

Research Article

In Vitro Antioxidant Activity and Anti Microbial Activity of Traditionally Designed Neutraceutical

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ABSTRACT

A traditionally designed indigenous drug was formulated in accordance with GMP, and it is evaluated for its in vitro antioxidant and anti microbial activity. Ayurvedic plants are included in this formulation. Medicinal plants are source of certain bio-active molecules which act as antioxidants and antimicrobial agents. The details of the formulation are under patent process. So it is not revealed in this stage. In vitro antioxidant effects of aqueous and ethanolic extracts of traditionally designed indigenous drug were evaluated. The aqueous and alcoholic extract exhibits anti oxidant property in high concentration. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health. There is a dynamic balance between the amount of free radicals produced in the body and antibodies to scavenge or quench them to protect the body against harmful effects. To neutralize the free radicals the amount of antioxidant principles present under normal physiological condition may be insufficient. So natural antioxidants from plant materials are included in the traditionally designed indigenous drug and it is evaluated for its anti oxidant activity. All the analysis was made by help of U.V. Visible spectrophotometer (Shimadzu). The reducing power of the drug carried out with ascorbic acid as a standard reducing agent. Extract of the formulation were subjected to in vitro antimicrobial assay against *E.coli*, *Salmonella typhi*, *Staphylococcus aureus* and this was determined with the help of agar well diffusion method and two fold broth dilution method against ampicillin, the standard drug. The present study evaluates the antioxidant and antimicrobial properties of the new formulation.

Keywords: Antioxidant property, antimicrobial activity, minimum bactericidal concentration.

INTRODUCTION

In the recent scientific developments the medicinal properties of the plants are investigated. As a result of this it is included in a new formulation and its activities are evaluated. For the treatment of various diseases whether it is old or new, medicines are directly or indirectly derived from plants. The safe form of the medicine is green medicine, and it is having less side effects. The assessment of antioxidant properties remains an interesting and helpful task, for finding the natural anti oxidants. The present study verified that the newly formulated drug could be a good source of anti oxidant substance. This antioxidant based drugs or formulations are used for various

serious diseases and also for minor complications. Due to the free radicals only majority of the diseases occur. As a part of aerobic life and metabolism, free radicals have an important role. Free radicals are the back bone of all bio chemical process. Plant and plant products are being used as a source of medicine now days. According to FDA regulations the manufacturer is permitted to say that a dietary supplement addresses the particular characteristics action if there is a research to support the claim. So we conducted antibacterial studies and in vitro studies for the newly formulated neutraceutical.

MATERIALS AND METHODS

Traditionally designed indigenous drug combination:

The formulation was under the patent process. So it is not possible to disclose the contents.

Plant materials

The plant materials for the formulated drug was collected from different parts of Kerala and Tamlnadu, India during month of july-December 2009 and got authenticated by Pradeep Kumar, Herbarium curator, Department of Botany, Calicut University, Kerala.

Preparation of Extract:

Aqueous extract

The dried powder 250 mg was kept for maceration with 1000 ml distilled water for 24 hrs. It was double filtered by using muslin cloth and whattman filter paper. It is then concentrated in a water bath. The extract was dried and used as powder. The yield was about 1.56%.

Alcoholic extract

The powdered material was extracted with 90% ethyl alcohol using soxhlet apparatus. The extract was concentrated in vacuum to syrup like nature. The yield was about 2.43%.

Anti oxidant studies

DPPH radical scavenging activity

The free Radical scavenging activity was done according to the method reported by (Gramfi et al, 2002).

Fifty micro liters of plant extract in methanol, yielding 100µg/ml respectively in each solution was mixed with 1 ml of 0.01mM DPPH in methanol solution and 450µl of 50mM Tris-Hcl buffer (ph7.4).Methanol (50µl) only was used as control of experiment. After 30minutes, the absorbance was measured at 517 nm.

In this, Ascorbic acid is used as (+) control. The % of inhibition was calculated from the following equation:

$$\text{Inhibition (\%)} = (A_0 - A_1 / A_0) * 100$$

Where A₀=is the absorbance of control.
A₁=is the absorbance of test.

