Phytochemical investigation and comparative evaluation of *in vitro* free radical scavenging activity of Triphala & Curcumin.

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ABSTRACT

Evaluation of *in vitro* free radical scavenging activity of Triphala extract containing gallic acid 10% (quantities by HPLC) & Curcumin 95 % (quantities by HPLC) was performed. Commercial sample of hydroalcoholic extract of Triphala (HAT) was obtained from Amruta Herbals Pvt. Ltd. Indore. Triphala extract (HAT) was compared against Curcumin to study the free radical scavenging activity by DPPH scavenging assay method. It was found that the commercial sample of Triphala extract (hydroalcoholic - HAT) contained gallic acids, tannins & β – sitosterol, showed marked inhibition in the DPPH scavenging model as compared to Curcumin. Hydroalcoholic extract of Triphala (HAT) showed more potent activity (94.99 ± 0.59 compared to the Curcumin extract 39 ± 0.32). Both extracts showed the dose and time-dependent inhibition of the DPPH scavenging activity.

Keywords: Triphla, Curcumin, DPPH scavenging, Antioxidant activity.

INTRODUCTION

Curcumin the chief component of turmeric also known as diferuloylmethane is a carotenoid pigment. Turmeric itself is originally obtained from the powdered rhizome of the plant *Curcuma longa* L. [1, 2]. Curcumin, the major bioactive component of turmeric, has been shown to exhibited wide range of interesting biological activities including anti-oxidant [3, 4] anti-inflammatory [5, 6] and anti-HIV properties [7, 8] most importantly, it has also exhibited significant anti-tumor activity which can correlate with antioxidant properties. Triphala is a traditional ayurvedic formulation consist fruits of Amalaki (*Embelica officinalis*), Haritaki (*Terminalia Chebula*) & Bibhitaki (*Terminalia belerica*) used as antiseptic, anti-inflammatory, cardiotonic & antihypertensive, also reported to exhibited significant anti-oxidant activity [9]. Anti-oxidant activity of Triphala may be due to presence of gallic-acid, thus present research work involved evaluation of antioxidant activity of gallic-acid as chief component of Triphala (consist 10% gallic-acid) with comparison to the natural reference antioxidant Curcumin.

Literature reveals that the carbonyl groups are responsible for free radical scavenging activity [10]. Free radicals are atoms or groups of atoms with an odd number of electrons and can be formed when oxygen interacts with certain molecules. Once highly reactive free radicals are formed, they can start chain reactions. Their major threat comes from the damage they can do when they react with important cellular components such as DNA or cell membranes. Cells may function poorly or die if this occurs. To prevent free radical damage, the body has a defense system of antioxidants [11]. Antioxidants can give free radicals which become companions to their unpaired electrons, thus eliminating the threat of gene alteration which can lead to cancer [12]. Medicinal plants have attracted the attention of not only professionals from various systems of medicine, but also the scientific communities belonging to different disciplines, plants are promising source of drugs. In continuing the search for potential free radical scavenging agents, the present investigation was aimed at determining the comparative free radical

scavenging activity of Triphla (gallic acid) & Curcumin. Free radical scavenging properties help in strengthening the immune system of the body, which helps to overcome cancer.

Materials and Methods

Collection, extraction and identification of the material:

All Individual herbs are well identified and collected from Amruta Herbals Pvt. Ltd. along with commercial sample of Triphala extract, batch no: DE-1033. Curcumin was obtained from Sigma Aldrich. The dried extracts were stored in an airtight container in a refrigerator below 10–20^{o}C.

Preliminary phytochemical screening:

The preliminary phytochemical screening was carried out on Curcumin and commercial sample of Triphala extract for the detection of various phytochemicals. Tests for common phytochemicals were carried out by standard methods [13].

DPPH scavenging activity:

Curcumin and hydroalcoholic extracts of Triphla (HAT) were screened for anti-oxidant activity using DPPH free radical scavenging activity in a dose and time dependent manner. Percentage inhibition and IC_{50} values were calculated (results are shown in Table 1).

Experimental protocol:

DPPH scavenging activity:

Extracts were screened for anti-oxidant activity, using DPPH free radical scavenging activity in a dose- and time-dependent manner. The antioxidant activity was assessed on the basis of radical scavenging effects of stable DPPH free radical. The DPPH radicals are reduce to corresponding hydrazine when it react with hydrogen donors, the DPPH radical is purple coloured compound & upon reaction with hydrogen donors it becomes colourless. It is discolouration assay which is evaluated by the addition of the sample drug (antioxidant) to DPPH solution in methanol & the decreasement in absorbance was measured on 517 nm. The reaction mixture contained 0.1 mM DPPH with methanol & different concentrations of hydroalcoholic extracts of Triphala (HAT) & Curcumin. Equal volume of methanol & DPPH was used as control. Incubation period was 30 min. (results are shown in Table 1).

Results and Discussion:

Phytochemical investigation:

It was found that the commercial sample of hydroalcoholic extract of Triphala (HAT) contained gallic acids, tannins & β – sitosterol. Curcumin itself a chief component obtained from the rhizomes of plant *Curcuma longa* L., have been used as reference antioxidant drug.

