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Research Article

STANDARDIZATION IN VITRO ANTIOXIDANT ACTIVITY AND BRINE SHRIMP LETHALITY OF A POLYHERBAL FORMULATION

Macha Vijay[1]*, K Ramakanth Reddy[1], M Ravi Kumar Reddy[1], K Gnananath[2], B Surendra Babu[2]

*[1]Source Natural Foods and Herbal Supplements Limited Plot No 22 & 23, SVCIE, Bachupally, Quthbullapur, RR District.
[2]Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram, West Godavari District, Andhra Pradesh, India.

ABSTRACT

Shakti drops an Ayurvedic polyherbal formulation is an effective medicine for immuno modulatory activity. The formulation was prepared in the laboratory with authenticated organic plant drugs. The standardization procedure was conducted on the basis of physicochemical parameters and antimicrobial studies as directed by Ayurvedic formulary of India. Antioxidant activity is further performed based on its prescribed dose in three In-vitro antioxidant models. Physical characters remained unaltered throughout study, In antimicrobial activity total aerobic count was found to be 18×10^1 CFU/mL and total fungal count was found to be <10 CFU/mL. It is devoid of all tested microorganism i.e. *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli* tested. In antioxidant activity the results of the study suggest that the Shiakti drops has potent antioxidant and free radical scavenging activity justifying its use in *Rasayana*.

KEYWORDS: *Shakti* drops, Antimicrobial, Antioxidant, Standardization, Cytotoxicity.

INTRODUCTION

Ayurveda is a systematized holistic medical system resting on codified theories, which imply "science of life", has its roots in the Veda [1]. Since aeon it is an integral part of Indian medical system, has unraveled its mystery to preserve life for a longer time. It offers you a range of numerous herbs that help to develop vigour, which has gained popularity worldwide nowadays a majority of the present day diseases are reported to be due to life style modifications and stress which lead to infirmity [2]. The disease preventive and health promotive approach of Ayurveda, which takes into contemplation of the whole body, mind and spirit for upkeep of health. There has been a surplus of research on the plants used as Rasyana drugs in order to rationalize them in the modern context [3]. Source Natural Foods and Herbal Supplements limited, Hyderabad came with an idea that if a few drops of poison can ruin our body then definitely a few drops of nectar can also rejuvenate it and invented a GMP approved formulation named Shakti drops which is charged with the goodness of *Embilica officinals, Withania somnifera, Bacopa monniera, Eclipta alba, Tinospora cordifolia, Asparagus racemosus, Evolvulus alsinoides and Glycyrrhiza glabra.* Shakti Drops is a fine blend of all the indispensable organic herbs into one, such that it can be used as an immunity booster as well as consider being panacea for ailments. Importance of each herb is enumerated in our blog [4]. The present studies were done to standardized and scientifically validate its potential by performing antioxidant, antimicrobial and brine shrimp cytotoxicity studies.

MATERIALS AND METHODS

Soya Bean Casein Digest Medium, Saboraud Dextrose Agar, 1, 1 diphenyl-2-picryl hydrazyl (DPPH) was obtained from Sigma Aldrich, Mumbai. Ascorbic acid was purchased from finer chemicals, Ahmedabad. Sodium nitropruside, Potassium dihydrogen ortho-phosphate from Molychem, Mumbai. N-1-Napthyl ethylene Diamine Dihdrochloride from LOBA chemie pvt Ltd. Sulfanilimide from HiMedia Lab pvt Ltd. All chemicals and solvents used were of analytical grade available commercially.

Determine the Total Aerobic Count

For Total aerobic Count accurately Weighed 2.98 gms Soya Bean Casein Digest Medium (SCDM) was dissolved the 100 ml of DM water in conical flask autoclaved at 121^0 C for 15 lbs for 15 min along with Petri plates for sterilization after cooling one ml of sample solution was added incubated for at 20^o C to 25^o C for 5 days. After incubation, the numbers of colonies formed are counted.

