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Evaluation of an Invitro Anticoagulant and Antioxidant Activity of Allium Sativum Rhizome Extract

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ABSTRACT

The study evaluates the anticoagulant and antioxidant activity by Allium sativum rhizome extract.Allium sativum Rhizome extract taken different samples and concentrate, set the different values of coagulation time in minutes.when the compared to standard drug heparin,test compound also having anticoagulant activity.Allium sativum Rhizome extract prevents coagulation upto 70mins and heparin is natural anticoagulant is prevent or reduce the coagulation.So the study is concluded as the Allium sativum extract has anticongulant property.In addition to that, the Allium sativum extract with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid at different concentrations,find out the test sample percentage inhibition of free radical scavenging action compared with standard.Hence the Allium sativum rhizome extract has anti oxidant activity.

Key words: Allium sativum,Rhizome,prothrombin,coagulation time,DPPH.

I.INTRODUCTION

Allium sativum belongs to Lilliaceae family and is described as perennial, erect, bulbous herb up to 70cm tall, with strong smelling odor when crushed. The main biological active constituents are allicin (diallylthiosulfate) and thiosulfonates that greatly participates in its great potential in inhibiting plate aggregation and enhancing thrombolytic activity.Studies have proven that garlic has a fibrinolytic activity.This significantly found property of garlic led to further developments using garlic in drug treatment of thromboembolic disorder.Studiesof garlic skins (peels) extract showed strong antioxidant activity, and some responsible constituents were isolated and identified. It has been used as herbal medicine, but there was no report on the health benefits of the skin or peel. Garlic (Allium sativum) is a species of bulbous flowering plant in the genus Allium. Its close relatives include the onion, shallot, leek, chive, Welsh onion and Chinese onion. It is native to South Asia, Central Asia and northeastern Iran and has long been used as a seasoning worldwide, with a history of several thousand years of human consumption and use. It was known to ancient Egyptians and has been used as both a food flavoring and a traditional medicine[1-5].

SCIENTIFIC CLASSIFICATION:

Genus:Allium
Subfamily:Allioideae
Family:Amaryllidaceae
Order:Asparagales
Clade:Monocots
Clade:Angiosperms
Clade:Tracheophytes

II.AIM AND OBJECTIVES

Aim:

To evaluate the anticoagulant and antioxidant activity by Allium sativum rhizome extract.

Objectives:

- It has fibrinolytic action and anti platelet function.
- Allium sativum rhizome extract produced the Anticoagulant activity.
- The Allium sativum rhizome extract produced the Antioxidant activity.
- It has anti hyperlipidemic property.

III.MATERIALS AND METHODS

Several materials used for Invitro anticoagulant and anti oxidant activities.

Drugs and Chemicals used for the activity:

For anticoagulant activity:

Garlic cloves-5g, Saline solution-2ml**,** Calcium chloride-2ml, Sodium citrate-2ml**,** Coagulation Test Kit**,** Heparin-2ml

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For antioxidant activity:
Garlic juice-1ml,Methanol-1ml,2,2 Diphenyl-1-picryl hydrazyl(DPPH)-24mg,Test tubes-5 Spectrophotometer,Cuvettes,Pipettes.

Methods:
Plant collection/Allium sativum rhizome collection:
From a retail store,Falaknuma,Hyderabad.

Preparation of Rhizome extract:
• Crush 5 grams of fresh garlic cloves using a morter and pestle.
•Add 10ml of distilled water to the crushed garlic and stir the mixture.
•Filter the garlic extract using filter paper to obtain a clear solution.
•2ml of garlic extract is taken in each test tubey[6-8].

Methanolic extract of Allium sativum Rhizome:
•Take some garlic cloves and crush them to extract the juice.
•Add 1ml of methanol to garlic juice to make a 1:1(v/v) mixture.