Phytochemical constituents of the formulation

Phytochemical constituents	Ethyl alcohol extract	Aqueous extract
Carbohydrate	(+)	(+)
proteins	(+)	(+)
lipids	(+)	(+)
flavanoids	(+)	(+)
saponins	(+)	(+)

Anti oxidant activity of aqueous extract of 517 nm

Conc.	OD 517 nm		% activity	
	Sample	Std	Sample	Std
50 µl	1.052	2 µl 0.928	14.47	24.55
100 µl	1.010	4 µl 0.880	17.88	28.45
150 µl	0.756	6 µl 0.787	38.53	36.01
200 µl	0.623	8 µl 0.532	49.34	56.74
250 µl	0.729	10 µl 0.331	40.73	73.08

Optical density of control at 517 nm=1.230 nm

Anti oxidant activity of alcoholic extract of the formulation

Conc.	OD 517 nm		% activity	
	Sample	Std	Sample	Std
50 µl	1.011	2 µl 0.926	10.61	18.12
100 µl	1.029	4 µl 0.881	9.018	22.10
150 µl	0.612	6 µl 0.789	45.88	30.23
200 µl	0.722	8 µl 0.536	36.16	52.60
250 µl	0.719	10 µl 0.329	36.42	70.91

Optical density of the control at 517 nm=1.131nm.

Anti bacterial activity

The newly formulated nutraceutical was evaluated for its antibacterial activity.

Test organism:

Gram (+) ve bacteria, Staphylococcus aureus, Bacillus subtilis and gram (-) ve bacteria E.coli, salmonella typhi, were utilised. The bacterial strains were collected from Microbiology department of Devaki Amma Memorial College of Pharmacy, Chelembra and it is reconfirmed by gram staining and it is subculture in appropriate selective media.

Preparation of the extract for antimicrobial tests

The formulated product of 5 gm was loaded in the thimble of soxhlet apparatus and was fitted with a round bottom flask with 250 ml DMSO (dimethyl sulphoxide)

and the upper part was fitted with the condenser. Constant heat was provided by Mantox heater. After complete extraction, the extract in the round bottom flask was transferred in to clean and pre-weighed universal tubes. Universal tubes containing extracts were weighed and noted down and finally the % yield was calculated.

% yield = initial wt of the raw material / Final wt of the extract.

Separation of active compounds from the nutraceutical suspension by preparative TLC

Preparation of chromo plates

The microscopic glass plates were cleaned and dried in hot air oven. Slurry was prepared by mixing silica gel (silica gel with calcium sulphate binder) adding the double the volume of distilled water in clean beaker with continuous stirring. One large drop of slurry was placed on the slide and makes a thin film. This procedure is applied for all the slides. These chromo plates were activated by heating them in the hot air oven at 120°C for 30 minutes.

Sample Loading

Plate was allowed to cool at room temperature and marked about 2 cm from the bottom. The work suspension was loaded at the center of the slide about 2 cm away from the edge.

Chromatogram development

The development tank was saturated with suitable solvent system (Hexane: ethyl acetate in the ratio of 8.5 : 0.5). The plate was kept in the tank with out touching the baseline by the solvent and left for the development. The final solvent front was marked and the plate was dried.

Spot visualization

Few drops of Iodine crystals were transferred to a tank and covered with a glass plate. Allow for saturation. Collected the above material in a beaker containing DMSO and left over night. The contents in the beaker was stirred and filtered through Whattmann filter paper no

1. The filtrate was collected in clean dry beaker. The filtrate containing active compounds was used for determining the antimicrobial effect.

Phytochemical constituents of the formulation

Phytochemical constituents	DMSO
Carbohydrate	(+)
proteins	(+)
lipids	(+)
flavonoids	(+)
saponins	(+)

Identification tests for active compounds

Test for carbohydrate determination

To 1 ml of water add 0.5 ml of extract solution and 5 drops of Fehling solution, boil for 2 minutes. A brick red color precipitate was obtained which shows the presence of reducing sugar in the formulation.

Test for proteins

Take 3ml of sample solution in a test tube. Make it alkaline by using 5 drops of 10% sodium hydroxide and 3 drops of 0.5% copper sulphate solution. Presence of violet colour indicates the presence of proteins.

Test for Lipids

In a clean and dry test tube, take 2ml of filtered chloroform extract; mix well till the samples are soluble in chloroform. Add 10 drops of acetic anhydride, 2 drops of concentrated sulphuric acid for 10 minutes. If fat is present in the extract the reaction gives green colour. But for this reaction we get negative result.

Test for Flavanoids

Extract solution of 5 ml was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavanoids.

Test for Saponins

To 0.5 gm of extract add 5 ml of distilled water in a test tube and shaken vigorously to obtain a stable persistence froth. The froth was mixed with 3 drops of olive oil and again shaken vigorously. Formation of an emulsion indicates the test positive.

