric oxide [3]. Chaulmoogra (Hydnocarpus wightianus) is an herb. Chaulmoogra powder, oil, emulsion, or ointment on the skin to treat skin problems including psoriasis and eczema. Chaulmoogra is given intravenously for leprosy [1-2]. *Psoralea corylifolia* is a plant used in Indian and Chinese traditional medicine. The seeds of this plant contain a variety of coumarins, including psoralen [3-5]. The opium poppy (*P. somniferum*) is grown as an ornamental and for its edible seeds. It is an important source of opiate drugs, including heroin, morphine, and codeine [6-8]. *Sesbania grandiflora*, commonly known as vegetable hummingbird. The leaf extract may inhibit the formation of advanced glycation end-products. The leaf extract contains linolenic acid and aspartic acid, which were found to be the major compounds responsible for the anti-glycation potential of the leaf extract [9-11]. Ruellia is used in the treatment of gonorrhea, syphilis, eye sore, renal infection, cough, wounds, scalds, toothache, stomach-ache and kidney stones [12-13]. *Cocos nucifera* is considered antipyretic and diuretic property. Milk of young coconut is diuretic, laxative, antidiarrhoeic and counteracts the effects of poison. The oil is used to treat diseased skin and teeth and mixed with other medicines to make embrocations. Beeswax is used for lowering cholesterol and for relieving pain. It is also used for swelling (inflammation), ulcers, diarrhea, and hiccups [14-15].

2. MATERIALS & METHODS

2.1. Preparation of extract

All eleven medicinal plants about 100 gm taken in equal ratio in one liter round bottomed flask and add 1000 ml of distilled water, boiled for 5 hours, cool the extract, and filtered. The filtrate was evaporated under vacuum, to get dried extract. This dried extract was used for the investigation

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of pharmacological activity. The extract was concentrated under vacuum and dried extract was used for antioxidant assay.

2.2. Determination of DPPH radical scavenging activity

Antioxidant activity in the sample Dermo herb was estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals (Odabasoglu et al., 2005) [16]. 100µL of AL-A extract was taken in the micro-titer plate. 100µL of 0.1% DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively and read the plate on Elisa plate reader at 490 nm. Standard ascorbic acid was used as reference. All the analysis was performed in triplicates and the average values were taken.
The Radical scavenging activity was calculated by using the below mentioned equation:

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] x 100

2.3. Anti-bacterial activity

Test organisms: *Streptococcus epidermidis MTCC 435* and *Pseudomonas aeroginosa MTCC424* are the bacteria used as test organisms.

Procedure: Agar cup-plate method was used to assess the antibacterial activity of successive extracts.0.1 mL of bacterial test organisms was seeded into 20 ml of the sterile nutrient agar medium and poured into sterile Petri-dishes (pour-plate technique) and allowed to solidify. The six mm cups were made on the medium and dried extracts were dissolved in 10% dimethyl sulfoxide (DMSO) to obtain a concentration of 500 µg mL^{-1} and sterilized by filtration, through a Whatman filter paper No. 1 and 0.1 mL of different concentrations of extract were added to the respective cups. 0.1 mL of streptomycin at a concentration of 50 µg mL^{-1} was taken as standard

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reference (PC-positive control). The Petri-dishes with different concentrations of extract were kept in refrigerator at 4°C for 1 h for diffusion. After the process of diffusion, the Petri-dishes were incubated at 37°C for 24 h and the zones of inhibition were measured for the assessment of antibacterial activity using DMSO as the control (N) [17].

3. RESULTS & DISCUSSION

Dermo herb investigated for *in vitro* antioxidant activity by DPPH assay and compared with standard ascorbic acid. Dermo herb had significant anti-oxidant activity (78.9%) (Table 1) when compared with standard ascorbic acid (1 mg/ml) by dose dependent manner. Dermo herb showed significant antibacterial against *Pseudomonas aeroginosa* MTCC424 than *Streptococcus epidermidis* MTCC 435 at 1mg, 5mg and 10mg concentrations.