Calculation for Allium sativum extraction:
5gm (5000mg) of Allium sativum(garlic)diluted in 1000ml of distilled water and make upto 1000ml.
1000ml(dis.water)=5000mg(Allium sativum)
Each ml contains=5mg

IV.METHODOLOGY

Evaluation of InvitroAnti Coagulant Activity
Procedure:
Crush 5 grams of fresh garlic cloves using a morter and pestle.Add 10ml of distilled water to the crushed garlic and stir the mixture.Filter the garlic extract using filter paper to obtain a clear solution.Take four test tube and 2ml of garlic extract to each test tube.To the first test tube,add 2ml of saline solution.To the second test tube,add 2ml of calcium chloride.To the third test tube,add 2ml of sodium citrate.To fourth test tube,add 2ml of coagulation test kit.Mix each test tube well.Incubate all test tube at 37°C for 30mins.After 30mins,add 1ml of blood to each test tube.Mix each test tube well and incubate them again at 37°C for 5mins.After 5mins,observe the clotting of blood in each test tube.Compare the clotting times of each test tube with the control of determine the anticoagulant activity of Allium sativum.The control in this experiment is a test tube containing only blood and coagulant reagent from the coagulantion test kit.The clotting time of control is used as reference for clotting times of the other test tube[9-12].

Procedure(2):
•Collect blood samples from study subjects using blood collection tubes according to standard phlebotomy procedures.
•Centrifuge the blood samples to obtain plasma.
•Prepare a series of dilutions of Allium sativum extract in an appropriate solvent.
•Add a small volume of each Allium sativum extract dilution to plasma samples, leaving one Sample without extract as a control.
•Incubate each sample at 37°C for 2-3 minutes.
•Add the reagents for PT assay (e.g., thromboplastin and calcium chloride) to each sample according to the manufacturer's instructions.
•Start the coagulation analyzer to measure the clotting time for each sample.
•Record the clotting time for each sample.
•Plot the clotting time versus the concentration of Allium sativum extract.
•Analyze the data to determine the anticoagulant activity of Allium sativum extract,such as the IC50 value (the concentration of Allium sativum extract that inhibits clotting by 50%).
•It is important to note that this procedure can be modified to measure the anticoagulant activity of Allium sativum using other laboratory tests such as activated partial thromboplastin time (aPTT)assay or thrombin time (TT) assay. Additionally, this procedure should be performed by trained professionals using appropriate safety precautions and controls[13-16].

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Anti coagualant activity of Allium sativum rhizome extract

Evaluation of Anti Oxidant Activity from Allium sativm Clove Extract

Procedure:

•Take some garlic cloves and crush them to extract the juice.

•Add methanol to garlic juice to make a 1:1(v/v)mixture.

•Prepare a stock solution of DPPH by dissolving 24mg of DPPH in 100ml of methanol.

•Take 1ml of the garlic-methanol mixture and mix it with 2ml of the DPPH stock solution in a test tube.

•Mix the solution thoroughly and keep it in dark for 30mins.

•After 30mins measure the absorbance of the solution at 517nm using a spectrophotometer.

•Repeat steps 4-6 with different concentrations of ascorbic acid.

•Prepare a calibration curve using different concentration of ascorbic acid[17-20].

•Calculate the percentage of inhibition of DPPH by using the following formula;

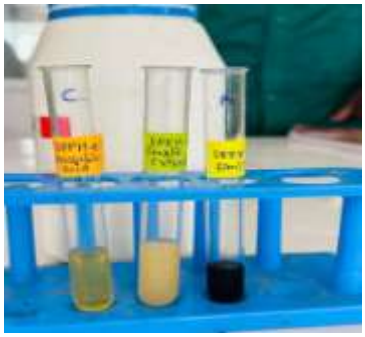
% inhibition = [(A_blank - A_sample) /A_blank]× 100

Where A blank is the absorbance of the DPPH solution without any sample ,and A-sample isabsorbance of the DPPH solution with the garlic methanol mixture.

•Plot the percentage of inhibition against the concentration of garlic methanol mixture and

•Calculation the IC50 value (concentration at which 50% inhibition of DPPH occurs) usimg the calibration curve.

•The lower the IC50 value the higher the antioxidant activity of Allium Sativum.



