ANALYSIS OF TRACE ELEMENTS AND ORGANIC CONSTITUENTS IN ARJUNA *(TERMINALIA ARJUNA)* BARK

A DISSERTATION

Submitted in partial fulfillment of the requirements for the award of the degree of

MASTER OF TECHNOLOGY

in

ADVANCED CHEMICAL ANALYSIS

By

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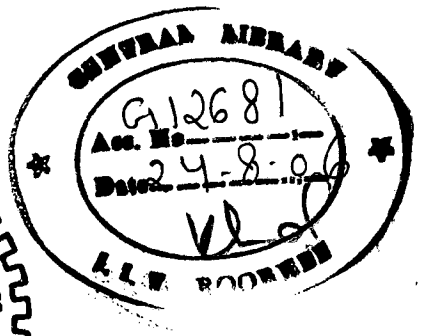

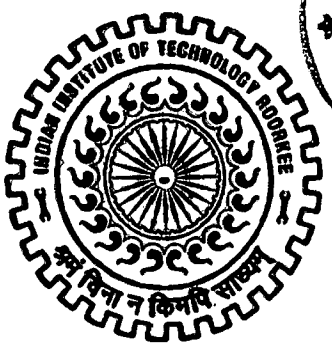

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'अर्जुनस्य त्वचा सिद्धं क्षीरं योज्यं हृदामये।
सितया पञ्चमूल्या वा बलया मधुकेन वा।।
घृतेन दुग्धेन गुडाम्भसा वा पिबन्ति चूर्णं ककुम्भत्वचो ये।
हृद्रोगजीर्णज्वरक्तपित्तं हृत्वा भवेयुश्चिरजीवनस्ते।।
भग्नः पिबेत्त्वक् पयसाऽर्जुनस्य गोधूमचूर्णं सघृतेन वाज्य।

(चन्द्रदत्त , भाव प्रकाश)

अर्जुन हृदय विकार एवम् दीर्घकालीन रोगों के लिये उपयोगी है। इसके सेवन से वात् , पित्त व कफ का समायोजन होता है तथा रोगी अमरत्व प्राप्त करता है। इसकी छाल का घी अथवा दूध के साथ सेवन, घावों तथा अस्थि टूटन में उपयोगी है।
श्वेत दुग्धवर्ण , अर्जुन की छाल एक उपयोगी हृदय शक्तिवर्धक है। छाल को जलाने के उपरान्त राख का घी , दुग्ध अथवा गुड़ के साथ सेवन करने से इसका प्रभाव पांच गुना हो जाता है।

Arjuna cures heart disorders, long term ailments, balances *Vatta, Pitta, Kapha* and the patient becomes immortal.

The bark taken with milk or ghee cures accidental wounds and fractures.

The white milk of *Arjuna* bark is a proven heart tonic.

The incineration of the bark is five times as powerful as honey.

Powdered bark taken with g*hee* or milk or jaggery cures skin ailments.

CANDIDATE'S DECLARATION

I hereby certify that the work being presented in the dissertation entitled **Analysis of Trace Elements and Organic Constituents in Arjuna *(Terminalia Arjuna)* bark** is an authentic record of my own work carried out by me under the supervision of Prof. A.N. GARG at the Department of Chemistry, Indian Institute of Technology, Roorkee during July, 2005 to June, 2006. The work forms a part of ***M.Tech. in Advanced Chemical Analysis*** and the matter presented in this dissertation has not been submitted by me for the award of any other degree of this or any other Institute.

Dated: 30 June 2006

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This is to certify that the above statement made by Ms Pratima Gajbhiye is correct to the best of my knowledge.

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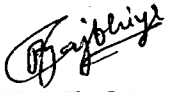
At last thanks to the God almighty who has given me spiritual support and courage to carry out this work.



(PRATIMA T. GAJBHIYE)

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Arjuna to be India's reply to Ginseng

By Kalpana Jain/TNN

New Delhi: India seems to have found its answer to the multi-billion Chinese stress buster, ginseng. The extract of the bark of a tree, Terminalia arjuna, commonly known as Arjuna, has been found to be extremely helpful in controlling stress and in early stages of coronary artery disease.

Scientists at the Central Drug Research Institute (CDRI) in Lucknow, after four years of painstaking research, have confirmed that the extract, used in ayurvedic preparations, does work on several animal models.

The Indian Council of Medical Research (ICMR) is now preparing to start clinical trials in at least four centres around the country. The All India Institute of Medical Sciences and the University College of Medical Sciences will be among the two centres chosen in Delhi. A senior ICMR scientist told TNN, "It seems to work as well as ginseng."

Ginseng is among some of the world's largest selling herbal products. In India, Revital, a multi-vitamin containing ginseng, has sales crossing $11 million annually. Ginseng is used to reduce stress, regulate blood and sugar levels and strengthen the cardiovascular system.

Scientists at ICMR said the preliminary results of arjuna showed that it has stress-busting properties. It also helps those in the early stages of coronary artery disease and patients of high blood pressure. The Arjuna tree is found all over India.

ICMR, in fact, for the first time is actively trying to validate traditional therapies and ayurvedic preparations with scientific research so as to create a global market for them. It has initiated trials to study a traditional remedy for diarrhoea, a plant extract for treating filariasis, another extract for treating liver diseases and a herbal preparation for treating prostate problems in men. At Nair hospital in Mumbai, trials are going on to study the use of dried ginger, curcumin and the extract of nutgram in the treatment of diarrhoea in children. The preparation is said to strengthen intestines and thus check recurrence of diarrhoea.

Nearly 220 patients have been enrolled for the study. One group has been put on the oral rehydration solution prescribed by the WHO and another on this preparation. Results so far have been encouraging, says ICMR, which hopes to push these alternative therapies into treatment regimens.

ABSTRACT

Arjuna (*Terminalia Arjuna*) bark powder is a widely used heart tonic as described in ancient Sanskrit texts. Five samples including two commercial brands, collected from different places have been analyzed for elemental contents by neutron activation analysis (NAA), atomic absorption spectrophotometry (AAS) and inductively coupled plasma-mass spectrometry (ICP-MS) for 27, 14 and 22 elements respectively. In all 23 elements have been determined by three different techniques and a maximum of 32 elements have been determined in *Arjuna* bark. All the elements show wide variation depending on their origin. A comparison for some elemental concentrations by different techniques suggests good agreement even though AAS and ICP-MS require sample dissolution. The bark powder is particularly enriched in K (5.87±2.11mg/g), Ca (34.1±10.5 mg/g), Mg (5.41±1.93 mg/g), Fe (2.99±1.77mg/g), Mn (75.5±24.8 μg/g) and Zn (11.9±8.9 μg/g). Further, Mo, Rb, Cs, Sr and V were also found in few μg/g amounts. Also Ca/P (105 ± 50) ratio is much higher compared to K/P (16.4 ± 4.6). NAA has the advantage of being non-destructive compared to AAS and ICP-MS where sample dissolution is required. Thin layer chromatography (TLC) and column chromatography were used to separate three new organic compounds; 1-phenylazo 2-naphthalenol, 2-isopropyl naphtho [2, 3, β], furan 4, 9-dione and tartaric acid with R_f = 0.79, 0.63 and 0.72 respectively from the methanolic extract of *Arjuna* bark. The compounds were identified by elemental analysis, infrared and NMR spectra and further confirmed by GC-MS where fragmentation pattern have been proposed. It is proposed that metal ions remain complexed with the naturally occurring organic ligands making them bioavailable. Thermogravimetric studies suggest three stage decomposition followed by ~16% non-volatile matter left at 900 °C

