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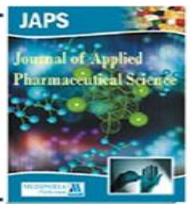

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Evaluation of Antioxidant activity of traditional formulation Giloy satva and hydroalcoholic extract of the *Curculigo orchioides* gaertn

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ABSTRACT

Herbal plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Thus, a proper scientific evidence or assessment has become the criteria for acceptance of herbal health claims. In the present study, the antioxidant effect of the traditional Ayurvedic formulation Giloy Satva (*Tinospora cordifolia* linn. known as Guduchi) and hydro alcoholic extract of the plant *Curculigo orchioides* linn. (Kali Musali) were examined. The present study was concentrated on the *in vitro* antioxidant methods where traditional formulation and hydro alcoholic extract screened for DPPH free radical scavenging activity, total reducing power assay and hydrogen peroxide scavenging activity assay. The results revealed potent scavenging activity when compared with standard. The plant extracts and formulation further phytochemically screened with thin layer chromatographic analysis indicating presence of flavonoids, alkaloids, glycosides, saponins and some amount of phytosterols. These active constituents alone or in combination may be responsible for the observed antioxidant activity. The findings indicated promising antioxidant activity of crude extracts needs further exploration for their effective use in both modern and traditional system of medicines.

Keywords: Antioxidant Activity, DPPH, H_2O_2 Assay, Reducing Power Assay.

INDUSTRIAL RELEVANCE

Giloy Satva *(Tinospora cordifolia* linn*. of family* Menispermaceae*) and Curculigo orchioides* gaertn. (family Amarylladaceae) are potent antioxidants found in ayurveda also reported in *Rasayana*. *Tinospora cordifolia* is one of the constituents of several ayurvedic preparations used in general debility, dyspepsia, fever and urinary diseases. The extract of its stem is useful in skin diseases. A bark of *T. cordifolia* has anti-spasmodic, anti-pyretic, anti-allergic, anti-inflammatory and anti-leprotic properties. It is an important ingredient of many Ayurvedic preparations and is considered to have aphrodisiac, immunostimulant, hepatoprotective, antioxidant, anticancer and antidiabetic activities. Hence present study explores the antioxidant activity of the plant extract of *Curculigo orchioides* gaertn. and traditional formulation.

INTRODUCTION

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals.

All these radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of human body rendering each cell to face out 10000 oxidative hits per second. when generation of ROS overtakes the antioxidant defense of the cell, the free radicals start attacking the cell proteins, lipids and carbohydrates and this leads to a number of physiological disorders. Free radicals are involved in the development of degenerative diseases, they have also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurological disorders, and in the process of aging. Many plant often contain substantial amounts of antioxidants including vitamin C and E, carotenoids, flavonoids and tannis etc., and thus can be utilized to scavenge the excess free radicals from human body.

In this research work the main objective is to study the antioxidant activity of traditional formulation Giloy satva and hydroalcoholic extract of *Curculigo orchioides* gaertn.

Antioxidants

The process of oxidation in the human body damages cell membranes and other structures including cellular proteins, lipids and DNA. When oxygen is metabolised, it creates 'free radicals' which steal electrons from other molecules, causing damage. The body can cope with some free radicals and needs them to function effectively. However, an overload of free radicals has been linked to certain diseases, including heart disease, liver disease and some cancers.

Giloy Satva *(Tinospora cordifolia* linn. *of family* Menispermaceae*) and Curculigo orchioides* gaertn. (family Amarylladaceae) is an endangered rasayana herb which is popularly known as "Guduchi" and "Kali Musli" respectively. The plants are native to India, and holds a special position as a potent adaptogen and aphrodisiac in Ayurvedic system of medicine. They are used as aphrodisiac, immunostimulant, antioxidant, anticancer and antidiabetic properties. *Curculigo orchioides* Gaertn. is a herbaceous tuberous geophilous perennial with rootstock bearing several fleshy lateral roots (rhizomes). The rhizomes of this plant possess medicinal properties and are sweet, cooling, diuretic, aphrodisiac, viriligenic and tonic which can be used against hemorrhoids, leucorrhoea, pruritis, skin diseases, asthma, bronchitis and jaundice etc. It is used extensively in ayurvedic formulations for a wide variety of ailments, specially as a general tonic and as an aphrodisiac. Thus, present study has been directed to investigate the antioxidant activity of Giloy satva and hydroalcoholic extract of *Curculigo orchioides* gaertn. plant in different *in vitro* models (Govindrajan, 2005).