Table 1: *In vitro* antioxidant activity of different doses of Dermo herb

S. No.	NM	C.OD	S.OD	% Inhibition
0.1				
1	520	0.95	0.17	**82.1%**
2	520	0.95	0.25	**73.6%**
3	520	0.96	0.18	**81.2%**
				78.9%
0.2				
1	520	0.95	0.25	**73.6%**
2	520	0.95	0.20	**79.5%**
3	520	0.96	0.18	**81.8%**
				78.3%
Vitamin C				
1	520	0.98	0.03	**96.9%**
2	520	0.95	0.09	**89.4%**
3	520	0.95	0.10	**89.4%**
4	520	0.98	0.11	**88.8%**
5	520	0.98	0.06	**93.8%**
				91.2

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Figure 2: Antibacterial assay of Dermo herb ointment

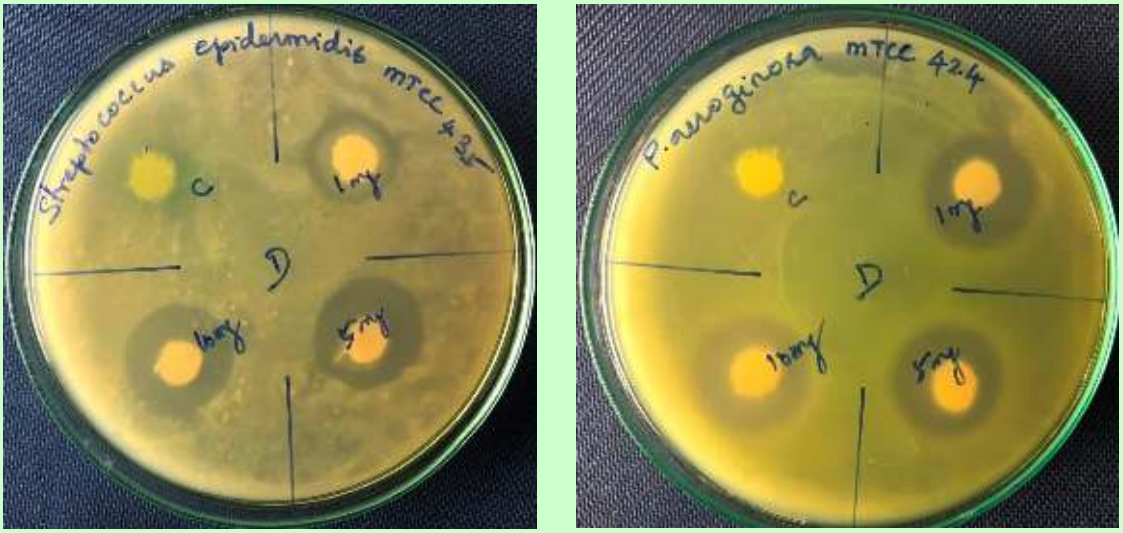

Table 2: Antibacterial assay of Dermo herb ointment

S. No.	Organisms	Concentrations			
		1 mg	5 mg	10 mg	Control
1	*Streptococcus epidermidis* MTCC 435	1635	2020	2065	887
2	*Pseudomonas aeroginosa* MTCC424	2050	2012	2095	771

4. CONCLUSION

Aqueous plant extracts and Dermo herb had significant antioxidant activity in dose dependent manner. Dermo herb exhibited significant antibacterial activity against selective skin pathogens, *Streptococcus epidermidis* MTCC 435 and *Pseudomonas aeroginosa* MTCC424. Further investigation needed to study more pharmacological potential of the plant species studied.

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EVIDENCE BASED HERBAL MEDICINE - PART - V

Investigation of pharmacological actions of Herbo green: A polyherbal formulation

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Abstract – *Herbo green oinment is a poly herbal formulation. Lead (II, IV) oxide, Lead (II) oxide, Copper Sulphate, Psoralea corylifolia, Semecarpus anacardium, Bee wax (Yellow) and Cocos nucifera used to heal skin disorders and venereal diseases. This ointment is made of effective herbs which has more medicinal properties for speedy recovery from skin disorders. In the present study we have investigated for the antioxidant activity Herbo green. Phytoconstituents of Herbo green is responsible for the antioxidant activities that can evaluated with suitable in vivo studies.*

Keywords – *Herbo green, antioxidant activity, skin disorders, venereal diseases*

