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Research Article

DEVELOPMENT OF A POLYHERBAL SOLID DOSAGE FORMULATION HAVING ANTIOXIDANT PROPERTIES

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ABSTRACT

Objective: The main objective of the present study was to develop a polyherbal solid dosage formulation and to evaluate antioxidant properties with respect to vitamin-E.

Methods: Polyherbal formulation was developed by using well documented medicinal plants, Amla, Withania and Tulsi. The extracts were prepared by using solvents like 60% ethanol for Amla, 50% ethanol for Withania and 99.9% ethanol for Tulsi. Formulation was evaluated by using different methods like nitric oxide scavenging activity, Free radical scavenging by DPPH method and scavenging of hydroxyl radical (Deoxyribose Method). Vitamin-E was used as standard.

Results: The formulated polyherbal chewable tablet was proved to show pronounced invitro antioxidant activity and its percentage inhibition was at 200µg concentration found to be 96.34 of DPPH inhibition, 69.22 of Nitric oxide, and 36.00 of Hydroxyl radical effect.

Conclusion: In conclusion, the present study shows that the polyherbal chewable tablet showed pronounced invitro antioxidant activity.
Keywords: Amla, Withania, Tulsi, Formulation, Antioxidant activity.

INTRODUCTION

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as ascorbic acid, or polyphenols[1]. Generally free radicals are produced in large amounts during metabolic disease conditions and have been implicated in the causation of several problems like atherosclerosis, ulcers, asthma, cancer, cataract, liver disease and other inflammatory process. Reactive oxygen species (ROS) are continuously produced during cell metabolism and under normal conditions; they are scavenged and converted to [2] nonreactive species by different intracellular enzymatic and non-enzymatic antioxidant system[3]. Antioxidants thus play an important role in the protection of human body against damage by reactive oxygen species[4,5]. Antioxidants may act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules and or activators of antioxidative defense enzyme system to suppress the radical damages in biological systems [6,7]. Therefore, inhibition of free radical-induced oxidative damage by supplementation of antioxidants has become an attractive therapeutic strategy for reducing the risk of these diseases. In recent years, it has been investigated that many plant species are serving as source of antioxidants and received therapeutic significance. Several anti-oxidants of plant origin are experimentally proved and used as effective protective agents against oxidative stress. They play an important role in major health problems such as cancer, cardio vascular diseases, rheumatoid arthritis, cataracts, Parkinson's disease, Alzheimer's disease and degenerative diseases associated with aging [8-10].

A great deal of research has been carried out to evaluate scientific basis for the claimed antioxidant activity of herbal agents as in the form of formulation. The selected plant materials; *Emblica officinalis*, *Withania somnifera* and *Ocimum sanctum* reported to have antioxidant activity [11]. The herbal formulation contains the extract of *Emblica officinalis*, *Withania somnifera* and *Ocimum sanctum*. The aim of the present study is to formulate the polyherbal formulation as chewable tablet and evaluate the antioxidant activity. By *in vitro* antioxidant methods like Nitric oxide, DPPH, Deoxyribose method.

MATERIALS AND METHODS

Procurement of plant materials

Dried fruits of Amla, dried roots of Withania and dried aerial part of Tulsi were procured from yucca enterprises Mumbai.

Preparation of extracts [12]

Procured dried plant materials (Amla pericarp, Withania root and Tulsi aerial part) were washed twice with tap water and one time with distilled water finally allowed to dry under shade, and then coarsely powdered in a blender. The coarse powder (500 gm each) was subjected to maceration for 72 hours, followed by exhaustive maceration for 48 hours by using various solvents like 60% ethanol for Amla pericarp, 50% ethanol for Withania root and 99.9% ethanol for Tulsi aerial part. The solvents were decanted and filtered with filter paper and recovered by distillation with help of rotary vacuum evaporator at 80^{0}C. The extracts were dried under desiccators.

Preparation of formulation

Poly herbal tablets containing known quantities of various extracts and selected excipients. Granules were prepared by wet granulation technique and tablets were punched by using a 16 station tablet punching machine. The quantity of extracts and the excipients used for formulating one tablet is tabulated below.

Formula for chewable tablet

Emblica officinalis extract - 49mg

Withania somnifera extract - 19.6mg

Ocimum sanctum extract - 31.4mg

Colloidal silicon dioxide - 19.5mg

Sodium sacchrin - 20mg

Mannitol - 493.52mg

Purified talc - 4.88mg

Magnesium stearate - 4.88mg

Polyvinylpyrolidone - 6.5mg

Methyl paraben - 0.65mg

Propyl paraben - 0.07mg

Isopropyl alcohol -(q.s)

The formulation development work includes preformulation and formulation evaluation.

