ROLE OF MEDICINAL PLANTS IN ARTERIOSCLEROSIS

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CERTIFICATE

This is to certify that the work entitled, "Role of medicinal plants in arteriosclerosis" submitted by Mr. Prashant kumar lathar (061720) and Ms. Kritika Sharma (061739) in partial fulfillment for the award of degree of Bachelors of Technology in Biotechnology of Jaypee University of Information Technology has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

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DECLARATION

I hereby declare that the work presented in this thesis has been carried out by me under the supervision of Dr. Chanderdeep Tandon, Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Waknaghat, Solan-173215, Himachal Pradesh, and has not been submitted for any degree or diploma to any other university. All assistance and help received during the course of the investigation has been duly acknowledged.

Prashant Kumar lathar (061720)

Kritika Sharma (061739)

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(Prashant Kumar lathar 061720) (Kritika Sharma 061739)

TABLE OF CONTENTS

1. Chapter 1 13-23

INTRODUCTION

1.1.1 Aorta

1.1.2 Arteriosclerosis

1.1.3 Terminalia arjuna

1.1.4 Buckwheat

2. Chapter 2 24-29

MATERIAL AND METHODS

2.1 Tissue Preparation : Extracted Abdominal and Thoracic aorta

2.2 Heterogeneous system

2.3 Calcium and Phosphate Estimation

2.4 Preparation of solvent extracts

3. Chapter 3 30-33

3.1 Selection of System

3.2 Results and Discussion

3.3 Conclusion

4. Chapter 4 34-43

4.1 Role of *Terminalia arjuna* in biomineralisation

4.1. a. Components

4.2 Results and Discussions

4.3. Effect of Aqueous extract of *T.arjuna* bark on abdominal aorta matrix mineralization.

4.4 Percentage inhibition of aqueous extract of *T.arjuna* bark on abdominal aorta matrix mineralization.

4.5. Effect of Aqueous extract of *T.arjuna* bark on thoracic aorta matrix mineralization.

4.6. Percentage inhibition of aqueous extract of *T.arjuna* bark on thoracic aorta matrix mineralization.

4.7. Effect of methanol extract of *T.arjuna* bark on thoracic aorta matrix mineralization.

4.8. Percentage inhibition of methanol extract of *T.arjuna* bark on thoracic aorta matrix mineralization.

5. Chapter 5 44-48

Effect of Buckwheat aqueous extract in Ca-P ion uptake on extracted thoracic aorta matrix preparations

5.1 Components

5.2 Results and Discussions

5.3 Conclusion

List of figures

Fig.1 Anterior (frontal) view of the human aorta
Fig2. Terminalia arjuna at Ananthagiri Hills, in Rangareddy district of Andhra Pradesh, India.
Fig. 3 Mineral composition Common buckwheat seed.
Fig4. *Fagopyrum esculentum* Moench var. *emarginatum* (Roth) Alef.: (a) flowering branch, (b) fruits and (c) flower (drawing by Ruth Kilian in Schultze-Motel 1986).
Fig 5. *Fagopyrum tataricum* (L.) Gaertn.: (a) fruiting branch, (b) inflorescence with fruits, (c) flower and (d) fruit (drawing by Ruth Kilian in Schultze-Motel 1986)
Fig.2.1.a Whole aorta sample in Normal saline.
Fig.2.1.b Whole aorta sample after removing adventitious tissue
Fig.2.1.c Abdominal Aorta pieces < $1cm^2$
Fig.2.1.d Washing of abdominal aorta pieces with 0.1% mM $CaCl_2$.
Fig.2.1.e Aorta pieces with extraction fluid.
Fig.2.1.f Lyophilization of aorta pieces
Fig 2.4.a Seeds of *F.tataricum*
Fig 2.4.b Seeds of *F.esculentum*
Fig.3.1.a Comparison of Phosphate ion uptake on extracted abdominal aorta matrix in different volumes of heterogeneous system.
Fig.3.1.b Comparison of Calcium ion uptake on extracted abdominal aorta matrix in different volumes of heterogeneous system.
Fig.4.a Effect of different concentration of aqueous *Terminalia arjuna* bark extract in calcium ion uptake on abdominal aorta matrix.
Fig.4.b Effect of different concentration of aqueous *Terminalia arjuna* bark extract in phosphate

ion uptake on abdominal aorta matrix.
Fig.4.c Percentage inhibition of different concentration of aqueous *Terminalia arjuna* bark extract in calcium ion uptake on abdominal aorta matrix.
Fig.4.d Percentage inhibition of different concentration of aqueous *Terminalia arjuna* bark extract in phosphate ion uptake on abdominal aorta matrix.
Fig.4.e. Effect of different concentration of aqueous *Terminalia arjuna* bark extract in calcium ion uptake on thoracic aorta matrix.
Fig.4.f. Effect of different concentration of aqueous *Terminalia arjuna* bark extract in phosphate ion uptake on thoracic aorta matrix.
Fig.4.g. Percentage inhibition of different concentration of aqueous *Terminalia arjuna* bark extract in calcium ion uptake on thoracic aorta matrix.
Fig.4.h. Percentage inhibition of different concentration of aqueous *Terminalia arjuna* bark extract in phosphate ion uptake on thoracic aorta matrix.
Fig.4.i. Effect of different concentration of methanol *Terminalia arjuna* bark extract in calcium ion uptake on thoracic aorta matrix.
Fig.4.j. Effect of different concentration of methanol *Terminalia arjuna* bark extract in phosphate ion uptake on thoracic aorta matrix.
Fig.4.k. Percentage inhibition of different concentration of methanol *Terminalia arjuna* bark extract in calcium ion uptake on thoracic aorta matrix.
Fig.4.l. Percentage inhibition of different concentration of aqueous *Terminalia arjuna* bark extract in phosphate ion uptake on thoracic aorta matrix.
Fig 5.a Comparison of Ca ion inhibiton by *F.tataricum* and *F. esculentum* extracts(0.08mg/ml)

Fig 5.d Comparison of Phosphate ion inhibiton by *F.tataricum* and *F. esculentum* extracts(0.08mg/ml)
Fig 5.e Comparison of Phosphate ion inhibiton by *F.tataricum* and *F. esculentum* extracts(0.16mg/ml)
Fig 5.f Comparison of Phospahte ion inhibiton by *F.tataricum* and *F. esculentum* extracts(0.32mg/ml)

LIST OF TABLES

TABLE 1. Ca-P Ion uptake on extracted abdominal aorta matrix with different system volumes.All values are mean ± SD of three replicates.
TABLE 2. Effect of T.arjuna aqueous extract in Ca-P ion uptake on extracted abdominal aorta matrix.All values are mean ± SD of three replicates.
TABLE 3. Effect of T.arjuna Aqueous extract in Ca-P ion uptake on extracted abdominal aorta matrix.All values are mean ± SD of three replicates.
TABLE 4. Effect of T.arjuna Methanolic extract in Ca-P ion uptake on extracted thoracic aorta matrix.All values are mean ± SD of three replicates.

LIST OF ABBREVIATIONS

Abbreviated terms	Full Form
Ca-P	Calcium-Phosphate
conc.	Concentration
Fig.	Figure
F. esculentum	*Fagopyrum esculentum*
F. tataricum	*Fagopyrum tataricum*
hrs	Hours
cm	Centimeters
mM	Milli Molar
ml	Milli litres
mins	Minutes
SD	Standard deviation
T.arjuna	*Terminalia arjuna*

ABSTRACT

It is well known that the calcium content of various blood vessel walls increases with age and that hydroxyapatite is a major component of aortic plaques. In vitro studies using heterogeneous system showed that abdominal/thoracic aorta matrix after their extraction with 3% Na_2HPO_4 and 0.1mM $CaCl_2$ were mineralized under physiological conditions of temperature, pH, and ionic strength to form matrix bound mineral phase resembling hydroxyapatite in nature and correspondingly the rate of ion uptake for both calcium and phosphate was studied for period of 24 hours.

The present study was carried out to evaluate the anti-calcifying properties of *Terminalia arjuna* bark extract and common/bitter buckwheat seed extract on organic matrices prepared from thoracic aorta pieces of goat.

Terminalia arjuna bark is an indigenous plant used in ayurvedic medicine in India, primarily as a cardio-tonic for many cardiac ailments. The addition of the aqueous extract of *Terminalia arjuna* bark in this assay system was found to inhibit the above mineralization process. Aqueous extract of *Terminalia arjuna* bark was found to inhibit the rate of ion uptake in a concentration dependent manner. A significant percentage inhibition of both calcium and phosphate ion uptake onto the matrix was observed at different intervals of time. A similar pattern was obtained with methanol extract of *Terminalia arjuna*.

Buckwheat has an excellent reputation for its nutritious qualities in the human diet. Its renewed popularity stems from its many bioactive components, which have been shown to provide various health benefits much sought after in natural foods. Rutin present in buckwheat can soften soften blood vessel and reduce blood fat, sugar and cholesterol to prevent and cure hypertension, diabetes and arteriosclerosis. Two species of buckwheat namely common-buckwheat (*F.esculentum*) and bitter- buckwheat (*F. tataricum*) were evaluated for their Ca-P ion uptake inhibition on extracted thoracic aorta matrix preparation. Aqueous seed extract of both the mentioned species was evaluated in the assay system in a concentration dependent manner. Comparison between the roles of both the species in inhibition rate of Ca-P ion uptake on extracted thoracic aorta matrix preparation is also drawn as both differ in their chemical

ingredient and nutritional character. The data concludes that *F.tataricum* extract aqueous extract is causing inhibition of Ca ion uptake completely as compare to *F. esculentum* extract whereas *F. esculentum* extract is causing inhibition of phosphate ion uptake completely as compare to *F. tataricum* extract.

These results throw light on medicinal value of *Terminalia arjuna* bark powder and common/bitter buckwheat seed extract in the control of arteriosclerosis, a common cause of coronary heart diseases.

CHAPTER 1

1. INTRODUCTION

1.1 Aorta

- The aorta is the largest artery in the body, originating from the left ventricle of the heart and bringing oxygenated blood to all parts of the body in the systemic circulation.
- The aorta is an elastic artery, and as such is quite distensible. When the left ventricle contracts to force blood into the aorta, the aorta expands. This stretching gives the potential energy that will help maintain blood pressure during diastole, as during this time the aorta contracts passively.

The aorta is usually divided into five segments/sections:

1. Ascending aorta—the section between the heart and the arch of aorta.
2. Arch of aorta—the peak part that looks somewhat like an inverted "U".
3. Descending aorta—the section from the arch of aorta to the point where it divides into the common iliac arteries.
4. Thoracic aorta—the half of the descending aorta above the diaphragm.
5. Abdominal aorta—the half of the descending aorta below the diaphragm.

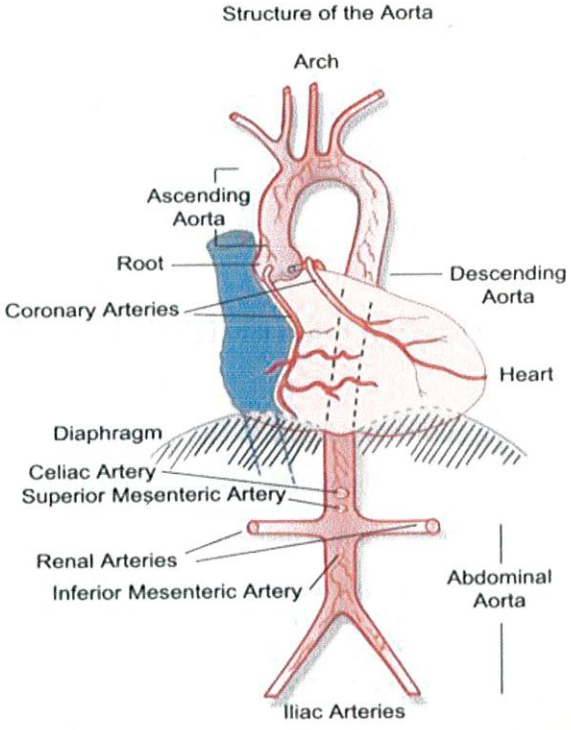


Fig.1 Anterior (frontal) view of the human aorta

1.2 Arteriosclerosis

1.2.a Definition

1. Arteriosclerosis refers to a stiffening of arteries.[*arteriosclerosis* at Dorland's Medical Dictionary]

Arteriosclerosis refers to several diseases in which the arterial wall thickens and loses its elasticity. Commonly confused with *atherosclerosis*, which is the formation of plaques consisting of cholesterol and other substances on the arterial walls, arteriosclerosis is the thickening and stiffening of the artery walls from too much pressure. Atherosclerosis can lead to arteriosclerosis, which comes from the Greek for "hardening of the arteries."

Arteriosclerosis is a general term describing any hardening (and loss of elasticity) of medium or large arteries (from the Greek Arterio, meaning *artery*, and sclerosis, meaning *hardening*)

1.2.b Types

i. Arteriolosclerosis is any hardening (and loss of elasticity) of small arteries and arterioles (small arteries). It is often associated with hypertension.

ii. Atherosclerosis is a hardening of an artery specifically due to an atheromatous plaque. Atherosclerosis is the most common form of arteriosclerosis. Atherosclerosis is characterized by a thickening of the intima with plaques that can contain lipid-laden macrophages ("foam cells"). The plaques contain free lipid (cholesterol, etc.) and are prone to calcification and ulceration.

iii. Arteriosclerosis obliterans is typically seen in medium and large arteries of the lower extremity. Characterized by fibrosis of the intima and calcification of the media. The lumen of the vessel may be obliterated or markedly narrowed.

iv. Medial calcific sclerosis (Monckeberg's calcific sclerosis) is seen mostly in the elderly, commonly in arteries of the thyroid and uterus. Characterized by calcification of the internal elastic lamina but without thickening of the intima or narrowing of the vessel

lumen. A similar form of an intramural calcification, presenting the picture of an early phase of arteriosclerosis, appears to be induced by a number of drugs that have an antiproliferative mechanism of action.[2]

The most common sites for arteriosclerosis are arteries in the brain, kidneys, heart, abdominal aorta, or legs. Symptoms of arteriosclerosis vary according to which arteries are affected. Leg pain when exercising might indicate peripheral arterial disease. Sudden weakness or dizziness could be caused by an obstruction in the carotid artery in the neck, which produces stroke-like symptoms. Chest pain or symptoms of a heart attack might indicate obstruction of the coronary arteries. Arteriosclerosis can also cause erectile dysfunction.

