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BIOLOGICAL EVALUATION OF LEAF AND BULB EXTRACT OF *ALLIUM CEPA VAR. AGGREGATUM*

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Abstract: The present study was aimed to biological evaluation of leaf and bulb extract of Allium cepa Var. aggregatum. Shallot (Allium cepa var. aggregatum), a variety species of onion which is a well-known traditional nutraceutical and medicinal plant that is cultured and used all around the world. It is also called by small onion, potato onion, underground onion, shallot, multiplier onion, nesting onion, ever-ready onion. The bulb and leaf both the part of plant shows medicinal use. The leaf and bulb extract of plant shows significant effect on anti-inflammatory and antioxidant activity. Apart from this, it is also used for diabetes, wound healing, cytotoxic activity, anti-parasitic, cardio-protective, anti-microbial, anti-pyretic, insecticidal and hepato-protective. It also rich in flavanoid, phenolic compound, tannins and polysaccharides which responsible for various therapeutic activity. The anti-inflammatory and antioxidant activity have been performed with bulb and leaf extract of the plant with aqueous solvent (Environment friendly) which showed good activity compared to standard.

Keywords: Allium cepa var. aggregatum, leaf and bulb extract.

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INTRODUCTION

Nature, the master of craftsman of molecules created almost an inexhaustible array of molecular entities. Natural products have been the backbone of traditional system of healing throughout the globe, and have also been an integral part of history and culture. Even though popularity of the synthetic products increased due to its production cost, time effectiveness, easy quality control, stringent regulation and quick effects, but their safety and efficacy was always remained questionable, resulting in the dependence on the natural products by more than 80% of the total population in the developing world. Until recently, plants were an important source of novel pharmacologically active compounds with many blockbuster drugs being derived directly or indirectly from plants[1].

The term "Natural products" is generally regarded as being synonymous with "secondary metabolite". Secondary metabolites have been the most successful sources of potential drug leads in new drug discovery. The secondary metabolites such as Alkaloids, Terpinoids, Flavanoids, Glycosides, Tannins, Carbohydrates etc., Produces wide ranges of pharmacological activity[2].

According to the World Health Organization (WHO), 80% of the people still rely on plant based traditional medicines for primary health care and 122 plant derived drugs were related to their original ethno-pharmacological purpose[1].

Shallot (Allium cepa L. var. aggregatum) is a close relative of onion (Allium cepa L. var. cepa) [3]. It is a well-known traditional nutraceutical and medicinal plant that is cultivated and used around the world [2]. Onion contain phenolics and flavanoids that have potential anti-inflammatory, anti-cholesterol, anti-cancer, anti-cancer, anti-diabetic, cardio-vascular property etc. It contain 89% water, 1.5% protein, vitamin B1, B2, and C, along with potassium and selenium. It also contain polysaccharides such as fructose, saccharose, peptides, flavanoid (mainly quercetin), and essential oil. Shallot contain numerous sulfur compounds including thiosulfinates and thiosulfonates: cepaenes, S-oxides, S-S dioxides, mono, di, and tri-sulfides and sulfoxides[4]. These compounds can act either directly as an antioxidant or indirectly by modulating the pro-apoptotic pathway or activating the endogenous antioxidant system [5]. Cyclooxygenase and lipoxygenase play an important role as inflammatory mediators. They are involved in the release of arachidonic acid, which is a starting point for a general inflammatory response. Selected phenolic compounds were shown to inhibit the both cyclooxygenase and lipoxygenase pathways, thus diminishing the formation of these inflammatory metabolites [6].

Based upon the chemical profile determined and to further explore new applications for the leaves and bulb while extending the traditional use, we investigated the leaves and bulb for their antioxidant and anti-inflammatory activity.

MATERIALS AND METHODS

Drugs and chemicals

All reagents procured were analytical grade.

Plant collection

Fresh plant specimen was collected from field of chinniyampalayam pudhur, near erode and authenticated by Dr. C. MURUGAN, Scientist D & Head office in charge, Botanical survey of India, southern regional center, TNAU campus, Coimbatore. (BSI/SRC/5/23/2018/Tech/2741). Voucher specimen has been deposited in the Department of Pharmaceutical Chemistry, SSM College of Pharmacy, Jambai, Tamilnadu, India.

The plant of *Allium cepa var. aggregatum* were washed and macerated then stored for further use.