Preparation of granules and punching of tablet

Granules were prepared by wet granulation method. All the solid fractions and excipients were passed through British standard sieves (BSS) 80 prior to use. Doses of individual extracts- required quantities of extracts were weighed accurately using an electronic balance and mixed with the diluents Colloidal silicon dioxide and mannitol to make it to dry powder form and then passed through 60 mesh. The Polyvinylpyrolidone paste was prepared by adding Isopropyl alcohol (q.s). The wet coherent mass was prepared by using Polyvinylpyrolidone paste then passed through sieve no. 10 and dried at 40ºC for 30 minutes in tray dryer. The dried granules were passed again through sieve no. 22. The granules were finally lubricated with purified talc and magnesium stearate and then characterized for the fines, angle of repose, bulk density and tap density. Round and biconvex shaped tablets, with average weight of 650mg were compressed using a Rotary tablet punching machine (RSP 16) and evaluated.

Physical evaluation

The prepared polyherbal tablets were subjected to determination of various physical parameters like hardness, thickness, friability and weight variation test as per the standard procedures.**[13]**

Methodology

The antioxidant activity of plant extracts were proved by different *In-vitro* methods which are given as follows -

1. Nitric Oxide Scavenging Activity.

2. Free Radical Scavenging by DPPH Method.

3. Scavenging of Hydroxyl Radical (Deoxyribose Method).

Procedure for Nitric Oxide Scavenging Activity [14]

The nitric oxide scavenging activity of plant extracts was determined according to the method (Green et al., 1982). Aqueous solution of sodium nitro prusside spontaneously generates nitric oxide (NO) at physiological pH, which interacts with oxygen to produce nitrate ions and which was measured colorimetrically. 3mL of reaction mixture containing 2mL of sodium nitro prusside (10mM) in phosphate buffer saline (PBS) and 1mL of various concentrations of the extracts were incubated at 37^{0}C for 4 hours. Control without test compound was kept in an identical manner. After incubation 0.5mL of Griess reagent was added. The absorbance of the chromophore formed was read at 546nm. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of control and those of test compounds. Vitamin-E (10, 50, 100, 200, 400, 800, 1000 µg/ml) was used as standard. The percentage nitric oxide inhibition was calculated from the following formula. The results were tabulated in table 3 and represented in figure 1.

$$\% \text{ Nitric Oxide Inhibition} = \frac{\text{OD of control-OD of test}}{\text{OD of control}} \times 100$$

Procedure for Free Radical Scavenging by DPPH Method [15]

DPPH scavenging activity was measured by the spectrophotometric method. A stock solution of 25mg of DPPH (150µM) was prepared in 100mL of ethanol. To the 0.2 ml of extract of different concentrations, 3.8 ml of DPPH was added. Control without test compound was prepared in an identical manner. In case of blank, DPPH was replaced by ethanol. The reaction was allowed to be completed in the dark for about 20 minutes. Then the absorbance of test mixtures was read at 517nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical. Vitamin-E (10, 50, 100, 200, 400, 800, 1000 µg/ml) was used as standard. The percentage DPPH inhibition was calculated from the following formula. The results are tabulated in table 3 and represented in figure 2.

$$\% \text{ DPPH Inhibition} = \frac{\text{OD of control-OD of test}}{\text{OD of control}} \times 100$$

Procedure for Scavenging of Hydroxyl Radical (Deoxyribose method) [16]

To the reaction mixture containing deoxyribose (3mM, 0.2ml), ferric chloride (0.1mM, 0.2ml), Ethylenediamine tetra acetic acid sodium salt (EDTA) (0.1mM, 0.2ml), ascorbic acid (0.1mM, 0.2ml), and hydrogen peroxide (2mM, 0.2mM) in phosphate buffer (pH 7.4, 20mM), was added to 0.2 ml various concentrations of extracts or standard in DMSO to give a total volume 1.2 ml. the solutions were then incubated for 30 min at 37° C. After incubation, trichloroacetic acid (0.2ml, 15%), and thiobarbituric acid (0.2ml, 1% w/v) in 0.25 N HCl were added. The reaction mixture was kept in a boiling water bath for 30 min, cooled, and the absorbance was measured at 532 nm. The results are tabulated in Table no. 3 and represented in figure 3.

