EVALUATING THE ROLE OF *TERMINALIA ARJUNA* IN CALCIFICATION OF AORTA.

BY-

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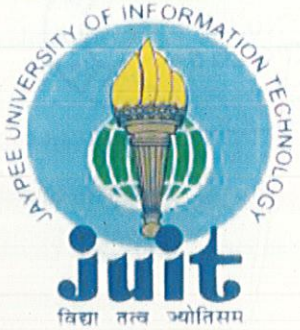
UNDER THE SUPERVISION OF PROF. C. TANDON



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CERTIFICATE

This is to certify that the work entitled, "**Evaluating the role of *Terminalia arjuna* in calcification of aorta**" submitted by Mr. Sushain Puri (071718) and Ms. Aujasvita Janmeja (071720) in partial fulfilment for the award of degree of Bachelor 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.

21/5/11

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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 Prof. Chanderdeep Tandon, Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Waknaghat, Solan-173215, Himachal Pradesh, and has not been submitted for this or any degree or diploma to any other university or institute. All assistance and help received during the course of the investigation has been duly acknowledged.

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SUMMARY

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 homogeneous system showed that with ionic precipitation of 50 mM $CaCl_2$ and 50 mM KH_2PO_4 under physiological conditions of temperature, pH, and ionic strength resulted in the formation of hydroxyapatite and correspondingly the rate of ion uptake for both calcium and phosphate was studied using different concentration of the plant extract. The present study was carried out to evaluate the anti-calcifying properties of *Terminalia arjuna* bark extract. *Terminalia arjuna* 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 was observed at different concentration of extract. A similar pattern was obtained with methanol extract of *Terminalia arjuna*. Also the addition of the ethanolic and aqueous extracts through successive solvent extraction of *Terminalia arjuna* in this assay system was found to inhibit the above mineralization process. These results throw light on medicinal value of *Terminalia arjuna* bark powder in the control of arteriosclerosis, a common cause of coronary heart diseases.

Signature of student

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Signature of Supervisor

Prof. Chanderdeep Tandon

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LIST OF ABBREVIATIONS

Abbreviated terms	Full Form
Ca-P	Calcium-Phosphate
conc.	Concentration
Fig.	Figure
Cm	Centimetres
Mm	Milli Molar
Ml	Milli litres
µg	Micro grams
Mins	Minutes
SD	Standard deviation

CHAPTER 1

INTRODUCTION

1.1 Mineralization

- Mineralization is of common occurrence throughout the animal and the plant kingdoms. It involves the deposition of inorganic ions in the form of a solid mineral phase in or onto the organic matrices. In other words mineralization refers to the process where an organic substance is converted to an inorganic substance. This may also be a normal biological process which takes place during the life of an organism such as the formation of bone tissue or egg shells, largely with calcium, known as calcification.

- Although under physiological conditions, the serum of mammals is known to be supersaturated with respect to mineral phase constituents yet, it is well known that only certain collagenous tissues e.g. bone, teeth etc. get mineralized under physiological conditions, whereas others e.g. tendon, cartilage, aorta, skin etc. get mineralized only under specific pathological conditions.

- In order to explain why all our collagenous tissues do not get mineralized under physiological conditions, so as to turn us into pillars of stones, scientists have been tempted from time to time to assign some of the biomolecules present in body fluids a possible role in the control of this selective tissue mineralization, by acting either as inhibitors or promoters of mineralization. Although during the last 25 years or so, a lot of work has been done on the chemical nature of the physiologically important inhibitors present in body fluids, yet even today it is far from clear what controls the physiological/pathological mineralization at the molecular level.

- Calcification is the process in which calcium salts build up in soft tissue, causing it to harden. Calcification of soft tissue (arteries, cartilage, etc) can be caused by Vitamin K deficiency or by poor calcium absorption due to a high calcium/vitamin D ratio. This can occur with or without a mineral balance.

- Ninety-nine percent (99%) of calcium entering the body is deposited in bones and teeth. The remaining calcium dissolves in the blood.

- When a disorder affects the balance between calcium and certain chemicals in the body, calcium can be deposited in other parts of the body such as arteries, kidneys, lungs, and brain. Calcium deposits in these parts of the body can cause problems with how these blood vessels and organs work. Calcifications can usually be seen on x-rays. A common example is calcium depositing in arteries as part of atherosclerosis. (Jian-su shao *et. al.* 2006)

- Calcifications may be classified on whether there is mineral balance or not.

- **Dystrophic calcification**, without a systemic mineral imbalance. This is where blood levels of calcium are normal, and abnormalities or degeneration of tissues result in mineral deposition. Dystrophic calcification is not associated with metabolic disorder. They have a prevalence of 95-98% out of all soft tissue calcification.

- **Metastatic calcification**, a systemic elevation of calcium levels. This calcification is deposition of calcium salts in otherwise normal tissue, because of elevated serum levels of calcium-phosphate in blood, which can occur because of deranged metabolism as well as increased absorption or decreased excretion of calcium and related minerals. Metastatic calcification is frequently associated with a metabolic disorder.

1.1.1 Hydroxyapaptite

- **Hydroxyapatite**, also called **hydroxylapatite** (HA), is a naturally occurring mineral form of calcium apatite with the formula $\mathbf{Ca_{10}(PO_4)_6(OH)_2}$ to denote that the crystal unit cell comprises two entities. Hydroxyapatite is the hydroxyl end member of the complex apatite group. It crystallizes in the hexagonal crystal system. Pure hydroxyapatite powder is white. Naturally occurring apatite's can, however, also have brown, yellow, or green colorations, comparable to the discolorations of dental fluorosis.

- Upto 50% of bone is made up of a modified form of the inorganic mineral hydroxyapatite (known as bone mineral) and it is also the primary mineral for teeth. Hydroxyapatite crystals are also found in the small calcifications.(Azari F. *et al* 2006)
- Abnormal accumulation of hydroxyapatite can occur in areas of tissue damage, in hypercalcemic or hyperparathyroid states, in chronic renal failure, hyperphosphatemia and in coronary heart disease.
- Hydroxyapatite may be released from exposed bone and cause the acute synovitis occasionally seen in chronic stable osteoarthritis. Hydroxyapatite deposition is also an important factor in an extremely destructive chronic arthropathy of the elderly that occurs most often in knees and shoulder. Joint destruction is associated with attenuation or rupture of supporting structures, leading to instability and deformity.
- In case of atherosclerotic calcification, the hydroxyapatite deposition occurs more commonly in the advanced athermanous plaques, especially in the aorta and the coronaries. Microscopically the calcium salts are deposited in the vicinity of necrotic area and in the soft lipid pool deep in thickened intima.

1.2 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.

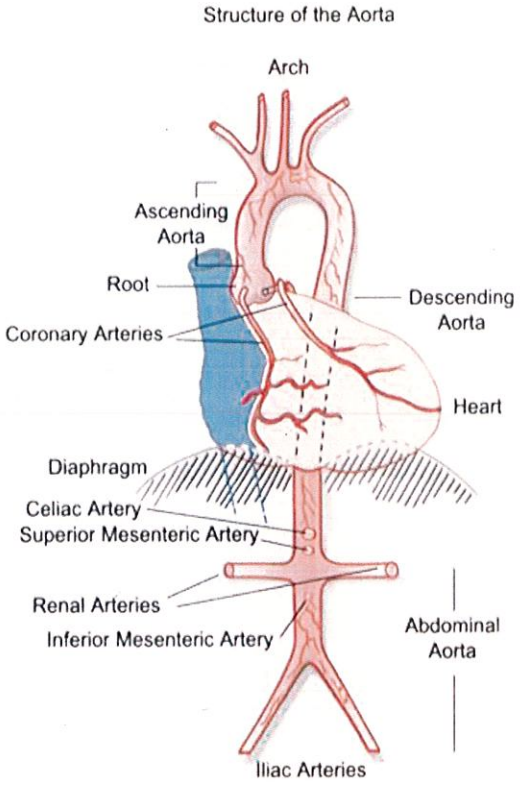

Fig.1 Anterior (frontal) view of the human aorta

1.3 Arteriosclerosis

1.3.1 Definition

- 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*)

1.3.2 Types

1. Arteriolosclerosis is any hardening (and loss of elasticity) of small arteries and arterioles (small arteries). It is often associated with hypertension.
2. 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.
3. 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.
4. 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.

5. 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.

1.3.3 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 (ECGs), 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.