Determine the Total Fungal Count

6.5g Saboraud Dextrose Agar (SDA) was dissolved the 100 ml of DM water in conical flask autoclaved at 121^0 C for 15 lbs for 15 min along with Petri plates for sterilization after cooling one ml of sample solution was added incubated for at 20^o C to 25^o C for 5 days. After incubation, the numbers of colonies formed are counted

Antimicrobial activity

Presence of different test organisms like *Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi* and *Escherichia coli* were tested accordingly by using standard protocol. [5]

DPPH Radical Scavenging Activity

The free radical scavenging activity *Shakti* drops and *Ojastiva* was measured using DPPH scavenging method[6] One ml of *Shakti* drops to 1 ml of 0.1 mM solution of DPPH in methanol. After 30 minutes, absorbance was measured at 517 nm. A 0.1mM solution of DPPH in methanol was used as control, where as ascorbic

acid was used as a reference material. Percent inhibition was calculated using equation.

$$\text{Percentage Inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

$A_{control}$ = Absorbance of control reaction and

A_{test} = Absorbance of sample

Nitric Oxide Scavenging Activity

In this method SNP (10mM) in phosphate buffer saline (PBS) was mixed 1ml of *Shakti* drops was incubated at 25°C for 150 minutes. To 1ml of incubated solution, 1ml of Griess reagent (1% sulphanilamide, 0.1% naphthyl-ethylenediamine dichloride and 3% phosphoric acid) was added[7]. The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine dichloride was read at 546 nm. Results were averaged and ascorbic Acid used as a positive control treated in the same way with Griess reagent. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance of control and test.

$$\text{Nitric Oxide scavenged} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where,

$A_{control}$ = Absorbance of control reaction and

A_{test} = Absorbance in the presence of the samples

Hydrogen Peroxide Scavenging Activity

One ml of *Shakti* drop was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the H_2O_2 without sample. Ascorbic acid used as a positive control treated in the same way with H_2O_2 solution. The percentage inhibition was measured by comparing the absorbance of control and test [8].

$$H_2O_2 \text{ scavenging activity} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where,

$A_{control}$ = Absorbance of control reaction and

A_{test} = Absorbance in the presence of the samples.

Brine shrimp lethality (BSL) assay

BSL assay was performed as per Mayer *et al*. [9] With minor modifications. A minimum quantity of brine shrimp eggs were added to glass bowl containing simulated sea water prepared by adding 30 g/L of sodium chloride (NaCl) in tap water which is aerated continuously. After 24 h incubation under illumination at room temperature, the nauplii hatched out were attracted towards a light source and collected using dropper. *Shakti* drops was dissolved as prescription 1ml in 150 mL distill water from these 5 ml was transferred. 15 nauplii were added to each petriplates.10 µg/ml of protein (1%) was added as feed to all plates. The set up was kept for incubation for 24 hrs. After 24 hrs, number of dead shrimps in each plate was counted.

RESULTS AND DISCUSSION

Physical characteristics and Microbiological tests

The physical characteristics of *Shakti* drops were also carried out along with microbiological test by storing at room temperature. All the characters were monitored batch wise it maintained its specification throughout the study were in microbiological tests Total Aerobic Count, Total fungal count and presence of microbes like *E. coli, Salmonella* spp, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were carried out Total Aerobic Count and Total Fungal Count is an microbial limit testing, which is performed on pharmaceutical product and medical products for quality control purposes. Products or components used in the pharmaceutical or medical field require control of microbial levels during processing and handling. Microbial limit testing on these products proves that these requirements have been met individual microbiological tests showed absence of test organism the results are shown in table no1.

Antioxidant activity

The antioxidant activity of *Shakti* drops was evaluated by three models DPPH radical scavenging method, Nitric Oxide Scavenging Activity method, Hydrogen Peroxide Scavenging Activity method. DPPH is a free radical compound that has widely been used to test the free radical scavenging abilities of pure sample and diluted once the results are shown as the relative activities against the standard ascorbic acid. The results showed that for the highest antioxidant activity was displayed by causing 66.8 % DPPH inhibition for pure formulation and 37.6 percent for diluted in case of nitric oxide model the percentage inhibition found to be 74.4 and 41.3 respectively and were as in Hydrogen Peroxide Scavenging Activity percentage inhibition was 81.5 and 45.8% respectively the results are shown in table no2.

