Research Article

In-Vitro Anti-Oxidant and Antibacterial, Antifungal Activity of Chloroform Extract of *Trichosanthes Tricuspidata* Lour Roots

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ABSTRACT

To investigate the *in vitro* Antioxidant, *In vitro* antimicrobial, antifungal activity chloroform extract of plant *Trichosanthes tricuspidata.* In vitro Antioxidant activity of chloroform extracts of *Trichosanthes tricuspidata* roots were screen by different in-vitro models i.e. nitric oxide, hydrogen peroxide and reducing power. In-Vitro evaluation of Anti-Bacterial and Anti-Fungal Activity of chloroform extracts of *Trichosanthes tricuspidata* roots were screen by agar- well diffusion method. The Nitric oxide radical concentration of extract and ascorbic acid needed for IC50 values was found to be 40.24 and 46.68 mcg/ml respectively. The reducing capacity of compound may serve as significant indicator of its potential antioxidant. Reducing power of selected diluted extract found to be significant and as good as L-Ascorbic acid. In H_2O_2 method extracts and ascorbic acid IC50 values 224.37 and 278.99 mcg/ml respectively. Chloroform ($CHCl_3$) extracts shows significant antioxidant and antibacterial, antifungal activity and so further studies are required to isolate and characterize the active phytochemical constituents responsible for activity.

Keywords: Antimicrobial, *Trichosanthes tricuspidata,* Vitro Antioxidant.

INTRODUCTION

Ayurveda is conventional medicinal systems of Indian's. Now the whole world is interested in India's *ayurveda* and other traditional medicine systems. The demand of medicinal plants is increasing day by day in both developing as well as developed countries as a result of recognition of the non-narcotic nature, lack of side effects and easily availability of many herbal drugs. Most often the medicinal plants are collected from the wild. There are numerous data on the uses of medicinal plants. The therapeutic potential of various herbal plant have need to be explore for its medicinal use. In this present paper we have attempted to briefly summarize the information available on the potency of *Trichosanthes tricuspidata* because of its immense medicinal potential it is a very important medicinal plant.[1]

MATERIALS AND METHODS

Plant material

Fresh roots of *Trichosanthes tricuspidata* collected from Kolhapure District, Maharashtra. The fresh roots were shade dried and ground into powder with the aid of blender and stored in air tight bottles at room temperature till use.

Extraction

Hot continuous extraction, Soxhlet process was used for the extraction of the plant material with Solvents were choose according to increasing order of its polarity like petroleum ether and chloroform. In Cold maceration procedure water is use as a solvent. For experimental study chloroform extract ($CHCl_3$) of roots of plant were used, shown in table 1.

In vitro antioxidant activity

The *in vitro* scavenging activities of the Chloroform ($CHCl_3$) extracts of *Trichosanthes tricuspidata* roots against different free radicals were performed. The results are expressed in terms of IC50, which is the concentration of the sample required to cause 50% inhibition of free radicals. Ascorbic acid was used as standard and the experiments were performed in triplicate.

Assay of Nitric oxide scavenging activity

Principle

The nitric oxide radical scavenging activity of extract was determined using the method of Sreejayan and Rao (1997) with minor modifications. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generate nitric oxide which interacts with oxygen to produce nitrite ions determined by the Griess reagent.

Procedure

For the experiment, sodium nitroprusside (10mM) in phosphate buffered saline was mixed with different concentrations of ($CHCl_3$) extract of *Trichosanthes tricuspidata* dissolved in ethanol The reaction mixture (6 ml) containing sodium nitroprusside (10mM, 4 ml), PBS (pH 7.4, 1 ml) and ($CHCl_3$) extract or standard solution (1 ml) were incubated at room temperature for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed and incubated at room temperature for 150 min. The same reaction mixture without the ($CHCl_3$) extract but the equivalent amount of ethanol served as the control. After the incubation period, 0.5ml of Griess reagent (1%

sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl) ethylene-diamine-dihydrochloride was added. The absorbance of the chromophore formed was read at 546 nm. (Sreejayan and Rao, 1997).[1-6] Result shown in table 2 and figure 1.

Preparation of reagents

The extracts solutions were prepared in ethanol and the standard ascorbic acid solutions were prepared in water.