Extraction

The onion bulb was washed with freshly prepared sterile distilled water. The outer covering of the bulb was manually peeled off and the fleshy part of the onion wash rewashed with freshly prepared sterile water. A part of 50.0 gm of the onion bulb was cut into small parts and squashed. The squashed preparation macerated at 50ml of water for 8hr with constant interval shaking. The extraction was filtered using muslin cloth and then Whatman no.1 filter paper. The filtrate was evaporated at 45°C to dryness and the dried substance was kept in sterile bottle under refrigerated condition until use.[7]

1. PRELIMINARY PHYTOCHEMICAL SCREENING TEST

The extracted of *Allium cepa var. aggregatum* was subjected to qualitative tests for the identification of various plant constituents.

1) TEST FOR ALKALOIDS [8]

• **Dragondroff's Test: -** 1 ml of the extract was added to 1 ml of dragondroff's reagent (potassium bismuth iodide solution). An orange, red precipitate indicates the presence of alkaloids.

• **Mayer's Test: -** 1 ml of the extract was added to 1 ml of Mayer's reagent (potassium mercuric iodide solution). Whitish yellow colored precipitate indicates the presence of alkaloids.

• **Hager's Test: -** 1 ml of the extract was added to 3 ml of Hager's reagent (saturated aqueous solution of picric acid), yellow colored precipitate indicates the presence of alkaloids.

• **Wagner's Test: -** 1 ml of the extract was added to 2 ml of Wagner's reagent (Iodide in potassium Iodide), formation of reddish brown precipitate indicates the presence of alkaloids.

• **Tannic acid Test: -** 1 ml of the extract was added to 1 ml of 10% tannic acid solution, buff colored indicates the presence of alkaloids.

2) TEST FOR SAPONINS [8]

• **Foam Test: -**The extract was diluted with 20 ml of distilled water and shaken in a graduated cylinder for 15 min lengthwise. A 1 cm layer of foam indicates the presence of Saponins.

• **Lead acetate Test: -** 1 ml of sample solution was treated with 1% lead acetate solution, formation of a white precipitate indicate the presence of saponins.

• **Hemolytic Test: -** The extract or dry powder was added one drops of blood placed on a glass slide. If hemolytic zone appears shows the presence of saponins.

3) TEST FOR GLYCOSIDES [8]

• **Legal's Test: -** Dissolved the extract in pyridine and added sodium nitroprusside solution to make it alkaline. The formation of pink, red to red color shows the presence of glycosides.

• **Baljet Test: -** 1 ml of the test extract was added to 1 ml of sodium picrate solution and the yellow to orange color shows the presence of glycosides.

• **Keller- killiani Test: -** The ethanol extract 0.5ml of strong solution of lead acetate was added and filtered. The filtrate is shaken with 5 ml of chloroform. The chloroform layer is separated in a porcelain dish and removes the solvent by gentle evaporation. Dissolve the cool residue in 3 ml of glacial acetic acid containing 2 drops of ferric chloride solution. Carefully transferred this solution to the surface of 2 ml of concentrated sulfuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening withstanding.

• **Borntrager's Test: -** Added a few ml of dilute sulphuric acid to 1 ml of the extract solution. Boiled, filtered and extracted the filtrate with chloroform the chloroform layer was treated with 1 ml of ammonia. The formation of the red color of the ammoniacal layer shows the presence of anthraquinone glycosides.

4) TEST FOR CARBOHYDRATES AND SUGARS [9]

• **Molisch's Test: -** 2 ml of the extract was added with 1 ml of α- napthol solution was added and also added concentrated sulphuric acid through the side of the test tube. Reddish violet color at the junction of the two liquids indicates the presence of carbohydrates.

• **Fehling's Test :-** 1 ml of the extract was added with equal quantities of Fehling solution A and B were added, upon heating formation of a brick red precipitate indicates the presence of reducing sugars.

• **Benedict's test: -** 1 ml of extract was added to 5 ml of Benedict's reagent, was added and boiled for 2 min. and cool. Formation of a red precipitate shows the presence of sugars.

• **Tollen's Test:-**1 ml of extract was added with 2 ml of tollen's reagent was added and boiled. A silver mirror is obtained inside the wall of the tube which indicates the presence of aldose sugar.

• **Seliwanoff's Test: -** The extract was treated with hydrochloric acid and resorcinol and heated. Formation of red color shows the presence of glucose.

• **Bromine water Test:-**The little quantity of test extract, bromine water was added. Bromine water decolorization indicates the presence of aldose sugar.