1.2.c Symptoms

- Risk factors for arteriosclerosis include smoking, obesity, high blood pressure and/or cholesterol, stress, and diabetes. A virus or allergic reaction, chronic kidney disease, irritants such as nicotine and drugs, or too much of the amino acid homocystine can also lead to arteriosclerosis. A family history of early heart disease is also a risk factor for developing arteriosclerosis.
- If you experience any signs of restricted blood flow, you should see your doctor. Those with poor blood flow in one area of the body are likely to have arteriosclerosis or atherosclerosis in another part of the body. During a physical exam, your doctor may find signs of either arteriosclerosis or atherosclerosis by several methods, including listening to your arteries through a stethoscope.
- Decreased blood pressure in a limb or lack of a pulse in a narrowed artery could indicate arteriosclerosis. Other warning signs include a bulge in the abdomen or behind the knee. The physician might also notice poor wound healing in an area with restricted blood flow. Blood tests, imaging, ultrasounds, electrocardiograms (EKGs), and other tests help a physician diagnose arteriosclerosis.
- Treatment varies according to the symptoms and severity of the condition, but can include exercise, medication, or surgery. Some treatments include reducing dietary

calcium and increasing magnesium intake. Cholesterol lowering drugs, aspirin therapy, anticoagulants, and vasodilators are used in some cases.

1.3 *Terminalia arjuna*

1.3.a About the Plant

Terminalia arjuna is a medicinal plant of the genus *Terminalia*, widely used by ayurvedic physicians for its curative properties in organic/functional heart problems including angina, hypertension and deposits in arteries. Arjuna bark (*Terminallia arjuna*) is thought to be beneficial for the heart. This has also been proved in a research by Dr. K. N. Udupa in Banaras Hindu University's Institute of Medical Sciences, Varanasi (India). In this research, they found that powdered extract of the above drug provided very good results to the people suffering from Coronary heart diseases.

Classification of *Terminalia arjuna*
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Myrtales
Family: Combretaceae
Genus: Terminalia
Species: T. arjuna
common name is arjuna.

Research suggests that *Terminalia* is useful in alleviating the pain of angina pectoris and in treating heart failure and coronary artery disease. Terminalia may also be useful in treating hypercholesterolemia[1]. The cardioprotective effects of terminalia are thought to be caused by the antioxidant nature of several of the constituent flavonoids and oligomeric proanthocyanidins, while positive inotropic effects may be caused by the saponin glycosides.

The bark leaves and fruits of *Terminalia arjuna* have been used in indigenous system of medicine for different ailments (Warrier et al., 1996). The bark is said to be sweet, acrid, cooling and heating, aphrodisiac, expectorant, tonic, styptic, antidysenteric, purgative and laxative. Its use has been advocated in urinary discharge, strangury, leucoderma, anemia, hyperhidrosis, asthma and tumors.

The use of bark powder as an astringent and diuretic finds mention in the works of Carak (Carak Samhita, 1941). The bark powder has been attributed to possess cardioprotective properties. Vagbhatta was the first to cite this in his book 'Astang Hridayam' written some 1200 years ago (Lal Chandra, 1963). Subsequently, Chakradutta and also Bhawa Mishra, described its use in chest pain (Bajpeyee, 1959; Bhawa Prakash, 1963). Traditional method of its administration was to prepare an alcoholic decoction of its bark stem (asava) or give it along with clarified butter (ghrita) or along with boiled milk (kshirpak) (Nadkarni and Nadkarni, 1954; Warrier et al., 1996).

- ✓ Having realised the potential atherogenic properties of clarified butter and whole milk it would be interesting to examine the role of such preparations in experimental model of atherosclerosis.

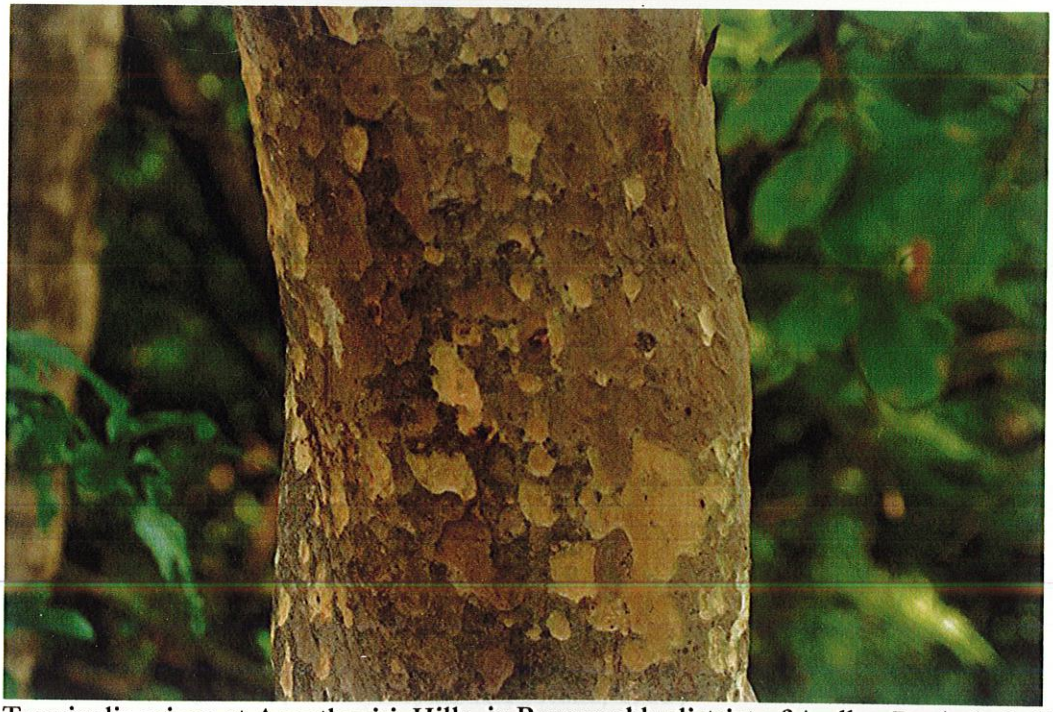


Fig2. Terminalia arjuna at Ananthagiri Hills, in Rangareddy district of Andhra Pradesh, India.

1.3.b Research

A number of clinical studies have shown *T.arjuna* extract's cardiac effectiveness.

i. In a double-blind, placebo-controlled trial of 58 males with chronic stable angina, arjuna-treated patients had a significantly-decreased frequency of angina, and significantly better treadmill parameters.

ii. (2) In another placebo-controlled, randomized trial of 105 patients with elevated cholesterol, 35 patients not given other lipid-treatment were treated with *T.arjuna* for 30 days. Total cholesterol decreased from 9.7-12.7%, LDL cholesterol decreasd from 15.8-25.6%, and lipid peroxide levels decreased significantly.

iii. (3) In an open study of ten patients with stable angina, *T.arjuna* treatment resulted in an 80% of patients having symptomatic relief.

iv. (4) In a placebo-controlled study of 12 patients with chronic congestive heart failure, patients treated with arjuna experienced an improvement of congestive heart failure symptoms, a decrease in echo-left ventricular endiastolic and endsystolic volume indices, increased left ventricular stroke volume index and increased left ventricular ejection fractions.(5,6)

v. In another study of 20 angina patients for 3 months, arjuna treatment resulted in a 50% reduction of angina episodes among stable angina patients, lowered systolic blood pressure and body mass index, slightly increased HDL-cholesterol and marginal improvement in left ventricular ejection fraction.(7). Safety was demonstrated in all studies.

1.3.c Major chemical constituents of various parts of Terminalia arjuna

(A)Stembark

1. Triterpenoids:

arjunin, arjunic acid, arjunolic acid, *arjungenin, **terminic acid (Row et al., 1970a; *Honda et al., 1976a; **Anjaneyulu and Prasad, 1983)

2. Glycosides:

arjunetin, *arjunosideI, *arjunosideII, **arjunaphthanoloside, ***terminosideA (Ghoshal, 1909; Ghosh, 1926; Row et al., 1970b; *Hondaet al., 1976b;**Alietal., 2003a; ***Alietal., 2003b)

3. Sitosterol (Ghosh, 1926; Anjaneyulu and Prasad, 1983)

4. Flavonoids:

arjunolone, arjunone, bicalein, *luteolin, gallic acid, ethyl gallate, quercetin, kempferol, pelorgonidin, oligomeric proanthocyanidins (Sharma et al., 1982; *Pettit et al., 1996; Anonymous,1999)

5. Tanins:

pyrocatechols, punicallin, punicalagin, terchebulin, terflavinC, castalagin, casuariin, casuarinn (Dymock etal.,1891; Ghoshal, 1909; Chopra and Ghosh, 1929; Takahashi etal., 1997; Linetal.,2001)

6. Minerals/trace elements:

Calcium, Aluminium, Magnesium, Silica, Zinc, Copper (Dwivedi and Udupa, 1989)

(B)Roots

1. Sitosterol (Anjaneyulu and Prasad,1983)

2. Triterpenoids: arjunic acid, arjunolic acid, oleanolic acid, terminic acid(AnjaneyuluandPrasad,1983)

3.Glycosides: arjunoside I, arjunoside II, arjunoside III, arjunoside IV, *2 ,19 -dihydroxy-3-oxo-olean-12-en28-oicacid28-O- -d-glucopyranoside (Anjaneyulu and Prasad, 1982 a,b; *Choubey and Srivastava, 2001)

(C)Leaves and fruits

1. Glycosides

2. Flavonoids: luteolin (Pettitetal., 1996)

1.4 BUCKWHEAT

1.4.a About the Plant

Common buckwheat (*Fagopyrum esculentum*) is a broad-leafed herbaceous annual. It belongs to the family Polygonaceae, which is generally referred to as the buckwheat, rhubarb or sorrel family. However, because its seed structurally and chemically resembles the cereal grains, buckwheat is usually handled and classed with the cereals. Shown to have originated in the mountainous regions of southern China, buckwheat is produced in many parts of the world and has long been an important part of the human diet. Buckwheat has been grown in Canada and the U.S. for many years.

Buckwheat has a triangular seed, which is covered by a hull (pericarp). The exact shape, size, and colour of the seed may vary depending on the species and variety. The hull may be a glossy or dull brown, black or gray. The dehulled buckwheat seed, called the groat, resembles the cereal kernel in its gross chemical composition and structure. The first layer of the groat is a one-cell thick testa layer (seed coat), which is light green in color. A large embryo and two cotyledons extending in the shape of letter AS are embedded in the center of the endosperm.

Buckwheat has gained an excellent reputation for its nutritious qualities in the human diet. Its renewed popularity stems from its many bioactive components, which have been shown to provide various health benefits much sought after in natural foods.

1.4.b Classification of the genus

Buckwheat belongs to the family Polygonaceae. This plant group is generally referred to as the buckwheat, rhubarb or sorrel family. There has been a great deal of interest generated over the past 10 years regarding the classificationof *Fagopyrum* species. Much of this has occurred as a result of Ohnishi's work in the finding of six new species in China and his work on their classification. Ye and Guo (1992) suggested a key to the classification of 15 species that occur in the temperate areas of Euro-Asia, with approximately 10 species occurring in China. However, the key to classification of the genus *Fagopyrum* by Ohnishi (1995) is more complete.

1.4.c Common buckwheat *(Fagopyrum esculentum)*

This is an annual herb, up to 1 m tall, branched, glabrous (Fig. 3). Leaves are petiolate, blades are ovate-triangular to triangular, 2-8 cm long, with acuminate tips, bases are cordate or approximately hastate; upper leaves are smaller, sessile. Inflorescences are terminal and auxiliary, branch in dense corymbose or paniculate cyme. Flowers are white or pink, 6 mm in diameter; pedicel is 2-3 mm long, articulate; perianths are 3 mm long; 8 nectaries are yellow, alternating with stamens; being heterostyly, capitate stigma. Achene is triquetrous, acute angle, longer than 5 mm, more than twice the length of the persistent perianths, brown or black-brown, lucid. There are many cultivars or landraces in this species. Their achene forms can vary greatly, some of them being winged on the angles.

Mineral	Content (mg/100 g)
Calcium	110
Iron	4
Magnesium	390
Phosphorus	330
Potassium	450
Copper	0.95
Manganese	3.37
Zinc	0.87

Source: Marshall and Pomeranz 1982.

Fig.3 Mineral composition (Common buckwheat seed).