1.4 *Terminalia arjuna*

1.4.1 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 (*Terminalia 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. (Udupa, N., *et al* 1989)

Classification of *Terminalia arjuna*-
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Myrtales
Family: Combretaceae
Genus: Terminalia
Species: *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. 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.

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

The use of bark powder as an astringent and diuretic finds mention in the works of Charak. 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. Subsequently, Chakradutta and also Bhawa Mishra, described its use in chest pain. Traditional method of its administration was to prepare an alcoholic decoction of its bark stem or give it along with clarified butter or along with boiled milk .(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.

1.4.2 Research

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

1. 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. (Bharani A. *et al.*, 2002)
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 decreased from 15.8-25.6%, and lipid peroxide levels decreased significantly.(Gupta R, *et al.*, 2001)
3. In an open study of ten patients with stable angina, *T.arjuna* treatment resulted in an 80% of patients having symptomatic relief.
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. (Dwivedi S. *et al.*,1997)
5. 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. (Dwivedi S. *et al.*,1994) Safety was demonstrated in all studies.

1.4.3 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)

1.5 Bioactivity guided successive solvent extraction of plants

The separation of materials of different chemical types and solubilities by selective solvent action; that is, some materials are more soluble in one solvent than in another, hence there is a preferential extractive action. This is known as solvent extraction. When this process of extraction is repeated successively using different polarity solvents it is known as successive solvent extraction. (S.Sarker - Natural product isolation)

Extraction Procedure

The typical extraction process, especially for plant materials incorporates the following steps-

1. Drying and grinding of plant material or homogenizing fresh plant parts.
2. Choice of solvents—
    a) Non polar extraction
    b) Medium polar solvent extraction
    c) Polar solvent extraction
3. Choice of extraction method

Choice of Solvents—

Solvents differ in their polarity, polarity index, is a relative measure of the degree of interaction of the solvent with various polar test solutes. A numerical index is proposed that ranks solvents according to their polarity. It is based entirely on structure, encoding the relative content of electrons in the molecule. The index is modified for the number of isolated functional groups in the molecule.

There are 3 polarity strength solvents and they are –

- **Low Polar Solvents/ Non polar solvents** – Petroleum ether, Hexane, Chloroform, etc.
- **Medium Polar Solvents** – Dichloromethane, acetone, Methane.
- **High Polar Solvents** -- Water, Aqueous acids bases, aqueous alkali.

In a selective extraction, the plant material is extracted using a solvent of an appropriate polarity following the principle of "like dissolves like".

- **Non polar solvents** solubilize – lipophilic compounds – alkanes, fatty acids, pigments, waxes, sterols, some terpenoids.
- **Medium polarity solvents** solubilize – alkaloids, flavonoids.
- **Polar solvents** solubilize – flavonoid glycosides, tannins, some alkaloids.

Extraction Methods

A range of methods using organic and aqueous solvents are employed in the extraction of natural products. Solvent extraction relies on the principle of either liquid-liquid or solid-liquid extraction. Solid-liquid extraction is the most commonly used solvent extraction method. In this method, the plant material is placed in contact with a solvent. While the whole process is dynamic, it can be simplified by dividing it into different steps.

Different methods used for solid-liquid solvent extraction are -

- Maceration
- Ultrasound-assisted solvent extraction
- Percolation
- Soxhlet extraction
- Pressurized solvent extraction
- Extraction under reflux and steam distillation

Maceration

Maceration is a simple and widely used method, which involves leaving the pulverized plant to soak in a suitable solvent in a closed container at room temperature. Occasional or constant stirring of the preparation can increase the speed of the extraction.

The extraction stops when equilibrium is reached between the concentration of the metabolites in the extract and that in the plant material. After extraction, the residual plant material has to be separated by decanting, which is usually followed by a filtration step. Centrifugation may be needed if the powder is too fine.

Ultrasound Assisted Solvent Extraction

This is a modified maceration in which the extraction is facilitated by the use of ultrasound (high frequency pulse- 20 kHz).The plant powder is placed in a vial, which is then placed in an ultrasonic bath, and ultrasound is used to induce a mechanical stress on the cells through the production of cavitations in the sample. This increases the solubilization of metabolites in the solvent and improves the extraction. The efficiency of the reaction depends on the instrument's frequency, length and temperature of sonication. Ultrasonification is rarely applied to large scale extraction; it is commonly applied to facilitate the extraction of intracellular metabolites from plant cell cultures.

Percolation

In percolation, the powdered plant material is soaked initially in a solvent percolator (container with a tap at the bottom).Additional solvent is then poured on top of the plant material and allowed to percolate dropwise out of the bottom of the percolator. Additional filtration of the extract is not required because there is a filter at the outlet of the percolator. Successive percolations can be performed to extract the plant material exhaustively by refilling the percolator with fresh solvent and pooling all extracts together.

Soxhlet extraction

Soxhlet extraction is a convenient and widely used extraction method, used for plant metabolite extraction. The plant powder is placed in a cellulose thimble in an extraction chamber, which is placed on top of a collecting flask beneath a reflux condenser. A suitable solvent is added to the flask, and the set up is heated under reflux. When a certain level of condensed solvent has accumulated in the thimble, it is siphoned into the flask beneath.

ADVANTAGES—The main advantage is that it is a continuous process. Also Soxhlet extraction is less time and less solvent consuming than maceration or percolation.

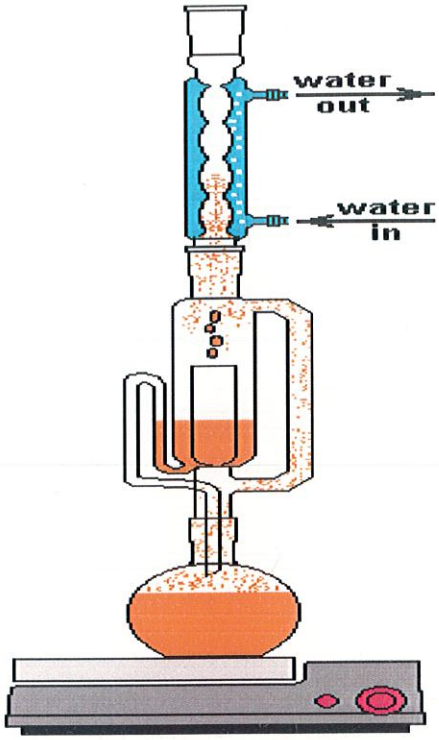

Fig 3. Soxhlet extraction apparatus

DISADVANTAGES—The major disadvantage is that the extract is constantly heated at the boiling point of the solvent used, and this damages the thermo-labile compounds and initiate the formation of artifacts.

Pressurized solvent extraction

It is also known as 'accelerated solvent extraction'. This employs high pressure to maintain the solvent in a liquid state at high temperatures. The powdered extract is loaded into an extraction cell, which is placed in an oven. The solvent is then pumped from a reservoir to fill the cell, which is heated and pressurized at programmed levels for a set period of time. The cell is flushed with nitrogen gas and the extract which is automatically filtered, is collected in a flask. Fresh solvent is used to rinse the cell and to solubilise the remaining components. A final purge with nitrogen gas is performed to dry the material. As the material is dried thoroughly after extraction, it is possible to perform repeated extractions with the same solvent or successive extractions with solvents of increasing polarity.

Extraction under reflux and steam distillation

In extraction under reflux, plant material is immersed in a solvent in a round-bottomed flask, which is connected to a condenser.. The solvent is heated until it reaches its boiling point. As the vapor is condensed, the solvent is recycled in the flask. Steam distillation is a similar process and is commonly applied to the extraction of plant essential oils. The plant is covered with water in a flask connected to a condenser. Upon heating, the vapors condense and the distillate is collected in a graduated tube connected to the condenser. The aqueous phase is re-circulated into the flask, while the volatile oil is collected separately.

Among all these extraction methods, we used the maceration method to obtain the extracts of *Terminalia arjuna.*

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.