Ch I

INTRODUCTION

I.1 GENERAL ASPECTS

The genus *Terminalia* consists of large hard wooded trees. Over 100 species of this genus are widely distributed in the tropics (*Terminalia* word originated from the Latin word *Terminus*, referring to the leaves being borne on the tips of the shoots). In India, *T. arjuna*, *T. chebula*, *T. bellrica* and *T. ciliata* are major related species. *Terminalia arjuna* or *Arjuna* in Hindi belongs to the family *Combretaceae* and it is a deciduous tree (60-70 feet or 25 meter in height),

abundantly found throughout the sub-Himalayan tracts, the Deccan region, Myanmar, Sri Lanka and in the southeastern countries [1]. It has long, cone shaped round leaves and white bark, which gives out a milky juice when cut. The tree and especially its bark has been found to be medicinally important, widely used in the preparation of Ayurvedic formulations for as a heart tonic. Leaves are sub-opposite, 5–14 × 2–4.5 cm., oblong or elliptic oblong, glabrous, often in equilateral, margin often crenulate, apex obtuse or sub-acute, base rounded or sometimes cordate; petioles 0.5–1.2cm. Flowers are small and white but fruit is 2.3–3.5 cm long, fibrous woody, glabrous with 5 hard wings, striated with numerous curved veins.

Flowering time is normally during spring. It can be artificially propagated through seeds, coppicing, pollarding, root-suckers, stumps and air layering. It is initially slow growing but later grows fast. It attains 2–3 m height in 3 years. *Arjuna* yields up to 45 kg dry bark chips on a three-year cycle without injury [2].

Nomenclature: It is known by various names in different Indian languages [3] as listed in Table I.1. However, it is also known as *Arjun Chhal* in trade circles. Both *Charaka* and *Susruta* have mentioned this plant in their *Samhitas*. It was *Acharya Vagbhata* who for the first time mentioned about this plant *Arjuna* [2], for its use in treating heart diseases. Because of its beneficial properties of strengthening

Table I.1. Nomenclature of Arjuna (Terminalia Arjuna) [3]

Language	***Nomenclature***
English	White Marudah
Hindi	Arjun, Arjuna, Koha, Kahu, Arjan
Gujarati	Arjun - Sadada, Sadado
Marathi	Arjuna, Arjun Sadada, Sadura, Ladada
Sanskrit	Arjuna, Dhanvi, Indradruma, Kakubha, Karvirak
Oriya	Arjuna, Sahajo
Tamil	Vellamatta
Kannada	Maddi
Telugu	Yerra maddi, Tellamaddi
Assam	Orjun
Bengali	Arjhan
Punjabi	Arjuna
Chinese	... San guo mu (Taiwan)
German	Echte Myrobalane
Russian	Терминалия арджуна, Терминалия аржуна
Nepalese	Arjun, Kaahuu

the heart muscles, it has attracted the attention of modern research workers all over the world. It is widely found in the terrains of Himalaya, Bengal, Bihar and Madhya Pradesh. Lines of trees can be seen in dry mountain areas, along rivers and ponds.

Though modern medical sciences have progressed a lot in its fight against heart ailments, the numbers of heart patients are continuously increasing. Large number of medications starting from aspirin to sophisticated open-heart surgeries is now a days common in developed countries. In spite of the ultramodern technologies and the awareness programs, heart diseases like *Angina pectoris* and *Myocardial Infraction* are creating alarming situation in the society. If we consider the traditional Indian herbal system of medicine - *Ayurveda*, we come to

know that Ancient medical scientists have mentioned the remarkable cardioprotective, heart muscle strengthening properties of *Arjuna* [3].

I.2 MEDICINAL IMPORTANCE

Every part of *Arjuna* tree has useful medicinal properties though its bark powder (*Churna*) is most used. *Arjuna* holds a reputed position in both Ayurvedic and Unani Systems of medicine [4]. It is used externally as well as internally. It alleviates *kapha* and *pitta doshas* but vitiates *vata dosha*. It is a very effective heart tonic and its blood coagulating properties are extremely useful in arresting the bleeding [2]. Indian *Arjuna* is being considered as an answer to the Chinese wonder drug *Ginseng,* the world's largest selling herbal product. Its bark ground to a paste with water and applied externally on fractures helps in early healing. It is also recommended in traumatic injuries associated with oedema swelling. The dressing of the wounds and ulcers with the decoction of *Arjuna* bark hastens healing and cleansing. Its juice effectively arrests bleeding from the wounds. It can be used with great benefit as a complementary herb with *Sariva, Lodhara, Yastimadhu, Manjistha, Chandana* etc in treating acne or pimples [2].

The fruit is a tonic and deobstruent. 14-28 mL of water decoction is useful in hemorrhage, sour tongue and failure to absorb fats. Some of the common formulations widely used are; *Arujanarishta, Arjunghruta, Arjunakhsirpak* besides *Arvindasava, Devadarvy-arishta* etc. *Arujanarishta,* a medicinal wine prepared from its bark and black raisins is the most popular formulation used to treat cardiac debility, convalescent patients, bleeding piles, diarrohea, leucorrhoea in

the dosage of 2-4 teaspoons twice a day [3. Some of its beneficial properties are [5,6]

(i) Healing Power: The bark of *Arjuna* tree is a cardiac stimulant and has a cooling effect. It is useful in arresting secretion or bleeding. It helps to relieve fever. It is also useful in removing calculi or stones formed in the urinary system, in promoting flow of bile, in wound healing and healing of fractured bones.

(ii) Heart Disorder: The bark and its preparations are known to have a marked stimulant action on the heart [6]. The practitioners of Indian system of medicine use it in conditions of cardiac failure and dropsy. As a heart stimulant, either a decoction of the thick portion of the bark made with milk should be taken every morning on an empty stomach or its powder in 1 to 2 g doses should be used with milk and jaggery. In case of heart attack, it cannot act against like streptokinase or eurokinase. Its regular use just recovers the patient from heart attack and reduces the chance of further attack to a great extent [7,8]. 2-3 g powdered bark soaked overnight in a cup of water, decanted next morning and the water taken in empty stomach helps bring down the blood pressure. Besides, no toxic or side effects have been found.

(iii) Asthma: In *Ayurveda*, the bark of *Arjuna* is considered beneficial in the treatment of asthma [9]. A fine powder of the bark is made and stored in a well - corked bottle. Infusion is made in hot water from 1 teaspoon of *Arjuna* bark powder and drunk as tea.