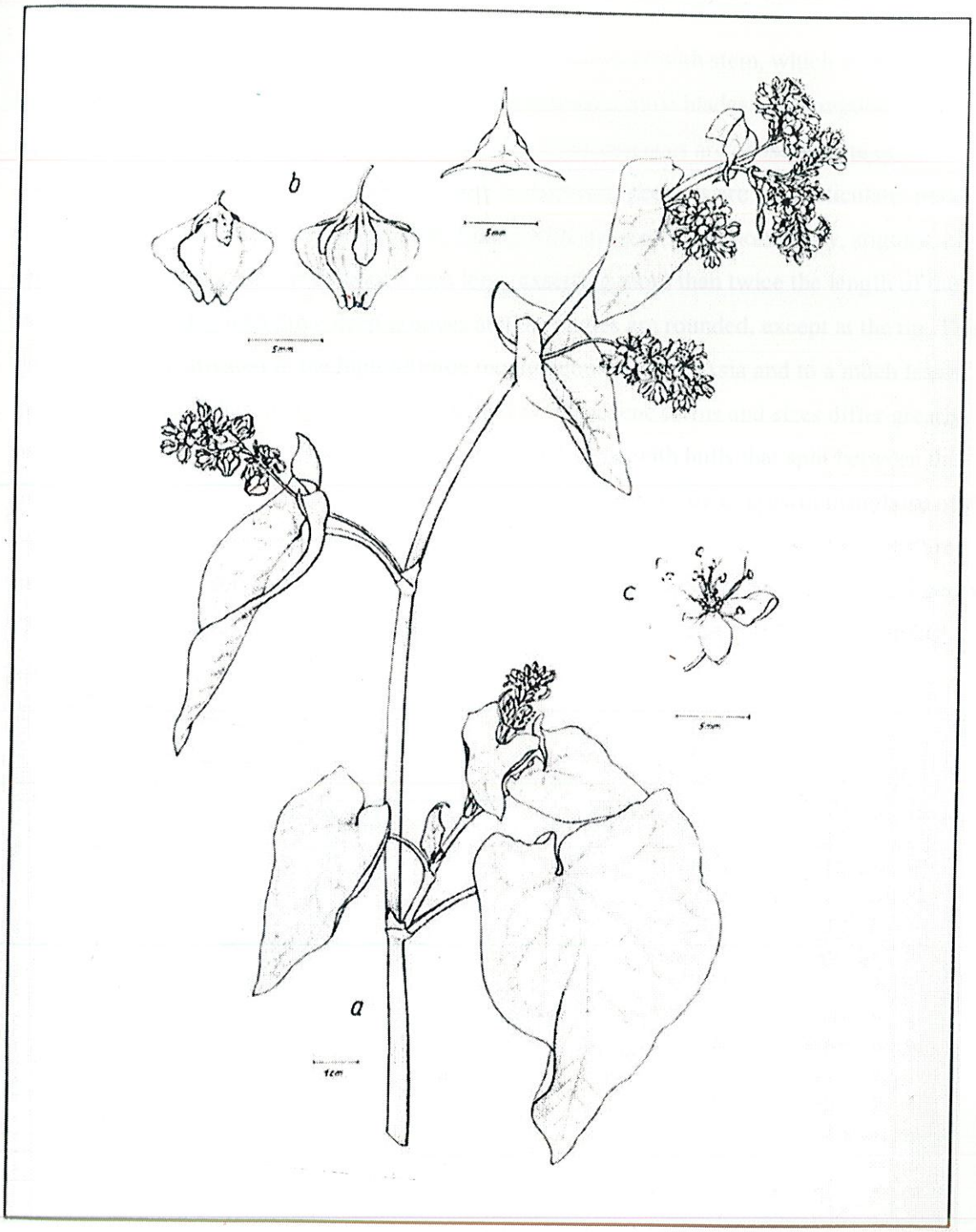


Fig4. *Fagopyrum esculentum* Moench var. *emarginatum* (Roth) Alef.: (a) flowering branch, (b) fruits and (c) flower (drawing by Ruth Kilian in Schultze-Motel 1986).

1.4.d *Fagopyrum tataricum*

This is an annual herb, up to 1 m tall, branched or unbranched with stem, which is striate, always having papillate on branchlet (Fig. 2). Leaves are petiolate, most blades are triangular, width equals length, 2-8 cm, bases are cordate or hastate. Inflorescences are dense spicate or corymbose. Flowers are yellow-green, 2.5 mm in diameter, pedicels are nonparticulate; perianths are 2 mm long; 8 nectaries are yellow, alternating with stamens being homostyly, stigmas are capitate. Triquetrous achene is about 5 mm long, exserting more than twice the length of the persistent perianths, with three deep grooves and the angles are rounded, except at the tip. This species is now cultivated in the high-altitude mountainous areas of Asia and to a much lesser extent elsewhere. It has many cultivars or landraces. Its achene forms and sizes differ greatly, some of them are winged or spinous on the angles and some with hulls that split between the angles on maturity. The plants of *F. tataricum* are usually less husky in growth than those of *F. esculentum*. They are more branched and the leaves are more arrow-shaped. The flowers are smaller, have inconspicuous greenish-white sepals and do not appear to be attractive to insects. The lowers are homomorphic, self-fertile and are cleistogamous, with pollination occurring before the flower opens.

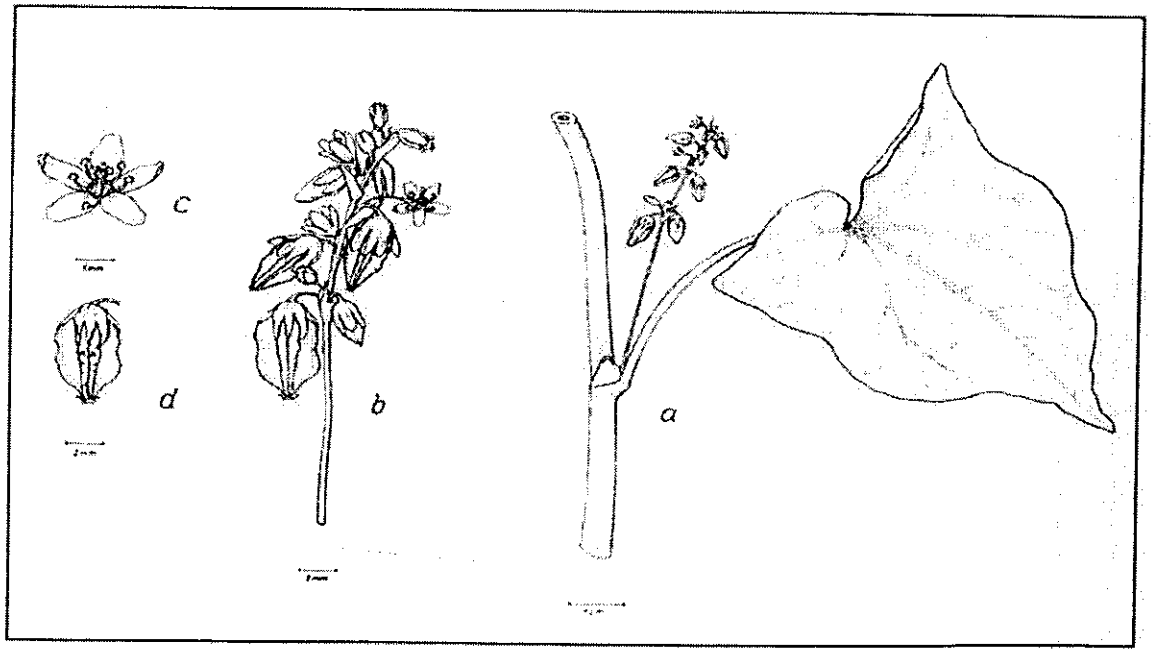


Fig 5. *Fagopyrum tataricum* (L.) Gaertn.: (a) fruiting branch, (b) inflorescence with fruits, (c) flower and (d) fruit (drawing by Ruth Kilian in Schultze-Motel 1986).

CHAPTER 2

MATERIAL AND METHODS

All reagents and chemicals were obtained from Merck Company and were of highest purity available. The source of *Terminalia arjuna* bark is Natural remedies, Bangalore, India. The seeds of *F. esculentum; (*common buckwheat) IC Accession number: 213682 and *F. tataricum* (tartary buckwheat) IC Accession number: 107895 were obtained from NBPGR, Phagli, Shimla, H.P.

2.1 Tissue Preparation: Extracted Abdominal and Thoracic aorta.

Materials:

Normal saline (0.9% NaCl), Extraction fluid: 3% Na_2PO_4 and 0.1mM $CaCl_2.2H_2O$ (250 ml). Conical flasks, measuring, cylinders, dH2O, beakers, surgical blades, gloves.

Procedure:

1. Took whole Aorta obtained from Slaughter House at Panchkula and keep it in normal saline. (Fig. 1)
2. Remove the adventitious tissue and separate abdominal aorta.(Fig. 2.)
3. Cut into small pieces not more than 1cm2. (Fig.3.)
4. Put the aorta pieces in extraction fluid/ solution in 100 ml.(Fig.5.)
5. Keep it at 4C for 5-6 days.
6. After incubation period take out the extracted abdominal aorta pieces and rinse them with 0.1 mM CaCl2. (Fig.4.)
7. Keep them at -80 °C for lyophilization. (Fig.6.)

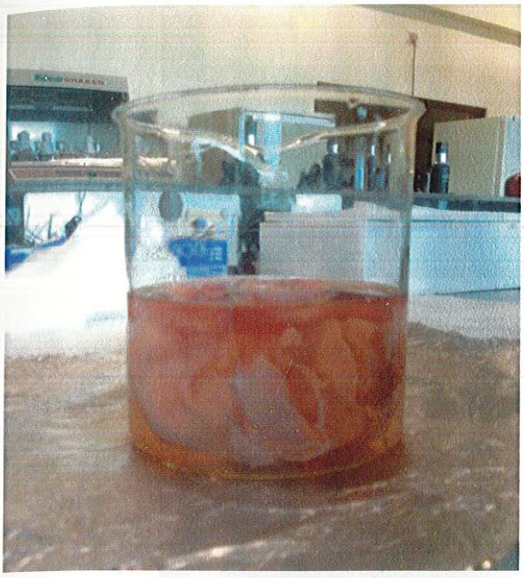


Fig.2.1.a Whole aorta sample in Normal saline.

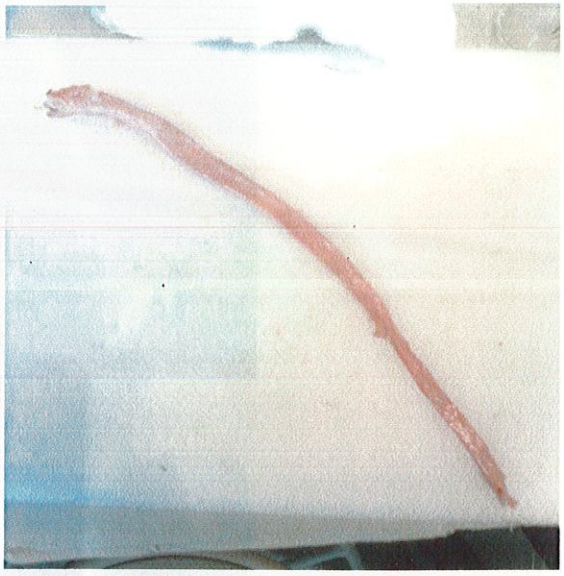


Fig.2.1.b Whole aorta sample after removing adventitious tissue.

Fig.2.1.c Abdominal Aorta pieces < 1cm^2.

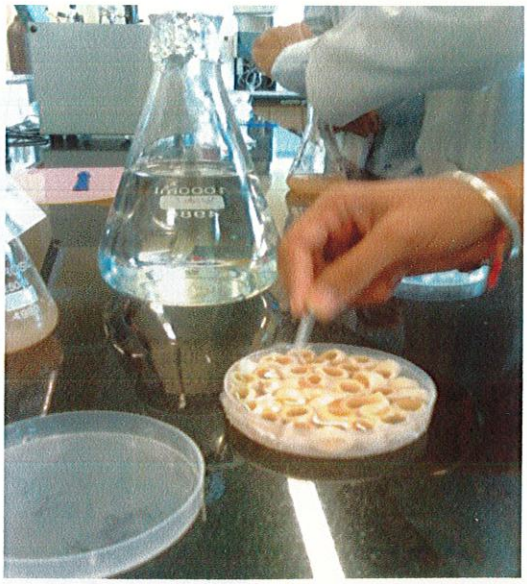


Fig.2.1.d Washing of abdominal aorta pieces with 0.1% mM $CaCl_2$.

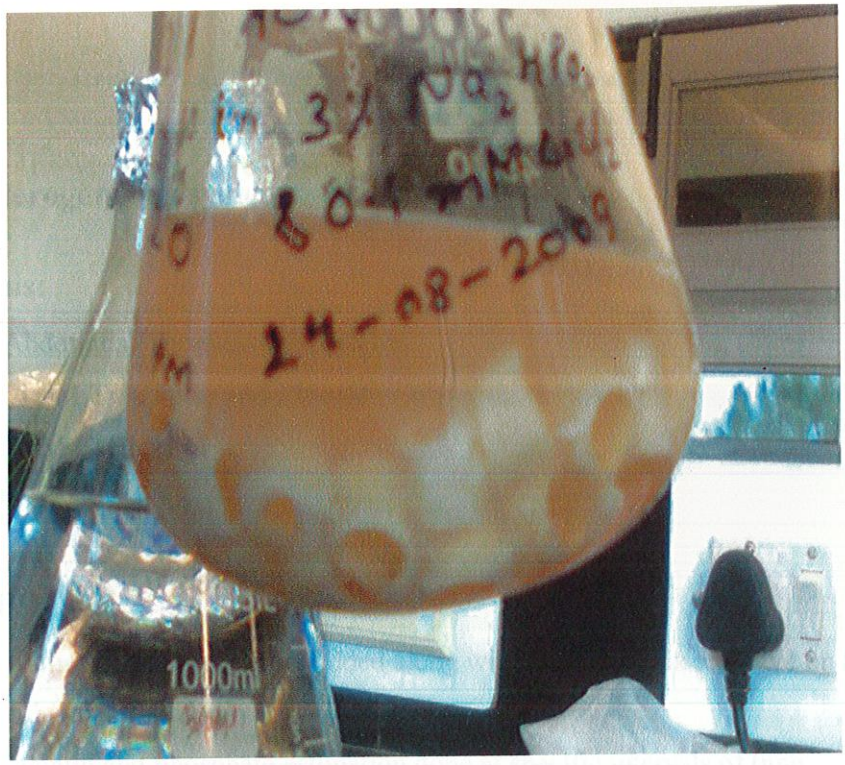


Fig.2.1.e Aorta pieces with extraction fluid.

