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Research Article

IN-VITRO ANTIOXIDANT ACTIVITY OF MEGAEXT OF TRIAMRIT

Singh Veena. D and Mishra R.N.*

Sagar Institute of Pharmaceutical Sciences, Sagar, Madhya Pradesh, India.

*Corresponding Author: rnmishr@gmail.com

ABSTRACT

The objective of study is to investigate the *In-Vitro* Antioxidant Activity of TriAmrit megaExtract. DPPH, Superoxide radical scavenging activity and reducing power method was determined by *in vitro* experiments. The sample possesses statistically significance DPPH free radical scavenging activity ($P<0.001$). Sample of 50µg/ml inhibited the production of Superoxide anion radical by 84.36% showing strong superoxide radical scavenging activity .The reducing power activity of TriAmrit megaExt (sample) and Ascorbic acid increases absorbance with increasing concentration dependent manner. TriAmrit megaExt has been found to be having high antioxidant activity and radical scavenging activity against various antioxidant systems in vitro.

Keywords: TriAmrit Reducing power, DPPH and Super-oxide radical scavenging antioxidant.

INTRODUCTION

The role of free radicals has been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, aging, arthritis, diabetes etc. and the compounds that can scavenge free radicals have great potential in ameliorating these disease processes. Antioxidants play an important role to protect the human body against damage by reactive oxygen species. Human body has endogenous mechanisms such as superoxide dismutase, glutathione peroxidase, catalase and vitamin E (α-tocopherol) to reduce free radical induced injury[1].

TriAmrit consists of the dried herbs of three medicinal plants, Terminalia *chebula* (combretaceae), *Allium sativum* (liliaceae) and *Tinospora cardifolia* (menispermiacee).These three medicinal plants are born out of Amrit (nector) as per Ayurveda*Terminalia chebula* Retz. (Family: Combretaceae, Ayurvedic name: 'Haritaki'). In 'Ayurveda', myrobalans are used in fevers, cough, asthma, urinary diseases, piles and worms. It is also useful in chronic diarrhea and dysentery, flatulence, vomiting, colic and enlarged spleen and liver. Chebulic myrobalans are extensively used in combination with belleric and embolic myrobalans under the name of 'Triphala' and also as adjuncts to other medicines in numerous diseases[2]. *Terminalia chebula* exhibited antioxidant activity at different magnitudes of potency for anti-LPO, anti-superoxide radical formation and free radical scavenging activities[3].

Tinospora cordifolia (Willd.) Miers. (Family: Menispermaceae, Ayurvedic name: 'Guduchi'). In 'Ayurveda', the starch obtained from the roots and stems of the plant is useful in diarrhea and dysentery, it is also a nutrient. the plant is commonly used in rheumatism, urinary diseases, dyspepsia, general debility, syphilis, skin diseases, bronchitis, spermatorrhea, and impotence .As 'Rasayana', juice is given with honey or raw sugar[4]. Extract of *Tinospora cordifolia* has been shown to inhibit the LPO and superoxide and hydroxyl radicals' in vitro[5].

Garlic (*Allium sativum* Linn.), used as a spice and medicinal herb, exhibits a wide range of properties including immunomodulatory, hepatoprotective, antioxidant, antimutagenic and anticarcinogenic effects [6,7].

Research envisaged (Justification- Aim-Objectives): In the literature we found some reports on the Antioxidant Activity of one or the other individual herb constituents of TriAmrit However, there is any report on the mixture of these 3 herbs for pharmacological screening of

such therapeutic activity to the Antioxidant .As there is synergistic effect or mutual potential of therapeutic action when such herbs of similar nature are mixed together. In the light of this, it was thought worthy to evaluate Antioxidant activity of Trikatu and their correlation with adoptogenic activity.

MATERIALS AND METHODS

Plant material and Drugs: The herbs of TriAmrit *(Allium sativum, Tinospora cardifolia and Terminalia chebula*) were collected from local market of Sagar, All three herbs dried in shade, coarsely powdered and all three powdered drugs were mixed in 1:1:1 w/w, to preparation of megaHerb. megaHerb subjected to soxhlet successive extraction, using non polar to polar solvent (Pet. ether, Benzene, Chloroform, Ethyl acetate, 70% ethanol and water). All six extract was concentrated by distilling the solvent and air dried. All six extracts were mixed to prepare megaExtract (megaExt).The megaExt was subjected to qualitative phytochemical analysis for presence of various constituents like Alkaloids, Carbohydrate, Glycosides, Terpanoids, Protein and Amino acids, Phenolic and Tannins, Flavanoids, Oils and Fats, Saponins etc.