(iv) Diarrhoea and dysentery: A decoction of the bark taken in doses of 15 to 30 g may relieve patients of diarrhoea or dysentery [10].

(v) Acne: An ointment made by mixing the bark and honey applied over the affected area, can treat acne successfully [11].

(vi) Aphrodisiac: The powder of the bark is an effective sex stimulant, if taken with milk regularly over a period of time.

(vii) Other disorders: The juice of fresh leaves can be used beneficially in earache. Ash of the bark is also prescribed in scorpion sting. The decoction of the herb is used as an astringent for cleaning sores, ulcer etc. It is useful as diuretic, incirrhosis (degeneration of liver), inflammatory conditions and drops [12,13]

(viii) Radioprotective Effect: Some researchers have found it as radioprotective with different model systems against γ- radiation [14].

(ix) Antioxidant Properties: Its antioxidant effects like prevention, radical scavenging and repair have been studied and mechanism well understood in terms of its high ability to react with and neutralize the free radicals and related species of biological importance such as superoxide, hydroxyl radical, singlet oxygen, thiyl radical and peroxyl radical derived from various sources. [14].

I.3 IMPORTANCE OF TRACE ELEMENTS

Realization of the importance of trace elements in nutrition began in 19th century with the chemical analysis of elements in biological systems. It was demonstrated that certain elements were essential for the growth of microorganisms and biochemical processes [15]. In addition to the organic elements C, H, N and O there are nine other elements required in relatively large quantities and are therefore called macronutrients. These are Na, K, Mg, Ca, S,

P, Cl, Si and Fe. Besides there are others, which are called micronutrients means, these are required in very small amounts. The name trace element means that these are needed by the body in microgram per gram (μg/g) or even nanogram per gram (ng/g) level [16, 17]. They include Cr, I, Mn, Cu, Se, Mo, Co, Zn. All the elements found in biological systems can be classified in three categories (Structural, Electrolytic and Trace) as shown in Table I.2.

Table I.2 Classification of elements of biological importance [18]

I. Structural Elements	C, H, O, P, N, S, Ca
II. Electrolyte Elements	Ca, Cl, K, Mg, Na
Anions	HCO_3^-, SO_4^{2-}, HPO_4^{2-}
III. Trace Elements	
Essential	
a. Biologically	Co, Cr, Cu, Fe, I, Mn, Mo, Se, Zn, As, F, Ni, Si, Sn, V
b. Clinically	Cr, Cu, Fe, I, Se, Zn
Toxic	As, Br, Cd, Hg, Pb, Tl, Th
a. Potentially Toxic	Cd, Hg, Pb
b. Environmental contaminants	As, Be, Cr, Hg, Ni, Mn, Pb, Sb,
c. Industrial Hazards	Si, Ba, Sr

Trace elements can be further divided into following two groups:

i) The elements whose essentiality have been established and accepted by scientific standards. These may be biologically or clinically essential.

ii) Those elements who's proof of essentiality does not exist but on the other hand their presence is harmful. Hence these are called toxic elements.

An essential element is defined as the one required for maintenance of life. Its absence results in deleterious effects causing death of the organism. Elements such as Fe, Mn, Co, Cu, and Zn in trace amounts are active in biological systems and biochemical processes. Their deficiency causes disturbances in metabolism and growth of tissue, whereas their excess amounts may cause harmful effects. Underwood and Mertz (1986) [16] have reviewed the role of trace elements in nutrition required for the development of purified diets and methods of animal care that limit exposure to contaminants. The science of human nutrition is mainly concerned with defining the optimum amounts of the constituents of food necessary to achieve or maintain health in all groups of the population. During last few decades, researchers have further added Se, Cr, Ni, V, Si, I, and As in the list of essential elements suggesting their essentiality at optimum concentration. These play a vital role in biochemical and enzymatic processes. Nutritional supply of these elements must be in sufficient quantity to maintain essential metabolic functions of enzymes and hormones.

Most people meet the daily requirement of trace elements from their food as a part of balanced diet [17, 18]. However in some cases deficiency may occur causing diseases. The supply status of a trace element may be divided into following five concentration ranges [18, 19] as illustrated in Fig. I.1:

- ***Severe deficiency***, characterized by clinical symptoms.
- ***Marginal deficiency***, where no conspicuous clinical symptoms are measurable and supplementation may work.

- ***Optimal concentration*** leading to metabolic functioning, promoting normal health and organ performance.
- ***Sub toxic***, where intake is in excess of the optimal supply and is characterized by biochemical changes with pharmacological aspects manifested by clinical symptoms.
- ***Toxic*** is characterized by clinical symptoms and finally acute toxicity may cause death.

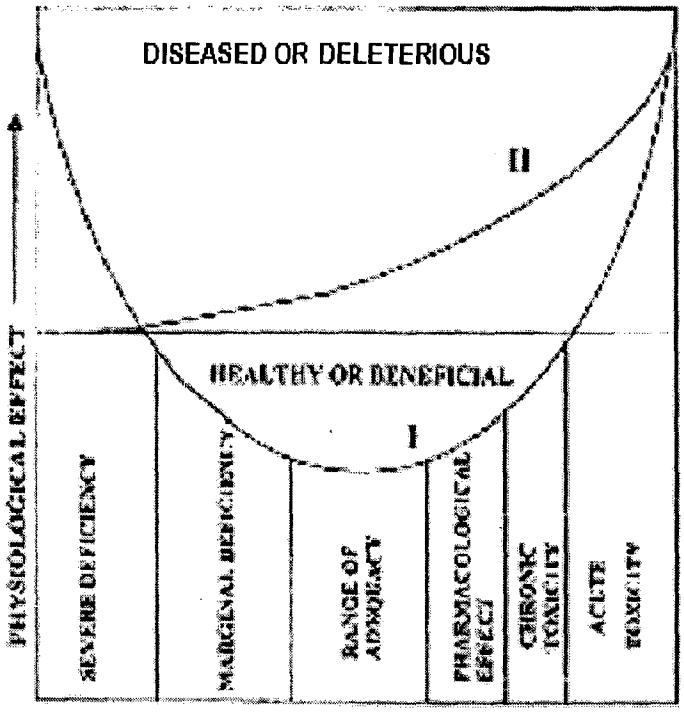


Fig. I.1 Correlation of elemental concentrations with its physiological effects

Trace elements are essential in the assimilation and utilization of vitamins, proteins and other nutrients. They aid in digestion and act as catalyst for many hormones, enzymes and essential body functions and biochemical reactions. They also aid in replacing electrolytes lost through heavy perspiration or extended diarrhea and protect against toxic reactions and heavy metal poisoning [19]. Excessive levels of non-essential mineral contaminants such as

Al, As, Cd, Pb may affect the adsorption and efficient use of minerals in the body and can have an imbalancing effect on the body cell [19]. For example Cd an air pollutant from cigarette smoke and industrial emission is experimentally known to cause hypertension, cancer and immune disorders. It acts as classical stress agent and has also been implicated in learning disabilities. Several diseases are known to be associated with the deficiency or excess amounts of particular elements in body systems such as:

- *Minimata* due to Hg.
- *Itai-itai* due to Cd.
- *Keshan* due to deficiency of Se.
- *Baritosis* due to Ba inhaling.
- *Goiter* due to I deficiency.
- *Parkinson's disease* due to Cu and
- Alzheimer's supposedly due to Al.