Fig.2.1.f Lyophilization of aorta pieces

2.2 Heterogeneous system

Materials:

i. Abdominal/Thoracic aorta matrix
ii. 0.1mM Tris buffer and 210mM $NaCl_2$ with Ph=7.4
iii. 2 mM $CaCl_2.H_2O$
iv. 1.6mM KH_2PO_4

PROCEDURE:

1. All the above reagents are added along with 10 mg matrix and kept on Shaker at 37 °C with 150 strokes/min for 24 hrs.
2. Calcium and phosphate estimation done at specific intervals of time.

2.3 Calcium and Phosphate Estimation:

Materials:

Calcium Reagent, EDTA + NaOH, Color reagent, Ammonium Molybdate, Distilled water, Metol reagent, Reagent bottle, beaker, flasks

Calcium Estimation Steps:

1. Take 0.1 ml. of Sample
2. Add 2.5 ml of Calcium Reagent (Yellow)
3. Wait for 20 mins.
4. Then after centrifuge for 20 mins. At 4500 rpm. Decant supernatant immediately .Make sure orange ppt. on wall of centrifuge tube does not drain.
5. Add 1 ml. EDTA +NaOH and boil for 10 mins. At 100°C
6. Add 3 ml. Color reagent. Shake well so that orange ppt. dissolves.
7. Take O.D at 450 nm.

Phosphate estimation Steps:

1. Take 0.1 ml of sample.
2. Add 0.24 ml of 2.5% Ammonium Molybdate and 10N H_2SO_4 mixed in ratio 10:4 resp.
3. Wait for 10 mins.
4. Add 3ml. Distilled water.
5. Then Add 0.1 ml Metol reagent
6. Keep it for 30 mins.
7. Take O.D at 660 nm.

2.4 Preparation of solvent extracts:

Terminalia arjuna aqueous extract:

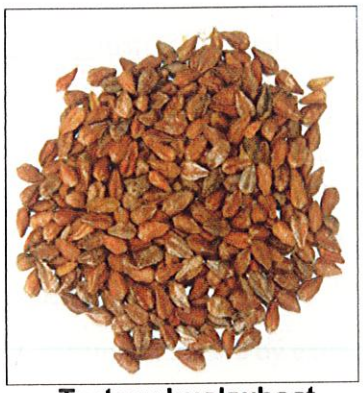
1. T.arjuna bark was dried and then crushed using an electric grinder.
2. The resulting powder was used for aqueous extract preparation.
3. 100 g of the fine powder was dissolves in 500 ml of distilled water and boiled for 10 min.
4. This mixture was incubated at 37 ± 1 °C for 72 hrs.
5. The aqueous extract was filtered and concentrated to a dry mass by using vacuum distillation and evaporation.
6. Then this extract was dried in an oven at 60 °C to obtain a dry mass.

Terminalia arjuna Methanolic extract:

1. Finely powdered stem bark (100 g) was extracted with 50% methanol in the cold for 72 hours.
2. The alcoholic extract was filtered and concentrated to a dry mass by using evaporation in an oven at 60 °C to obtain a dry mass.
3. A dark brownish/red shiny crystal like residue was obtained.

Buckwheat aqueous extract:

1. Buckwheat seeds (Fig.2.4.a and 2.4.b) were dried and then crushed using an electric grinder.
2. The resulting powder was used for aqueous extract preparation.
3. 100 gm of the fine powder was dissolves in 500 ml of distilled water and boiled for 10 min.
4. This mixture was incubated at 37 ± 1 °C for 72 hrs.
5. The aqueous extract was filtered and concentrated to a dry mass by using vacuum distillation and evaporation.
6. Then this extract was dried in an oven at 60 °C to obtain a dry mass.



Tartary buckwheat

CHAPTER 3

3.1 Selection of System

❖ Different ways :

1. Changing the system volume.
2. Changing the Aorta matrix conc.
3. Changing both system volume and Matrix conc.

✓ We adopted change in system volume method.

❖ Different systems were experimented with Abdominal Aorta matrix.

1. 10 mg matrix in 5 ml heterogeneous system.
2. 10 mg matrix in 10 ml heterogeneous system.
3. 10 mg matrix in 15 ml heterogeneous system.

*with same calcium and phosphate ion concentration.

- We used extracted abdominal matrix for the selection and standardisation of the heterogeneous system.
- Phosphate ion uptake by extracted abdominal matrix at 24 hour after system incubation.

  10 mg/5ml system 702.22 nMoles/mg.

  10mg/10ml system 382.34 nMoles/mg.

  10mg/15ml system 782.43 nMoles/mg.
- Calcium ion uptake by extracted abdominal matrix at 24 hour after system incubation.

  10 mg/5ml system 97.72 nMoles/mg.

  10mg/10ml system 115.45 nMoles/mg.

  10mg/15ml system 110.45 nMoles/mg.

TABLE1. Ca-P Ion uptake on extracted abdominal aorta matrix with different system volumes. All values are mean ± SD of three replicates.

Mean Ion uptake (nMoles/mg.) ± SD (Three replicate) in heterogeneous system for Calcium ion uptake (nmoles/mg) on extracted abdominal aorta matrix.			
Time in hrs.	Std. Deviation (10mg/5ml)	Std. Deviation (10mg/10ml)	Std. Deviation (10mg/15ml)
2	15.90±17.239	35.10±17.0254	28.69±14.891
4	22.27±15.468	61.36±16.901	30.45±11.432
6	33.18±12.653	75.45±14.517	32.72±14.1274
8	57.72±19.571	86.81±20.893	54.09±15.697
24	97.72 ±5.237	115.45 ±5.0356	110.45 ±3.861
Mean Ion uptake (nMoles/mg.) ± SD (Three replicate) in heterogeneous system for Phosphate ion uptake (nmoles/mg) on extracted abdominal aorta matrix.			
Time in hrs.	Std. Deviation (10mg/5ml)	Std. Deviation (10mg/10ml)	Std. Deviation (10mg/15ml)
2	44.45±12.35894	17.77±11.5683	25.56±8.329
4	57.78±13.957	26.67±14.364	151.11±7.625
6	93.33±13.426	75.56±12.483	280.05±7.018
8	186.67±15.603	253.33±17.903	360.27±10.473
24	702.22 ±3.214	382.34 ±4.691	782.43±3.094

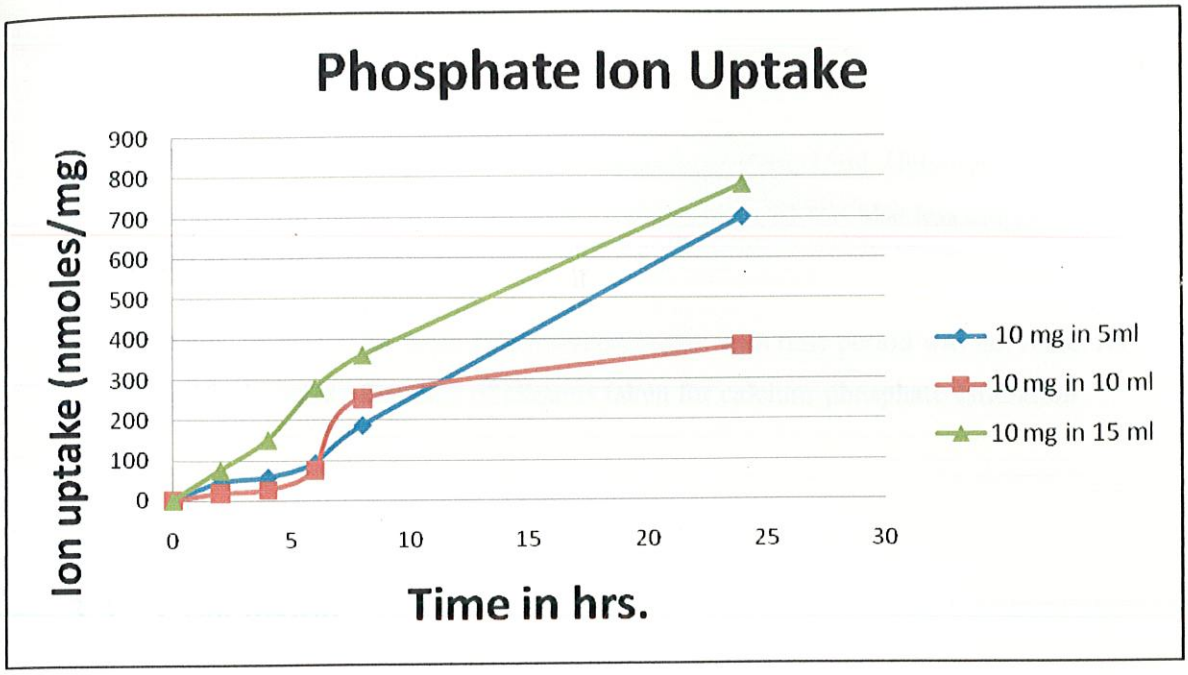


Fig.3.1.a Comparison of Phosphate ion uptake on extracted abdominal aorta matrix in different volumes of heterogeneous system.

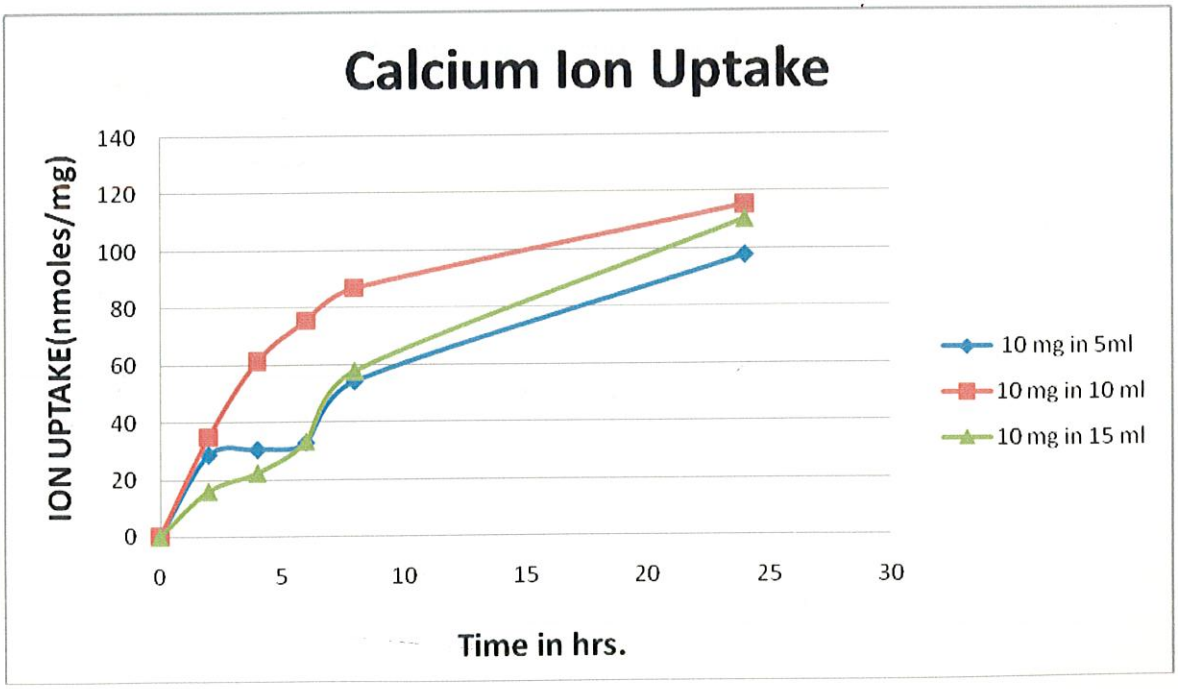


Fig.3.1.b Comparison of Calcium ion uptake on extracted abdominal aorta matrix in different volumes of heterogeneous system.

3.2 Results and Discussion

Much better ion uptake pattern were obtained from 10mg/15ml. Heterogeneous system of abdominal aorta matrix on comparison to others.Even Sd was also less compared to other system as TABLE 1 indicates.

Problems related to haziness of incubation buffer with time period was not there. As 10mg/15ml. require flirtation of aliquots taken for calcium-phosphate estimation.

3.3 Conclusion

- Using 10mg/15ml. Heterogeneous system for Thoracic Aorta Matrix for kinetic study in-vitro.
- To develop control for study with medicinal plant extract in respect to thoracic aorta matrix.

CHAPTER 4

4.1 Role of *Terminalia arjuna* in biomineralisation

Aqueous and Methanolic extract of Terminalia arjuna bark were used to study the biomineralisation on extracted thoracic and abdominal aorta matrix.

4.1. a. Components

Bark of *Terminalia arjuna* used.

Source: Natural remedies, Banglore, India.

i. 10 mg/15ml heterogeneous system is used for study.

ii. Standard Calcium and Phosphate methods were used for determining ion uptake on matrices.

iii. Different concentrations i.e 0.08mg/ml, 0.16mg/ml, 0.32mg/ml of aqueous and methanol extract of *Terminalia arjuna bark* were used in the same heterogeneous system.

4.2 Results and Discussions

The aqueous extract of *Terminalia arjuna* show significant calcium and phosphate ion uptake inhibition (Fig.4.a & 4.b) on extracted abdominal aorta matrix at 6 , 8 and 24 hour interval. The maximum percentage inhibition of aqueous extract on calcium ion uptake at 8 and 24 hr intervals was 95 % and 100% respectively (Fig.4.c). The percentage inhibition of aqueous extract on phosphate ion at 8 and 24 hour intervals was 85% and 93% respectively (Fig.4.d). Graphs clearly indicate that *Terminalia arjuna* extract is able to inhibit Calcium ion uptake completely to 100% at 24 hr. interval in the standardized heterogeneous system (10mg/15ml). On increasing the concentration of aqueous extract in the heterogeneous system (10mg/15ml) the inhibition increases.