1. Sodium nitroprusside: 10 mM solution of sodium nitroprusside was prepared in distilled water.

2. Naphthyl ethylene diamine dihydrochloride (NEDD, 0.1%): 0.1g of NEDD was dissolved in 60 ml of 50% glacial acetic acid by heating and then diluted to 100 ml with distilled water.

$$\text{Nitric Oxide scavenged (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where,

$A_{control}$ = Absorbance of control reaction and

A_{test} = Absorbance in the presence of the samples of extracts.

Reducing power test

Principle

Reduction ability of the extract has been investigated from the Fe+++ to Fe++ transformation using the method followed by (Oyaizu, 1986).[1-5] Earlier authors (Tanaka et al. 1988; Duh, 1998) have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductones (Duh, 1998) which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation.

Procedure

The reducing power of the extract was determined according to the method of Oyaizu. Different amounts of ($CHCl_3$) extracts (50-300mcg/ml)was dissolved in ethanol and mixed with phosphate buffer (2.5ml, 0.2mol/l, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged (650 x g at room temperature) for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5ml, 0.1%), and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. Results shown in table 3 and figure 2.

Scavenging of hydrogen peroxide

Principle

Hydrogen peroxide is generated *in vivo* by several oxidase enzymes. In this method, when an antioxidant is incubated with hydrogen peroxide, the decay or loss of hydrogen peroxide is measured spectrophotometrically. Hydrogen peroxide is a weak oxidizing agent which inactivates enzymes by oxidation of the essential thiol (SH-) groups. It rapidly transverses cell membranes and once inside the cell interior, interacts with Fe2+ and Cu2+ to form hydroxyl radicals, which is harmful to the cell.[6-9]

Procedure

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4) and concentration was determined spectrophotometrically at 230 nm (Schimadzu UV-Vis 1700). concentrations of ($CHCl_3$) extract of *Trichosanthes tricuspidata* dissolved in ethanol (25-300 µg/ml) dissolved in ethanol was added to a hydrogen peroxide solution (0.6 ml, 40mM) and the absorbance of hydrogen peroxide at 230 nm was determined after 19 min against a blank solution in phosphate buffer without hydrogen peroxide.

The percentage of scavenging of hydrogen peroxide of ($CHCl_3$) extract and standard compounds was calculated using the following equation:

$$\% \text{ scavenged } [H_2O_2] = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control, and A_1 was the absorbance of standards. Results shown in table 4 and figure 3.

Evaluation of Anti-Bacterial and antifungal Activity

Cultivation of microorganisms

Microorganisms: All the strains of micro- organism from the stock cultures of microorganisms (Govt. College of Pharmacy, Aurangabad and Department of Microbiology) were used, obtained from National chemical Laboratory; Pune.[17]

The following bacterial cultures were used for the study

a. Bacteria		**Strain Type**
Bacillus subtilis	Gram-positive organism,	ATCC 6633
Staphyllococcus aureus	Gram-positive organism,	ATCC 6538
Escherichia coli	Gram-negative organism,	ATCC 10535
Pseudomonas aeuroginosa	Gram-negative organism.	ATCC 27853
b. Fungi		
Aspergillus niger.		ATCC 16404
Candida albicans.		ATCC 10231

Preparation of culture:

a. Preparation of nutrient broth

The composition of nutrient broth media;

Ingredient	**Quantity**
Beef extract	10 gm
Peptone	10 gm
NaCl	0.5 gm
Purified water	1000 ml

pH	7.2±0.2
Yeast extract	1.5gm

The media was prepared by dissolving the nutrient broth in purified water. The medium and the test tubes were closed with cotton plugs and sterilized by autoclaving at 121°c (15 lbs Psig) for 15 minutes.

b. Preparation of Nutrient agar medium

The composition of nutrient agar media,

Ingredient	Quantity
Beef extract	10 gm
Peptone	10 gm
NaCl	0.5 gm
Purified water	1000 ml
Agar	20.0gm
pH	7.2 ± 0.2

c. Preparation of Bacterial culture

Now that sterile Nutrient broth media added into the test tube and microorganism inoculate on it by using nicromium wire loop then kept that test tube into incubator for 24 hours at 37°C.