MATERIALS AND METHODS

Plant Collection and Extraction

The formulation of Giloy Satva collected from market and fresh part of the *Curculigo Orchioides* gaertn. were authentified from university of Pune. The powder of rhizomes was extracted by Maceration using Hydro alcoholic mixture for 72 hours. This extract was concentrated under vacuum and then subjected to preliminary phytochemical screening.

Screening for the preliminary phytoconstituents. (Khandelwal, 2003)

Ethanol extract was evaluated for presence of various phytoconstituents by performing different qualitative chemical tests.

Screening for Thin Layer Chromatography (Wagner, 2004)

The various solvent systems selected for TLC analysis are listed in the following table

Table. 1: Various solvent system.

no	Detection	Solvent system	Ratio	Locating reagent
1	Glycosides	Ethylacetate: Methanol : Ethanol : Water	81:11:4:8	5% KOH
2	Flavonoids	Chloroform: Acetone :Formic acid	75:16.5:8.5	Vanillin-sulfuric acid
3	Alkaloids	Tolune:ethyl acetate:diethylamine	70:20:10	Dragendroff's reagent

Screening for in-vitro Antioxidant models (Jayaprakash *et al*, 2001).

The following models are performed for antioxidant activity

DPPH Assay (1, 1 diphenyl 2, picryl hydrazyl) (Vani *et al*., Sanchez-Moreno, 1999., Navarro *et al*., 1993): 0.3 ml solution of DPPH in 100% ethanol was prepared.5 ml of this solution + 1 ml of the fraction dissolved in ethanol at different concentrations (50-250 µg/ml), mixture was shaken and allowed to stand at room temperature for 30 min Absorbance was measured at 517 nm using a spectrophotometer. The % scavenging activity at different concentrations was determined and compared with that of Butylated hydroxy toluene, which was used as the standard.

Determination of reducing power. (Jayaprakash *et al*., 2001): 2.5 ml of solution of different concentrations of extract (50, 100, 150, 200, 250 µg/ml). 2.5ml PO_4 buffer solution (pH 6.6). 2.5 ml potassium ferricyanide solution (1 %w/v). Mixture placed in water bath/Incubate at 50^0C for 20 min. After incubation resulting solution cooled & mixed with 2.5 ml 10% Trichloro acetic acid to each test tube. The mixture was centrifuged at 650 rpm for 10 min.2.5 ml upper solution layer was mixed with 5 ml of deionised water & 0.5 ml ferric chloride (1%w/v). Absorbance was measured at 700 nm.

Hydrogen peroxide scavenging activity: Hydrogen peroxide 2mm/L solution prepared with standard (PO_4 buffer pH-7.4).Different concentrations of extract (50, 100, 150, 200, 250 µg/ml) prepared in distilled water.1ml of solution of different concentrations of extract (50, 100, 150, 200, 250 µg/ml). 0.6 ml hydrogen peroxide solution. After 10 min Absorbance was measured at 230 nm against blank solution containing PO_4 Buffer without hydrogen peroxide.

Comparing with standard Statistical analysis: The relative antioxidant activity results were compared using Standard deviation mean and Standard error mean method. P values less than 0.05 were considered as indicative of significance.

RESULT

There is an increasing evidence that different plant extracts was done according to indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress. There is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs and medicinal plants and reducing the risk of degenerative diseases associated with aging.

1. Screening for the preliminary phytoconstituents: Ethanolic extract was evaluated for presence of various phytoconstituents by performing different qualitative chemical tests reported. It showed the presence of flavonoids, anthraquinone/cardiac glycosides, saponins, tannins and phytosterols (Khandelwal; 2003).
2. Screening for Thin Layer Chromatography Giloy Satva – The RF value for alkaloids was found to be 0.63 and 0.76 and 0.80 and 0.27 for tannins respectively.
3. Screening for *in-vitro* Antioxidant models

FOR TRADITIONAL FORMULATION GILOY SATVA
(Values for all expressed as Mean±SEM),
*p<0.05,**p<0.01,*** p<0.001

DPPH (1, 1 diphenyl 2, picryl hydrazyl) Assay

This is the most widely reported method for screening of antioxidant activity of many plant drugs. DPPH assay method is based on the reduction of methanolic solution of colored free radical DPPH by free radical scavenger. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 516 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. The activity is expressed as effective concentration EC_{50}. Different concentrations of extract (50, 100, 150, 200, 250 µg/ml).

Table.1: DPPH method.

Sr.no.	Groups	Absorbance (A^0)
1	Standard	0.694±0.0023
2	50 µg/ml HE	0.448± 0.0030
3	100 µg/ml HE	0.440±0.0030
4	150 µg/ml HE	0.386±0.0010
5	200 µg/ml HE	0.216±0.0040
6	250 µg/ml HE	0.159±0.0011

Table. 2: Reducing Power Assay.

