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Bioactivity Guided Chromatographic separation of *Allium sativum*

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Abstract:

The current availablesynthetic drugs have certain limitations/undesired effects in some cases of treatments. Attempts have been made to discover new drugs of plant origin. In continuation to this effort,bioactivity guided chromatographic separation of *Allium sativum*was performed against plasma re-calcification time. The isolated active fraction of the plant could be used as a processed bio-drug having enhanced efficacy and lesser undesired effects.

(**Keywords**: Bioactivity guided separation, re-calcification time, bio-drug, *Allium sativum*)

Introduction:

Due to the adverse effects of many synthetic drugs, the search for new improved drugs is being shifted towards bio-resources in recent years.

Cardiovascular disease is a leading cause of death throughout the world. This disease is caused due to the abnormal blood coagulation.Blood clots that develop in the arteries can cause heart attack/stroke[1].The current available drugs used for the prevention of undesired clots have shownsome adverse effects like bleeding, adverse skin manifestations[2-3],background intracranial haemorrhage[4].Limitations of existing anticoagulants have accelerated a search for new anticoagulants with improved pharmacological and bio-safety profile[5].

Plants produce a variety of natural products. The natural products have been found to be a significant source of commercial medicines[6].

There is a growing focus on the importance of medicinal plants in the traditional health care system[7]. Plant extracts can be used as a tool in the development of plant derived drugs[8].
One of the most common limitationsofmedicinal plants is that they havelowlevel of efficacyto cure a disease.A plant extract may not be effective to serve as a high potency drug because of

other inactive constituents which are present along with the active chemical principle. The efficacy of such plant can be increased by separation of its active fraction or by eliminating inactive constituents to maximum possible level,by performing some analytical/separation methods, to use it as an effective drug.

It is reported that the plant *Allium sativum*[9-14]exhibits anticoagulant activity. The present study is an attempt to increase efficacy of *Allium sativum*by separation of biologically active fraction from it, so that it could be used as effective biopharmaceutical drug.

Materials and method:

Human blood was collected by making venipuncture. To the 9 ml volume of blood, 1 ml of 3.8 % tri-sodium citrate (prepared in 0.85% saline solution) was added to cease the natural coagulation.The plant *Allium sativum*was purchased from local market.Tri-sodium citrate was purchased from Sisco Research Lab.

The ext of the plant was prepared by dissolving 10g of crushed bulb part in to 50 ml methanol. The plant ext was subjected to column packed with silica gel- G slurry prepared in methanol. The Column was eluted by using 1-butanol:aceticacid:water:formic acid as mobile phase (28:9:9:2). Each fraction was tested for the anticoagulant activity by mixing 100µl of the fraction with 100 µl plasma. The coagulation/plasma re-calcification time was measured after addition of 100 µl $CaCl_2$ (0.025 moles/L) into the plasma–plant ext mixture, using Lee and White method[15]. The active fractions (which prolonged the re-calcification time) were mixed and evaporated to dryness. The residue (74mg) was dissolved in methanol (1:5 w/v) and subjected to silica gel 60-120 column and eluted with methanol:acetic acid (95:5). The active fractions were mixed and rechromatographed using same combination yielding residue (12 mg) after solvent evaporation.

Results and Discussion:

The bio-active fraction (having positive anticoagulant activity) of *Allium sativum*was separated using two stage CC. This isolated active fraction of the plant could be used as a

processed bio-drug having enhanced efficacy which could prove as an option to whole plant/synthetic drugs in future.

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