A chronological order of the establishment of essentiality of trace elements and their normal metabolic functions including deficiency symptoms are listed in Table I. 3.

Physical illness can raise demands for many trace elements. For example, the need for Mg increases in heart disease and eating disorders. The demand of zinc increases under psychological stress. In following lines are described the importance of some elements whose essentiality or toxic effects have been proved.

Table I.3: List of essential elements, their metabolic functions and deficiency symptoms [18]

Element	Function	Deficiency Symptoms
Fe	Oxygen and electron transport, constitutes hemoglobin	Anaemia, Stomatitis, Dysphasia, brittle nails
Cu	Constituent of oxidative enzymes, interaction with iron, cross linking of elastin	Anaemia, Change of ossification, elevated serum cholesterol, diarrhea, nervous system damage
Mn	Mucopolysaccharide metabolism, constituent of super oxide dismutase	Disturbance in bones and cartilage formation, deafness, dizziness
Zn	Constituent of enzymes involved in energy metabolism and transcription	Growth depression, poor healing of wounds, sexual immaturity, skin lesions, menstrual problems, change of taste, acuity
Co	Constituent of vitamin B_{12}	Loss of appetite, vitamin B_{12} deficiency
Cr	Potentiation of insulin	Relative insulin resistance, impaired glucose tolerance, elevated serum lipids, fatigue and lack of energy, indigestion. Cancer and heart problems.
As	DNA repair mechanism	Growth depression
V	Gene regulations and in various enzymatic systems	Several physiological malfunctioning including thyroid, glucose and lipid metabolism, poor bone development..
Ni	Interaction with iron absorption, activates enzymes	Anaemia, inconsistent growth
Ca	Essential for formation of healthy bones and teeth, regulates blood clotting, muscle function, nerve transmission	Muscle cramps, brittle bone disease, dental problems, Osteoporosis.
Mg	Helps in absorption of other minerals, stimulates bone growth and promotes the body use of vitamin B, C, and E	Lack of energy, muscle spasms, weakness asthma, cardiovascular disorders
K	Essential for growth, stimulates nerve impulses, promotes healthy skin, boosts kidney function, In combination with Na regulates heart beats	Irregular heart beats, dry skin, nervous disorders
P	Cell repairs, vital to growth of bones and teeth, helps digest proteins, fats and carbohydrates	Poor growth, arthritis, loss of appetite, Bone weakening

Chromium (Cr): It is an essential trace nutrient required for normal sugar and fat metabolism [22]. It occurs in the body with highest concentrations in the liver, kidney, spleen and bone and works with insulin in the metabolism of sugar and stabilizes blood sugar levels, cleans the arteries by reducing cholesterol and triglyceride levels, helps transport amino acids and control appetite. It has been shown that persons with low levels of Cr are more susceptible to having cancer and heart problems and becoming diabetic. Toxic effects are due to industrial exposure of Cr (VI), whereas Cr (III) is essential [23].

Manganese (Mn): It helps nourish the nerves and brain and aids in the coordination of nerve impulse and muscular action. It helps eliminate fatigue and reduces nervous irritability [24]. Manganese is found in citrus fruits, the outer covering of nuts, grains, green leaves of edible plants, fish and raw egg yolk.

Iron (Fe): It exists chiefly as hemoglobin in the blood and transports oxygen inhaled into the lungs [25]. It is the master mineral which creates vitality and stamina, required for the healthy complexion and for building up resistance in the body. Its chief sources are grapes, raisins, spinach, all green vegetables, whole grain, cereals, dried beans, dark colored fruits, beets, dates, liver and egg yolk.

Cobalt (Co): It is a component of vitamin B_{12}, a nutritional factor necessary for the formation of red blood cells. Recent researches have shown that its pink colour is attributed to the presence of cobalt. The presence of this mineral in foods helps the synthesis of hemoglobin and the absorption of food-iron [26].

Copper (Cu): It helps in the conversion of iron into hemoglobin and stimulates the growth of red blood cells. It is also an integral part of certain digestive

enzymes. It makes the amino acid tyrosine usable, enabling it to work as the pigmenting factor for hair and skin. It is also essential for the utilization of vitamin C [27]. Copper is found in most foods containing iron, especially in almonds, dried beans, peas, lentils, whole wheat, prunes and egg yolk.

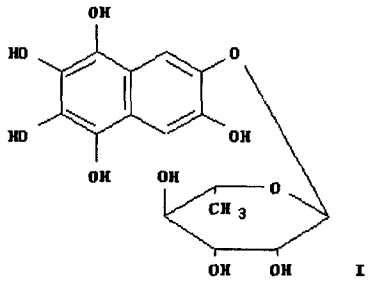
Zinc (Zn): It is needed for healthy skin and hair, proper healing of wounds, successful pregnancy and male virility. It plays a vital role in guarding against diseases and infection [28]. It is needed to transport vitamin A to the retina. Large number of enzymes requires zinc for their functioning.

Selenium (Se): It slows down ageing and hardening of tissues through oxidation. Males seem to have a greater need for this mineral. It is useful in keeping youthful elasticity in tissues. It alleviates hot flushes and menopausal distress and helps in the prevention and treatment of dandruff. Its deficiency can cause premature loss of stamina and may be a cause for breast cancer [29,30]. In Its deficiency was first noted in soil from Keshan province of China resulting in the cause of a disease named after that province.

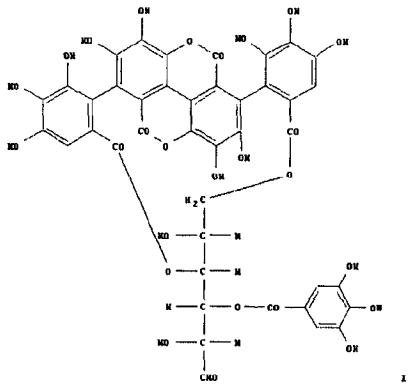
I.4 ORGANIC CONSTITUENTS

The phytochemistry of *Terminalia arjuna* has been extensively worked up in terms of a large number of organic compounds and reviewed [31,36]. Various constituents of the dried bark extract include acids such as arjunic acid, gallic acid, ellagic acid, arjunin [31] and terminic acid, glycosides such as arjunaphthanoloside [32], the flavone arjunolone, tannins, oligomeric proanthocyanidins (OPCs), colouring matter, essential oils and minerals such as

Ca, Mg, Zn and Cu. A novel naphthanol glycoside, arjunaphthanoloside, was isolated and its structure established as 2,3,6,7,8,9-hexahydroxynaphthalene 2-O-β-L(-)-rhamnoside by spectroscopic methods. Ali et al. [32] showed that arjunaphthanoloside [32] had potent antioxidant activity and inhibited NO production in lipopolysaccharide (LPS)-stimulated rat peritoneal macrophages.