Sr.no.	Groups	Absorbance (A^0)
1	Standard	0.377±0.0023
2	50 µg/ml HE	0.230± 0.0030
3	100 µg/ml HE	0.300±0.0030
4	150 µg/ml HE	0.320±0.0010
5	200 µg/ml HE	0.400±0.0040
6	250 µg/ml HE	0.480±0.0011

Determination of reducing power

This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. In this method antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700 nm.

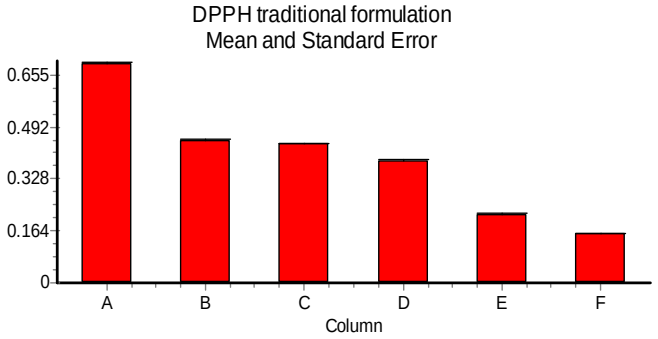

Fig. 1: DPPH Method SEM

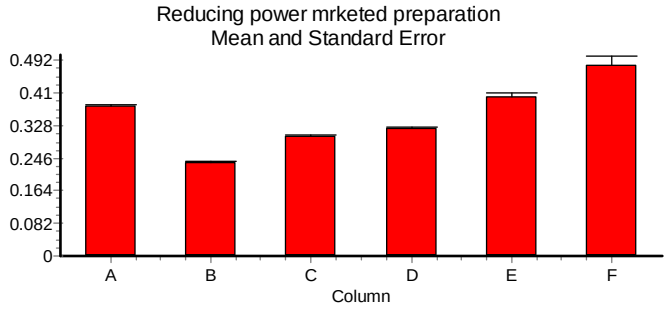

Fig. 2: Reducing Power Assay SEM

Hydrogen peroxide scavenging activity

Table. 3: Hydrogen Peroxide Scavenging Assay.

Sr.no.	Groups	Absorbance (A^0)
1	Standard	
2	50 µg/ml HE	1.125± 0.0030
3	100 µg/ml HE	1.122±0.0030
4	150 µg/ml HE	1.143±0.0010
5	200 µg/ml HE	1.153±0.0040
6	250 µg/ml HE	1.162±0.0011

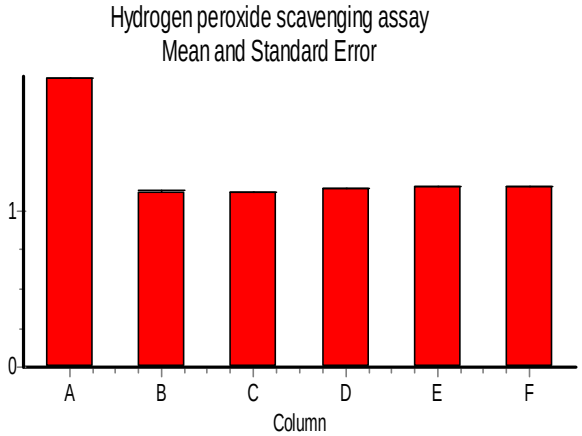

Fig. 3: Hydrogen Peroxide Scavenging Assay SEM .

FOR HYDROALCOHOLIC EXTRACT OF *C.ORCHIOIDES* GAERTN.

(Values for all expressed as Mean±SEM),*p<0.05,**p<0.01,***p<0.001

DPPH Assay

Table. 4: DPPH Assay

Sr.no.	Groups	Absorbance (A^0)
1	Standard	0.694±0.0023
2	50 µg/ml HE	0.462± 0.0030
3	100 µg/ml HE	0.440±0.0030
4	150 µg/ml HE	0.296±0.0010
5	200 µg/ml HE	0.216±0.0040
6	250 µg/ml HE	0.150±0.0011