Antibacterial assay

Agar-well diffusion method

The agar diffusion method was used to screen the antibacterial activity of ($CHCl_3$) extracts of *Trichosanthes tricuspidata* roots. 0.2 ml of each of the seeded broth containing 10^{-7} test organisms was inoculated on the plates of solidified agar and spread uniformly. Then 3 wells were cut in the agar layer of each plate with an aluminum bore of 6mm diameter. In every plate 2 different concentration of extracts. Concentration 10mg/ml, 20mg/ml dissolved in DMSO were added while in 3rd well standard chloramphenicol was added. Then all plates were incubated at 37°C ± 1 for 18 hrs. After the incubation period the mean diameter of the zone of inhibition in mm obtained around the well was measured.[1] Which has been shown in Table 5 and figure 4.

Antifungal assay

Anti-fungal study was carried out through same procedure as used in antibacterial study. Results are shown in Table 5 and figure 4.

RESULTS

Percentage of Extracts as follows,

Table 1: Selection of appropriate extraction method for plant material

Solvent	Percentage of extracts
Pet ether extract	2.32 % w/w
Chloroform extract	5.59 % w/w
Water extract	6.22 % w/w

In vitro antioxidant study results of Nitric oxide, reducing power and H_2O_2 scavenging activity method has cleared that extracts possesses significant antioxidant properties and antimicrobial effect may be correlated to this property.

Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by extracts. The concentration of extract and ascorbic acid needed for IC50 values was found to be 40.24 and 46.68 mcg/ml respectively.

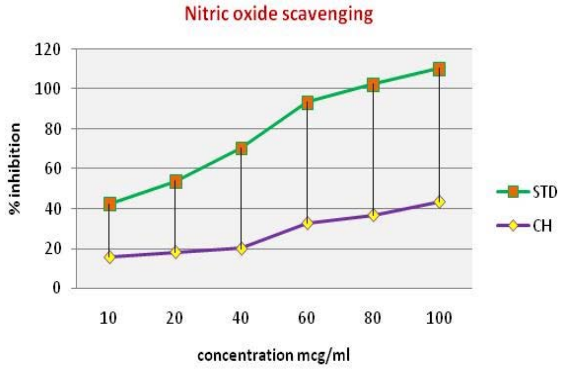

Figure 1: *In vitro* antioxidant activity of ($CHCl_3$) extract by Nitric Oxide method

For measurements of the reductive ability, we investigated the Fe3+ to Fe2+ transformation in presence of different extracts of *Trichosanthes tricuspidata.* The reducing capacity of compound may serve as significant indicator of its potential antioxidant. Reducing power of selected diluted extract found to be significant and as good as L-Ascorbic acid. In H_2O_2 method extracts and ascorbic acid IC50 values 224.37 and 278.99 mcg/ml respectively.

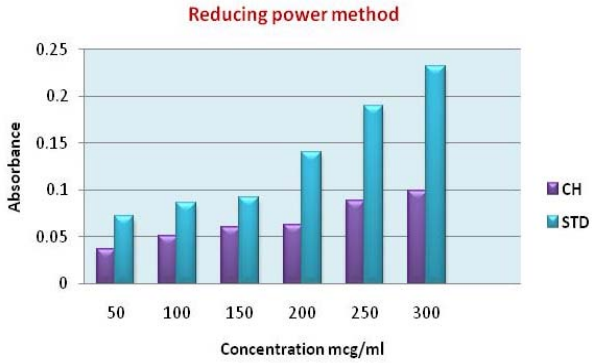

Figure 2: *In vitro* antioxidant activity of ($CHCl_3$)) extract by reducing power method

CONCLUSION

($CHCl_3$) extracts shows significant antioxidant and antibacterial, antifungal activity and so active phytochemical constituents responsible for activity.