DPPH free radical scavenging activity

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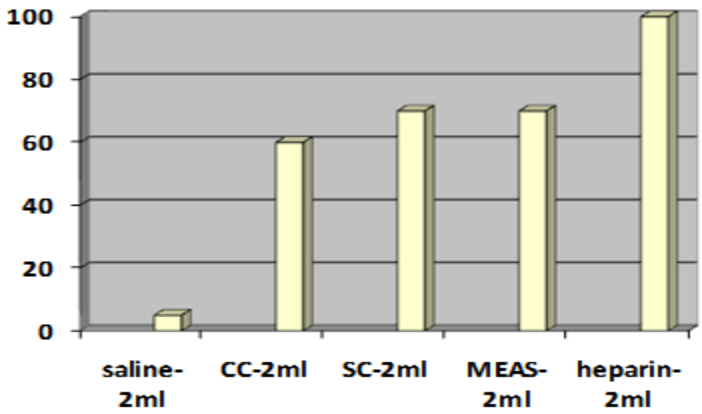
Samples of anti oxidant activity

V.RESULTS AND DISCUSSION

Table: 1 Anti coagulant activity

S.NO	Sample	Concentration in ml	Amount of blood plasma	Time of coagulation in minutes
1.	Saline solution	2ml	1ml	5mins
2.	Calcium chloride	2ml	1ml	60mins
3.	Sodium citrate	2ml	1ml	70mins
4.	Aqueous extract of Allium sativum Rhizome	2ml (5mg/ml)	1ml	70mims
5.	Heparin	2ml (80 IU)	1ml	100mins



Graph1:anti coagulant activity of A.sativum rhizome extract and standard Heparin

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• Saline-2ml(5mins),Calcium chloride-2ml(60mins),Sodium citrate-2ml(70mins),Aqueous extract of Allium sativum Rhizome-2ml(70mins),Heparin-2ml(100mins).

Evaluation of anti oxidant activity:

Table: 1 DPPH samples only

S.NO	Sample	Concentration in (100µg/ml)	Absorbance
1.	**DPPH(0.1mg) + Methanol (100µg/ml)**	200µg/ml	0.070
2.		300µg/ml	0.190
3.		400µg/ml	0.330
4.		500µg/ml	0.410

Table:2DPPH+ascorbic acid(standard)

S.NO	Sample (standard)	Concentration in µg/ml	Absorbance	Percentage(%) inhibition
1.	**DPPH (2ml) + Ascorbic acid (standard)**	100 µg/ml	0.8115	18.85%
2.		200 µg/ml	0.6877	31.23%
3.		300 µg/ml	0.592	40.80%
4.		400 µg/ml	0.425	57.5%

Table:3DPPH + Allium sativum Rhizome extract(Test sample)

S.NO	Sample (Test solution)	Concentration in mg/ml	Absorbance	Percentage(%) Inhibition
1.	**DPPH+ Allium sativum(Rhizome)**	5mg	0.712	28.8%
2.		10mg	0.60	40%
3.		15mg	0.42	58%

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4.		20mg	0.31	69%

Calculation:[DPPH with Ascorbic acid(standard)]

1. [DPPH(2ml) and Sample (100µg/ml)]

% inhibition = [(A_control - A_sample) /A_control]× 100

=1.000-0.8115/1.000×100

= 0.1885/1.000×100

= 0.1885×100

= 18.85%

2.[DPPH(2ml) and Sample(300µg/ml)]

% inhibition = [(A_control - A_sample) /A_control]× 100

= 1.000-0.6877/1.000×100

= 0.3123/1.000×100

= 0.3123×100

=31.23%

3. [DPPH(2ml) and Sample(400µg/ml)]

% inhibition = [(A_control - A_sample) /A_control]× 100

= 1.000-0.592/1.000×100

= 0.408×100

= 40.80%

4. [DPPH(2ml) and Sample(500µg/ml)]

% inhibition = [(A_control - A_sample) /A_control]× 100

=1.000-0.425 /1.000×100

= 0.575/1.000×100

= 57.5%

Calculation:[DPPH with Allium sativum extract(Test sample)]

1. [DPPH(2ml) and Sample (5mg/ml)]

% inhibition = [(A_control - A_sample) /A_control]× 100

= 1.000-0.712/1.000×100

= 0.288/1.000×100

= 28.8%

2. [DPPH(2ml) and Sample(10mg/ml)]

% inhibition = [(A_control - A_sample) /A_control]× 100

= 1.000-0.60/1.000×100

= 0.4×100

= 40%

3. [DPPH(2ml) and Sample(15mg/ml)]