Similar inhibition pattern was seen on extracted thoracic aorta with different concentrations of aqueous extract of *Terminalia arjuna* on calcium and phosphate ion uptake (Fig.4.e & 4.f). However on thoracic aorta matrix percentage inhibition on calcium ion uptake (fig.4.g) was less as compared to phosphate ion uptake where 100% inhibition is seen at 8 and 24 hour interval (Fig.4.h).

Continuing with activity guided fractionation of *Terminalia arjuna* bark the effect of methanol extract on calcium and phosphate ion uptake on thoracic aorta matrix was seen. The methanol extract showed increased inhibition on calcium and phosphate ion uptake on extracted thoracic aorta matrix starting from 4 hour interval itself (Fig.4.i & 4.j). 100 % inhibition was achieved at 6 hour interval by methanol extract in both calcium and phosphate ion uptake (Fig.4.k & Fig.4.l).

4.3 Conclusion

The data concludes that both aqueous and methanol *Terminalia arjuna* bark extract is causing inhibition of Ca-P ion uptake on extracted thoracic matrix. However more inhibition in case of methanol extract is achieved. Hence the bark of *Terminalia arjuna* contain potent polar components that inhibits the uptake of Ca-P ions on the extracted thoracic matrix. Further isolation of the active components of *Terminalia arjuna* must be done to know the inhibitors of Ca-P ions that will be very beneficial in the treatment of arteriosclerosis.

4.3. Effect of aqueous extract of *T.arjuna* bark on extracted abdominal aorta matrix mineralization.

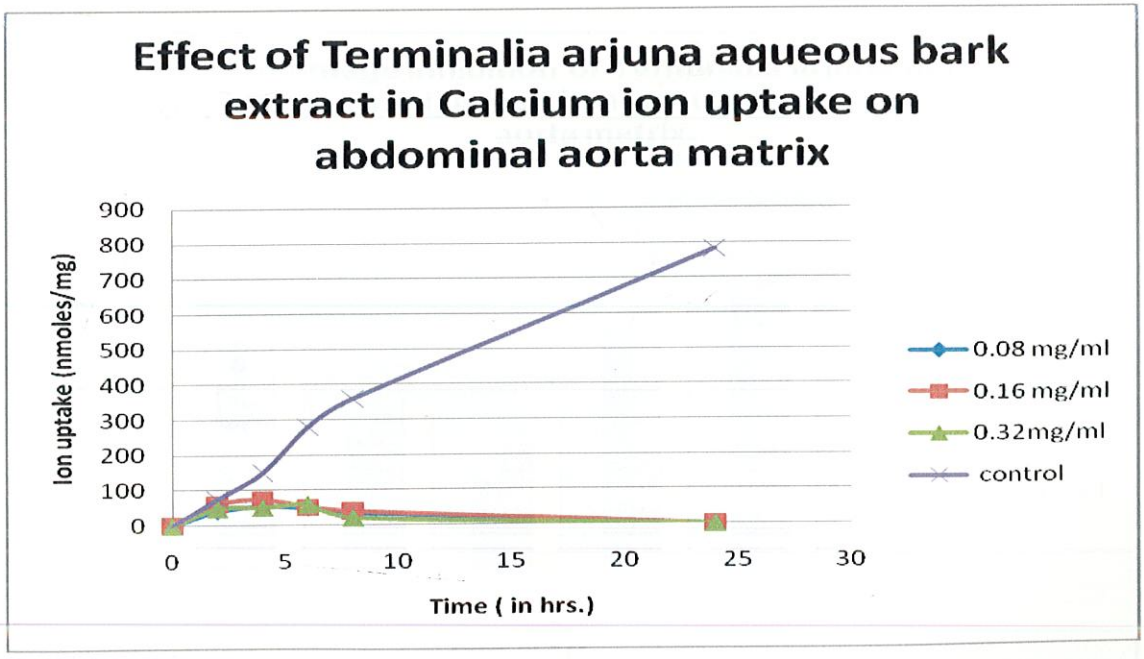


Fig.4.a Effect of different concentration of aqueous extract of *Terminalia arjuna* bark in calcium ion uptake on extracted abdominal aorta matrix.

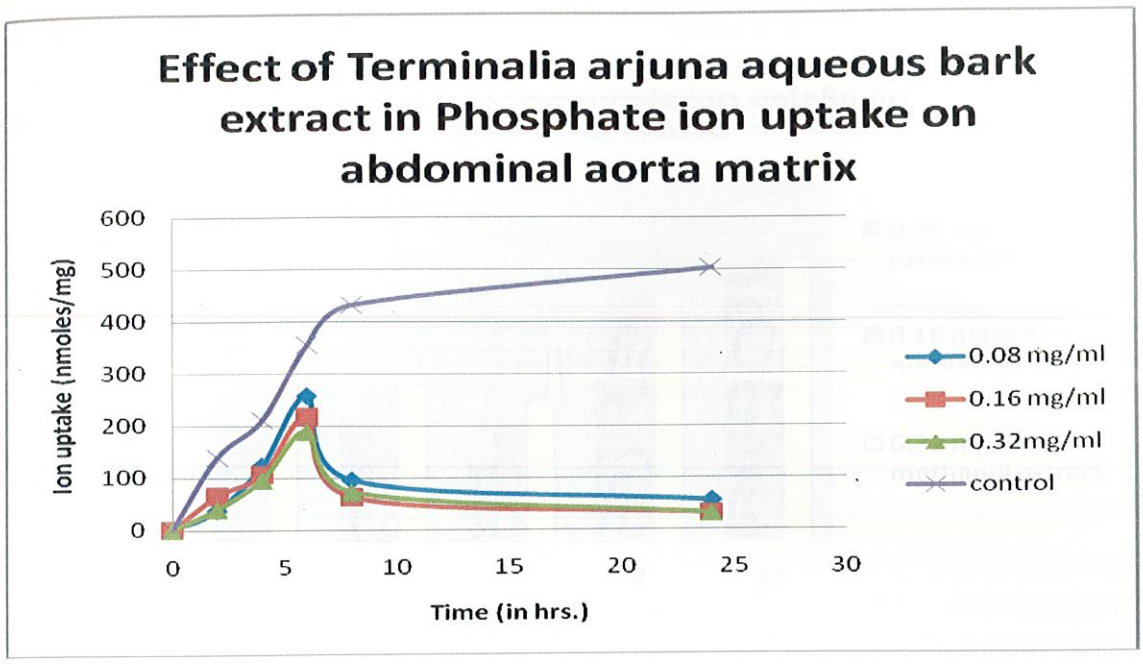


Fig.4.b Effect of different concentration of aqueous extract of *Terminalia arjuna* bark in phosphate ion uptake on abdominal aorta matrix.

4.4 Percentage inhibition of aqueous extract of *T.arjuna* bark on extracted abdominal aorta matrix mineralization.

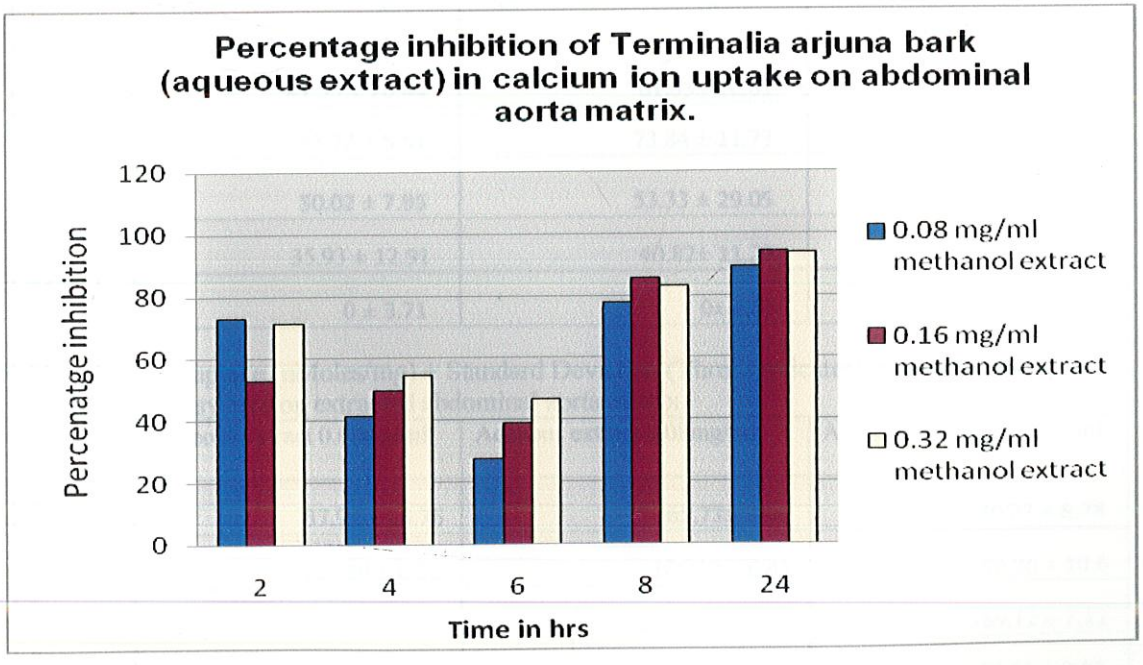


Fig.4.c Percentage inhibition of different concentration of aqueous extract of *Terminalia arjuna* bark in calcium ion uptake on extracted abdominal aorta matrix.

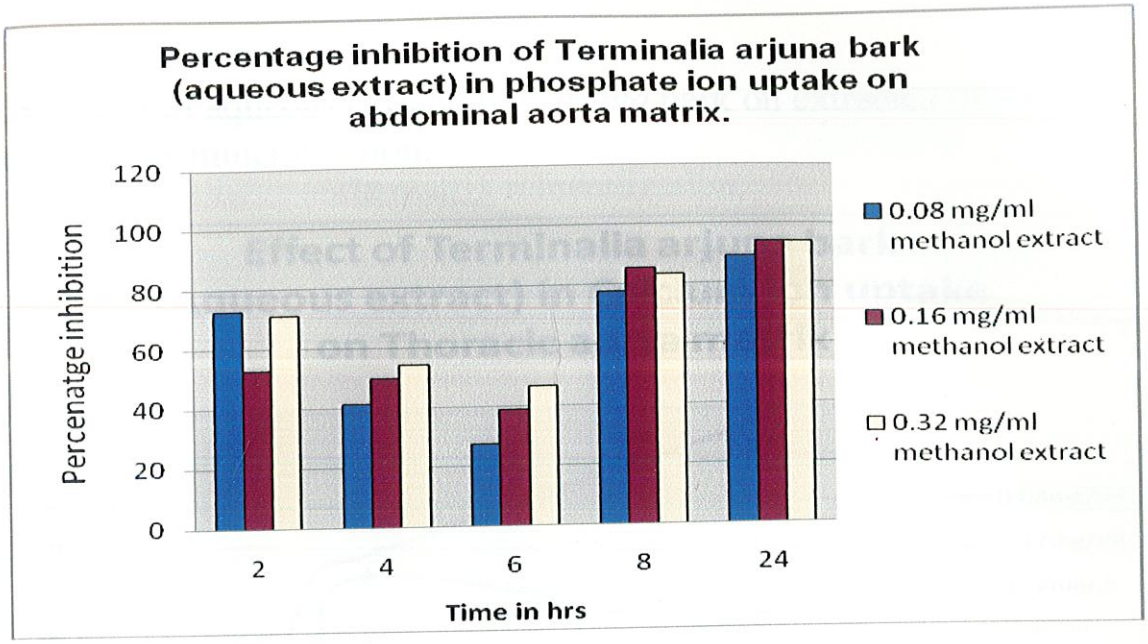


Fig.4.d Percentage inhibition of different concentration of aqueous extract of *Terminalia arjuna* bark in phosphate ion uptake on extracted abdominal aorta matrix.

Table 2. Effect of T.arjuna aqueous extract in Ca-P ion uptake on extracted abdominal aorta matrix.All values are mean ± SD of three replicates.