5) TEST FOR TANNINS [9]

• **Gelatin Test: -** 1 ml of extract was added with 1% gelatin solution containing 10% sodium chloride. Formation of white precipitate indicates the presence of tannins.

• **Ferric chloride Test: -**1 ml of extract was added to 1ml ferric chloride solution, formation of dark blue or greenish black product shows the presence of tannins.

• **Vanillin hydrochloride Test: -**1 ml of extract was added with vanillin hydrochloride. Formation of purplish red color indicates the presence of tannins.

• **Lead acetate Test:** - Taken a little quantity of test solution was taken and mixed with a basic lead acetate solution. Formation of white precipitate indicates the presence of tannins.

• P**otassium dichromate Test:** - The sample solution was treated with 1ml of 10% Potassium dichromate solutions give yellowish brown precipitate indicates the presence of tannins.

6) TEST FOR FLAVONOIDS [8]

• **Shinoda's Test: -** The extract solution, a few fragments of magnesium ribbon were added and add concentrated HCL drop wise gives cherry red color appears after a few minutes, shows the presence of Flavonoids.

• **Alkaline reagent Test: -** The extract was treated with sodium hydroxide; formation of yellow color indicates the presence of Flavonoids. Less quantity of the extract was treated with lead acetate, a yellow color solution formed, disappears on addition of an acid indicates the presence of Flavonoids.

• The extract was treated with concentrated sulphuric acid, formation of yellow or orange color indicates the presence of flavonoids.

7) TEST FOR STEROIDS [9]

• **Libermann- Burchard's Test:-** 2 ml of extract was added with chloroform solution, 1-2 ml of acetic anhydride and 2 drops of concentrated sulphuric acid was added along the sides of the test tube. The appearance of bluish-green color shows the presence of steroids.

• **Salkowsky's Test:-**Dissolve the extract in chloroform solution, 2 ml conc. sulphuric acid was added. If the chloroform layer appears red color indicate the presence of steroids.

8) TEST FOR PROTEINS AND AMINO ACIDS [10]

• **Biuret Test: -** 1 ml of the extract was treated with 4% NaOH and a few drops of CuSO4 solution, Formation of purple violet color indicate the presence of proteins.

• **Ninhydrin Test: -** 1 ml of the extract was treated with 3 drops of 5% Ninhydrine solutions in boiling water bath for 10 min; formation of purplish or bluish color appearance indicates the presence of proteins, peptides or amino acid.

• **Xanthoproteic Test: -** 1 ml of the extract was treated with 1 ml of concentrated nitric acid. A white precipitate formed, it was boiled and cooled. Then 20% of sodium hydroxide or ammonia is added. Orange color indicates the presence of amino acids.

• **Millon's Test: -** 1 ml of the extract was treated with millions reagents (mercuric nitrate in HNO3) white precipitate turns to brick red indicates the presence of proteins.

9) TEST FOR TRITERPENOIDS [10]

• **Knoller's Test: -** Dissolved 2 or 3 granules of tin metal in 2 ml thionyl chloride solution. Then added 1 ml of the extract into the test tube and warm, the formation of pink color indicates the presence of Triterpenoids.

10) TEST FOR FIXED OILS AND FATS [10]

• **Spot Test: -** Pressed a small quantity of extract between two filter papers, the stain on the filter paper indicates the presence of fixed oils.

• **Saponification Test: -** Added a few drops of 0.5 N of alcoholic potassium hydroxide to the small quantity of various extracts along with a drop of phenolphthalein separately and heat on a water bath for 1 to 2 hrs. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

11) TEST FOR GUMS AND MUCILAGE [11]

10 ml of ethanol extract was slowly added 25ml of absolute alcohol with constant stirring filter the precipitate and dried in air. The precipitate for its swelling property indicates the presence of carbohydrates.

2. Determination of *in vitro* anti-inflammatory activity

Effect of protein denaturation

Requirements:

- Herbal plant extract
- Indomethacin
- Bovine albumin
- UV-Visible Spectrophotometer
- Petroleum ether
- Distilled water
- Test tubes

Preparation:

Test solution (1ml) containing concentrations of herbal plant extracts (10µ-50µg) or indomethacin (10µ-50µg) was mixed with 1ml of bovine albumin solution (1mM) and incubated at 27± 1°C for 15min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10min. After cooling, the turbidity was measured by spectrophotometer, at 517nm. Percentage inhibition of denaturation was calculated from the control, where no drug was added.