Table 2: Nitric oxide scavenging activity of extracts

Extract	Concentration (mcg/ ml) and % inhibition						IC50 (mcg /ml)
	10	20	40	60	80	100	
($CHCl_3$) extract	15.691 ± 0.06	18.191±0.003	19.946±0.006	32.712±0.0008	36.70±0.0004	43.351±0.0001	40.24±0.05
Std (ascorbic acid)	26.80±0.0002	35.79±0.0006	50.63±0.0004	60.74±0.0008	65.76±0.0002	67.02±0.0003	46.68±0.86

Table 3: Results of reducing power method

Extract	Concentration (mcg/ ml) and absorbance					
	50	100	150	200	250	300
($CHCl_3$) extract	0.0364±0.001	0.0503±0.004	0.0594±0.0007	0.0623±0.004	0.0880±0.0005	0.0983±0.00003
Std (ascorbic acid)	0.0710±0.0008	0.0858±0.001	0.0911±0.0005	0.1394±0.001	0.1893±0.06	0.2313±0.015

Table 4: Results of H_2O_2 scavenging activity of extracts

Extract	Concentration (mcg/ ml) and % inhibition						IC50 (mcg /ml)
	50	100	150	200	250	300	
($CHCl_3$) extract	18.06±0.005	28.26±0.0001	35.06±0.005	40.07±0.005	53.47±0.0009	68.59±0.0003	224.37±0.24
Std (ascorbic acid)	80.53±0.0001	85.39±0.004	91.27±0.0007	94.27±0.004	94.64±0.005	95.78±0.0007	278.99±0.0004

Table 5: *In-vitro* anti-bacterial and anti-fungal assay

Extracts Microbial Strain	Chloroform extract concentration 10mg/ml 20mg/ml Zone of inhibition in cm.	Standard 2.5mg/ml (chloramphenicol)
Bacteria		
E. Coli	15.5 27.0	33.25
Staphylococcus aureus	16.5 23.4	37.6
Bacillus subtilis	18.5 25.75	44.25
Proteus valgaris	9.75 12.25	22.25
Fungi		
Aspergillus niger	18.75 25.5	32.25
Candida albicans	17.82 21.43	31.2

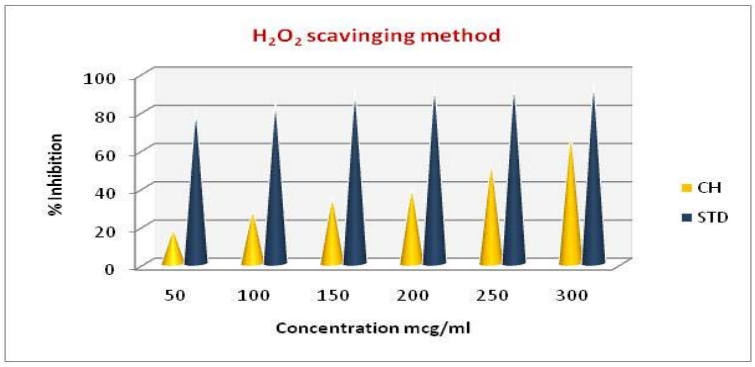

Figure 3: *In vitro* antioxidant activity of ($CHCl_3$) extract by H_2O_2 scavenging method

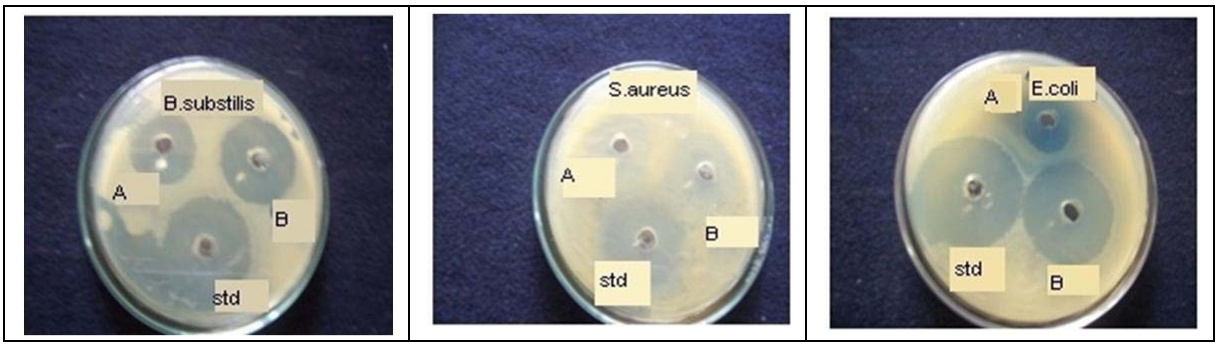

Figure 4: Photographs of zone of inhibition

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