Calcium ion uptake (nMoles/mg) ± Standard Deviation (Three replicates) in 10 mg/15ml heterogeneous system on extracted abdominal aorta matrix.			
Time in hrs.	Aqueous extract 0.08mg/ml	Aqueous extract 0.16mg/ml	Aqueous extract 0.32mg/ml
2	41.55 ± 20.44	61.53± 10.81	47.76± 11.94
4	55.72 ± 5.51	73.84 ± 11.73	50.38 ± 11.91
6	50.02 ± 7.95	53.33 ± 29.05	59.25 ± 34.24
8	35.93 ± 12.91	40.82± 11.29	21.43 ± 5.14
24	0 ± 3.71	0± 2.86	0 ± 4.27
Phosphate ion uptake (nMoles/mg) ± Standard Deviation (Three replicates) in 10 mg/15ml heterogeneous system on extracted abdominal aorta matrix			
Time in hrs.	Aqueous extract 0.08mg/ml	Aqueous extract 0.08mg/ml	Aqueous extract 0.08mg/ml
2	37.92 ± 16.76	65.73± 3.08	40.23 ± 8.78
4	123.89 ± 4.96	106.19 ± 6.41	96.26 ± 10.6
6	257.90± 4.29	217.44 ± 10.57	189.12 ± 7.12
8	96.08 ± 8.24	63.21 ± 9.26	72.65 ± 8.83
24	55.62± 2.70	30.34± 3.00	31.14± 2.86

4.5. Effect of aqueous extract of *T.arjuna* bark on extracted thoracic aorta matrix mineralization.

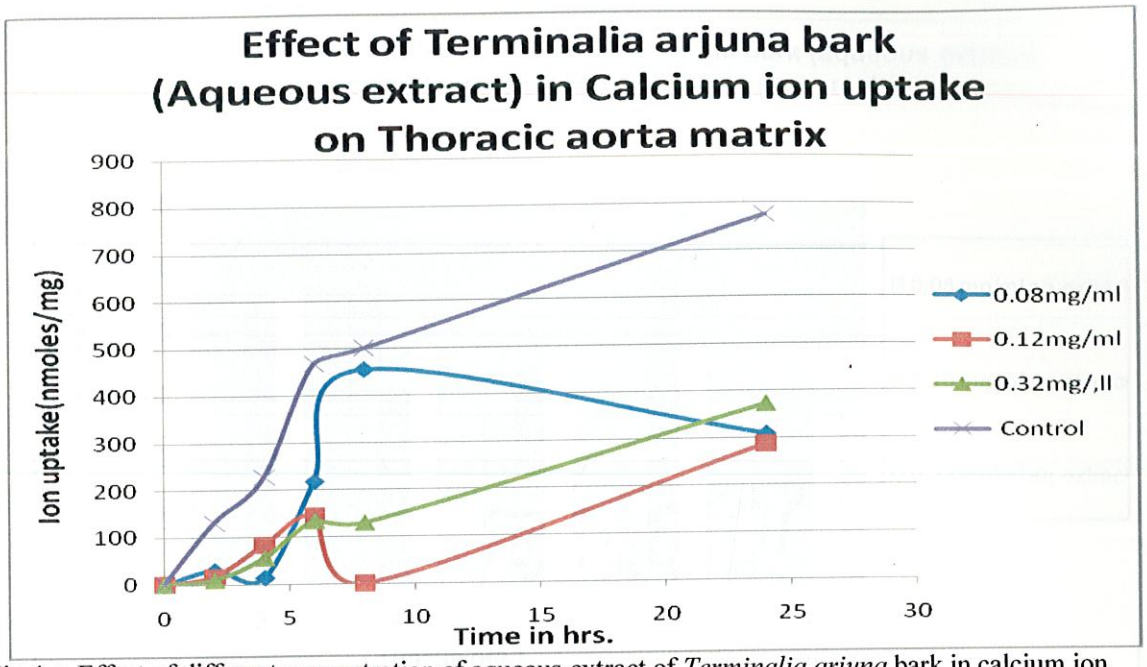


Fig.4.e. Effect of different concentration of aqueous extract of *Terminalia arjuna* bark in calcium ion uptake on extracted thoracic aorta matrix.

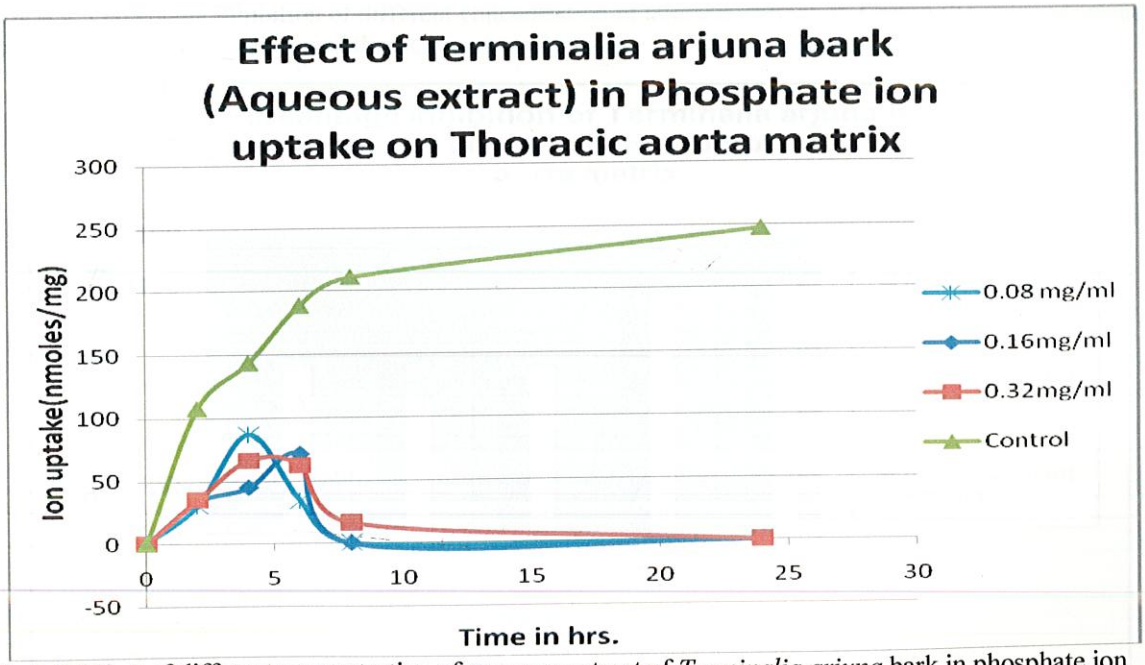


Fig.4.f. Effect of different concentration of aqueous extract of *Terminalia arjuna* bark in phosphate ion uptake on thoracic aorta matrix.

4.6. Percentage inhibition of aqueous extract of *T.arjuna* bark on extracted thoracic aorta matrix mineralization.

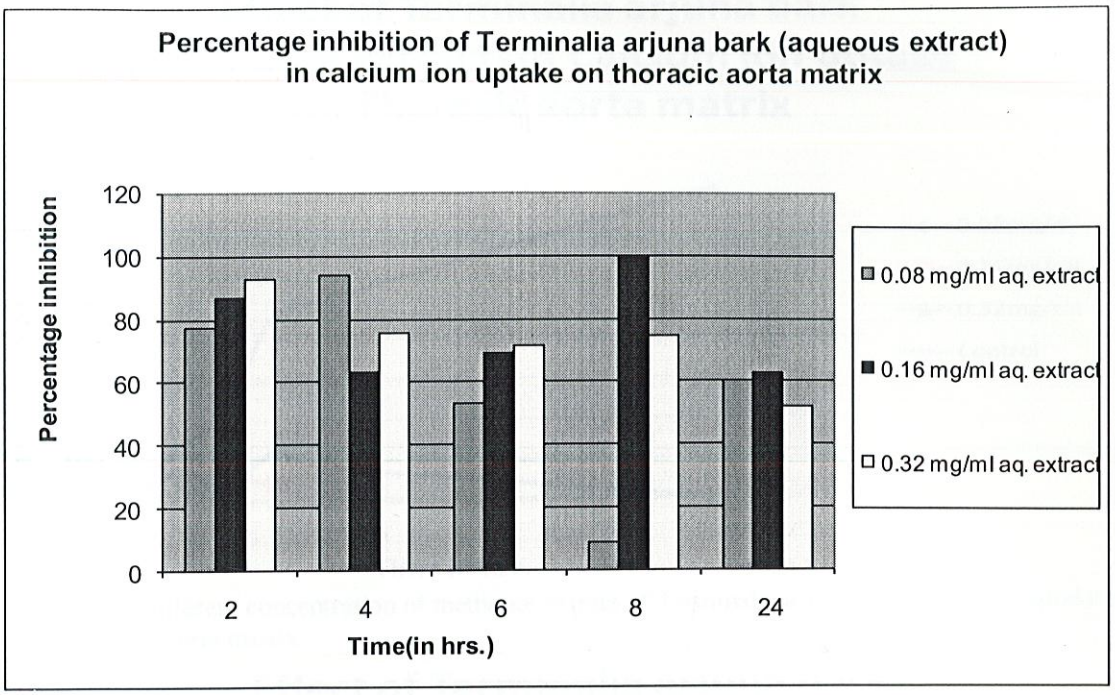


Fig.4.g. Percentage inhibition of different concentration of aqueous extract of *Terminalia arjuna* bark in calcium ion uptake on extracted thoracic aorta matrix.

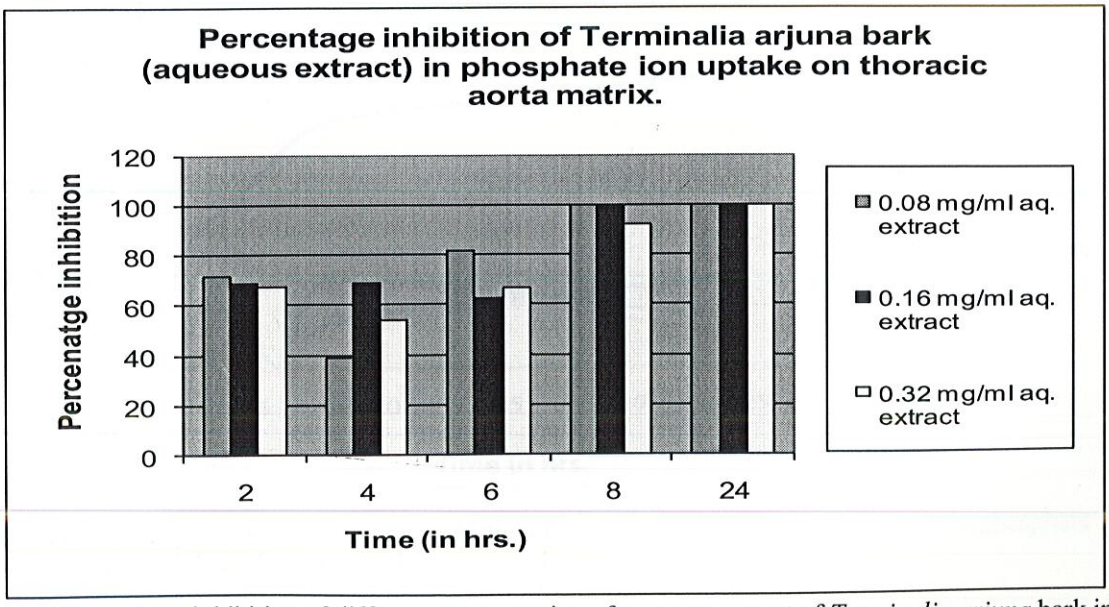


Fig.4.h. Percentage inhibition of different concentration of aqueous extract of *Terminalia arjuna* bark in phosphate ion uptake on thoracic aorta matrix.

4.7. Effect of methanol extract of *T.arjuna* bark on extracted Thoracic aorta matrix mineralisation.

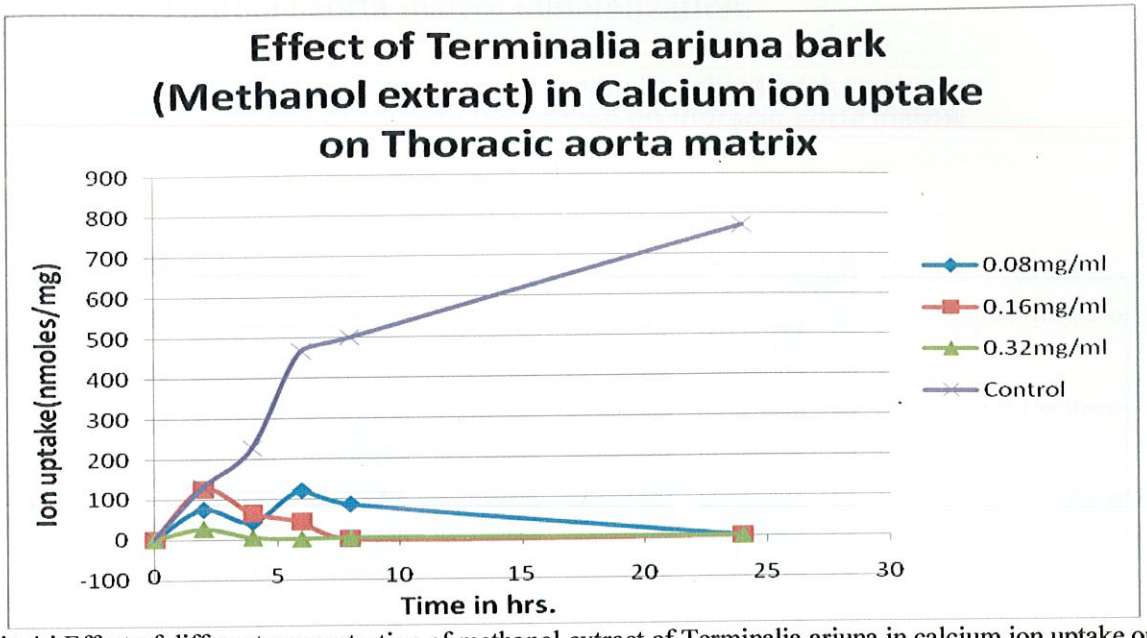


Fig.4.i Effect of different concentration of methanol extract of Terminalia arjuna in calcium ion uptake on extracted thoracic aorta matrix.

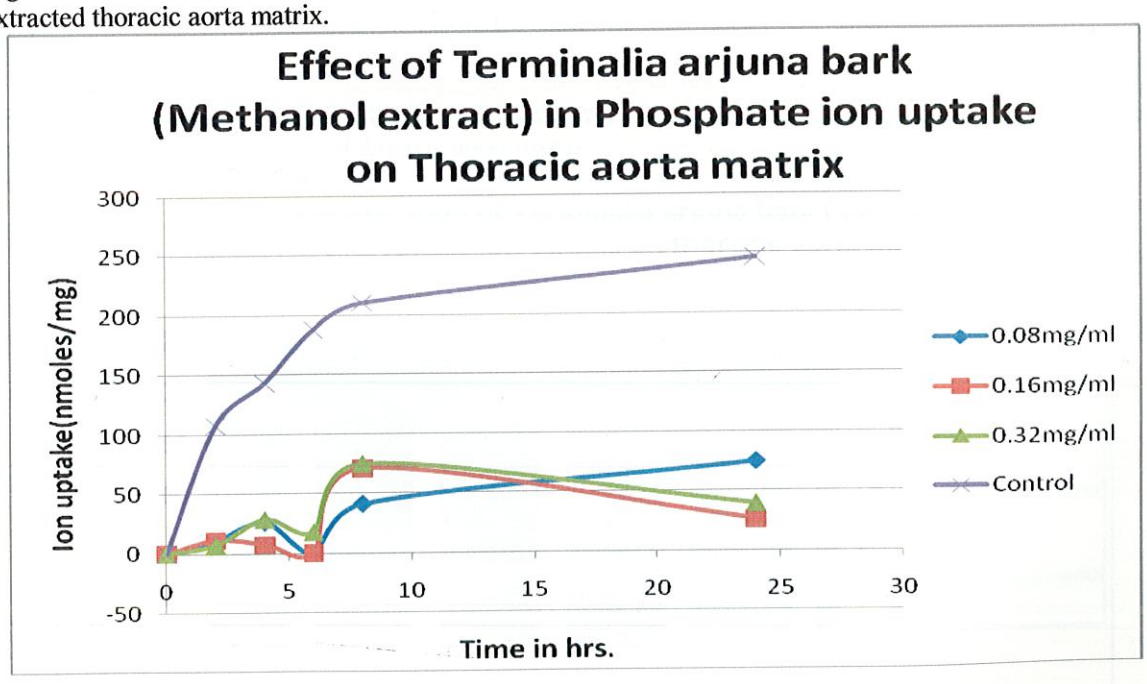


Fig.4.j. Effect of different concentration of methanol extract of *Terminalia arjuna* bark in phosphate ion uptake on extracted thoracic aorta matrix.