2.1Homogeneous System of Mineralization

$CaCl_2$ (50mM), KH_2PO_4 (50mM), Tris buffer and 210mM $NaCl_2$ (0.1Mm with pH=7.4) and distilled water were added in the glass test tubes and the tubes were incubated at 37°C for 30 min. After incubation, the tubes were centrifuged at 4,000 rpm for 20 min to separate the precipitates. Each precipitate was then dissolved in 5 ml of 0.1 N HCl. Calcium and phosphate estimation was done. (Tandon CD *et al.*, 1997)

2.2 Calcium and Phosphate Estimation:

2.2.1Calcium Estimation – Trinder Method

0.1ml of Sample is taken, and 2.5 ml of calcium reagent (yellow) is added. Wait for 1hr. Then setup centrifuge for 20 mins at 4500 rpm. Supernatant was immediately discarded, such that orange precipitate on wall of centrifuge tube does not drain. 1 ml. EDTA + NaOH is added and boiled for 10 mins, at 100°C. Then 3 ml. Color reagent was added. Shake well so that orange precipitate dissolves and take O.D at 450 nm.

2.2.2 Phosphate estimation – Gomori Method

0.1 ml of sample was taken. 0.24 ml of 2.5% ammonium molybdate and 10N H_2SO_4 mixed in ratio 10:4 respectively were added to each test tube. Wait for 10 mins then 3ml Distilled water was added. Next 0.1ml Metol reagent was added. This setup was kept for 30 mins and O.D at 660 nm.

2.3 Preparation of solvent extracts:

(Tandon CD *et al.*, 1997; *R Chander *et al.*, 2004)

2.3.1 Terminalia arjuna Methanolic extract:

Finely powdered stem bark (100 g) was extracted with 50% methanol in the cold for 72 hours. 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. A dark brownish/red shiny crystal like residue was obtained.

2.3.2 *Terminalia arjuna* aqueous extract:

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

2.3.3 *Terminalia arjuna* successive ethanol extract

Finely powdered stem bark (100gm) was extracted with 500ml of 95% ethanol (spirit) and incubated at 37 ± 1 °C for overnight. Next day the alcoholic extract was filtered and concentrated to a dry mass by using evaporation in an oven at 60°C to obtain the dry mass. A dark brown shiny residue was obtained.

2.3.*4Terminalia arjuna* successive aqueous extract

The residue obtained after filtration of the ethanol extract, was successively extracted with a higher polarity solvent i.e. 500ml of distilled water. This setup was again incubated at 37 ± 1 °C for overnight. The aqueous extract was then filtered and concentrated to a dry mass by using evaporation in an oven at 60°C. A dark brown residue was obtained.

2.4 Qualitative Tests

All the four extracts – methanol, aqueous, successive ethanol and successive aqueous of *Terminalia arjuna* bark were qualitatively tested for the presence of secondary metabolites, mainly saponins, flavonoids, tannins, alkaloids and terpenoids.

The following tests were used to test the secondary metabolites presence.

- ***Test for Flavonoids:*** (NaOH\HCl test by DN Onwukaeme *et al.,* 2007) Water extract of the sample was reduced to dryness on the boiling water bath. The residue was treated with dil. NaOH, followed by addition of dilute HCl, solubility and colour was noted. A yellow solution with NaOH, which turns colorless with dil. HCl confirms flavonoids.

- ***Test for Alkaloids:*** (Dragondroff 's test by Adegoke AA *et al.*, 2010) About 0.5 g of the extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. A few drops of Dragendorff's reagent were used to treat 1 ml of the filtrate. Turbidity or precipitation with this reagent was taken as evidence for the presence of alkaloids.

- ***Test for Saponins:*** (Foaming test by sazada siddiqiu *et al.*, 2009) To 5 ml. of extract, add water and shake vigorously, then set aside for two minutes. If frothing occurs and stays for some time then presence of Saponins is confirmed. Also, to 5 ml. of solution of the extract add 1 ml. ammonia solution and 1 ml. lead acetate solution. Appearance of white precipitate confirms the presence of Saponins.

- ***Test for Tannins:*** (Ferric chloride test by P. S. Das and A. D. Talukdar, 2010) To 5 ml. of solution of the extract, 1 ml.of 10% potassium dichromate solution is added. Appearance of Yellowish –brown precipitate confirms the presence of tannins. Also, to 5 ml of the extract, 1 ml.10% lead acetate solution is added. Appearance of yellow precipitate confirms the presence of tannins.

- ***Test for Terpenoids***: (Salkowski test by H.O. Edeoga *et al.*, 2005) Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of Terpenoids.

CHAPTER 3

STANDARDIZATION

3.1 Calcium Standard

Calcium estimation protocol was used to obtain a calcium standard curve. Varying concentration of $CaCl_2$ from 0.5mM to 10Mm were used and respective absorbances were recorded.

The graph was plotted Conc. *vs* Absorbance.

CALCIUM CONCENTRATION (mM)	MEAN ABSORBANCE	STANDARD DEVIATION
0.5	0.127	0.02
1	0.244	0.06
2	0.301	0.11
3	0.383	0.13
5	0.C585	0.16
10	0.776	0.22

Table 1. Calcium standardization

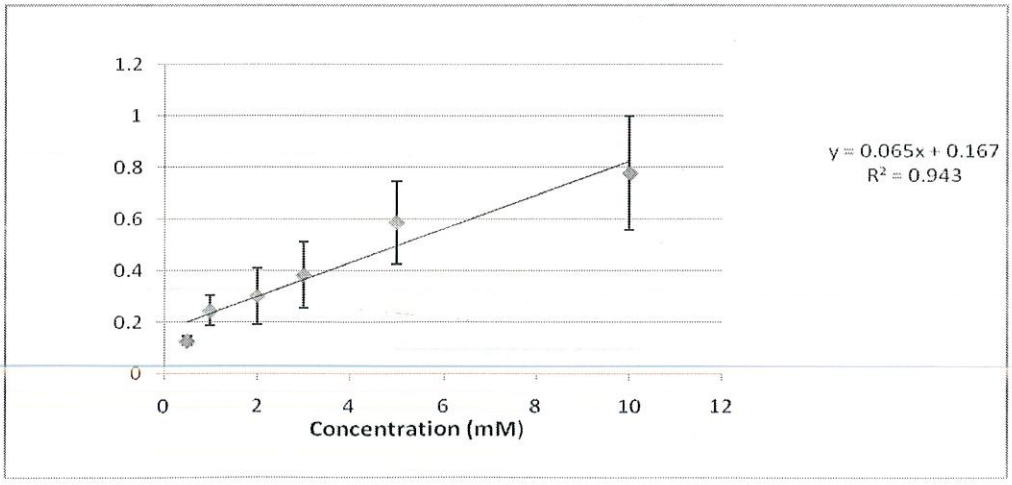

Fig 4. Calcium standard plot. Conc. *vs* Absorbance

3.2 Phosphate Standard

Phosphate estimation protocol was used to obtain a phosphate standard curve. Varying concentration of KH_2PO_4 from 0.5mM to 10Mm were used and respective absorbances were recorded.

The graph was plotted Concentration *vs* Absorbance.

PHOSPHATE CONCENTRATION	MEAN ABSORBANCE	STANDARD DEVIATION
0.5	0.048	0.02
1	0.096	0.04
2	0.197	0.05
3	0.299	0.07
5	0.509	0.10
10	0.991	0.07

Table 2. Phosphate Standardization

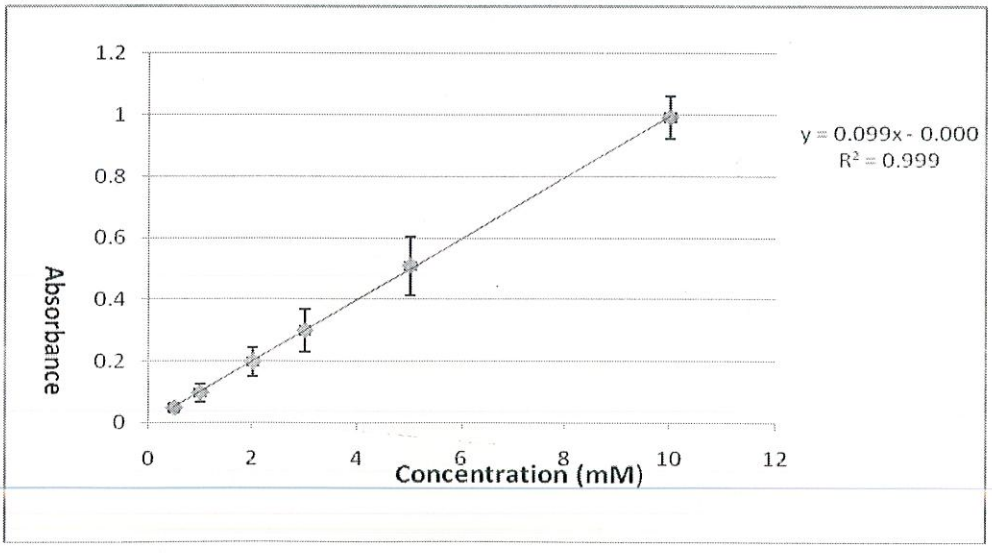

Fig 5. Phosphate Standard Plot. Conc.*vs* Absorbance

3.3 *In vitro* Mineralization of Calcium and Phosphate

Mineralization system i.e. 5ml homogeneous system already standardized in our laboratory was used to study the extent of *In vitro mineralization.*

Ca-P concentrations were obtained, using these concentrations, **calcium: phosphate ratio** was calculated.