Reagents and chemicals: Nitro blue tetrazolium, and all the solvents used in the study were of analytical grade and were procured from S D Fine Chemicals Limited, Mumbai, India. 1, 1-diphenyl-2-picrylhydrazyl, Ascorbic acid, and other chemicals were obtained from Sigma Chemical Company (St. Lousis, MO).

EXPERIMENTAL/ METHOD

In-vitro antioxidant activity

TriAmrit megaExt was evaluated for *in vitro* antioxidant activity by DPPH (1, 1- diphenyl-2-picryl hydrazyl) radical, Superoxide radical scavenging, Reducing Power methods.

1. DPPH radical scavenging activity

The method is based on the reduction of colored solution of DPPH (1, 1-diphenyl-2picryl hydrazyl) in presence of test drug measured at 517 nm. The free radical scavenging capacity of the TriAmrit megaExtract was determined using DPPH method of DPPH solution (0.004% w/v) was prepared in 95% methanol. Ascorbic acid solution preparation: 1000µg/ml stock solution was prepared by dissolving 10mg of ascorbic acid in 10 ml of methanol. From this 5, 10, 20, 30, 40 and 50µg/ml ascorbic acid solutions were prepared. The megaextracts were mixed with 95% methanol to prepare the stock solution (10 mg/100mL). The concentration of this megaExtract solution was 10 mg /100 mL. Stock solution 2ml, 4ml, 6ml, 8ml & 10ml of this solution were taken in five test tubes & by serial dilution with same solvent were made the final volume of each test tube up to 10 ml whose concentration was then 5, 10, 20, 30, 40 and 50µg/ml respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing megaExtract (5, 10, 20, 30, 40 and 50µg/ml) and after 10 min, the absorbance was taken at 517 nm using a spectrophotometer. Control sample was prepared containing the same volume without any extract was used as blank. % scavenging of the DPPH free radical was measured using the following equation. Results are shown in table and graphically

% inhibition = [(Ao-At) / Ao x 100]

Where Ao was the absorbance of the control (blank, without extract) and At was the absorbance in the presence of the extract.(8)

2. Superoxide radical scavenging activity

This activity was measured using NBT (Nitro blue tetrazolium reagent). The method is based on generation of superoxide radical (O_2^-) by auto oxidation of hydroxylamine hydrochloride in presence of NBT, which gets reduced to nitrite. Nitrite in presence of EDTA gives a color that can be measured at 560 nm. Various concentrations (5, 10, 20, 30, 40 and 50µg/ml) of test solutions were taken in test tube. To this, reaction mixture consisting of 1 ml of 50 mM sodium carbonate, 0.4 ml of 24 mM NBT 0.2 ml of 0.1 mM EDTA solution were added to the test tube and zero minute reading was taken at 560 nm. The reaction was initiated by the addition of 0.4 ml of 1 mM hydroxylamine hydrochloride to the above solution. Reaction mixture was incubated at 25^0C for 15 minute; the reduction of NBT was measured at 560 nm. Absorbance was recorded and % inhibition was calculated according to the following equation.

% inhibition = [(Ao-At) / Ao x 100]

Where Ao was the absorbance of the control (blank, without extract) and At was the absorbance in the presence of the extract[9].

3. Reducing power

The reducing power of the megaExt was

determined according to the method .Various concentrations of the mega Ext (5, 10, 20, 30, 40 and 50µg/ml) in 1.0 ml of demonized water were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and 1% potassium ferricynide (2.5 ml). The mixture was incubated at 50°C for 20 min. aliquots of trichloroacetic acid (2.5 ml, 10%) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared $fecl_3$ solution (0.5 ml, 1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power[10].

STATISTICAL ANALYSIS

The data were analysed using one way analysis of variance (ANOVA) followed by student 't'-test .p values <0.001 were considered significant.

RESULT

Phytochemical screening reveals that the major constituents of TriAmrit megaExtract are phenolic compound, glycosides alkaloid, and flavanoid were, phenolic compounds which may be responsible for the activities of antioxidant.

1. DPPH radical scavenging activity

TriAmrit megaExt had significant scavenging effect on the DPPH free radical which increased with increasing concentration from 5-50µg/ml. The scavenging effect of sample was lower than that of Ascorbic acid. The sample possess statistically significance DPPH free radical scavenging activity (P<0.001).