1. INTRODUCTION

Herbo green ointment is a siddha proprietary medicine, made of pure Indian herbs helps to heal skin disorders and venereal diseases. This ointment is made of effective herbs which has more medicinal properties for speedy recovery from skin disorders. Herbo green ointment contains combination of a versatile medicinal plants such as Lead (II, IV) oxide, Lead (II) oxide, Copper Sulphate, *Psoralea corylifolia*, *Semecarpus anacardium*, Bee wax (Yellow) and *Cocos nucifera* (Figure 1) with high therapeutic values and pharmacological actions due to their enriched phytochemical constituents, which is used for the treatment of skin and venereal diseases [1-15]. Poly herbal formulation with antioxidant, anti-inflammatory, anti-bacterial activity essential

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requirement for the treatment of skin and venereal diseases [13-16]. Current study involves the preparation of an aqueous extract from Herbo green poly herbal formulation by using hot continues extraction method. Present work involves the study of the antioxidant activity of aqueous extract. Antioxidant activity by DPPH method was compared with standard ascorbic acid under similar conditions. Antibacterial activity of the extract was assessed against *Streptococcus epidermidis* (MTCC 435) and *Pseudomonas aeroginosa* (MTCC424).

Figure 1: Constituents of Herbo cream ointment

COMPOSITION OF HERBO GREEN OINTMENT

Composition of Herbo green ointment is listed in the below mentioned table [1-15].

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Table 1: Composition of Herbo green

S. No.	Tamil name	Technical name	Part used	Form used	Quantity
1	Vanga chendooram	Lead (II, IV) oxide	(Metal)	Pwd.	0.135 gms.
2	Miruthan sirungi	Lead (II) oxide	(Metal)	Pwd.	0.135 gms.
3	Mayilthutham	Copper Sulphate	(Metal)	Pwd.	0.135 gms.
4	Karpoga Arisi	*Psoralea corylifolia*	(Dr.Sd.)	Dc.	0.135 gms.
5	Sengkottai	*Semecarpus anacardium*	(Dr.Sd.)	Dc.	0.135 gms.
6	Bee wax	Bee wax (Yellow)	Wax	Wax.	3 gms.
7	Coconut oil	*Cocos nucifera*	*(Dr.Sd.)*	Oil.	12 gms.

Lead (II, IV) oxide, also called lead monoxide, is the inorganic compound with the molecular formula PbO. PbO occurs in two polymorphs: litharge having a tetragonal crystal structure, and massicot having an orthorhombic crystal structure. Modern applications for PbO are mostly in lead-based industrial glass and industrial ceramics, including computer components. It is an amphoteric oxide [3]. Copper (II) sulfate, also known as copper sulphate, are the inorganic compounds with the chemical formula $CuSO_4(H_2O)x$, where x can range from 0 to 5. The pentahydrate (x = 5) is the most common form. Copper sulphate is widely used as a fungicide, either alone or in association with other fungicides (maneb, zineb), for the treatment of orchards and vineyards. *Psoralea corylifolia* is a plant used in Indian and Chinese traditional medicine. The seeds of this plant contain a variety of coumarins, including psoralen [3-5]. *Cocos nucifera* is considered antipyretic and diuretic property. Milk of young coconut is diuretic, laxative, antidiarrhoeic and counteracts the effects of poison. The oil is used to treat diseased skin and teeth and mixed with other medicines to make embrocations. Beeswax is used for lowering cholesterol and for relieving pain. It is also used for swelling (inflammation), ulcers, diarrhea, and hiccups [14-15].

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2. MATERIALS & METHODS

2.1. Preparation of extract

All eleven medicinal plants about 100 gm taken in equal ratio in one liter round bottomed flask and add 1000 ml of distilled water, boiled for 5 hours, cool the extract, and filtered. The filtrate was evaporated under vacuum, to get dried extract. This dried extract was used for the investigation of pharmacological activity. The extract was concentrated under vacuum and dried extract was used for antioxidant assay.

2.2. Determination of DPPH radical scavenging activity

Antioxidant activity in the sample Herbo green was estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals (Odabasoglu et al., 2005) [16]. 100µL of AL-A extract was taken in the micro-titer plate. 100µL of 0.1% DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively and read the plate on Elisa plate reader at 490 nm. Standard ascorbic acid was used as reference. All the analysis was performed in triplicates and the average values were taken.
The Radical scavenging activity was calculated by using the below mentioned equation:

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] x 100

2.3. Anti-bacterial activity

Test organisms: *Streptococcus epidermidis MTCC 435* and *Pseudomonas aeroginosa MTCC424* are the bacteria used as test organisms.