% inhibition = [(A_control - A_sample) /A_control]× 100

= 1.000-0.42/1.000×100

= 0.58×100

= 58%

4. [DPPH(2ml) and Sample(20mg/ml)]

% inhibition = [(A_control - A_sample) /A_control]× 100

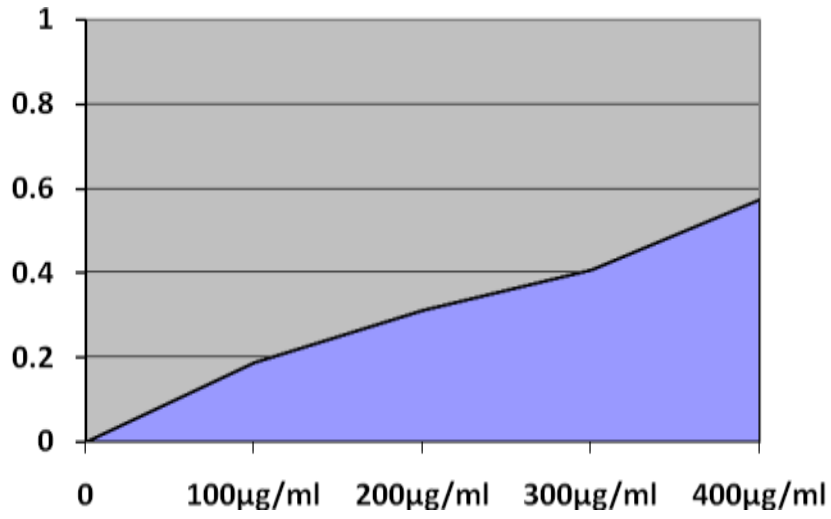
= 1.000-0.31/1.000×100

= 0.69×100

= 69%

Graph2 :

1.[DPPH with Ascorbic acid(standard)]



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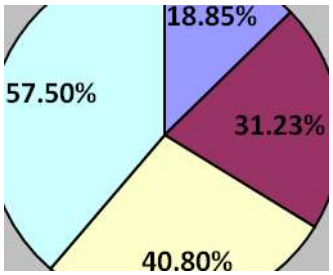
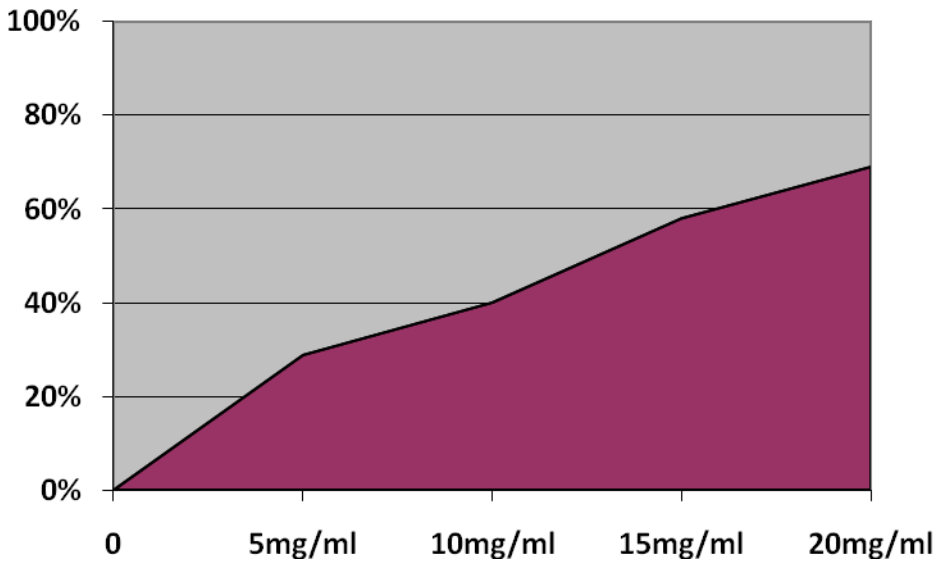
Concentration in µg/ml

Pie chart:

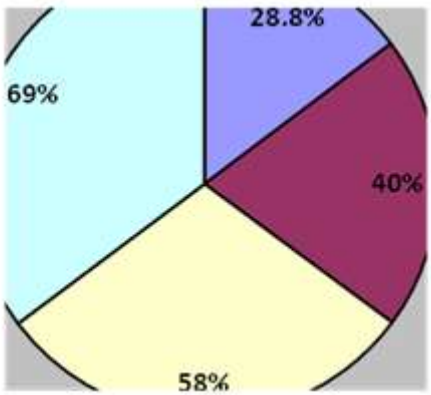


2.[DPPH with Allium sativum extract(Test sample)]



Concentration in mg/ml

Pie chart:



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Discussion:

Table: DPPH+ascorbic acid(standard)

S.NO	Sample (standard)	Concentration in µg/ml	Absorbance	Percentage(%) inhibition
1.	**DPPH (2ml) + Ascorbic acid (standard)**	100 µg/ml	0.8115	18.85%
2.		200 µg/ml	0.6877	31.23%
3.		300 µg/ml	0.592	40.80%
4.		400 µg/ml	0.425	57.5%

Table: DPPH + Allium sativum Rhizome extract(Test sample)

S.NO	Sample (Test solution)	Concentration in mg/ml	Absorbance	Percentage(%) Inhibition
1.	**DPPH+ Allium sativum(Rhizome)**	5mg	0.712	28.8%
2.		10mg	0.60	40%
3.		15mg	0.42	58%
4.		20mg	0.31	69%

Anti coagulant activity:

The study was carried out to evaluate the effect of Garlic (Allium sativum)as an anticoagulant in blood samples of normal individuals by using principles of coagulation time.Several materials used for intro anticoagulant activity which were collected from different places and kept under suitable temperature until used in experiment.Materials such as Allium sativum Rhizome (Garlic clove),saline solution,calcium chloride,sodium citrate and heparin. After collecting the materials the procedure for the experiment of anti coagulant were taken under consideration,5grams of fresh Allium sativum(garlic clove) taken and crushed by using a equipment known as motor and pestle.Add 10ml of distilled water to the crushed Garlic and stir the mixture.Then the mixture is filtered by using a filter paper to get a clear solution,hence the clear solution is the Allium sativum Rhizome extract. The following extraction is used 2ml in each test tube and 4 test tube are taken.The saline solution is added to the test tube containing Allium sativum extraction.2ml of saline solution is added.The 2ml of calcium chloride is added to second test tube containing Allium sativum.

The 2ml of sodium citrate is added to third test tube containing Allium sativum.

- In fourth test tube we are adding 2ml of coagulation test kit(Heparin).
- All of this test tube should mixed well after mixing this test tube should be incubated for 30mins under 37°C.
- After 30mins of incubation now adding 1ml of blood[normal human individuals with normal prothrombin time (14.6±0.7 seconds) were randomly selected to participate in blood donation for experiment. The participants were either sex was selected for studies. Their average age is 21 ±2SD year] to each test tube.
- Mix each of the test tubes well and incubate these test tubes again at 37°C for 5mins.
- After 5mins, observe the clotting of blood in each test tube After that compare the clotting time of each test tube with the control of determination the anticoagulant activity of Allium sativum.
- after comparing the test tubes the values for blood to get clot in each test tube are saline solution clotting time is 5mins, calcium chloride clotting time is 60mins, sodium citrate clotting time is 70mins,heparin clotting time is 100mins and only the aqueous extract of Allium sativumclotting time is 70mins.
- the graph plotted by percentage of coagulation with the concentration.the plotted graph is in increasing order.
- Hence from the above study we could say that anticoagulant does containing heparin,andthe extraction having anticoagulant activity.
- Heparin, anticoagulant drug that is used to prevent

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blood clots from forming during and after surgery and if treat Various heart, lung, and circulatoly disorders in ultch there is an increased risk of blood clot formation[21-24].

Anti oxidant activity:

•This study was carried out to evaluate the effect of Garlic (Allium sativum) as an antioxidant activity by using DPPH.

•for the evaluation of the effect of Garlic (Allium sativum) as an antioxidant activity by using DPPH some procedure have been followed.

• The materials and equipments required for the experiment are collected first before the procedure begins.