2 Superoxide radical scavenging activity

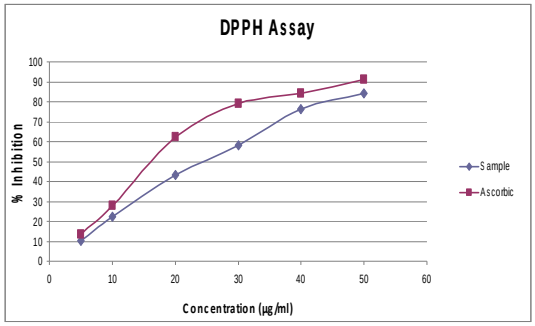
TriAmrit megaExt is found to posses scavenging effect on superoxide anion in a concentration dependent manner % of inhibition. Sample of 50µg/ml inhibited the production of superoxide anion radical by 84.36% showing strong superoxide radical scavenging activity .However the activity was lesser than the Ascorbic acid.

3. Reducing power

The reducing power activity of TriAmrit megaExt (sample) and Ascorbic acid increases absorbance with increasing concentration dependent manner.

Table 1: Effect of megaExt of TriAmrit in DPPH Antioxidant model

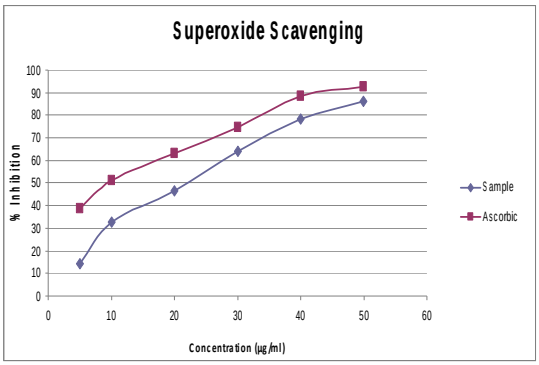
S. No.	Conc. µg/ml	% inhibition	
		Sample	Ascorbic Acid
1.	5	10.28	13.42
2.	10	22.24	28.04
3.	20	43.22	62.32
4.	30	58.08	79.14
5.	40	76.20	84.32
6.	50	84.36	91.06



Graph 1: Comparative effect of megaExt of TriAmrit (sample) and Ascorbic acid on DPPH assay

Table 2: Effect of megaExt of TriAmrit in Superoxide Antioxidant model

S. No.	Conc. µg/ml	% inhibition	
		Sample	Ascorbic acid
1.	5	14.26	38.63
2.	10	32.84	51.28
3.	20	46.32	63.36
4.	30	64.27	74.52
5.	40	78.22	88.38
6.	50	86.38	92.66

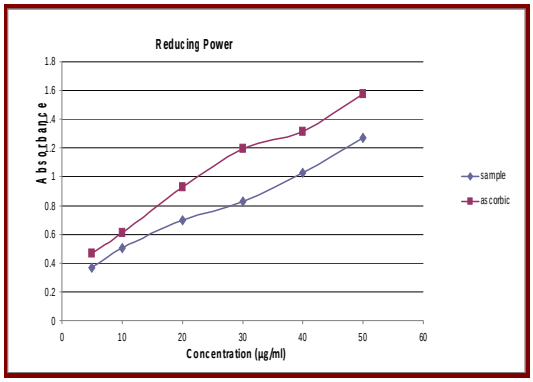


Graph 2: Comparative effect of megaExt of TriAmrit (sample) and Ascorbic acid on Superoxide scavenging activity

Table 3: Effect of megaExt of TriAmrit in reducing power

S. No.	Conc. (µg/ml)	Absorbance (Mean ± SEM)	
		Sample	Ascorbic acid
1	5	0.3751 ±0.005	0.4681±0.0008
2	10	0.5050 ±0.0001	0.6126±0.001
3	20	0.6952 ±0.005	0.9311±0.0005
4	30	0.8287±0.0009	1.1972±0.001
5	40	1.0246±0.0003	1.3157±0.06
6.	50	1.2702±0.01	1.5784±0.015

All value expressed, mean ± SEM



Graph 3: Comparative effect of megaExt of TriAmrit (sample) and Ascorbic acid on reducing power

DISCUSSION

The results of this study, it is clearly indicate that TriAmrit megaExt have high antioxidant activity and radical scavenging activity against various antioxidant systems in vitro. These assays have important applications for the food and pharmaceutical industry. Moreover, TriAmrit megaExt can be used as an easily accessible source of natural antioxidants and as a possible food supplement.

CONCLUSION

In our present study we conclude that megaExtract of TriAmrit has good antioxidant property and could be attributed to the presence of flavonoids, alkaloids, tannins, saponin glycosides and phenolic compounds. It was already reported that naturally occurring phenolic compounds have free redical scavenging property.

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