Procedure: Agar cup-plate method was used to assess the antibacterial activity of successive extracts.0.1 mL of bacterial test organisms was seeded into 20 ml of the sterile nutrient agar

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medium and poured into sterile Petri-dishes (pour-plate technique) and allowed to solidify. The six mm cups were made on the medium and dried extracts were dissolved in 10% dimethyl sulfoxide (DMSO) to obtain a concentration of 500 µg mL^{-1} and sterilized by filtration, through a Whatman filter paper No. 1 and 0.1 mL of different concentrations of extract were added to the respective cups. 0.1 mL of streptomycin at a concentration of 50 µg mL^{-1} was taken as standard reference (PC-positive control). The Petri-dishes with different concentrations of extract were kept in refrigerator at 4°C for 1 h for diffusion. After the process of diffusion, the Petri-dishes were incubated at 37°C for 24 h and the zones of inhibition were measured for the assessment of antibacterial activity using DMSO as the control (N) [17].

3. RESULTS & DISCUSSION

Herbo green investigated for *in vitro* antioxidant activity by DPPH assay and compared with standard ascorbic acid. Herbo green had significant anti-oxidant activity (78.9%) (Table 1) when compared with standard ascorbic acid (1 mg/ml) by dose dependent manner. Herbo green showed significant antibacterial against *Pseudomonas aeroginosa* MTCC424 than *Streptococcus epidermidis* MTCC 435 at 1mg, 5mg and 10mg concentrations.

Table 1: *In vitro* antioxidant activity of different doses of Herbo green

S. No.	NM	C.OD	S.OD	% Inhibition
0.1				
1	520	0.87	0.38	**56.3%**
2	520	0.88	0.06	**93.1%**
3	520	0.89	0.11	**87.6%**
				79.0%
0.2				
1	520	0.89	0.18	**79.7%**
2	520	0.90	0.25	**72.2%**
3	520	0.92	0.21	**77.1%**
				78.3%

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Vitamin C				
1	520	0.98	0.03	**96.9%**
2	520	0.95	0.09	**89.4%**
3	520	0.95	0.10	**89.4%**
4	520	0.98	0.11	**88.8%**
5	520	0.98	0.06	**93.8%**
				91.2