• as the collection of Allium sativum is done from the local market and the other materials such as DPPH and ascorbic acid were also been collected with the apparatus required for the procedure.

• Apparatus such as test tubes,cuvettes,spectrophotometer,glassrod,etc.

•by taking some Allium sativum(Garlic)and crush them to extract the juice of the Allium sativum as in anticoagulant procedure.

• after extraction of Allium sativum clear solution.Add methanol to the Allium sativum extract to make up the ratio 1:1(v/v)mixture(Allium sativum 1ml with methanol 1ml both are added).

• prepare a stock solution of DPPH by dissolving 24mg of DPPH in 100ml of methanol.

• take 1ml of Allium sativum extract-methanol mixture and mix it with 2ml of DPPH stock solution in a test tube.

•the solution was mixed thoroughly and keep it in the dark for 30mins.

• after 30mins we measured the absorbance of the solution at 517nm using a spectrophoto meter.

• repeating the steps 4-6 with different concentration of ascorbic acid with DPPH.

• calculating the percentage of inhibition of DPPH by using the following formula:

% inhibition = [(A_control - A_sample) /A_control]× 100

Where,

A_control is the absorbance of the DPPH solution without any sample

A_sample is the absorbance of the DPPH solution with the Allium sativum(garlic) methanol mixture.

•As different values are shown in table: 1, table:2,table:3 from the above tables

• From table:1 the only DPPH value is 1.000… as it is A_control value

•From table:2 we got the absorbance value of DPPH with ascorbic acid

• From table:3 we got the absorbance value of DPPH with Allium sativum Rhizome extract

•The absorbance values are used to calculate the percentage of DPPH with ascorbic acid and DPPH withAllium sativum extract(Test sample) by using the above formula to get this percentage.

After finding the percentage value a graph is been plotted. The plotted graph is in increasing order[25-26].

VI.CONCLUSION

Allium sativum Rhizome extract taken different samples and concentrate, set the different values of coagulation time in minutes.when the compared to standard (heparin)test also having anticoagulant activity.Allium sativum Rhizome extract prevents coagulation upto 70mins and heparin is natural anticoagulant is prevent or reduce the coagulant.So the study is concluded as the Allium sativum extract has anticongulantproperty.The Allium sativum extract with DPPH and ascorbic acid at different concentration get the absorbance readings and determined the percentage(%) inhibition of free radical scavaring activity(anti oxidant activity).

VI.REFERENCES

[1]. Amagase, H. (1997) Antioxidant and radical scavenging effects of aged garlic extract and its constituents. In: Antioxidants and Disease, 6th Congress on Clinical Nutrition. Banff, Alberta, Canada, p. 28 (abs.).

[2]. Amagase, H., Schaffer, E. M. & Milner J. (1996) Dietary components modify the ability of garlic to suppress 7,12,-dimethyl(a)anthracene induced DNA adducts. J. Nutr. 126: 817–824.

[3]. Awazu, S. &Horie, T. (1997) Antioxidants in garlic. II. Protection of heart mitochondria by garlic extract and diallyl polysulfide from the doxorubicininduced lipid peroxidation. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice (Lanchance, P. P., ed.), pp. 131–138. Food & Nutrition Press, Trumbull, CT.

[4]. Beckman, J. S. (1991) The double edged sword of nitric oxide in brain function and superoxide mediated injury. J. Dev. Physiol. 15: 53–59.

[5]. Block, E. (1985) The chemistry of garlic and onion. Sci. Am. 252: 114–119.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 842-851 www.ijprajournal.com ISSN: 2249-7781

[6]. Borek, C., Ong, A., Mason, H., Donahue, L. &Biaglow, J. E. (1986) Selenium and vitamin E inhibit radiogenic and chemically induced neoplastic transformation in vitro by different mechanisms. Proc. Nat. Acad. Sci. U.S.A. 83: 1490–1494.

[7]. Chung, H. T., Burnham, B., Robertson, L. K. &Daynes, R. A. (1986) Involvement of prostaglandins in the immune alterations caused by the exposure of mice to ultraviolet radiation. J.Immunol. 137: 2478–2484.

[8]. Cox, D. A. & Cohen, M. L. (1996) Effects of oxidized low density lipoproteins on vascular contraction and relaxation: clinical and pharmacological implications in atherosclerosis. Pharmacol. Rev. 48: 36.