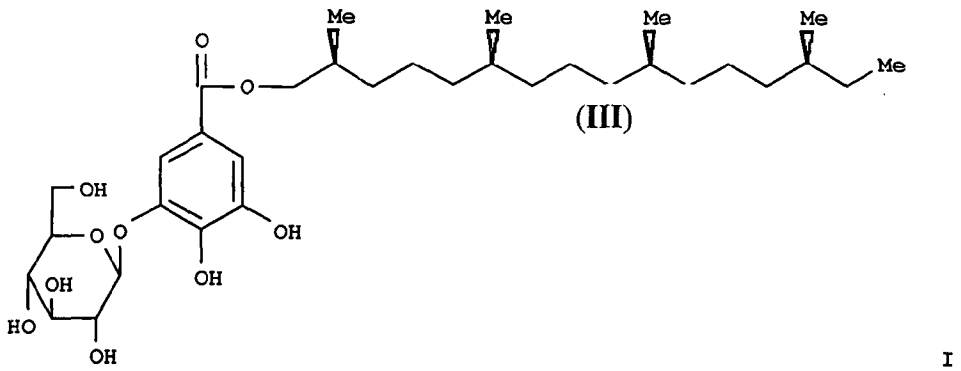


Arjunaphthanoloside (I)



Arjunin (II)

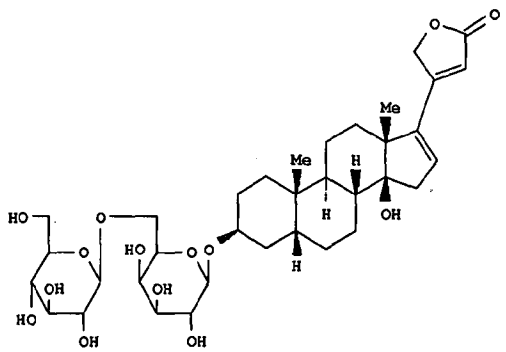
Siddiqui et al. [33] isolated a new phytoconstituent (III) responsible for hypotensive activity and its structure was elucidated from spectral evidence.



(III)

Crude ethanolic extract of the bark of *Terminalia arjuna* (Combretaceae)

and its three compounds arjunic acid, arjungenin and arjunetin were evaluated for antifeedant, growth inhibitory and oviposition-deterrent activities against a lepidopterous insect Spilarctia oblique [33]. Two pentacyclic triterpenoid glycosides from the bark of Terminalia arjuna, have been characterized as olean-3α, 22β-diol-12-en-28-oic acid-3-O-β-D-glucopyranosyl (1-4)- β-D-glucopyranoside and olean-3β,6β,22β-triol-12-en-28-oic acid-3-O-β-D-glucopyranosyl (1-4)- β-D-glucopyranoside [34]. Terminoside A, a oleanane-type triterpene was isolated from the acetone fraction of the ethanolic extract. The structure was established as olean-1α,3β,22β-triol-12-en-28-oic acid-3β-D-glucopyranoside. Chang et al. [35] isolated casuarinin [35], a hydrolyzable tannin from the bark and investigated its antiviral activity on herpes simplex type 2 (HSV-2) in vitro. It possesses anti-herpesvirus activity in inhibiting viral attachment and penetration, and also disturbing the late event(s) of infection. Yadav and Rathore [36] isolated a new cardenolide 16,17-dihydroneridienone 3-O-β-d-glucopyranosyl-(1 6)-O-β-d-galactopyranoside (**IV**) from its roots.



IV

I

The main purpose of studying the organic compounds is that these may act as probable ligands to the metals making them bioavailable.

I.5 ANALYTICAL TECHNIQUES

The composition of biological systems is so complex that the essential and trace elements are totally masked by the major constituents of C, H, N, O etc. Hence, their determination requires dissolution followed by sophisticated analytical methods. The techniques used here for the determination of essential and trace elements Arjuna bark are as follows-

(i) Neutron Activation Analysis **(NAA)** is one of the most sensitive, accurate and precise technique used for qualitative and quantitative elemental analysis of multiple major, minor, and trace elements in samples from almost every conceivable field of scientific or technical interest [37-42]. For many elements, NAA offers sensitivities that are superior to those possible by any other technique. Moreover, the accuracy and precision of the technique is such that it is still one of the primary analytical methods used by the National Institute of Standards and Technology (NIST) to certify the elemental concentrations in Standard Reference Materials (SRMs). For many elements, NAA offers sensitivities that are superior to those attainable by other methods of the order of ppb or better. In addition, NAA is generally recognized as the "reference method" of choice when new procedures are developed or when other methods yield results that do not agree.

George de von Hevesy and Hilde Levi discovered NAA in 1936 and found that samples containing certain rare earth elements became highly radioactive after exposure to a source of neutrons (^{222}Rn-Be). The sequence of events

occurring during NAA, namely the neutrons capture or (n,γ) reaction is illustrated in Fig. I.2.

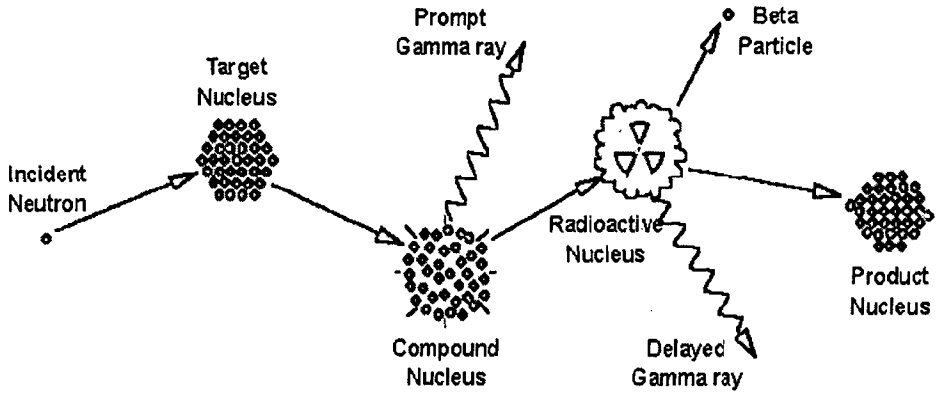


Fig. I.2 Schematic representation of neutron activation process followed by decay

When neutrons interact with the target nuclei, a compound nucleus is formed in an excited state. The excitation energy of the compound nucleus is due to the binding energy of the neutron with the nucleus. The compound nucleus will almost instantaneously de-excite into a more stable configuration through emission of one or more characteristic prompt γ rays. In many cases, this new configuration yields a radioactive nucleus which also de-excites (or decays) by emission of one or more characteristic delayed γ rays, but at a much slower rate according to the unique half-life of the radioactive nucleus. Depending upon the particular radioactive species, half-lives can range from fractions of a second to several years.