Figure 2: Antibacterial assay of Herbo green ointment

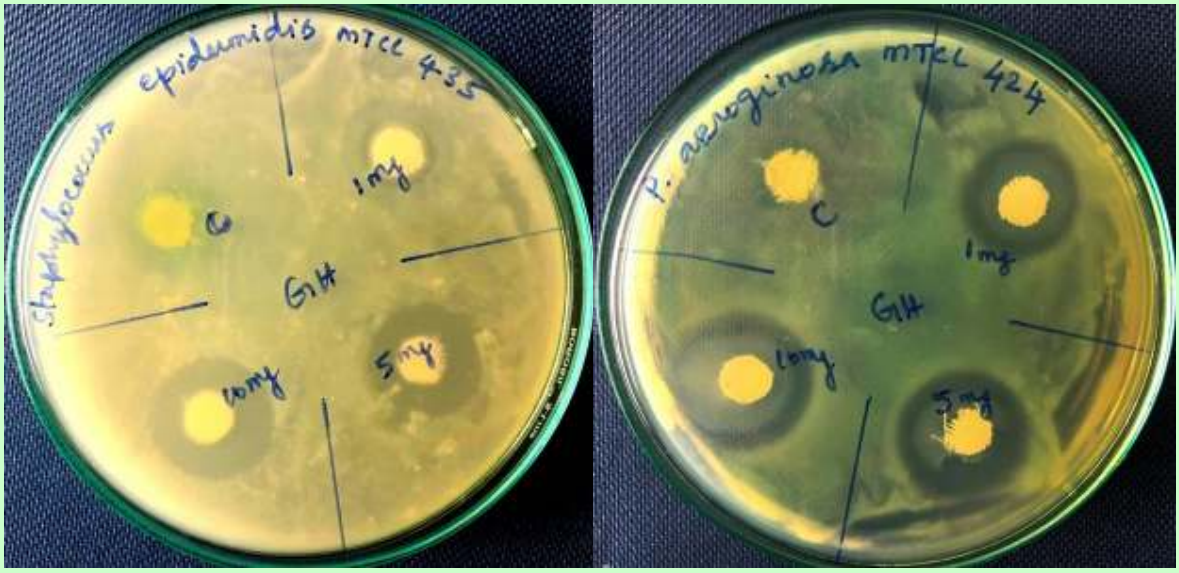

Table 2: Antibacterial assay of Herbo green ointment

S. No.	Organisms	Concentrations			
		1 mg	5 mg	10 mg	Control
1	*Streptococcus epidermidis* MTCC 435	1768	1924	2129	909
2	*Pseudomonas aeroginosa* MTCC424	1872	2183	2331	895

 ISBN: 978-93-5408-448-5

4. CONCLUSION

Aqueous plant extracts and Herbo green had significant antioxidant activity in dose dependent manner. Herbo green exhibited significant antibacterial activity against selective skin pathogens, *Streptococcus epidermidis* MTCC 435 and *Pseudomonas aeroginosa* MTCC424. Further investigation needed to study more pharmacological potential of the plant species studied.

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EVIDENCE BASED HERBAL MEDICINE - PART - V

Investigation of anticancer and antimicrobial activities of Kabasura Kudineer: A polyherbal formulation

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Abstract – *Phytochemicals are ecologically derived plant secondary metabolites that plants make to protect themselves against environmental stress and pathogenic microbial invasion. These phytochemicals have been shown to have both positive and pharmacological effects in the treatment of human illnesses. It is a well-known fact that the active principles present in the medicinal plants act synergistically to alleviate the primary and secondary complications of several diseases. Kaba Sura Kudineer (KSK) is a polyherbal preparation comprising of several medicinal plants such as Zingiber officinale (Rhizome), Piper longum (Fruit), Syzygium aromaticum (Bark), Tragia involucrate (Root), Anacyclus pyrethrum (Root), Hygrophilla auriculata (Root), Terminalia chebula (Fruit), Adathoda vasica (Leaf), Coleus ambonicus (Leaf), Saussurea lappa (Root), Tinospora cordifolia (Stem), Clerodendron serratum (Root), Andrographis paniculata (Whole plant), Cissampelo spareira (Root), Cyperus rotandus (Rhizome). It is widely used as a therapeutic agent. In this study, to investigate the anti-cancer and anti-microbial activities were tested.*

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Keywords *– Kaba sura kudineer, in vitro anti-oxidant activity*

1. INTRODUCTION

Kabasurakudineer (KSK) includes the medicinal plants such as *Zingiber officinale* Rhizome [1], *Piper longum* Fruit [2], *Syzygium aromaticum* Bark [3], *Tragia involucrate* Root [4], *Anacyclus pyrethrum* Root [5], *Hygrophilla auriculata* Root [6], *Terminalia chebula* Fruit [7], *Adathoda vasica* Leaf [8],*Coleus ambonicus* Leaf [9], *Saussure alappa* Root [10], *Tinospora cardifolia* Stem [11], *Clerodendron serratum* Root [12], *Andrographis paniculata* whole plant [13], *Cissampelos pareira* Root [14], *Cyperus rotandus* Rhizome [15]. It is widely used as a therapeutic agent as well as prophylactic supplement in lung diseases and other respiratory diseases approved by ministry of AYUSH. Excellent pharmacological and therapeutic benefits of KSK is due their enriched phytochemical constituents and micronutrients. KSK is reported with broad spectrum of pharmacological activity due their chemical compounds and combination of medicinal plants with the high therapeutic values. KSK recommended for treatment of respiratory diseases.