SAMPLE	CONCENTRATION OF CALCIUM	CONCENTRATION OF PHOSPHATE	RATIO
A	2.48	1.683	1.474
B	1.961	1.274	1.539
C	1.981	1.2882	1.538

MEAN RATIO = 1.517

Table 3. *In vitro* mineralization of calcium phosphate

- This value of **1.517** of calcium phosphate concentration ratio was acceptable, as it is closer to **1.67** which is the actual calcium phosphate ratio in **human body**.
- This 1.67 value comes from hydroxyapatite.
- **[Ca]/[PO4] = 10/6 = 1.67**

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1Role of *Terminalia arjuna* in biomineralisation

Aqueous and Methanolic extract of *Terminalia arjuna* bark and the successive ethanolic and aqueous extract of Terminalia arjuna were used to study the biomineralisation.

4.1. 1. Components

Bark of *Terminalia arjuna* used.

Source: Natural remedies, Bangalore, India.

1. 5ml homogeneous system is used for study.
2. Standard Calcium and Phosphate methods were used for determining ion uptake inhibition.
3. Different dose concentrations i.e. 40ug/ml, 80ug/ml, 160ug/ml & 240ug/ml of aqueous, methanol, successive ethanol and successive aqueous extract of *Terminalia arjuna bark* were used in the same homogeneous system.

4.2 Results and Discussions

The methanol extract of *Terminalia arjuna* shows significant calcium and phosphate ion uptake inhibition. The maximum percentage inhibition on calcium ion uptake is 90.43% with 240 µg/ml as the dose conc.(Fig.6). The maximum percentage inhibition on phosphate ion uptake is 37.34% with 240µg/ml dose conc.(Fig.7).

The aqueous extract of *Terminalia arjuna* show significant calcium and phosphate ion uptake inhibition but lesser than that shown by methanolic extract. The maximum percentage inhibition of aqueous extract on calcium ion uptake is 83.65% (Fig.8) and that by phosphate ion uptake with 240 µg/ml dose conc. is 35.47% (Fig.9).

Similar inhibition pattern was seen when bioactivity guided successive solvent extracts of *Terminalia arjuna* were used to study the percentage inhibition. The successive ethanol extract also show significant percentage inhibition on calcium and phosphate ion uptake.

The maximum percentage inhibition of successive ethanol extract on calcium ion uptake is and that of phosphate ion uptake is 90.79% with 240 µg/ml dose conc. and 38.28% with 240µg/ml respectively (Fig10 &Fig.11).The successive aqueous extract of *Terminalia arjuna* shows significant percentage inhibition on calcium and phosphate ion uptake as 83.51% and 34.46% respectively (Fig.12 & Fig.13).

Qualitative tests for secondary metabolites of all the extracts inferred the presence of saponnins, alkaloids, terpenoids, tannins and flavonoids.

4.2.1 Percentage inhibition shown by methanol extract of *T.arjuna* bark on calcium ion uptake.

DOSE CONC. (µg/ml)	% INHIBITION – I	% INHIBITION - II	% INHIBITION - III	MEAN INHIBITION ± STANDARD DEVAITION
40	27.96	31.93	32.8	30.90 ± 2.58
80	44.3	50.72	45.03	46.69 ± 3.52
160	62.87	69.92	62.07	64.95 ± 4.32
240	90.29	94.93	86.07	90.43 ± 4.43

Table 4. Percentage inhibition shown by methanol extract on calcium ion uptake

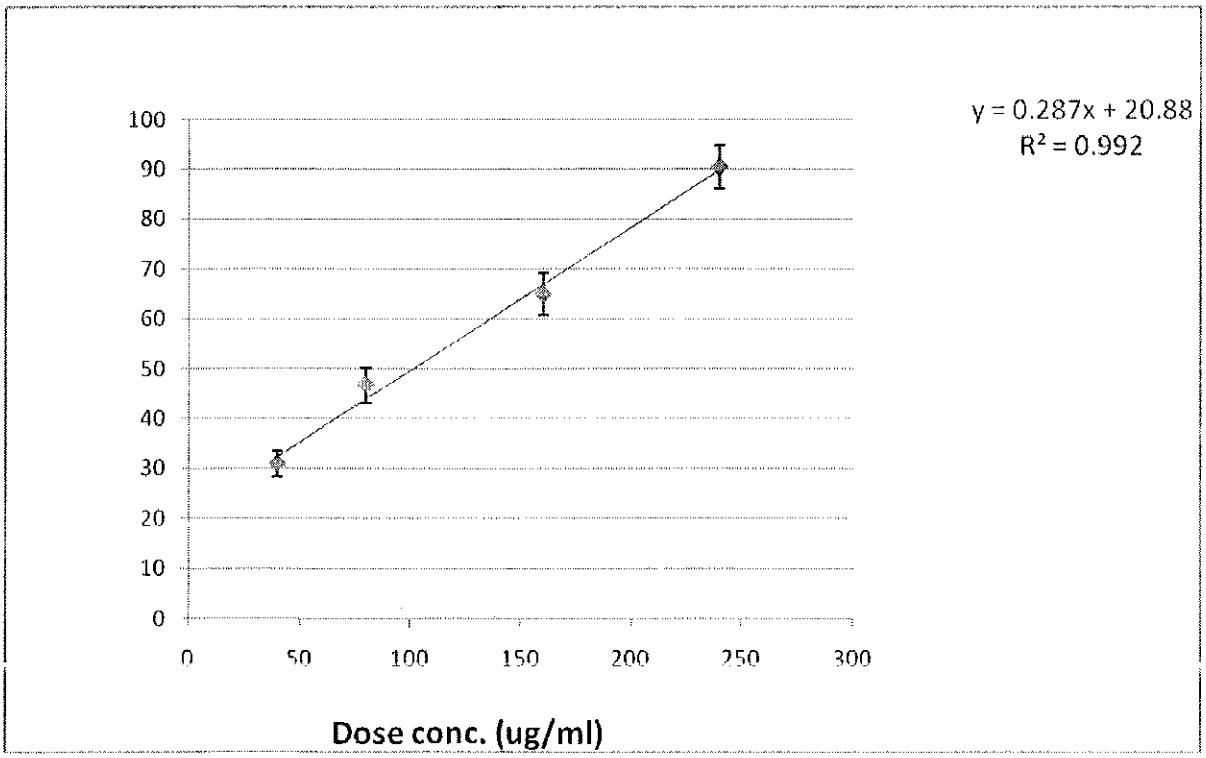

Fig 6. Methanol extract percentage inhibition on calcium ion uptake plot, Dose conc. *vs* Percentage Inhibition

4.2.2 Percentage inhibition shown by methanol extract of *T.arjuna* bark on phosphate ion uptake.

DOSE CONC. (µg/ml)	% INHIBITION - I	% INHIBITION - II	% INHIBITION - III	MEAN INHIBITION ± STANDARD DEVIATION
40	18.62	17.3	14.48	16.8 ± 2.12
80	23.99	24.22	19.47	22.56 ± 2.68
160	27.31	30.55	26.74	28.2 ± 2.06
240	38.41	37.93	35.68	37.34 ± 1.46

