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Research Article

EVALUATION OF ANTIOXIDANT AND ANTI BACTERIAL ACTIVITY OF POLYHERBAL FORMULATION *ALLIUM OPHIOSCORODON*

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ABSTRACT:

The active properties of poly herbal formulation Allium ophioscorodon contain not less than 0.2% of allicin which enhances the microbial and antioxidant activity. The allicin in raw garlic has been shown to kill 23 types of bacteria .At present study an attempt was done on Allium ophioscorodon to extract by hand power rotation by ethanol and separated by centrifugation at 3000 rpm and the filtrate was collected and stored at -4^{0}c, phytochemical tests, anti-bacterial activity by disc diffusion method, in vitro antioxidant activity by hydrogen peroxide scavenging method were done.

Keywords: *Allium ophioscorodon, disc diffusion method, hydrogen peroxide scavenging method.*

INTRODUCTION:

Allium ophioscorodon commonly called as garlic is among the oldest cultivated plant, which is used for therapeutic purposes. Garlic is the member of lilliaceae family which consist more than 250 genera. These are tolerable to unfavorable conditions due to its bulb structure[1].The active ingredient of *Allium ophioscorodon* was allicin, obtained when fresh garlic is chopped or crushed[6].The enzyme allinase converts allin into allicin which is responsible for aroma of garlic. The allicin generated is unstable and quickly changes into a series of other sulfur-containing compounds such as diallyl disulfide the biological and medical functions are due to the presence of organo sulfur compounds.[2]

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There is a report of therapeutic effects such as Hypolipidemic, Anti atherosclerotic, Hypo glycemic,Anti-Coagulant[5], Antihypertensive[7], Antidote Anticancer and Chemo preventive activities[3].A wide range of Anti-microbial and Anti-oxidant properties[4] are also exhibited by garlic. Due to the presence of sulfur compound it has a property of anti-microbial agent [8]. Garlic is with an amazing list of healing properties The potential antioxidant properties of garlic is due to its phenolic flavanoids [9].it is a great source of vitamin B6 which is needed for a healthy immune system and regulates blood sugar as it enhances the level of insulin in the blood

Materials and methods:

Extraction of *Allium ophioscorodon* by general maceration method with a solvent like ethanol.

Procedure for Extraction of Garlic:

Garlic was soaked in 450ml of 95% ethanol.250gm sliced garlic pieces were crushed in blender for 1min. Garlic paste was

prepared and the juice was transferred into centrifuge tubes for centrifugation at 3000 rpm, the supernatant was stored at -4^0 C.

Phytochemical screenings of garlic extract [10]:

Phytochemical screening was done in order to detect the presence of bioactive Constituents such as alkaloids, tannins, saponins, phenols, glycosides, flavanoids using the methods described by sofowora(1978),Trease and Evans (1989).

Test for Saponins:

2ml of the aqueous and ethanolic extract in a test tube was shaken for two minutes. On vigorus shaking frothing will be persisted it is taken as evidence for the presence of saponins.

Test for alkaloids:

3ml of the ethanolic extract was stirred with 5ml of 1% Hcl on a steam bath for 20min.The solution obtained was cooled and filtered and few drops of Mayers reagent, picric acid were added to the filtrate. A cream precipitate indicates the presence of alkaloid.

Test for phenolics:

2 drops of 5% ferric chloride were added to 5ml of crude ethanolic extract in a test tube. A greenish precipitate was taken as a indication of phenolics.

Test for Tannins:

A volume of 1ml of freshly prepared 10% pottasium hydroxide was added to a volume of 1ml of ethanolic extracts. Presence of a dirty white precipitate was taken as a indication of tannins

Test for Steroids:

To a volume of 1ml of the extract, five drops of concentrate tetra-oxo-iso sulphate VI acid was added. Red coluration indicates the presence of steroids.

Test for Flavonoids:

To a volume of 3ml of the ethanolic extract add 1ml of 10% sodium hydroxide was added. Yellow coloration indicates the presence of flavonoids.

Test for Glycosides:

To a volume of 3ml of the ethanolic extract, 2ml of chloroform was added. Tetra-oxo-iso sulphate VI acid was carefully added to form a lower layer. A reddish brown colour at interface indicates the presence of glycosides.

Anti-Bacterial Activity:[11]

The following conditions must be accomplished for the determination of proper antibacterial activity:

There should be intimate contact between the test organism and substance to be evaluated.
Micro-organism should be provided with the required condition for growth.
Measurement of activity should be done correctly.
Aseptic environment should be maintained.
Condition should be maintained unchanged throughout the study.
Various methods with their own advantages and limitations have been used from time to evaluate the microbial activity of the drug. The anti-bacterial activity can be evaluated by the following technique [12]

Agar streak dilution method.

Serial dilution method.

Agar diffusion method.

- Cup plate method
- Cylinder method
- Paper disc method
- Turbid metric method.

Study of Anti-bacterial activity:

Strains can be used are:

- *Staphylococcus aureus* (Gram positive)
- *Micrococcus variance* (Gram negative)

Method: Disc diffusion method [13]

Dilution of the compounds: 0.1,0.2,0.3,0.4,0.5µg/ml

Composition of the Media:

Bacterial medium: Nutrient Broth Medium was prepared by adding Peptone-5gm, Beef Extract-3gm, Sodium chloride-5gm, Agar-20gm, Distilled water up to 1000ml

Preparation of standard as Ciprofloxacin

Ciprofloxacin tablet 150mg was collected and then calculated their equivalent by following formula:

Equivalent of tablet

$$= \frac{\text{weight of drug taken}}{\text{label claim weight}} \times \text{average}$$

= 100/150 x 0.169

=0.112gm powder.