2. MATERIALS & METHODS

2.1. Preparation of extract

Kaba Sura Kudineer (KSK) is a poly herbal formulation containing 15 medicinal plants such as *Zingiber officinale* (Rhizome), Piper longum (Fruit), *Syzygium aromaticum* (Bark), *Tragia involucrate* (Root), *Anacyclus pyrethrum* (Root), *Hygrophilla auriculata* (Root), *Terminalia chebula* (Fruit), *Adathoda vasica* (Leaf), *Coleus ambonicus* (Leaf), *Saussurea lappa* (Root), *Tinospora cordifolia* (Stem), *Clerodendron serratum* (Root), *Andrographis paniculata* (Whole plant), *Cissam pelospareira* (Root), *Cyperus rotandus* (Rhizome) collected from Aravindh herbal laboratory, Rajapalayam, Tamilnadu for this investigation. 100 gm of KSK powder packed in soxhlet apparatus and extracted by using hot continues extraction process with three different solvents ethanol, methanol and chloroform for 48 hours to achieve complete extraction. Solvent is

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collected and subjected to distillation to concentrate the extract. Extracts (KSK ET, KSK Me & KSK CH) were dried under vacuum and stored under 5°C until further use.

2.2. Anticancer activity

The minimum essential medium (Eagle) with 2 mM I glutamine and Earle's BSS adjusted to contain 1.5 g/L Na bicarbonate, 0.1 mM non-essential amino acids and 1.0 mM Na pyruvate 90%; fetal bovine serum, 10%. The human lung cancer cell line (A549) and vero cell lines were incubated in Growth Conditions: 37°C temperature at 5% Carbon-dioxide atmosphere. Remove growth medium from flask. Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the cell sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cells come off substratum. Aspirate with fresh medium and dispense cell suspension into new flasks. KSK extract (Chloroform) was studied for anticancer activity against human lung cancer cell line (A549) and vero cell lines through MTT assay [16]. The parameters such as inhibitory concentration (IC_{50}-concentration required to inhibit the growth of 50% cancer cells) and cytotoxic concentrations were measured for the predictions of anticancer activity and the cytotoxicity potential of prepared extracts.

2.3. In-vitro MTB MABA assay

MABA assay to determine the MIC (minimum inhibitory concentration) of compounds against *Mycobacterium tuberculosis* H37Rv. The inoculum was prepared from fresh LJ medium re-suspended in 7H9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase [OADC]), adjusted to an OD 590 1.0, and diluted 1:20; 100 µl was used as inoculum. Each drug stock solution was thawed and diluted in 7H9-S at four-fold the final highest concentration tested. Serial two-fold dilutions of each drug were prepared directly in a sterile 96-well microtiter plate using 100 µl 7H9-S. A growth control containing no antibiotic and a sterile control were also prepared on each plate. Sterile water was added to all perimetre wells to avoid evaporation during the incubation. The plate was covered, sealed in plastic bags and

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incubated at 37°C in normal atmosphere. After 7 days incubation, 30 µl of alamar blue solution was added to each well, and the plate was re-incubated overnight. A change in colour from blue (oxidized state) to pink (reduced) indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in colour [17, 18]. Isoniazid, Rifampicin and Ethambutol were used as control.

2.4. Anti-fungal activity

Test organisms: *Mucor species, Candida albicans* and *Aspergillus fumigatus* were the fungal species used as test organisms.

Procedure: Agar cup-plate method was used to assess the antibacterial activity of successive extracts.0.1 mL of bacterial test organisms was seeded into 20 ml of the sterile nutrient agar medium and poured into sterile Petri-dishes (pour-plate technique) and allowed to solidify. The six mm cups were made on the medium and dried extracts were dissolved in 10% dimethyl sulfoxide (DMSO) to obtain a concentration of 500 µg mL^{-1} and sterilized by filtration, through a Whatman filter paper No. 1 and 0.1 mL of different concentrations of extract were added to the respective cups. 0.1 mL of streptomycin at a concentration of 50 µg mL^{-1} was taken as standard reference (PC-positive control). The Petri-dishes with different concentrations of extract were kept in refrigerator at 4°C for 1 h for diffusion. After the process of diffusion, the Petri-dishes were incubated at 37°C for 24 h and the zones of inhibition were measured for the assessment of antibacterial activity using DMSO as the control (N) [19, 20].

3. RESULTS & DISCUSSION

KSK extracts (CH, Me & ET) were investigated for Anti-cancer activity, MIC (Minimum Inhibitory Concentration) and Antifungal activity. The results showed Chloroform extract of KSK exhibit significant activity against Lung cancer cells (A549) (IC50 134.75) when compared to vero cell line (normal cell line) (IC50 233.16). The methanolic extract of KSK showed significant inhibitory activity against *Mycobacterium tuberculosis* (H37Rv). The ethanolic extract of KSK

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exhibited significant activities against the selected fungal strains such as *Mucor sp., C. albicans* and *A. fumigatus*.