4.8. Percentage inhibition of methanol extract of *T.arjuna* bark on extracted Thoracic aorta matrix mineralization.

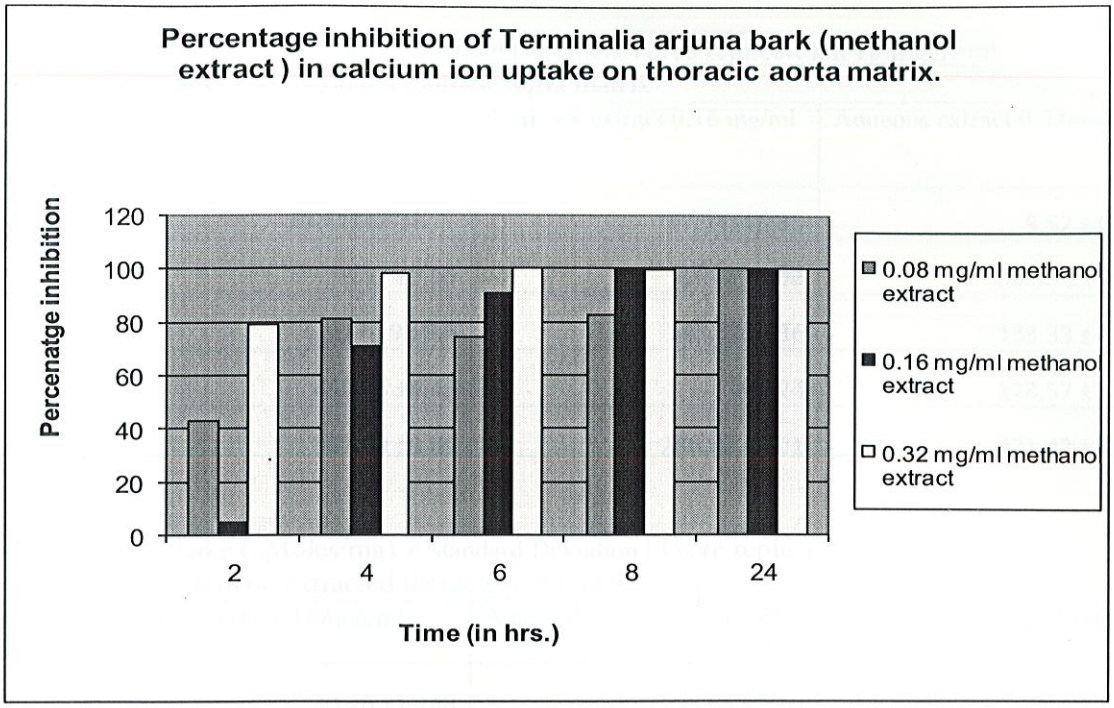


Fig.4.k. Percentage inhibition of different concentration of methanol extract of Terminalia arjuna bark in calcium ion uptake on extracted thoracic aorta matrix.

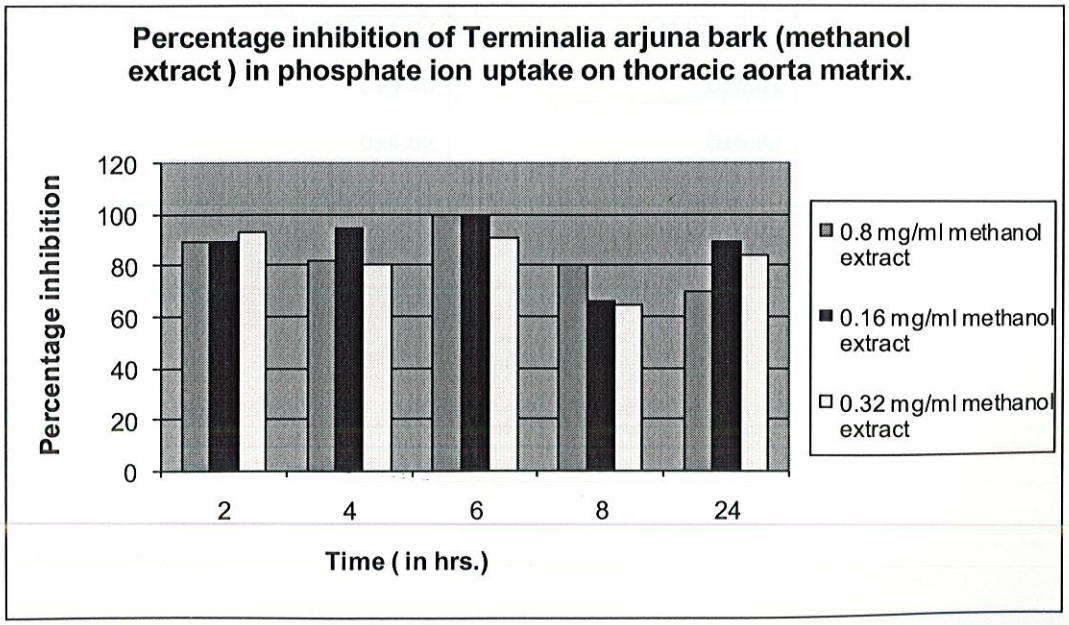


Fig.4.l. Percentage inhibition of different concentration of aqueous extract of *Terminalia arjuna* bark in phosphate ion uptake on extracted thoracic aorta matrix

TABLE 3. Effect of T.arjuna Aqueous extract in Ca-P ion uptake on extracted abdominal aorta matrix.All values are mean ± SD of three replicates.

Calcium ion uptake (nMoles/mg) ± Standard Deviation(Three replicate) in 10 mg/15ml heterogeneous system on extracted thoracic aorta matrix			
Time in hrs.	Aqueous extract 0.08mg/ml	Aqueous extract 0.16 mg/ml	Aqueous extract 0.32mg/ml
2	29.41±12.38	17.11±10.95	9.52 ±12.8
4	13.57 ±9.65	83.42 ±8.32	55.38 ±10.98
6	217.19 ±9.6	143.31±8.36	133.33 ±4.68
8	454.75 ±8.45	0±6.24	128.57 ±9.24
24	307.69 ±3.89	286.63 ±2.01	371.42 ±4.87

Phosphate ion uptake (nMoles/mg) ± Standard Deviation (Three replicate) in 10 mg/15ml heterogeneous system on extracted thoracic aorta matrix.			
Time in hrs.	Aqueous extract 0.08mg/ml	Aqueous extract 0.16mg/ml	Aqueous extract 0.32mg/ml
2	30.26 ±1.248	33.64 ±2.10	35.10 ±2.3
4	86.99 ±5.24	44.85 ±8.45	66.18 ±1.89
6	34.04 ±1.02	71.02 ±2.13	62.28 ±5.21
8	0±3.46	0±4.41	16.18 ±5.7814
24	0±4.62	0±5.83	0±6.5381

TABLE 4. Effect of T.arjuna Methanolic extract in Ca-P ion uptake on extracted thoracic aorta matrix. All values are mean ± SD of three replicates.

Calcium ion uptake (nMoles/mg) ± Standard Deviation (Three replicates) in 10 mg/15ml heterogeneous system on extracted thoracic aorta matrix.			
Time in hrs.	methanol extract 0.08mg/ml	methanol extract 0.16mg/ml	methanol extract 0.32mg/ml
2	74.66±9.24	124.43±10.234	27.14±9.14
4	42.98±7.23	65.61±7.014	4.52±8.2345
6	120.41±9.12	42.98±6.245	0±7.2145
8	85.97±11.023	0±10.523	2.26±9.564
24	0±2.234	0±3.245	0±3.1245
Phosphate ion uptake (nmoles/mg) ± Standard Deviation (Three replicates) in 10 mg/15ml heterogeneous system on extracted thoracic aorta matrix.			
Time in hrs.	methanol extract 0.08mg/ml	methanol extract 0.16mg/ml	methanol extract 0.32mg/ml
2	11.24 ±5.83	11.24±7.45	7.12±5.6
4	26.22±3.65	7.49±4.693	28.50±4.65
6	0±3.1462	0±4.0289	17.81±6.21
8	41.21±4.17	71.19±4.96	74.83±4.68
24	74.94±2.65	26.22±3.49	39.19±3.12

CHAPTER 5

Effect of Buckwheat aqueous extract in Ca-P ion uptake on extracted thoracic aorta matrix preparations

5.1 Components

Two Species of buckwheat plant used:

Source: NBPGR, Phagli, Shimla, H.P.

1. Common Buckwheat (*Fagopyrum esculentum)*
   IC Accession number: 213682
2. Bitter Buckwheat (*Fagopyrum tataricum)*
   IC Accession number: 107895

i. 10 mg/15ml heterogeneous system is used for study.

ii. Standard Calcium and Phosphate methods were used for determining ion uptake on matrices.

iii. Different concentrations i.e 0.08mg/ml, 0.16mg/ml, 0.32mg/ml of aqueous extract of both the mentioned species were used in the same heterogeneous system.

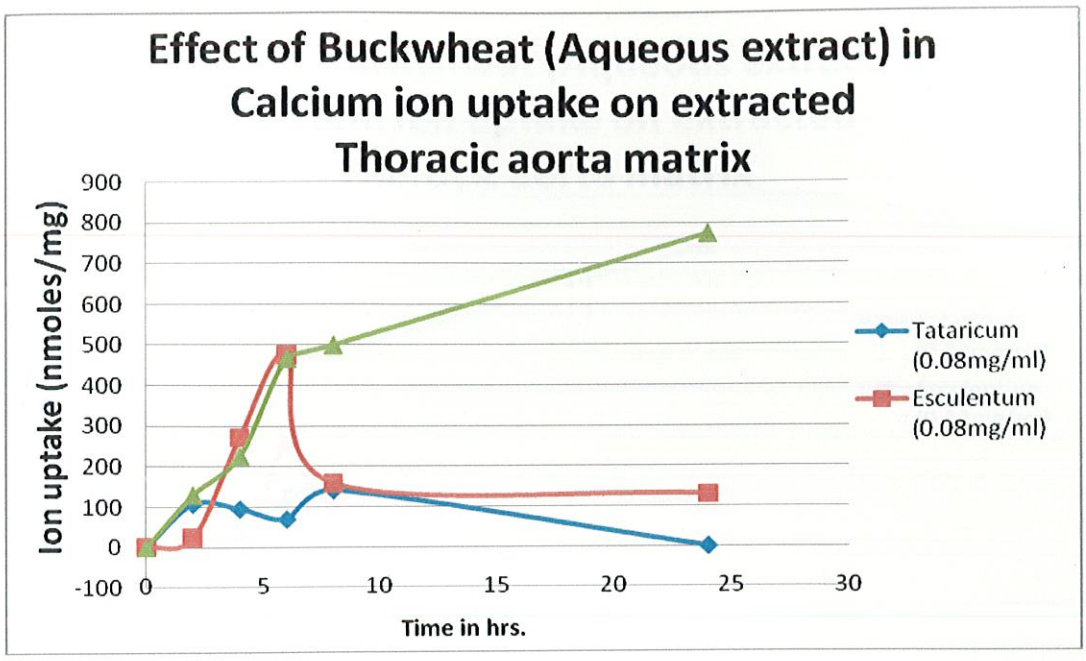


Fig 5.a Comparison of Ca ion inhibiton by *F.tataricum* and *F. esculentum* extracts(0.08mg/ml)

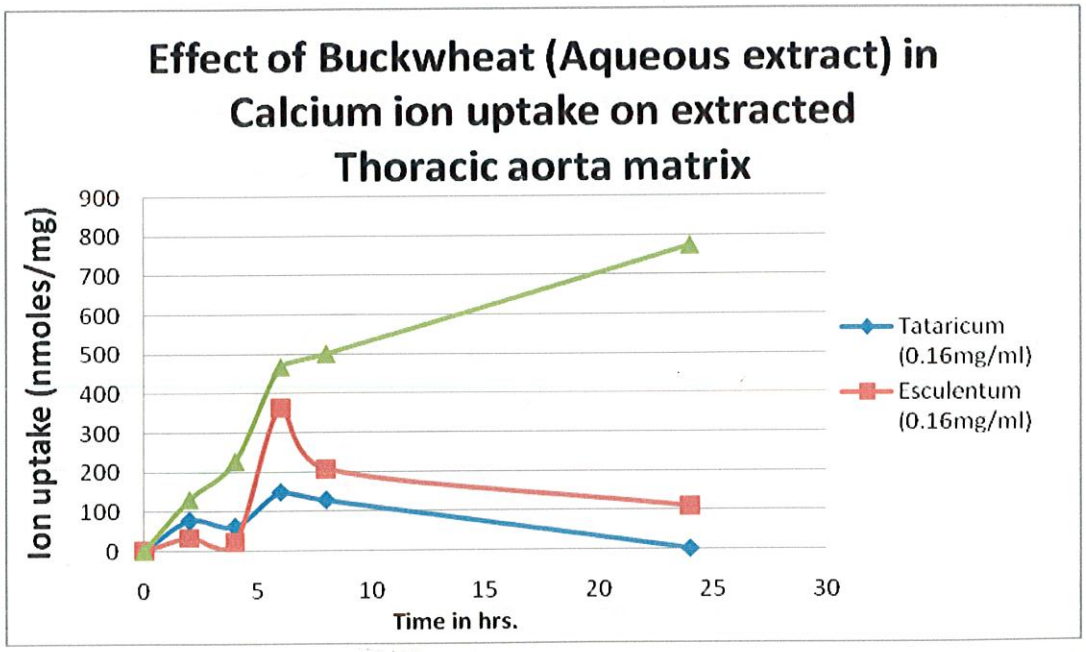


Fig 5.b Comparison of Ca ion inhibiton by *F.tataricum* and *F. esculentum* extracts(0.16mg/ml)