RESULTS AND DISCUSSION

Table 1: Evaluation of granules

S. No.	Evaluation	Observation/ Results
1.	Physical appearance of granules	Brownish coloured granules
2.	Flow ability	Free flow
3.	Fines	20.52%
4.	Loss on drying	1.2%
5.	Bulk density	0.564gm/cc
6.	Tap density	0.656gm/cc
7.	Hausner ratio	0.86
8.	Carr's index	16.28
9.	Angle of repose of granules	25

Table 2: Evaluation of Tablets

S. No.	Evaluation	Observation/ Results
1.	Physical appearance of tablets	Brownish coloured Tablet
2.	Thickness of tablets	1.30±0.01cm
3.	Weight variation	650 ± 5 mg
4.	Hardness	5 kg/cm^2
5.	Friability	0.45%

Table 3: *In vitro* antioxidant Activity of Tablet

Tablet	Conc. 10µg	Conc. 50µg	Conc. 100µg	Conc. 200µg	Conc. 400µg	Conc. 800µg	Conc. 1000µg
Nitric oxide (%inhibition)	58.87	63.05	65.76	69.22	68.71	61.20	58.34
DPPH (%inhibition)	45.50	62.63	80.98	96.34	95.84	94.63	93.31
Hydroxyl radical (%inhibition)	29.69	30.26	30.56	36	46.35	61.99	79.60

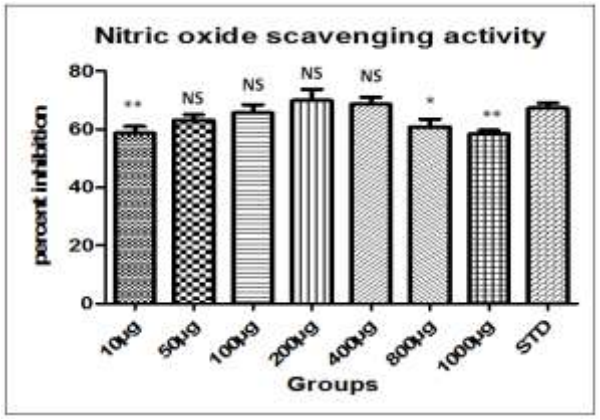

Fig. 1: Effect of Tablet on Nitric oxide scavenging activity

Each value represents **Mean ± SD**; n = 8. * * P < 0.01: * P < 0.05: NS = Nonsignificant

The tablet concentration Equivalent to 50, 100, 200 and 400µg showed no significant change in the percent nitric oxide inhibition when compared to standard. Concentration of 800µg showed slightly significant (p = 0.05) decrease in the activity when compared to standard. The concentration 10 and 1000 showed lesser nitric oxide inhibitory activity.

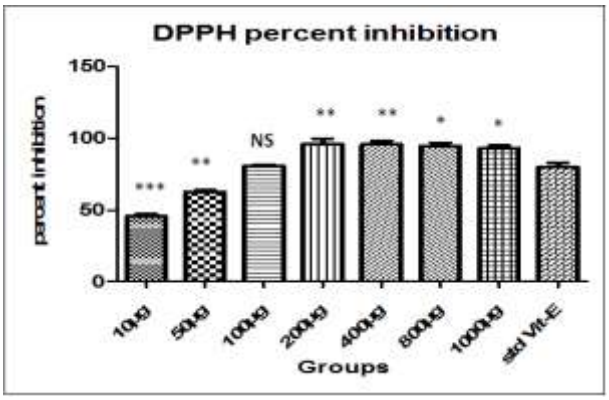

Fig. 2: Effect of Tablet on DPPH scavenging activity

Each value represents **Mean ± SD**; n = 8. * * P < 0.01: * P < 0.05: NS = Nonsignificant

The concentration of tablet at 200, 400,800 and 1000µg should increased percentage inhibition when compared to standard.

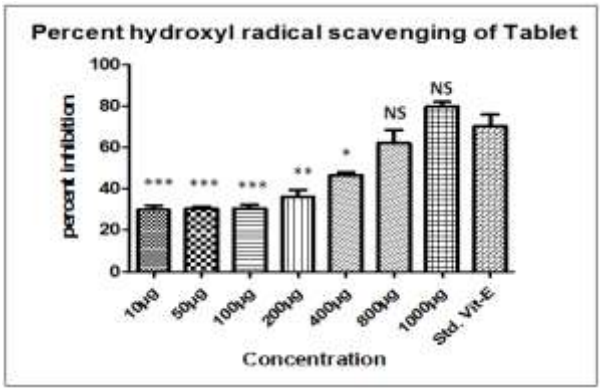

Fig. 3: Effect of Tablet on Hydroxyl radical scavenging activity

Each value represents **Mean ± SD**; n = 8. * * * P < 0.001: * * P < 0.01: * P < 0.05: NS = Nonsignificant

DISCUSSION

Concentration 800 and 1000 showed no significant increase in the percent hydroxyl radical inhibition when compared to standard. Concentration 200 and 400 showed slightly significant (p = 0.05) decrease in the activity when compared to standard. Almost all the groups showed good percent inhibition of hydroxyl radical.

CONCLUSION

The formulated poly herbal chewable tablet was proved to show pronounced invitro antioxidant activity and its percentage inhibition was at 200µg concentration found to be 96.34 of DPPH inhibition, 69.22 of Nitric oxide, 36.00 of Hydroxyl radical effect. Further after proving its safety and efficacy of the tablet anti stress can be carried out in animal models.

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