Table 1: Anti-cancer activity of KSK-CH against A549 (Lung cancer cell line)

S. No.	Concentration	% inhibition	IC_{50}
1	62.5	23.34	**134.75**
2	125	41.2	
3	250	61.27	
4	500	69.63	

Table 2: Anti-cancer activity of KSK-CH against Vero cells (Normal cell line)

S. No.	Concentration	% inhibition	IC_{50}
1	62.5	22.93	**233.16**
2	125	30.48	
3	250	43.77	
4	500	56.19	

Figure 1: Anti-cancer activity of KSK-CH against A549 (Lung cancer cell line)

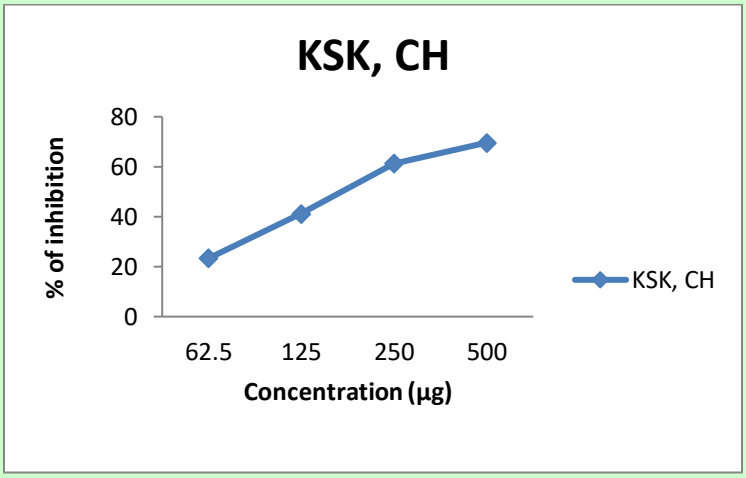

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Figure 2: Anti-cancer activity of KSK-CH against Vero cells (Normal cell line)

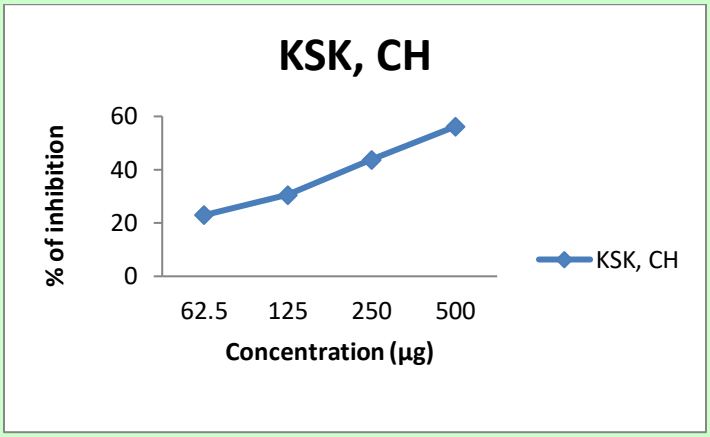

Table 3: *In-vitro* MTB MABA assay of KSK Me against *M. tuberculosis* (H37Rv)

S. No.	Compound code	MIC (µg/mL)
1	KSK Me	25
2	Isoniazid	0.05
3	Rifampicin	0.1
4	Ethambutol	1.56

Figure 3: Anti-fungal activities of KSK ET extract

Mucor sp.

C. albicans

A. fumigatus

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4. CONCLUSION

The different solvent extracts of KSK significant anti-cancer activities against Lung cancer cell line (A549). KSK extract also against *M. tuberculosis* bacterium. Hence these results strongly proved that the KSK has potent activities against respiratory diseases. COVID-19 viral infection cause secondary infections including opportunistic fungal infections. The important fungal species cause secondary infections are *Mucor species*, *Candida albicans* and *Aspergillus fumigatus*. The present study proved that the KSK extract significantly inhibit the growth of fungal species. Further investigation needed to study more pharmacological potential of the KSK.

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