Table 5. Percentage inhibition shown by methanol extract on phosphate ion uptake

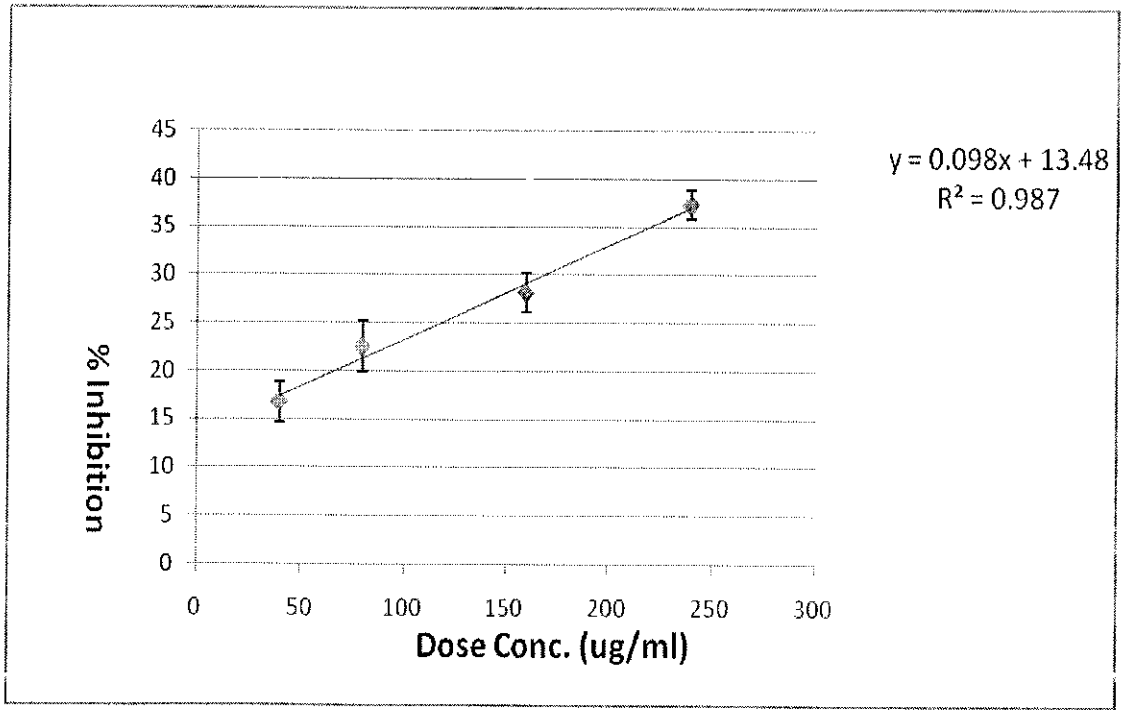

Fig 7. Methanol extract percentage inhibition on phosphate ion uptake plot, Dose conc. *vs* Percentage Inhibition

4.2.3 Percentage inhibition shown by aqueous extract of *T.arjuna* barks on calcium ion uptake.

DOSE CONC. (µg/ml)	% INHIBITION – I	% INHIBITION - II	% INHIBITION - III	MEAN INHIBITION ± STANDARD DEVIATION
40	22.03	24.07	24.3	23.47 ± 1.25
80	37.55	38.98	34.41	36.98 ± 2.34
160	61.16	58.98	56.99	59.04 ± 2.09
240	82.98	84.16	83.82	83.65 ±0.61

Table 6. Percentage inhibition shown by aqueous extract on calcium ion uptake

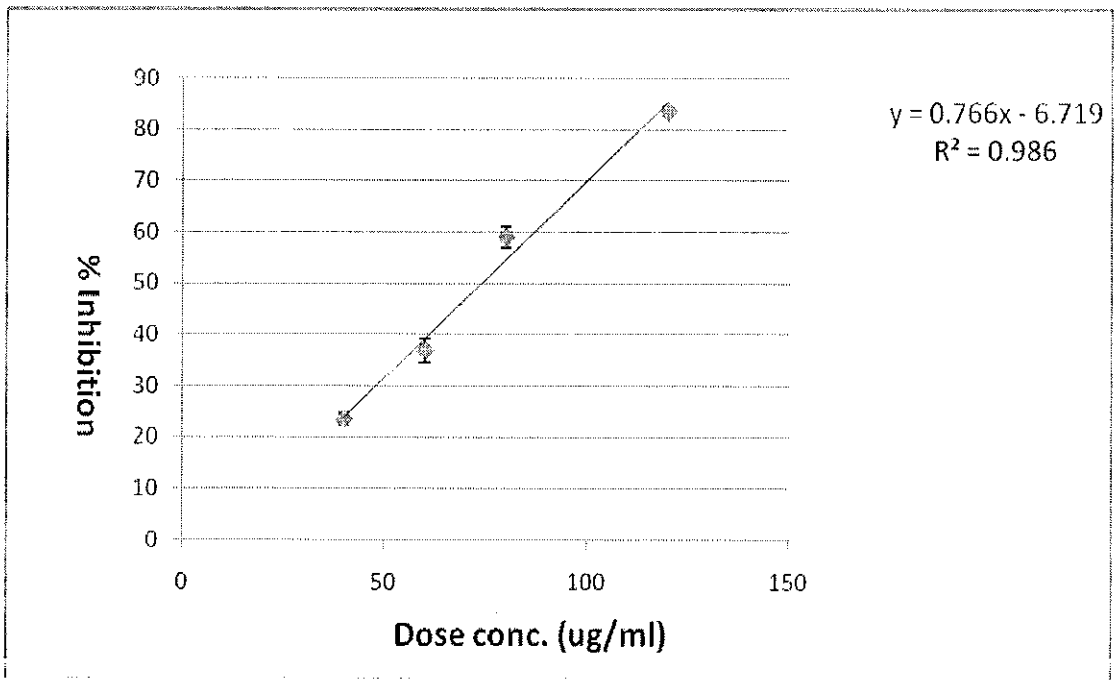

Fig 8. Aqueous extract percentage inhibition on calcium ion uptake plot, Dose conc. *vs* Percentage Inhibition

4.2.4 Percentage inhibition shown by aqueous extract of *T.arjuna barks* on phosphate ion uptake.

DOSE CONC. (µg/ml)	% INHIBITION - I	% INHIBITION - II	% INHIBITION - III	MEAN INHIBITION ± STANDARD DEVIATION
40	12.42	13.51	13.76	13.23 ± 0.71
80	22.62	22.17	20.99	21.93 ±0.84
160	27.55	28.7	27.55	27.93 ± 0.66
240	34.77	35.96	35.69	35.47 ± 0.62

Table 7. Percentage inhibition shown by aqueous extract on phosphate ion uptake

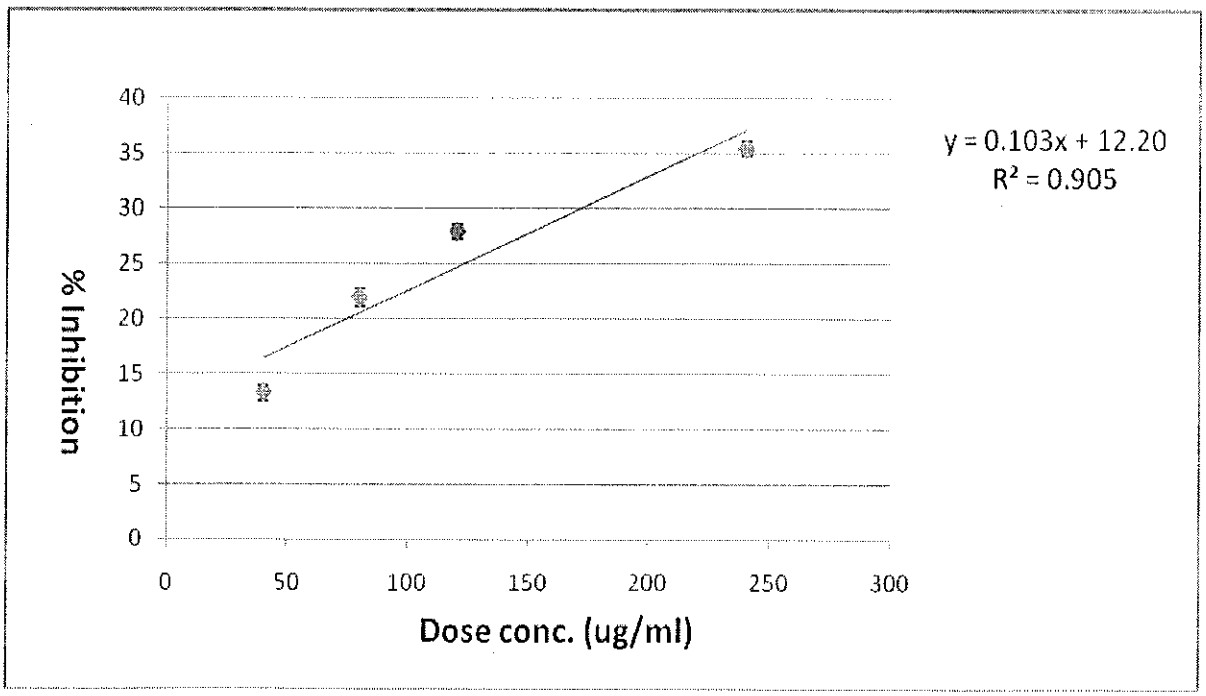

Fig 9. Aqueous extract percentage inhibition on phosphate ion uptake plot, Dose conc. Vs Percentage Inhibition