Subsequent to irradiation, the activity can be measured instrumentally by a high-resolution gamma ray spectrometry, or for still better sensitivity, chemical

separations can be applied to reduce interferences. The procedure generally used to calculate concentration (i.e., ppm of element) in the unknown sample is to irradiate the unknown sample and a comparator standard containing a known amount of the element of interest together in the reactor. If the unknown sample and the comparator standard both are measured on the same detector, then one needs to correct the difference in decay between the two. One usually corrects the measured counts (or activity) for both samples back to the end of irradiation using the half-life of the measured isotope. The eqn. used to calculate the mass of an element in the unknown sample relative to the comparator standard is

$$\frac{A_{sam}}{A_{std}} = \frac{m_{sam}}{m_{std}} \frac{(e^{-\lambda T_d})_{sam}}{(e^{-\lambda T_d})_{std}} \qquad \text{.....(I.1)}$$

where A = activity of the sample (sam) and standard (std),

m = mass of the element,

λ = decay constant for the isotope and

t_d= decay time.

When performing short irradiations, the irradiation, decay and counting times are normally fixed the same for all the samples and standards such that the time dependent factors cancel. Thus the above equation simplifies into

$$C_{sam} = C_{std} \frac{W_{std}}{W_{sam}} \frac{A_{sam}}{A_{std}} \qquad \text{.....(I.2)}$$

where C = concentration of the element in sample and standard and

W = Weight of the sample and standard comparator.

In principle, therefore, with respect to the time of measurement, NAA falls into two categories:

i. Prompt gamma-ray neutron activation analysis (PGNAA), where measurements take place during irradiation, or

ii. Delayed gamma-ray neutron activation analysis (DGNAA), where the measurements follow radioactive decay.

The latter operational mode is more common. Thus, NAA is generally assumed as the measurement of the delayed gamma rays. About 70% of the elements have properties suitable for measurement by NAA.

Although there are several types of neutron sources (reactors, accelerators, and radioisotopic neutron emitters) one can use for NAA, nuclear reactors with their high flux of neutrons from U fission offer the highest available sensitivities for most elements. Different types of reactors and different positions within a reactor can vary considerably with regard to their neutron energy distributions and fluxes due to the materials used to moderate (or reduce the energies of) the primary fission neutrons.

Owing to the high neutron flux, experimental ***nuclear reactors*** operating in the maximum thermal power region of 100 kW-10 MW with a maximum thermal neutron flux of ~10^{14} neutrons cm^{-2} s^{-1} are the most efficient neutron sources for high sensitivity activation analysis induced by epithermal and thermal neutrons. The reason for the high sensitivity of the majority of the elements is that the neutron absorption cross-section is high in the thermal region. There is a wide distribution of neutron energy in a reactor and, therefore, interfering reactions must be considered. In India, following nuclear reactors located at the Bhabha Atomic Research Centre (BARC), Mumbai are available for NAA experiments

Table I.4 Nuclear Reactors available for NAA at BARC, Mumbai

Reactor	*Type*	*Flux (ϕ)*	*Power (MW)*	*Moderator/Coolant*
APSARA (1956)	Swimming Pool	~10^{11}	1.0	Light water/ light water
CIRUS (1960)	Tank	~10^{12}	40	Heavy water/ heavy water
DHRUVA (1985)	Tank	~10^{13}	100	Heavy water/ heavy water

Apart from high sensitivity for many elements, NAA has the advantage that it is purely a nuclear method of analysis and the results are not influenced by the chemical state of the element under investigation. Moreover, contamination after irradiation is no longer a threat, a major advantage in trace element analysis. The modern γ measuring systems consist of a gamma detector, usually a HPGe type and sometimes NaI (Tl) scintillation crystals. The detectors are connected to a computer based multichannel analyzer (MCA) by an appropriate electronic system (preamlifier, spectroscopy amplifier, etc.).

Recent applications include:

- ✓ **Environmental** studies to characterize pollutants and determine their source and methods of reduction.
- ✓ **Semiconductor materials** analysis to measure ultra trace-element *impurities* and to determine methods for reducing or eliminating impurities from final products.
- ✓ **Pharmaceutical materials analysis** to measure ultra trace-element *impurities* and to determine methods for reducing or eliminating impurities from final products.

- ✓ **Forensic** studies as a non-destructive method to analyze *evidence* as an aid in the investigation and prosecution of criminal cases.
- ✓ **Archaeological** studies to fingerprint *artifacts* and determine the place of origin as a way to understand the activities of humans in the past.
- ✓ **Nutritional epidemiological** studies to investigate the contribution of diet, occupation, and lifestyle on *chronic diseases*.

(ii) Atomic Absorption Spectrometry (AAS) is now a well-established technique for the determination of trace elements covering a wide range of analyte types including metals and a few of the nonmetals [43, 44]. In their elemental form, metals will absorb UV light when they are excited by heat. Each metal has a characteristic wavelength that will be absorbed. AAS consists of a light source, a sample compartment and a detector. In this method, light source is directed through the sample to a detector. A schematic is shown in Fig. I. 3. The source of light is Hollow Cathode Lamp (HCL) whose cathode is composed of the element being determined. The lamp is housed inside the lamp compartment, which is really the flame since it absorbs radiation from the source. In the sample compartment an atomic sample vapor is generated in the light beam from the source. This is usually done by introducing the sample into a burner system (Flame AAS) or electrically heated furnace or platform, aligned in the optical path of the spectrophotometer. The sample of interest is aspirated into the flame. The flame height is controlled by regulating the flow of fuel mixture A monochromator is required to disperse several wavelengths of light that are emitted from the light source to isolate a particular line of interest. A detector

produces an electrical current that is dependent on the light intensity. . The instrument measures the change in intensity..

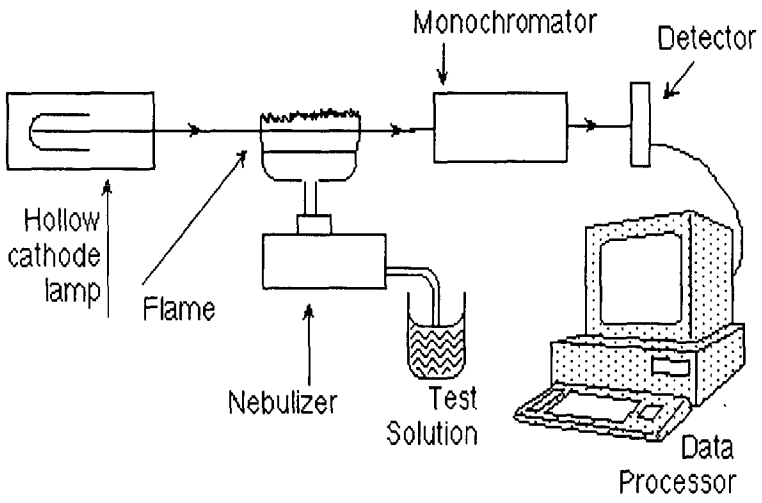


Fig. I. 3 Schematic of an Atomic Absorption Spectrophotometer (AAS)

The electrons of the atoms in the flame can be promoted to higher orbital for an instant by absorbing energy, which is specific to a particular electron transition in a particular element. As the quantity of energy put into the flame and the quantity remaining at the detector can be measured, concentration of the element may be calculated. A calibration plot is drawn by running standards of varying concentrations on the AAS and observing the absorbance. The computer data system draws the curve and samples are tested and measured against this curve.