Free radical scavenging activity:

The commercial sample of Triphala extract (HAT) showed marked inhibition (94.99 ± 0.59 % inhibition) in the DPPH model. The Curcumin extract showed poor inhibition at similar concentration (39 ± 0.32 % compared to the Triphala extract) thus it was found that commercial sample of Triphala extract showed potent free radical scavenging activity even in low concentration as compared to Curcumin. Curcumin showed potent inhibition of the DPPH assay but in high conc. (85.22 ± 0.71 % at conc. 250 µg mL $^{-1}$**).** Both extracts showed the dose and time-dependent inhibition of the DPPH assay. (The results are shown in Table 1).

Nowadays, traditional medicine all over the world is being revalued by extensive research into different plant species and their therapeutic principles. Experimental evidence suggests that free radicals and reactive oxygen species can be involved in a high number of diseases [14]. As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity. In this study commercial sample of hydroalcoholic extract of Triphala collected from Amruta Herbals Pvt. Ltd. (Bath-No: DE-1033, Claimed as Gallic Acid 10%) found comparable DPPH activity, hydroalcoholic extracts were selected as they contain gallic-acid, tannins and phenolic compounds. They may have active constituents for producing the free radical scavenging effect. Free radicals are produced under certain environmental conditions and during normal cellular function in the body. These molecules lose an electron when an electric charge is given to them. To neutralize this charge, free radicals try to withdraw an electron from, or donate an electron to, a neighboring molecule. The newly created free radical, in turn, looks out for another molecule and withdraws or donates an electron, setting off a chain reaction that can damage hundreds of molecules. Antioxidants halt this chain reaction. Some antioxidants are free radicals themselves, donating electrons to stabilize the dangerous free radicals, others antioxidants work against the molecules that form free radicals, destroying them before they can begin the domino effect that leads to oxidative damage.

Scientific literature reveals that the carbonyl groups present in the flavonoids and phenolic compounds were responsible for the free radical scavenging activity [10]. This investigation revealed that commercial sample of Triphala extract (HAT) showed potent DPPH scavenging activity as compared to Curcumin, may be due to the presence of pharmacologically active substances such as gallic-acid, tannins and phenolic compounds which are considered to be responsible for the antioxidant activity. Thus research concluded that commercial sample of Triphala extract (HAT) can be used as potent antioxidant even in low dose which can play vital role against the disease i.e; cancer. Potent antioxidant activity of Triphala increased its width as useful traditional medicine.

Statistical analysis

The results are expressed as mean values ± S.E.M. (standard error of mean). Statistical comparison was carried out by analysis of variance (ANOVA).

Table 1. % Inhibition of commercial sample of Triphla extracts (HAT) & Curcumin by DPPH Assay

	% Inhibition Hydroalcoholic Extract of Triphala		% Inhibition Hydroalcoholic Extract of Curcumin	
S.No.	Concentrations (µg mL $^{-1}$)	% Inhibition	Concentrations(µg mL $^{-1}$)	% Inhibition
1.	10	91.8 ± 0.69	50	39 ± 0.32
2.	20	92.94 ± 0.67	100	71.45 ± 0.44
3.	30	92.37 ± 0.49	150	74.29 ± 0.56
4.	40	94.19 ± 0.77	200	81 ± 0.68
5.	50	94.99 ± 0.59	250	85.22 ± 0.71

Note: Data are the mean ± SD of three measurements.

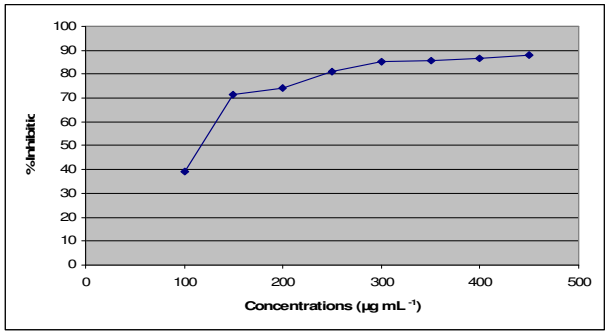

Fig.-1; % Inhibition of Curcumin Extract (DPPH Assay).

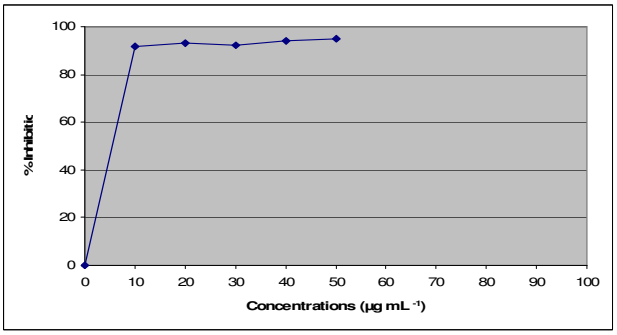

Fig.-2; % Inhibition of Triphala Extract (DPPH Assay)

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