4.2.5 Percentage inhibition shown by successive ethanol extract of *T.arjuna barks* on calcium ion uptake.

DOSE CONC. (µg/ml)	% INHIBITION - I	% INHIBITION - II	% INHIBITION - III	MEAN INHIBITION ± STANDARD DEVIATION
40	23.38	29.62	31.49	28.16 ± 4.25
80	44.5	48.7	44.4	45.87 ± 2.45
160	58.6	66.38	61.42	62.13 ± 3.94
240	88.32	92.31	91.75	90.79 ± 2.16

Table 8. Percentage inhibition shown by successive ethanol extract on calcium ion uptake

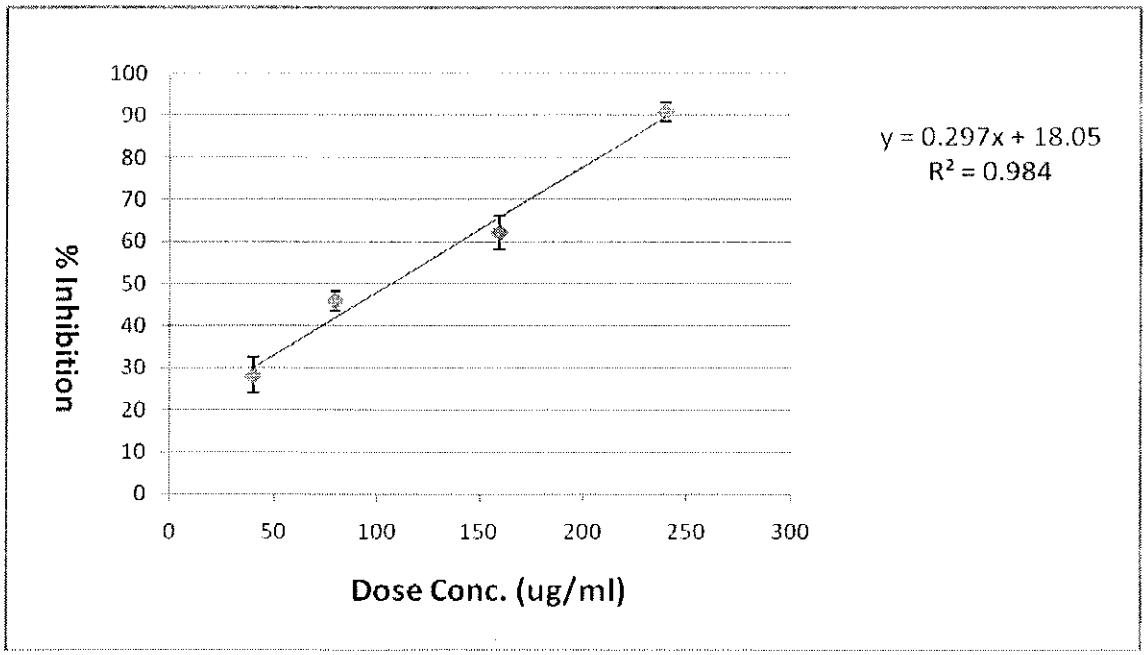

Fig 10. Successive ethanol extract percentage inhibition on calcium ion uptake plot, Dose conc. *vs* Percentage Inhibition

4.2.6 Percentage inhibition shown by successive ethanol extract of *T.arjuna barks* on phosphate ion uptake.

DOSE CONC. (µg/ml)	% INHIBITION - I	% INHIBITION - II	% INHIBITION - III	MEAN INHIBITION ± STANDARD DEVIATION
40	12.17	17.16	14.35	14.56 ± 2.50
80	20.26	23.53	20.12	21.30 ± 1.93
160	26.9	28.53	27.3	27.58 ± 0.85
240	39.37	38.03	37.45	38.28 ± 0.99

Table 9. Percentage inhibition shown by successive ethanol extract on phosphate ion uptake

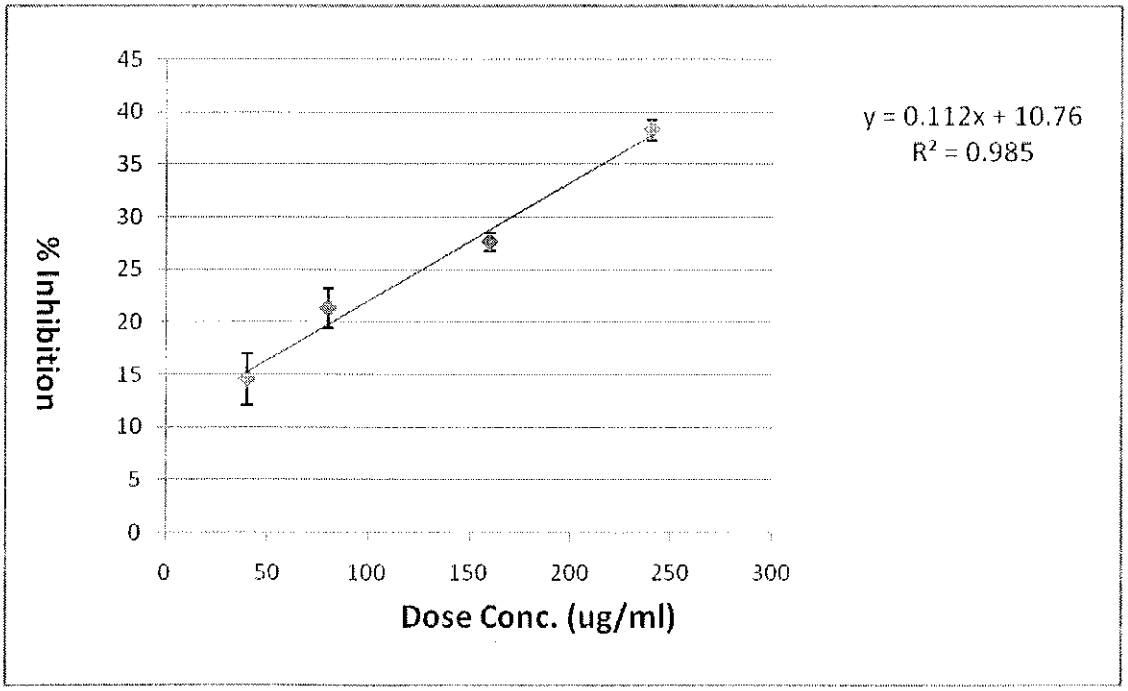

Fig 11. Successive ethanol extract percentage inhibition on phosphate ion uptake plot, Dose conc. *vs* Percentage Inhibition.

4.2.7 Percentage inhibition shown by successive aqueous extract of *T.arjuna barks* on calcium ion uptake.

DOSE CONC. (µg/ml)	% INHIBITION - I	% INHIBITION - II	% INHIBITION - III	MEAN INHIBITION ± STANDARD DEVIATION
40	22.17	26.58	22.1	23.62 ± 2.57
80	36.89	38.31	35.86	37.02 ± 1.23
160	59.24	60	57.6	58.95 ± 1.23
240	82.31	86.74	81.49	83.51 ± 2.83

Table 10. Percentage inhibition shown by successive aqueous extract on calcium ion uptake