[9]. Dimitrov, N. V. &Bennink, M. R. (1997) Modulation of arachidonic acid metabolism by garlic extract. In: Nutraceuticals:Designer Foods III Garlic, Soy and Licorice (Lanchance P.P., ed.), pp. 199–120. Food & Nutrition Press, Trumbull, CT.

[10]. Freeman, F. & Kodera, Y. (1995) Garlic chemistry: stability of S-(2-propenyl) 2-propene-1-sulfinothioate (allicin) in blood, solvents and simulated physiological fluids. J. Agric. Food Chem. 43: 2332–2338.

[11]. Geng, Z. & Lau, B.H.S. (1997) Aged garlic extracts modulate glutathione redox cycle and superoxide dismutase activity in vascular endothelial cells. Phytother. Res. 11: 54–56.

[12]. Geng, Z., Rong, Y. & Lau, B.H.S. (1997) S-Allyl cysteine inhibits activation of nuclear factor kappa B in human T cells. Free Radic. Biol. Med. 23: 345–350.

[13]. Gutteridge, J.M.C. (1993) Free radicals in disease processes: a compilation of cause and consequence. Free Radic. Res. Commun. 19: 141–158.

[14]. Horie, T., Murayama, T., Mishima, T., Fumiyaki, I., Minamide, Y., Fuwa, T. &Awaxu, S. (1989) Protection of liver microsomal membranes from lipid peroxidation by garlic extract. Planta Med. 55: 506–508.

[15]. Horie, T., Awazu, S., Itakura, Y. &Fuwa, T. (1992) Identified diallyl polysulfides from an aged garlic extract which protects the membranes from lipid peroxidation. Planta Med. 58: 468–469.

[16]. Ide, N. & Lau, B.H.S. (1997) Garlic compounds protect vascular endothelial cells from oxidized low density lipoprotein-induced injury. J. Pharm. Pharmacol. 49: 908–911.

[17]. Ide, N., Nelson, A. B. & Lau, B.H.S. (1996) Aged garlic extract and its constituents inhibit Cu12 -induced oxidative modification of low density lipoproteins. Planta Med. 63: 263–264.

[18]. Imai, J., Ide, N., Nagae, S., Moriguchi, T., Matsuura, H. &Itakura, Y. (1994) Antioxidants and free radical scavenging effects of aged garlic extract and its constituents. Planta Med. 60: 417–420.

[19]. Kojima, R., Epstein, C. J. Mizui, T., Carlson, E. & Chan, P. H. (1994) Protective effects of aged garlic extracts on doxorubicin-induced cardiotoxicity in the mouse. Nutr. Cancer 22: 163–173.

[20]. Lau, B.H.S. (1989) Detoxifying, radioprotective and phagocyte-enhancing effects of garlic. Int. Nutr. Rev. 9: 27–31.

[21]. Lau, B.H.S., Lam, F. & Wang-Ceng, R. (1987) Effect of an odor modified garlic preparation on blood lipids. Nutr. Res. 7: 139–149.

[22]. Lawson, L. D., Ransom, D. K. &Hughs B. G. (1992) Inhibition of whole blood platelet aggregation by compounds in garlic clove extracts and commercial garlic products. Thromb. Res. 65: 141–156.

[23]. Liu, J. Z., Lin, X. Y. & Milner, J. A. (1992) Dietary garlic powder increases glutathione content and glutathione S-transferase activity in rat liver and mammary tissues. FASEB J. 6: A3230 (abs.).

[24]. Milner, J. A. (1996) Garlic: its anticarcinogenic and antimutagenic properties. Nutr. Rev. 54: S82–S86.

[25]. Moriguchi, T., Saito, H. & Nishiyama, N. (1997) Anti-aging effect of aged garlic extract in the inbred brain atrophy mouse model. Clin. Exp. Pharmacol. Physiol. 24:235–242.

[26]. Nagae, S., Ushijima, M., Hatono, S., Imai, J., Kasauga, S., Itakura, Y. & Higashi, Y. (1994) Pharmacokinetics of the garlic compound S-allylcysteine. Planta Med. 60: 214–217.