3. Determination of *in vitro* antioxidant activity

Nitrous oxide method:

Scavenging of Nitric oxide Radical:

Nitric oxide was generated from sodium nitroprusside and measured by Griess' reaction (Green LC). Sodium nitroprusside (5 mM) in standard phosphate buffer saline solution (0.025 M, pH 7.4) was incubated with different concentrations (100-500 µg/mL) of the test extract dissolved in phosphate buffer saline (0.025 M, pH 7.4) and the tubes were incubated at 25°C for 5 h. Control experiments were conducted in the identical manner using the equivalent amounts of buffer. After 5 h, 0.5 mL of the sample was diluted with 0.5 mL of Griess' reagent (1% sulphanilamide, 2 % O-phosphoric acid and 0.1 % napthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with suphanilamide and its subsequent coupling with napthyl ethylene diamine was read at 546 nm. The experiments were repeated in triplicate. The percentage of sodium nitro prusside scavenging is calculated as follows:

% Scavenged = (A0 – A1 / A0) X100

Where; A0 is the absorbance of control,

A1 is the absorbance of test.

Ascorbic acid was used as a positive control.

RESULT

1. Phytochemical screening of extract

Table - 1

CONSTITUENT	OBSERVATION	
	LEAF	BULB
Flavonoids	+	+
Shinoda Test\ Alkaline test NH4OH test Zn test	+	+
	+	+
Glycosides	+	+
Keller Killani test Modified Borntragers test	-	-
Steroids	+	+
Salwoski test Libberman test	-	-
Protein	-	-
Biuret test Million test		
Xanthoprotein test	+	+
	-	-

"+" indicates Presence

"-" indicates Absence

2. Determination of anti-inflammatory activity

TABLE- 2

Absorbance of control – 0.15

TEST SAMPLE	CONCENTRATION (mg/ml)	ABSORBANCE	PERCENTAGE OF INHIBITION
L1	0.1	0.090	**40 %**
L2	0.2	0.072	**52%**
L3	0. 3	0.052	**60.6%**
L4	0.4	0.083	**78%**
L5	0.5	0.028	**81.3%**
STD	**1**	**0.015**	**90%**

L - LEAF **STD –** STANDARD

TABLE - 3

Absorbance of control – 0.15

TEST SAMPLE	CONCENTRATION (mg/ml)	ABSORBANCE	PERCENTAGE OF INHIBITION
B1	0.1	0.019	**73.3 %**
B2	0.2	0.023	**76%**
B3	0. 3	0.032	**78.6%**
B4	0.4	0.036	**84.6%**
B5	0.5	0.040	**87.3 %**
STD	**1**	**0.015**	**90%**