- 0.112gm ciprofloxacin tablet powder was weighed.
- Diluted to 10ml with distilled water.

Preparation of stock solution of garlic extract:

1ml of garlic extract was measured and then diluted to 10ml with distilled water.

Preparation of garlic dilution:

Dilution of concentration 0.1 to 0.5 µg/ml is prepared using distilled water.

Disc Diffusion Method: [13]

The *in vitro* antibacterial activities of the test samples (garlic extract) against are carried out by disc diffusion method against the standard ciprofloxacin concentration (0.1 to 0.5) .In the disc diffusion method, nutrient agar was used as culture media and the discs were placed aseptically over the bacterial culture on nutrient agar plates and incubated at 37 ^{0}c for 24hrs. After incubation plates are observed for development of zone of inhibition around the disc.

Antioxidant studies:[14]

The materials used were hydrogen peroxide, phosphate buffer. The solvents and the other chemicals were of analytical grade.

Instruments:

Absorbance was measured in UV-Visible spectrophotometer. P^H of buffer was measured in P^H meter.

Preparation of phosphate buffer:

Dissolve 2.38gms of disodium hydrogen phosphate, 0.19gm of potassium dihydrogen phosphate and 8.0gm of sodium chloride in sufficient water to produce 1000ml adjust the P^H if necessary.

Antioxidant activity by hydrogen peroxide scavenging method:

0.6 ml of hydrogen peroxide prepared solution is added to test tube with 1 ml of different concentration of extract 0.1, 0.2, 0.3, 0.4µg/ml. Test tube are incubated for 10 min . Absorbance of above solution is read at 230 nm against blank. Ascorbic acid is used as standard Hydrogen peroxide scavenging activity is then calculated using equation;

$$\frac{\text{Absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100$$

RESULTS AND DISCUSSION:

Table no: 01 phytochemical constituents present in Allium ophioscorodon

Phytochemical - constituents	Extract
Alkaloids	++
Glycosides	++
Flavonoids	++
Tannins	--
Steroids and terpenoids	++
Carbohydrates	++
Proteins	++
Fixed oils, fats & waxes	++

(++) Presence of phytochemical constituents in particular extract

(--) Absence of phytochemical constituents in particular extract

Anti-bacterial activity:

The extract is evaluated for antibacterial activity by using Gram positive and Gram negative bacteria against the standard ciprofloxacin. Compared to standard, ethanolic extract of garlic has shown activity on micrococcus Variance, while no effect on Staphylococcus Aureus.

Table No: 02 Result for standard drug and ethanolic extract of garlic

concentration µg/ml	Ciprofloxacin (std)		Ethanolic extract of garlic	
	Staphylococcus Aureus	*Micrococouus Variance*	*Staphylococcus Aureus*	*Micrococouus Variance*
0.1	+	+	+	_
0.2	_	+	+	+
0.3	_	+	+	+
0.4	_	+	+	+
0.5	_	+	+	+

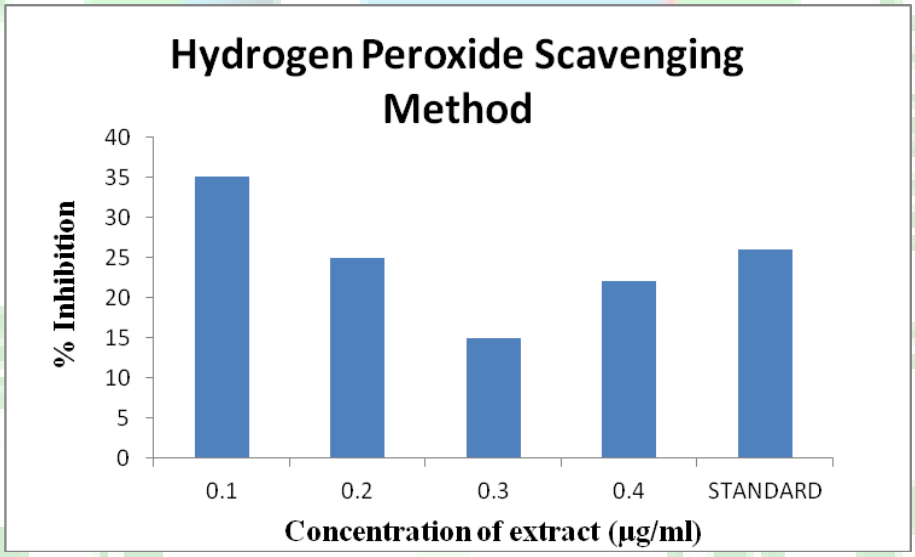
(+) indicates presences of growth, (-) indicates absences of growth

Anti-oxidant activity:

The extract of garlic was evaluated *in vitro* for anti-oxidant activity using Hydrogen peroxide scavenging method and Ascorbic acid is as standard. The extract at lower concentration 0.1 µg/ml had shown greater scavenging activity compared to the standard. Absorbance of control was found to be 0.876.

Table No: 03 Percentage Inhibition of Hydrogen peroxide scavenging method

Concentration(µg/Ml)	Garlic Extract(Abs)	Percentage Inhibition
0.1	0.568	35
0.2	0.654	25
0.3	0.744	15
0.4	0.856	22
Standard ascorbic acid	3.057	26



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