Brine shrimp cytotoxicity tests

Brine shrimp lethality assay allows use of smaller quantity of test material for fast and bulk screening than using other in vitro toxicity screening tests the present study reveals that showed no sign of toxicity.

Table 1: Physical and Microbiology results

Test	Specification	Results
Physical Tests:		
Description	Clear liquid	Clear liquid
Color	Color less	Color less
Odour	Characteristic	Characteristic
Taste	Sour and Characteristic	Sour and Characteristic
pH	3-4	3.24
Microbiology Tests:		
E. coli	Absent	Absent

Salmonella spp.	Absent	Absent
Staphylococcus aureus	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent
Total Aerobic Count	1000 CFU/ml	$01x10^1$ CFU/ml
Total fungal count	100 CFU/ml	01 CFU/ml

Table 2: Antioxidant activity

Concentration	% inhibition DPPH Method	% inhibition Nitric oxide scavenging	% inhibition H_2O_2 radical scavenging activity
Pure liquid (1 ml)	66.8	74.4	81.5
Diluted (6 drops in 150 mL)	37.6	41.3	45.8

CONCLUSION

Our formulation showed no signs of alteration in physical characters and devoid of microbes. It is having a potent antioxidant activity and whereas in brine shrimp cytotoxicity reveals no signs of toxicity justifying its use in human consumption.

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***Address for correspondence**
Macha Vijay
Quality Control
Source Natural Foods and Herbal Supplements Limited
Plot No 22 & 23, SVCIE,
Bachupally, Quthbullapur Mandal,
RR District, and Hyderabad-50009
Mobile no: +91-9059530954
Email: vijaymachares@gmail.com

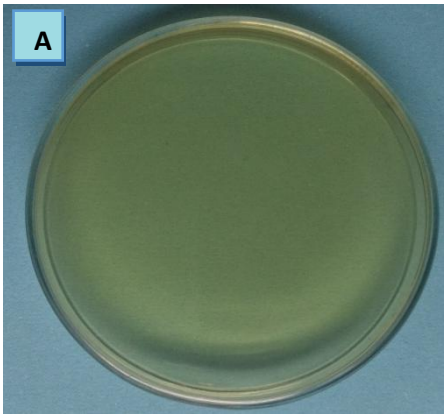

Figure A: Soya Bean Casein Digest Medium plate

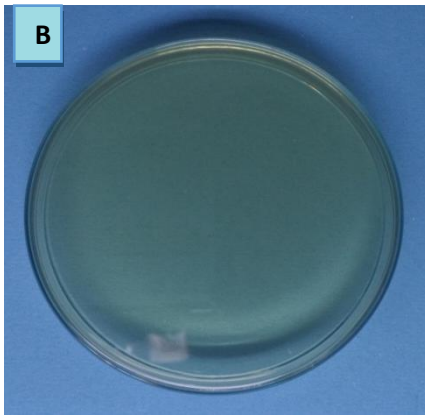

Figure B: Saboraud Dextrose Agar plate

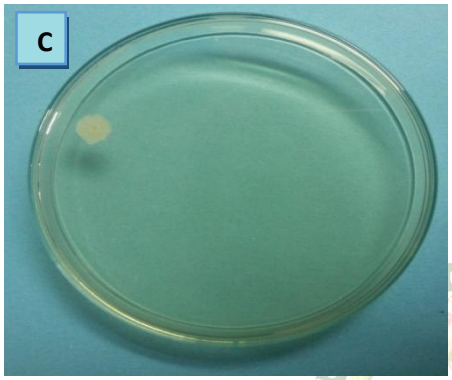

Figure C: Total Aerobic Count

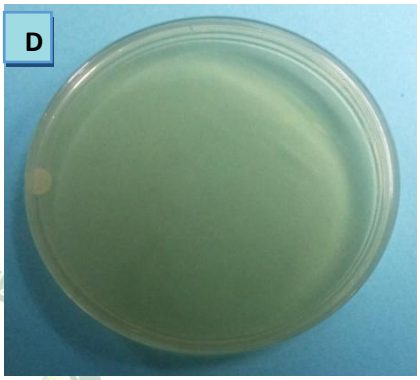

Figure D: Total Fungal Count

Figure E: Brine shrimp cytotoxicity tests