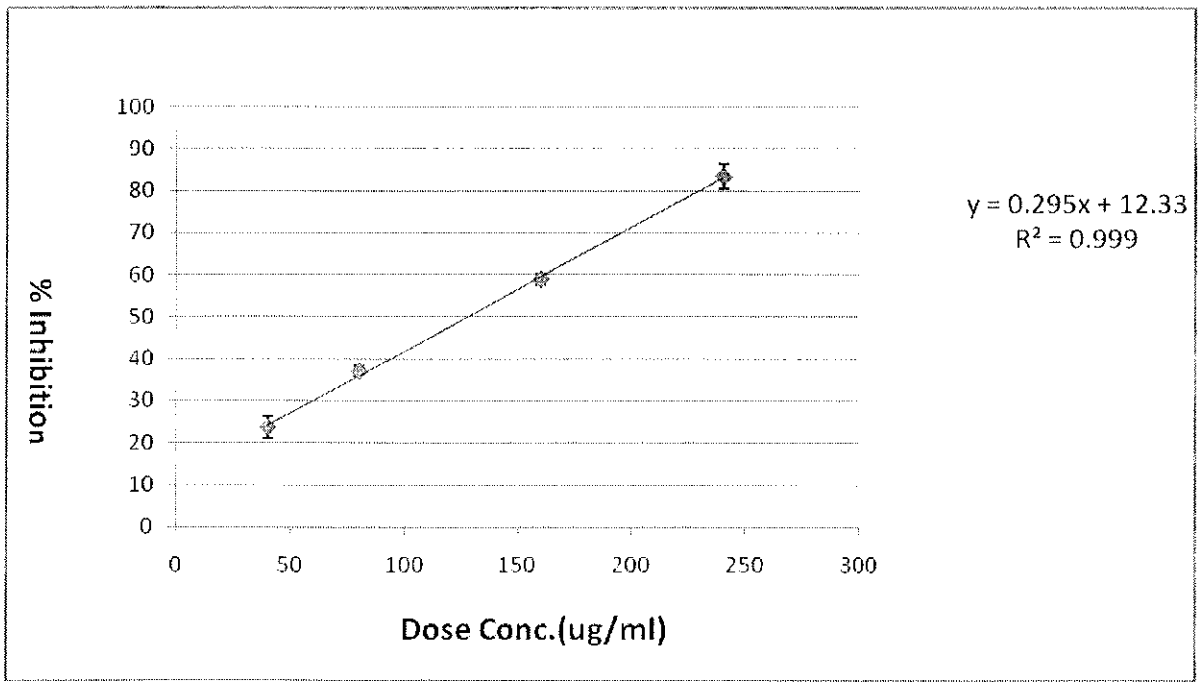

Fig 12. Successive aqueous extract percentage inhibition on calcium ion uptake plot, Dose conc. *vs* Percentage Inhibition

4.2.8 Percentage inhibition shown by successive aqueous extract of *T.arjuna barks* on phosphate ion uptake.

DOSE CONC. (µg/ml)	% INHIBITION - I	% INHIBITION - II	% INHIBITION - III	MEAN INHIBITION ± STANDARD DEVIATION
40	11.86	12.36	11.73	11.98 ± 0.33
80	21.95	20.12	21.8	21.29 ± 1.02
160	27.71	28.2	26.58	27.50 ± 0.83
240	34.05	36.43	32.89	34.46 ± 1.81

Table 11. Percentage inhibition shown by successive aqueous extract on phosphate ion uptake

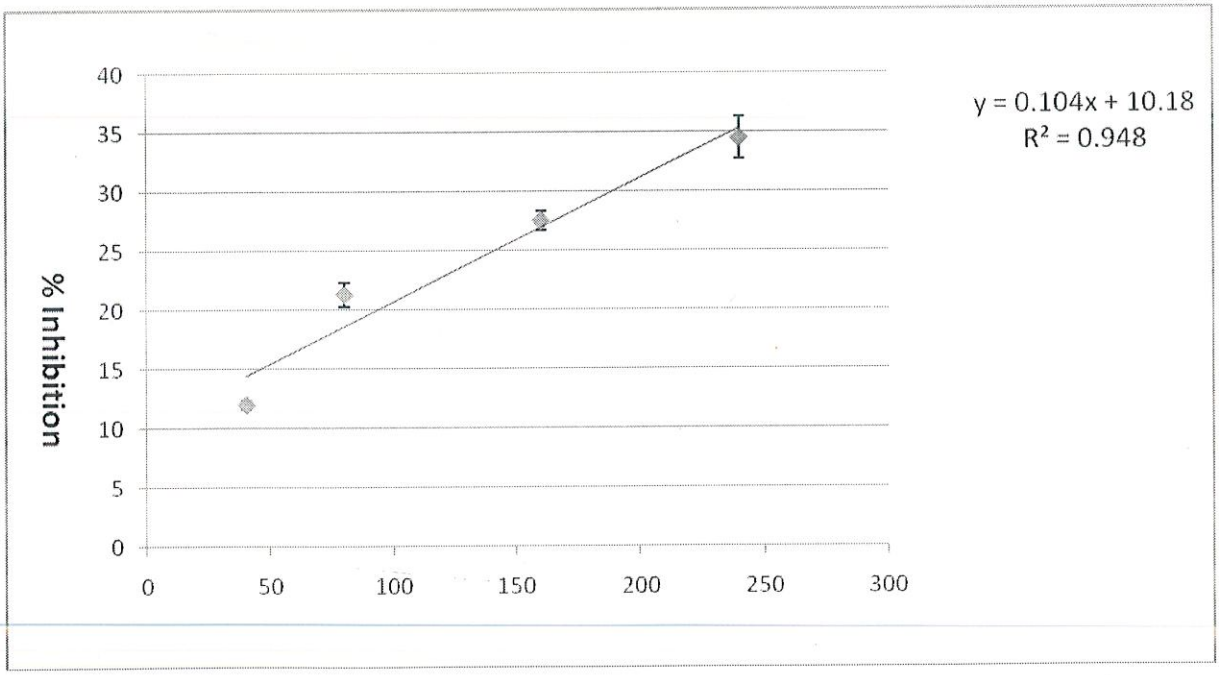

Fig 13. Successive aqueous extract percentage inhibition on phosphate ion uptake plot, Dose conc. *vs* Percentage Inhibition

4.2.9 Qualitative Tests confirming the presence of Secondary metabolites

S NO.	SECONDARY METABOLIE	METHANOLIC EXTRACT	AQUEOUS EXTRACT	SUCCESSSIVE ETHANOLIC EXTRACT	SUCCESSIVE AQUEOUS EXTRACT
1.	**Saponins**	Present	Present	Present	Present
2.	**Flavonoids**	Present	Present	Present	Present
3.	**Tannins**	Present	Present	Present	Present
4.	**Alkaloids**	Present	Present	Present	Present
5.	**Terpenoids**	Present	Present	Present	Present

Table 12. Qualitative tests confirming the presence of secondary metabolites in all the extracts

4.3 Conclusion

The data concludes that all aqueous, methanol, successive ethanol and successive aqueous *Terminalia arjuna* bark extract is causing inhibition of Ca-P ion uptake.

However more inhibition in case of successive ethanol and methanol extract is achieved. Hence the bark of *Terminalia arjuna* contains potent polar components that inhibit the uptake of Ca-P ions. Further qualitative tests for secondary metabolites on the extracts were done, which reflected the presence of saponins, terpenoids, flavonoids, tannins and alkaloids in the extracts.

Therefore 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.

4.4 Future work to be done

Further we will be setting up heterogeneous system using the same four extracts of *Terminalia arjuna.*

References.

1. Jian-su shao *et al* (2006) - Molecular Mechanisms of Vascular calcification Lessons learned from the Aorta by Journal of the American Heart association, Arteriosclerosis Thromb. Vasc. Biol; 26:1423-1430.
2. Fereshteh Azari, Hojatollah Vali' Jean-Luc Guerquin-Kern, Ting-Di Wu, Alain Croisy, S. Kelly Sears' Maryam Tabrizian and Marc D. McKee (2006) - Intracellular precipitation of hydroxyapatite mineral and implications for pathologic calcification; Journal of structural biology; volume 162, issue 3.
3. Dwivedi, S., Udupa, N (1989) - *Terminalia arjuna*: pharmacognosy, phytochemistry, pharmacology and clinical use. A review. Fitoterpia 60, 413–420.
4. Warrier PK, Nambiar VPK, and Ramankutty C (1996) - *Terminalia arjuna* In: Indian Medicinal Plants - A Compendium of 500 Species, (Eds. Warrier PK, Nambiar VPK, Ramankutty C) Vol. 5, 1st Ed. Orient Longman Limited, Madras, India. 253-257
5. Bharani A, Ganguli A, Mathur LK, Jamra Y, Raman PG (2002 Mar-Apr) - Efficacy of *Terminalia arjuna* in chronic stable angina: a double-blind, placebo-controlled, crossover study comparing Terminalia arjuna with isosorbide mononitrate - Indian Heart J.; 54(2):170-5.
6. Gupta R, Singhal S, Goyle A, Sharma VN (2001 Feb) - Antioxidant and hypocholesterolaemic effects of *Terminalia arjuna* tree-bark powder: a randomised placebo-controlled trial. J Assoc Physicians India; 49:231-5
7. Dwivedi S, Jauhari R. (1997) - Beneficial effects of *Terminalia arjuna* in coronary artery disease - Indian Heart J; 49:507-510.
8. Dwivedi S. Agarwal MP (1994) `- Antianginal and cardioprotective effects of *Terminalia arjuna*, an indigenous drug, in coronary artery disease – JAPI; 42:287-289.
9. Anjaneyulu, A.S.R., Prasad, A.V.R., (1983) - Structure of terminic acid, a dihydroxy triterpene carboxylic acid from *Terminalia arjuna*. Phytochemistry 22, 993–998.
10. Ali , A Kaur, G.Hamid, H Abdulla, T Ali, M Niwa, M Alam, M.S (2003b)