(iii) Inductively Coupled Plasma Mass Spectrometry (ICP-MS). This technique developed in the late 1980's combines the easy sample introduction and quick analysis of ICP technology with the accurate and low detection limits of a mass spectrometer [42,45]. The resulting instrument is capable of trace

multielemental analysis, often at the part per trillion levels. ICP-MS has been used widely over the years, finding applications in a number of different fields including drinking water, wastewater, natural water systems/hydrogeology, geology and soil science, mining/metallurgy, food sciences and medicine.

This technique is used to measure the concentrations ranging from ng/mL to mg/mL of inorganic elements from the liquid samples such as water, oil, and metal ion solutions. It consists of four main processes, including sample introduction and aerosol generation, ionization by an argon plasma source, mass discrimination and the detection system as shown schematically in Fig. I. 4.

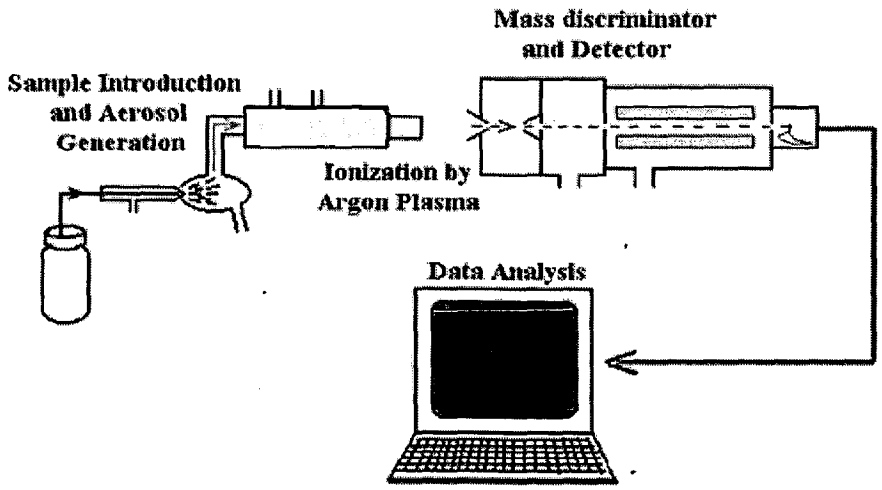


Fig. I. 4 Schematic of ICP-MS main process

Aqueous samples are introduced by way of a Nebulizer, which aspirates the sample with high velocity Ar gas forming a fine mist. The aerosol then passes into a spray chamber where larger droplets are removed via a drain. The liquid sample is pumped into a Nebulizer at atmospheric pressure where the aerosol mist is created. The mist droplets are separated by size in a spray chamber and only the finest particles are carried into the plasma where temperature varies

from 6000 to 10000 K. The aerosol is dried and ions are created which are extracted from the plasma into the ion lens through sample and skimmer cones interface by applying differential pressure. The pressure at the interface is ~ 10^{-3} torr. The ionized and atomized particles are extracted from the cone interface into the ion lens and quadrupole mass filter by applying high vacuum of 10^{-6} torr. The shadow stop in front of the ion lens removes the photons and the ion lens removes the atomized particles and allows mainly the ionized particles to the quadrupole mass filter by applying suitable electrical charge to the lens. However, all the ionized particles of various elements having different mass try to enter the mass filter under an electromagnetic field applied to the quadrupole. Only one mass passes at a time, which goes to the detector and produces electrons after collision, which is counted electronically. The measurement is done in pulse and analog mode for increased dynamic range. Therefore the measurement can be done together for low and high concentration in the same sample.

I.6 LITERATURE SURVEY

Most studies on medicinal plants reported in literature pertain to the organic contents viz. essential oils, alkaloids and their antioxidant properties, polyphenols, vitamins etc. A thorough literature survey shows only scanty reports on essential trace element contents in *Arjuna* bark. Garg and Khanduja [46] were the first to report the mineral composition in terms of calcium, magnesium and aluminum oxides in *Arjuna* leaves growing on an alkaline soil. Singh and Garg [47] reported 20 elements (As, Ba, Br, Ca, Cl, Co, Cr, Cu, Fe, K, Mn, Mo, Na, P,

Rb, Sb, Sc, Se, Sr and Zn) in *Arjuna* bark. An essential ingredient of *Pragya-peya*, an herbal drink is *Arjuna* and in our earlier work, we analyzed *Arjuna* bark along with 11 other herbal constituents for 7 minor (Al, Ca, Cl, Mg, Na, K, P) and 15 trace (Ba, Br, Co, Cr, Cs, Fe, Hg, La, Mn, Rb, Sc, Se, Th, V, Zn) elements [48]. Naidu and Swamy [49] observed seasonal variation in Ca, Mg, S and Na contents of *Terminalia arjuna*.

Comparatively speaking, organic constituents of *Arjuna* bark have been widely studied by a large number of workers in India and abroad. Pawar and Bhutani [50] isolated oleanane triterpenes arjunic acid, arjungenin and their glucosides, arjunetin and arjunglucoside from the bark. Cheng et al [35] isolated casuarinin, a hydrolyzable tannin and investigated its antiviral activity. This compound has been further studied by Kuo et al [51] who found it to inhibit human non-small cell lung cancer A549 cells. Kaur et al [52] studied the antimutagenic effect of benzene, chloroform, acetone and methanol extracts as well as that of ellagic acid isolated from *Terminalia Arjuna*. Kandil et al [53] isolated arjunin, four known tannins and two phenolic acids and determined their structures by spectroscopic analyses. Gupta et al [54] reported antioxidant action of the bark powder and found it to be comparable to vitamin E. In addition, it also has a significant hypocholesterolaemic effect. Nagpal et al [55] investigated the effects of acetone and methanol extracts on the growth of human normal fibroblasts. Bharani et al. [56] observed improvement in symptoms of heart by bark. Dwivedi et al. [57] studied the effect of *Terminalia Arjuna* on angina pectoris. Singh et.al. [31] evaluated the insect feeding-deterrent and growth

inhibitor activity of Arjunetin from the ethanolic extract. Upadhyay et al [58] isolated new triterpene glycoside, arjunetoside, together with oleanolic and arjunic acid in the root by chemical and spectral data. Sivalokanathan [59] evaluated the antioxidant nature of ethanolic extract of the bark on N-nitrosodiethylamine (DEN) induced hepatocellular carcinoma in rats. Rane [60] studied the comparative effect of oral administration and topical application of alcoholic extract the bark on incision and excision wounds in rats. Patel et al [61] extracted tannin from the bark of *Arjuna* tree. Bhuyan et al [62] extracted and identified the color components from the barks of *Terminalia arjuna* and also evaluated their wool dyeing characteristics on wool. Bhatia et al [63] isolated oxalic acid by digesting the bark with water and 20% H_2SO_4 at 100°C. Nagar et al [65] isolated cerasidin, β-sitosterol, friedelin, methyl oleanolate, gallic acid, ellagic acid, and arjunic acid from the fruits by successive extraction with light petroleum and ethanol followed by column chromatography over silica gel. Colabawalla et al [65] found that the alcoholic extract of the bark contained CaO (0.33 %), MgO (0.078%), and Al_2O_3 (0.076%). The powdered bark was found to contain 38% CaO. Mummert et al [66] reported the use of aqueous-alcoholic extracts of its bark for the production of cosmetic or dermatological preparations for the treatment of ignitable skin conditions and/or to the skin protection with sensitively determined dry skin. Honda [67] isolated arjungenin, arjunglucoside I, and arjunglucoside II, a new triterpene and new triterpene glucosides from Terminalia Arjuna bark.