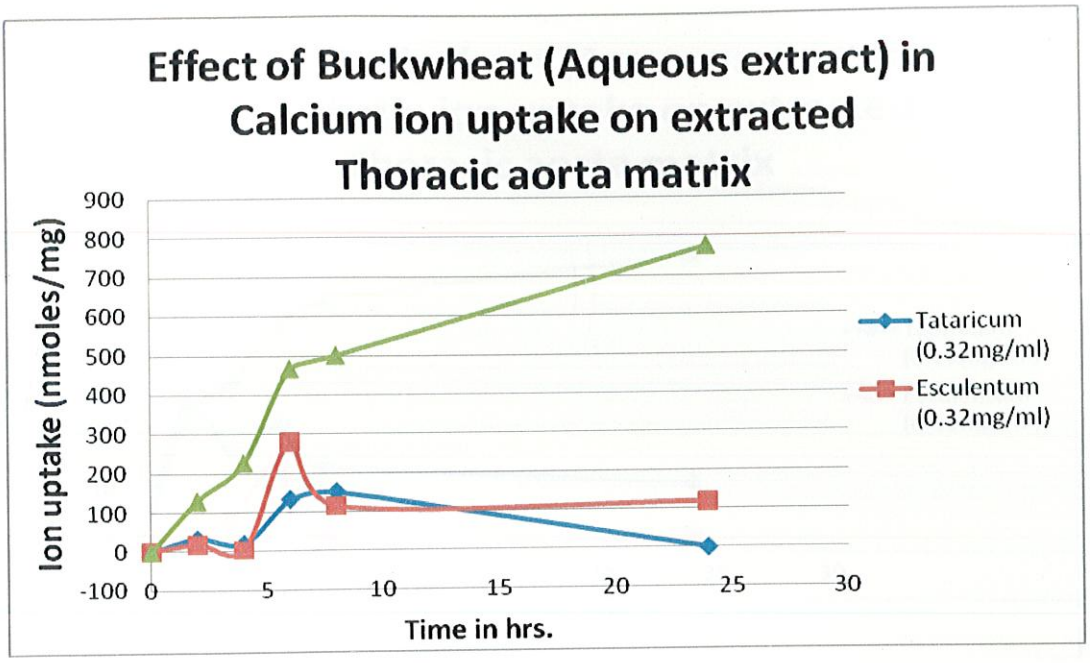


Fig 5.c Comparison of Ca ion inhibiton by *F.tataricum* and *F. esculentum* extracts(0.32mg/ml)

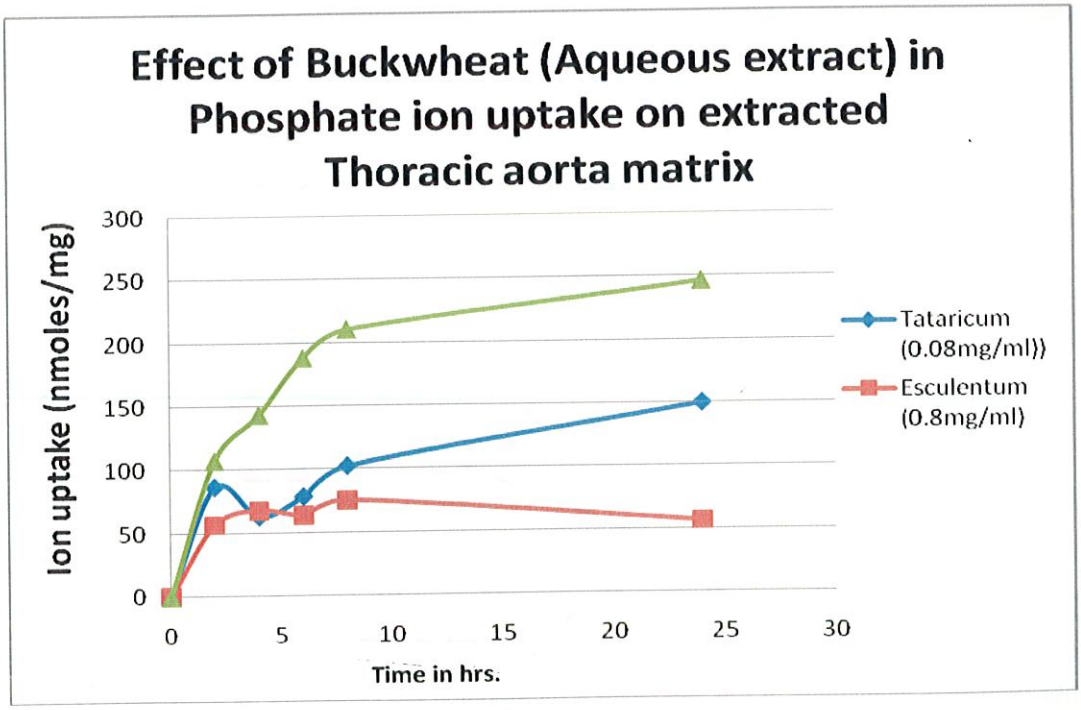


Fig 5.d Comparison of Phosphate ion inhibiton by *F.tataricum* and *F. esculentum* extracts(0.08mg/ml)

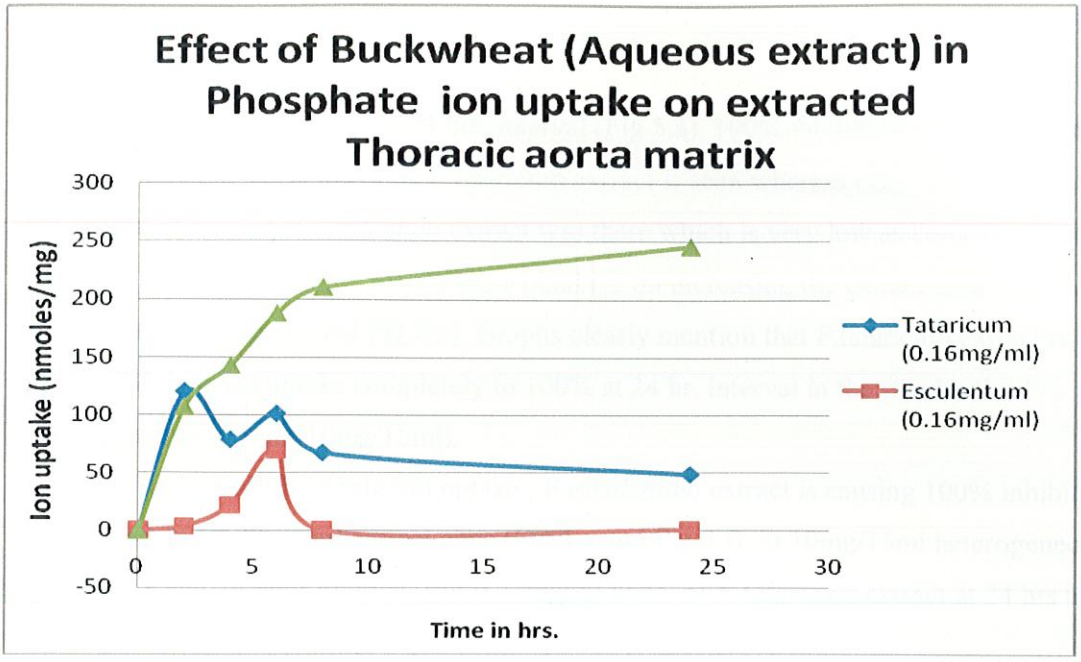


Fig 5.e Comparison of Phosphate ion inhibiton by *F.tataricum* and *F. esculentum* extracts(0.16mg/ml)

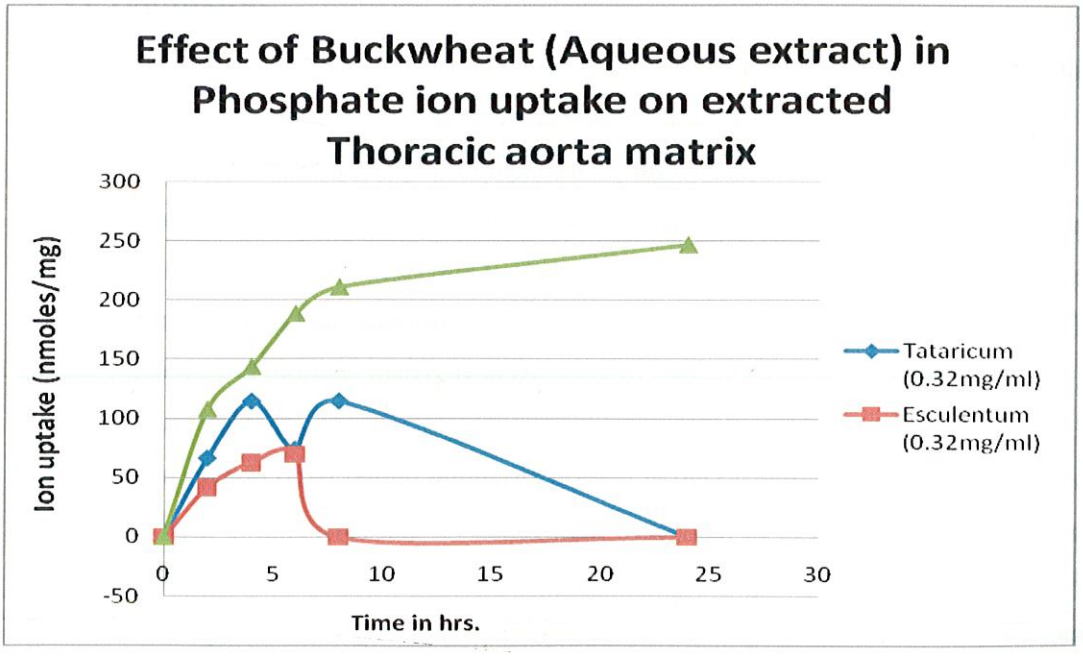


Fig 5.f Comparison of Phospahte ion inhibiton by *F.tataricum* and *F. esculentum* extracts(0.32mg/ml)

5.2 Results and Discussions

In 0.08mg/ml extract system at 24 hrs. interval (Fig 5.a), 100% inhibition of Ca ion uptake on extracted thoracic aorta matix by F.tataricum extract is seen whereas only 129.973 nMoles/mg of ion uptake in case of F.*esculentum* extract was there which is very low as compare to control i.e 773.109nMoles/mg. Similar results were obtained on increasing the extract conc. to 0.16mg/ml and 0.32mg/ml(Fig 5.b and Fig 5.c). Graphs clearly mention that F.tataricum extract is able to inhibit Calcium ion uptake completely to 100% at 24 hr. interval in the standardized heterogeneous system (10mg/15ml).

However in case of Phosphate ion uptake , F.*esculentum* extract is causing 100% inhibition with 0.16mg/ml and 0.32mg/ml extract conc.(Fig 5.e and Fig 5.f) in 10mg/15ml heterogeneous system at 8 and 24 hour interval. But 0.32mg/ml conc. of *F.tataricum* extract at 24 hrs interval is causing 100% inhibition of phosphate ion uptake. (Fig 5.f)

5.3 Conclusion

The data concludes that *F.tataricum* aqueous extract is causing inhibition of Ca ion uptake completely as compare to *F. esculentum* whereas *F. esculentum* is causing inhibition of phosphate ion uptake completely as compare to *F. tataricum.*

The inhibition of Ca-P ions on extracted thoracic matrix by both *F.tataricum* and *F. esculentum* aqueous extracts can be primarily due to presence of Magnesium as one of the constituents (Fig3.). Studies reveal that magnesium is a well known inhibitor of mineralization and Ca-P ion uptake on extracted thoracic matrix is in the form of hydroxyapatite [11], which is a mineralization process.

There can be several other potent inhibitors present in the different solvent polarity based extracts of both *F.tataricum* and *F. esculentum* which can be of great importance in treatment of arteriosclerosis. Activity guided fractionation of active ingredients from both *F.tataricum* and *F. esculentum* species having role in inhibition of Ca-P ion uptake on extracted aorta matrix will provide better understanding of role of buckwheat in arteriosclerosis. Extract preparation of different plant parts of namely leaves, stem and seeds and further experimentation under similar conditions will give better comparison between the two species and their role.

Paper presented in a Conference

Prashant kumar Lathar, Kritika Sharma, Dr.C.Tandon; Oral presentation on paper title "Role of *Terminalia arjuna* bark extract on *in vitro* thoracic aorta calcification"; Symposium on "Natural products in health & disease:Biochemical and molecular mechanisms"; organised by department of biochemistry Panjab University, Chandigarh, March 5-6, 2010. (DST-FIST & UGC-SAP Supported).

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This is to certify that Dr./Mr./Ms. Prashant Kumar Lathar has attended/presented a paper/poster entitled Role of Terminalia arjuna bark ..... in vitro thoracic aorta calcification in the symposium organized by the Department of Biochemistry, Panjab University, Chandigarh.

Dr. Rajat Sandhir
Organizing Secretary

Prof. S. Ojha
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Dr. Rajat Sandhir
Organizing Secretary

Prof. S. Ojha
Chairperson

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