- TerminosideA, a newtriterpine glycoside from the bark of *Terminalia arjuna* inhibits nitric oxide production in murine macrophages. Journal of Asian Natural Product Research 5, 137–142

11. Pettit, G.R., Hoard, M.S., Doubek, D.L., Schmidt, J.M., Pettit, R.K., Tackett, L.P., Chapuis, J.-C., (1996) - Antineoplastic agents 338. The cancer cell growth inhibitory constituents of *Terminalia arjuna* (Combretaceae). Journal of Ethnopharmacology 53, 57–63
12. Lin, T.C., Chien, S.C., Chen, H.F., Hsu, F.L., (2001) - Tannins and related compounds from Combretaceae plants. Chinese Pharmaceutical Journal 52,1-26.
13. Choubey, B.K., Srivastava, S.K., (2001). Antifungal agents from *Terminalia arjuna*. Indian Journal of Chemistry 40B, 354–356.
14. Satyajit D. Sarker, Zahid Latif, Alexander I. Gray – Natural Products Isolation, II Edition.
15. Tandon CD, Forouzandeh M, Agarwal S, Jethi RK(1997) - Inhibitors of *In vitro* mineralisation from flexor tendons of rabbits and their role in biological mineralisation - Mol Cell Biochem; 171.29-35
16. Ramesh Chander, Kavita Singh, A K Khanna, S M Kaul, Anju Puri, Rashmi Saxena, Gitika Bhatia, Farhan Rizvi and A K. Rastogi Kavita Singh (2004) - Antidyslipidemic and antioxidant activities of different fractions of *Terminalia arjuna* stem bark - Indian Journal of Clinical Biochemistry; 19 (2), 141-148.
17. Trinder P. Colorometric micro-determination of calcium in serum. Analyst 1960; 85:889-94.
18. Gomori HD. A modification of colorimetric phosphorous determination for use with photoelectric colorimeter. J Lab Clin Med 1941;27:955-60
19. D N Onwukaeme T B Ikuegbvweha and C C Asonye (June 2007) - Evaluation of hytochemical Constituents, Antibacterial Activities and Effect of Exudate of *Pycanthus Angolensis* Weld Warb (Myristicaceae) on Corneal Ulcers in Rabbits. Tropical Journal of Pharmaceutical Research; 6 (2): 725-730.
20. Adegoke A A, Iberi P A, Akinpelu D A, Aiyegoro O A, Mboto CI (Sep-Oct 2010) - Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. International Journal of Applied Research in Natural Products Vol. 3 (3), pp. 6-12.

21. P. S. Das and A. D. Talukdar (2010) - Department Phytochemical Screening and Bioactivity Evaluation of *Homalonema aromatica* (Roxb.) Schott. Assam University Journal of Science & Technology: Biological and Environmental Sciences Vol. 6 Number I 71-74.
22. H.O. Edeoga, D. E. Okwu and B.O Mbaebie (2005) - Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology Vol. 4 (7), pp. 685-688 July 2005 Available online at http://www.academicjournals.org/AJB ISSN 1684 5315 © 2005 Academic Journals.
23. www.google.com
24. www.wikipedia.com
25. www.pubs.acs.org
26. www.endo-society.org

BIO-DATA

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CARRER OBJECTIVE

To develop myself as a professional while working in a challenging and growing environment.

EDUCATION

Pursuing Bachelor of Technology in Biotechnology from Jaypee University of Information Technology, Waknaghat, Distt. Solan (H.P.) with the following marks till date:

Standard	College/School	Year	CGPA/Percentage
B.Tech (B.T)	Jaypee University of Information Technology, Solan.	2011 (Expected)	7.7 (Up till 7th semester)
High School (C.B.S.E)	Delhi Public School, Ghaziabad.	2007	78.8%
Intermediate (C.B.S.E)	Delhi Public School, Ghaziabad	2005	90%

TECHNICAL SKILLS

- Computer proficiency: operating system-windows/vista/xp, Programming languages and software's-C, MS office, Data structures (DS).
- Bioinformatics tools: BLAST, FASTA, primer designing, MSA, GENSCAN.
- Performed basic wet lab experiments like electrophoresis, worked in LAF, etc.
- Worked with PCR, Sonicator, lyophilizer, centrifuges, and spectrophotometers.

AREA OF INTEREST

- Genetics, Proteomics, plant and animal tissue culture, comparative and functional genomics and immunology.
- Also have a keen interest in professional development courses including financial management, project management and also principles of management.

PROJECT UNDERTAKEN

- Currently working on a project titled "Evaluating the role of Terminalia arjuna in calcification of aorta".
- Also done a project based on the preparation of cheese in our laboratory.

SUMMER INTERNSHIP

Organization – M/s AB Mauri India Pvt. Limited

This was a yeast manufacturing organization. Here I learnt about the large scale production of yeast using various fermentors. Also learnt about the quality testing and effluent treatment.

STRENGTHS

Good communication skills, willing to learn new things, sense of responsibility, sincere and hardworking, down to earth.

CO-CURRICULAR ACTIVITIES / ACHIEVEMENTS

- Was a member of the invitation committee of the JYC (Jaypee Youth Club) in the college fest of 2009 and 2008.
- Was a member of the event management club of the JYC (Jaypee Youth Club) in the college fest of 2009 and have been actively involved in the organization of various events.
- Was a member of Synapse (bio club) in college, an active member of the club and have been a part of various activities of the club like the blood donation camp.
- Member of college anti ragging squad in college.
- Have been an active participant in various literary events in the college and school. Have been receiving academic distinction certificate from school.
- Also have participated in activities regarding leprosy and cancer awareness in school.

PERSONAL DETAILS

Father's name : Mr. Sudhir Janmeja

Mother's name : Mrs. Guri Janmeja

Date of birth : June 3rd, 1989.

Hobbies : Listening to contemporary music, reading fiction novels.

Languages known : English, Hindi, German.

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EDUCATION

Course	Specialization	Year of Passing	%/Grade
B.Tech.	Biotechnology	2011	63(7^{th} Sem)
H.S.C.	Science	2007	74.4
S.S.C.	General	2005	74.4

TECHNICAL SKILLS

- Computer proficiency: operating system-windows/vista/xp.
- Programming languages and software's-C, MS office, Data structures (DS).
- Performed basic wet lab experiments like electrophoresis, worked in LAF, etc.
- Worked with PCR, Sonicator, lyophillizer, centrifuges, spectrophotometers.

PROJECT UNDERTAKEN

- Currently working on a project titled "Evaluating the role of Terminalia arjuna in calcification of aorta".
- Also done a project based on the preparation of cheese in our laboratory.

SUMMER INTERNSHIP

Organization - *Jackson Laboratories, Amritsar*

CO-CURRICULAR ACTIVITIES / ACHIEVEMENTS

- Participated in state institution of science education,sciencefair and got a consolation prize
- Silver medal in Avantika essay writing completion under the theme food and health.
- Bronze medal in swimming at district level.

INTERESTS AND HOBBIES

- Adventure sports like rafting, paragliding.
- Playing Cricket, football and Table-tennis.
- Listening to music.

PERSONAL ATTRIBUTES

- Team Player
- Energetic with zeal to learn and perform well in life.
- Highly adaptable.

PERSONAL DETAILS

Father's name : Mr. Atul Puri

Mother's name : Mrs. Vandana Puri

Date of birth : May 25th, 1989

Hobbies : Listening to music, travelling

Languages known: English, Hindi, French

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