Evaluation of Antimicrobial activity:

Preparation of standard culture inoculums of test organism

Three or four colonies were inoculated in the two ml nutrient in accordance to WHO recommended standard (Mac-Farland standard 0.5%) and it is incubated till the growth equals to the prescribed standards.

Determination of Zone of Inhibition

The freshly prepared inoculums were swabbed all over the surface of the plate using sterile cotton swab. Three wells of 6mm diameter were bored in the medium with the help of sterile cork borer having 6mm diameter and 50 μ litres of the working solution of the newly formulated

drug, same volume of extraction solvent for control, and a standard antibiotic (ampicillin) filled in the wells with the help of micropipette.

Give time for the extract to diffuse in to the medium, and then closed the lid and incubated at 37°C for 24 hrs and measured using scale and after incubation mean were recorded. Plates were observed for Zone of Inhibition.

Determination of Minimum Bactericidal concentration

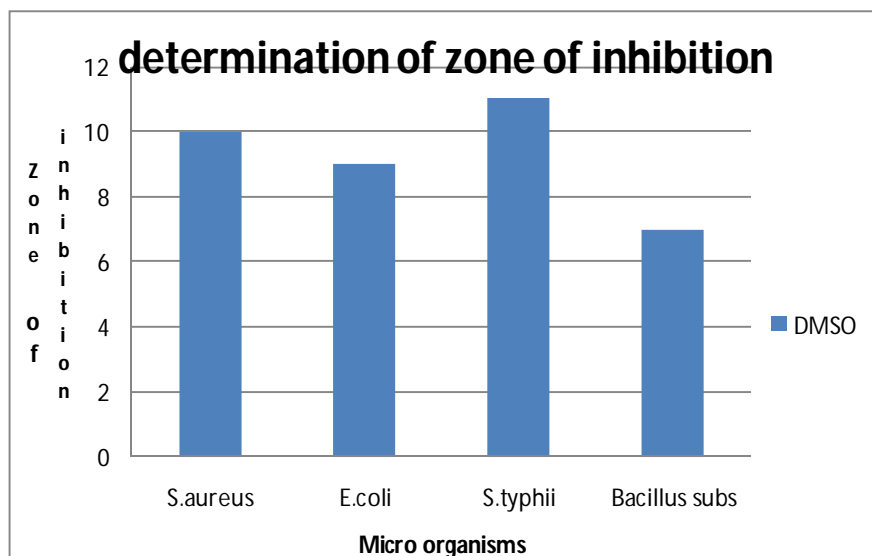
Procedure for determining Minimum Bactericidal concentration in the formulation:

Freshly prepared nutrient broth was used as diluents. Extract of the formulation was diluted by two fold serial dilution method. Inoculum of 50 μ liter was added to each test tube except control. All tubes were incubated at 37°C for 24 hrs. In fresh nutrient agar the tube contents were subculture separately and minimum bactericidal concentration was determined showing no growth.

Bactericidal concentration was determined by two fold broth dilution method

S. No	organism	solvent	Zone of Inhibition(mm)	Minimum bactericidal concentration(mg/ml)
1.	Staphylococcus aureus	DMSO	10	>10
2.	E.coli	DMSO	9	5
3.	S.typhi	DMSO	11	>2.5
4.	Bacillus substilis	DMSO	7	2

Graph:



RESULTS AND DISCUSSIONS

For determining the antioxidant property the alcoholic as well as aqueous extracts was collected and both of them are tested for the presence of carbohydrates, proteins, flavanoids etc. by qualitative phytochemical analysis. Free radical scavenging activity of aqueous extracts and ethanolic extract was quantitatively determined using DPPH assay.

Ethyl alcohol and aqueous extract of the formulation exhibited good reducing power. By comparing with ascorbic acid the reducing power of both extracts is between 100-500 mg/l. The results in Table 1 and Table 2 shows there is a decrease in absorbance of DPPH at its absorption maxima of 517 nm, which is proportional to the concentration of free radical scavenger added to DPPH reagent solution.

Evaluation of antimicrobial activity was performed by Agar well diffusion method and two fold broth dilution method. Estimation of potency of the formulated drug was measured by diameter of the Zone of Inhibition. In agar medium, due to variable diffusability the anti bacterial property does not show its efficacy in zone of inhibition. So we performed minimum bacterial concentration value for this

study. The largest zone of inhibition (14mm) was against E.coli followed by Salmonella typhi(11mm) and staphylococcus aureus(10 mm).The minimum bactericidal concentration was about >2.5 against S.typhii.

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