B- BULB **STD -**STANDARD

Figure - 1

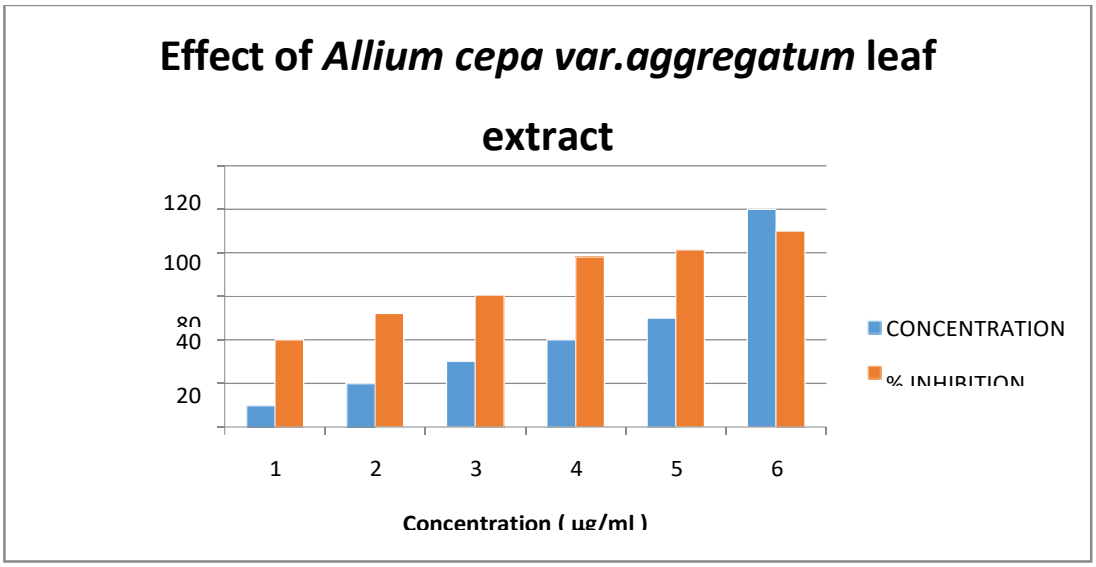


Figure - 2

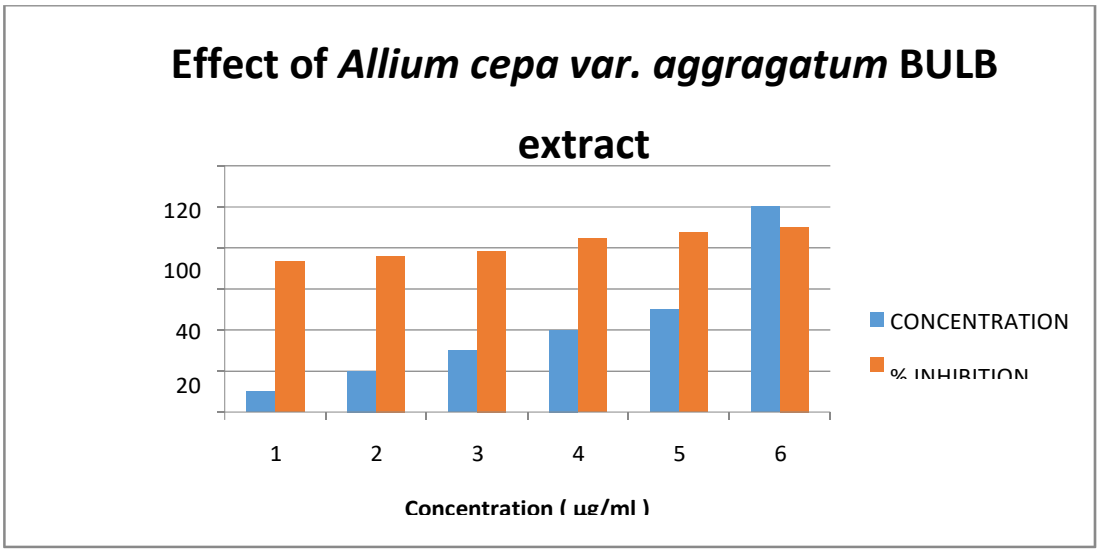


TABLE- 4

Absorbance of control – 1.02

TEST SAMPLE	CONCENTRATION (mg/ml)	ABSORBANCE	PERCENTAGE OF INHIBITION
L1	0.1	0.767	**24.8 %**
L2	0.2	0.737	**27.7%**
L3	0. 3	0.620	**39.2%**
L4	0.4	0.510	**50%**
L5	0.5	0.420	**58.8 %**
STD	**1**	**0.220**	**78.4%**

L- LEAF **STD –**STANDARD

TABLE - 5

Absorbance of control – 1.02

TEST SAMPLE	CONCENTRATION (mg/ml)	ABSORBANCE	PERCENTAGE OF INHIBITION
B1	0.1	0.72	**29.4 %**
B2	0.2	0.69	**32.3%**
B3	0. 3	0.58	**43.1%**
B4	0.4	0.44	**58%**
B5	0.5	0.39	**61.7 %**
STD	**1**	**0.22**	**78.4%**