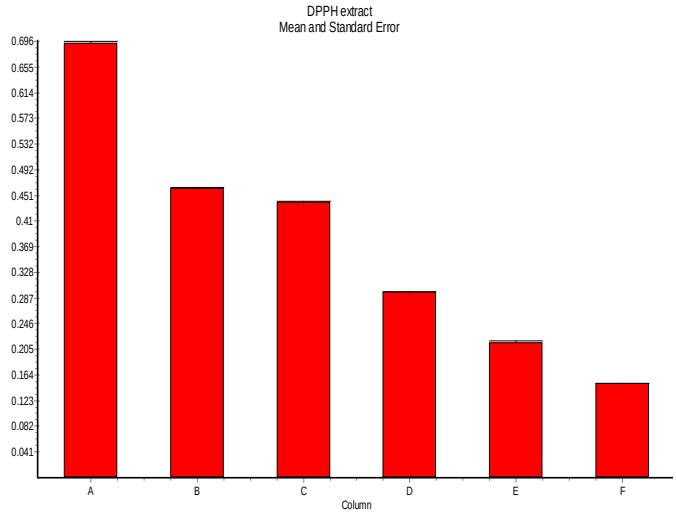

Fig. 4: DPPH Method Mean ± SEM

Table. 5: Determination of reducing power

Sr.no.	Groups	Absorbance (A^0)
1	Standard	0.377 ± 0.003
2	50 µg/ml HE	0.111 ±0.001
3	100 µg/ml HE	0.142 ± 0.002
4	150 µg/ml HE	0.237 ± 0.004
5	200 µg/ml HE	0.322 ± 0.007
6	250 µg/ml HE	0.466 ± 0.003

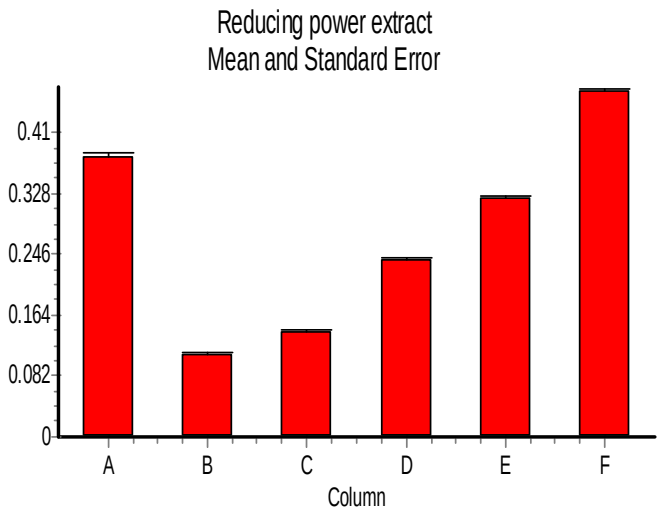

Fig. 5: Reducing Power Assay SEM.

HYDROGEN PEROXIDE SCAVENGING ASSAY

Table. 6: Hydrogen Peroxide Scavenging Assay.

Sr.no.	Groups	Absorbance (A^0)
1	Standard	1.852 ± 0.001
2	50 µg/ml HE	0.596±0.0005
3	100 µg/ml HE	1.350 ± 0.0011
4	150 µg/ml HE	1.482 ± 0.0023
5	200 µg/ml HE	1.589± 0.0005
6	250 µg/ml HE	1.895 ± 0.0017

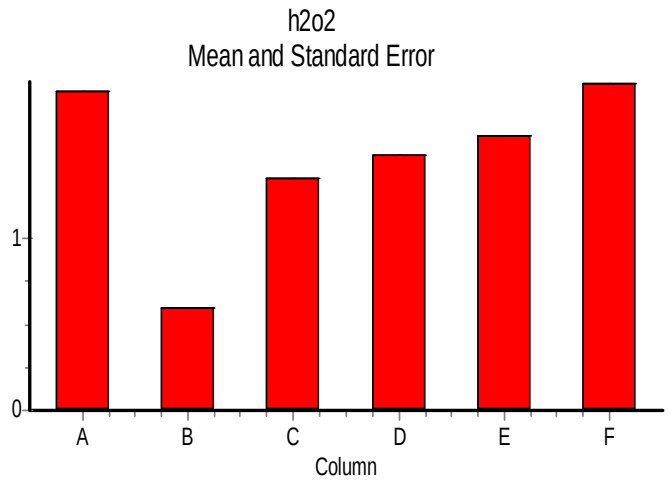

Fig. 6: Hydrogen Peroxide Scavenging Assay SEM

DISCUSSION

The results revealed that the extract shows potent scavenging activity when compared with standard Butylated hydroxy toluene. The plant extracts further studied for the phytochemical screening and thin layer chromatographic analysis with various solvent systems. It contains Flavonoids, glycosides, saponins and some amount of phytosterols. These active constituents alone or in combination may be responsible for the observed antioxidant activity. Further study on the active components and the mutual effect of these plant extract machinery may provide a better understanding about plant with a goal of elucidating their active potential compounds.

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