B- BULB **STD** -STANDARD

Figure - 3

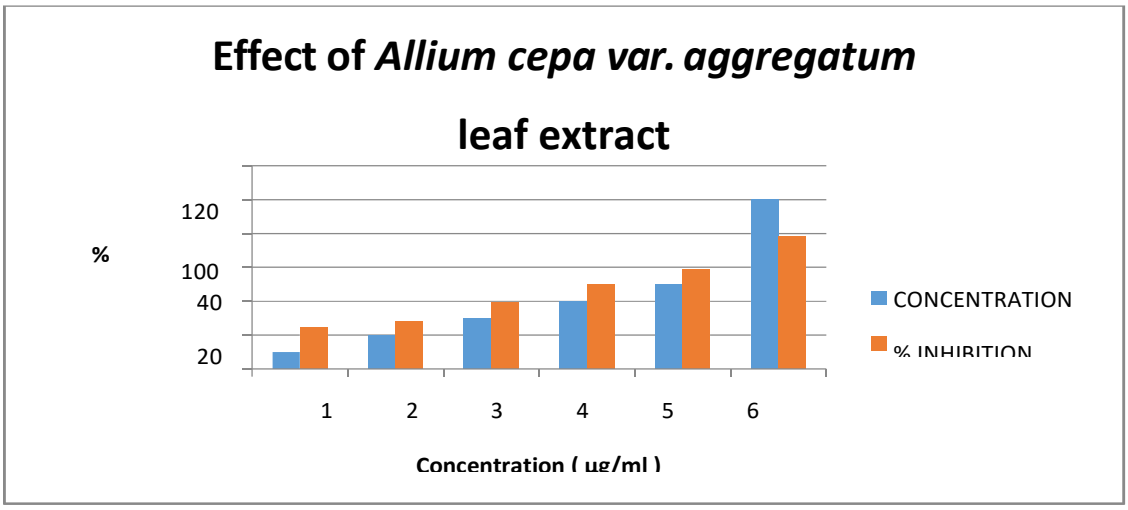
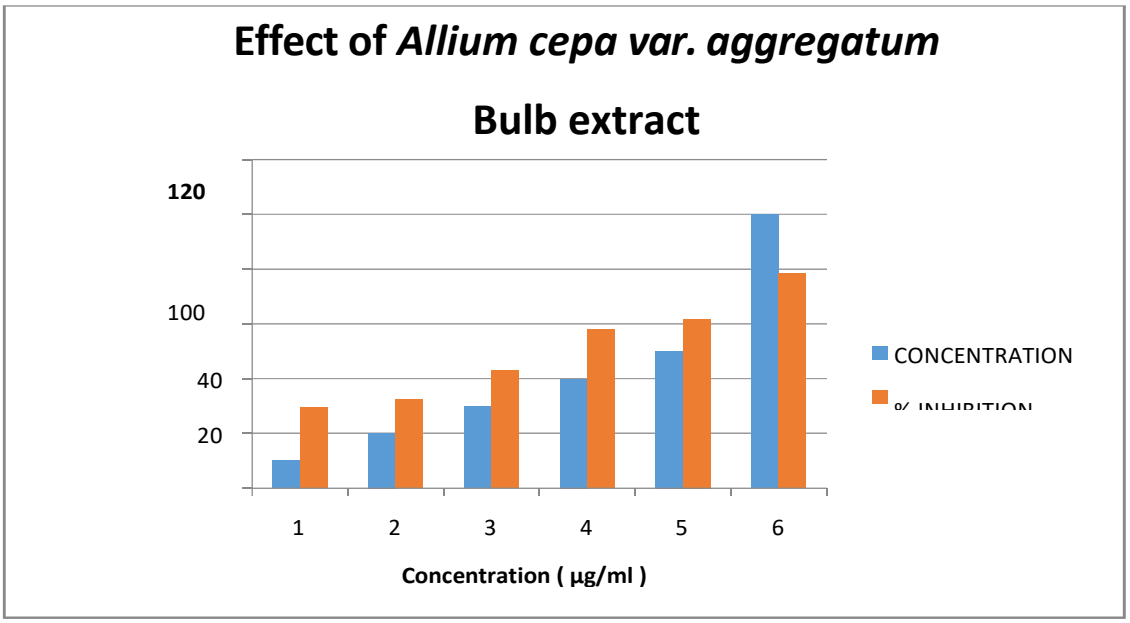


Figure - 4



DISCUSSION

EXTRACTION

The aqueous extract of shallot (leaf & bulb) were prepared by simple maceration using equal parts of water. Further the extraction were subjected to phytochemical screening.

Pharmacological evaluation

In vitro Anti-inflammatory activity

In vitro anti-inflammatory activity of the extracted compounds were evaluated by protein denaturation method. When compared to the standard (Indomethacin) both extraction at different concentration showed good activity.

In vitro Anti-oxidant activity

In vitro Anti-oxidant activity (nitric oxide radical scavenging) the extracted compounds were evaluated by nitrous oxide method. When compared to the standard (Ascorbic acid) both the extraction at different concentration showed moderate activity.

CONCLUSION

The aqueous extract of shallot were evaluated for their *invitro* pharmacological activity at different concentration. This present study reveals that the extracted compounds shows moderate anti-oxidant activity & good anti-inflammatory activity. The future plan of work is to do the *invivo* pharmacological evaluation to find out the potent natural drug for the above said activity.

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