I.7 AIM AND SCOPE OF THE PRESENT WORK

Plants have been a rich source for therapeutics in all world civilizations. *Ayurveda*, the ancient Indian system of medicine fully recognizes and utilizes medicinal properties of plants. *Arjuna (Terminalia Arjuna)* is a well-known heart tonic used extensively in cardiac debility. It is being used since the days of *Charak* and *Susruta*. It has attracted the attention of many research workers around the world essentially because of its beneficial properties of strengthening heart muscles. According to newspaper reports (see in front) *Arjuna* is considered as a wonder drug in reply to china's *Ginseng*. This prompted us to undertake present work to determine essential and trace elements composition along with organic constituents in the bark of *Terminalia Arjuna*. Though many workers have isolated organic compounds but no studies have been reported on elemental composition. *Arjuna* has blood-coagulating properties and can arrest bleeding and is also used in various problems like diabetes mellitus, diarrhoea, dysentery, obesity and leucorrhea. Following work has been carried out in the present dissertation.

- Determination of 7 minor (Na, K, Ca, Mg, Cl, P and Fe) and 20 trace (As, Ba, Br, Co, Cr, Cs, Cu, Eu, Fe, Hg, La, Mn, Rb, Sb, Sc, Sm, Sr, Th, V, Zn) elements by INAA
- SRMs from INCT Poland and NIST, USA were analyzed for quality assurance and data validation.
- Determination of 14 elements including Ni, Cd, Pb and Mo by AAS.
- Determination of 22 elements by ICP-MS including Sn, which could not be determined by other methods.

- Comparison of some essential trace elemental concentrations by different techniques of NAA, AAS and ICP-MS
- Geographical variation was studied by analyzing five samples collected from Mumbai, Nagpur and Roorkee including two commercial brands (Himalaya Drugs, Bangalore and Vyas Pharmacy, Indore).
- Three new organic compounds were isolated from the methanolic extract by TLC and column chromatography.
- Structures of the compounds were confirmed by elemental analysis, infrared spectra and GC-MS.
- Fragmentation pattern has been proposed for three compounds.
- Thermal decomposition studies were carried out in air and N_2 atmosphere and correlate the data with elemental analysis.

An attempt has been made to correlate elemental contents with the therapeutic properties of *Terminalia Arjuna*.

Ch II

METHODS & INSTRUMENTATION

We have primarily used NAA for elemental analysis of *Arjuna* bark though AAS and ICP-MS were also used for data validation. Further, three new organic compounds were separated from the methanolic extract by thin layer and column chromatography and identified by elemental analysis, spectral methods and GC-MS studies. Also thermal decomposition studies were performed. All the details of various instrumental methods used in this study are included here.

II. 1 SAMPLING

All the bark samples for analysis were procured in powder form and purchased from different chemist shops in cities of Roorkee, Nagpur and Mumbai. The other two brands were from Vyas Pharmacy, Indore, and capsules from the Himalaya Drug Company, Bangalore. For the analysis of organic constituents bark was collected from a tree in the campus. It was dried, and powdered in an agate mortar. Following three standard reference materials (SRMs) of biological origin were used as comparator standards and for quality assurance/data validation.

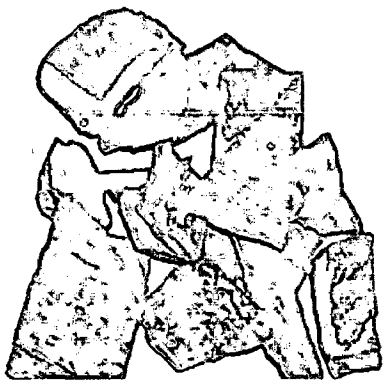


Fig. II.1 Arjuna bark

(i) Mixed Polish Herbs (INCT-MPH-2) from the Intitute of Nuclear Chemistry & Technology (INCT), Warsaw, Poland [68]

(ii) Peach Leaves (SRM 1547) from the National Institute of Standards and Technology (NIST), USA [69]

(iii) Pine Needles (SRM 1575a) from NIST USA [70]

(iv) Apple leaves (SRM 1515) from NIST, USA [71]

All the samples along with SRMs were dried in oven for 2 h before packing for irradiation.

II.2 PACKING AND IRRADIATION

About 30 mg each of the powdered samples along with RMs were accurately weighed and heat-sealed in Alkathene bags. In each batch five samples including two SRMs were packed for 1 min irradiation using pneumatic carrier facility (PCF) in DHRUVA reactor at a thermal neutron flux of $5x10^{13}$ n cm^{-2} s^{-1} at BARC, Mumbai. Activity measurements were carried out at the reactor site of BARC and later at the Radiochemistry Division of BARC, Mumbai. 40-50 mg each of samples and three RMs were accurately weighed and packed in aluminum foil to make a second batch of 12 samples. It was irradiated for 3 d in CIRUS reactor at a thermal flux of ~10^{13} n cm^{-2} s^{-1}. Afterwards these were brought to Roorkee for activity measurement. In both cases, irradiated packets were decontaminated by swiping with tissue paper and cotton soaked in acetone.

II.3 MEASUREMENT OF ACTIVITY

Activity of short lived radionuclides was measured using HPGe detector (EG & G, ORTEC) coupled with 8k multichannel analyzer (MCA) at the reactor site in BARC, Mumbai. My colleague Mr. R. Paul Choudhury carried out all these measurements at BARC. However, long-lived nuclides were counted at our laboratories in Roorkee. A coaxial HPGe detector with 8k MCA (Canberra, USA) and GENIE 2000 software was used as shown in the photograph (Fig. II.2). Following are the specifications and performance data of the detection system; Detector Model GC 2018; Cryostat Model 7600 SL; Serial No. 05017312; Preamplifier Model 2002 CSL; Resolution: 1.8 keV (FWHM) at 1332 keV of ^{60}Co and 0.9 keV (FWHM) at 122 keV of ^{57}Co,

Relative Efficiency; 20% w.r.to 3"x3" NaI(Tl) detector, Peak to Compton ratio 50:1

Physical characteristics: 60.5 mm dia x 29.5 mm length (Volume = 80 cm^3), Distance from window = 5 mm, Recommended Bias voltage = +4 kV dc obtained through 5 kVA UPS system (On line) from Stellar, New Delhi.

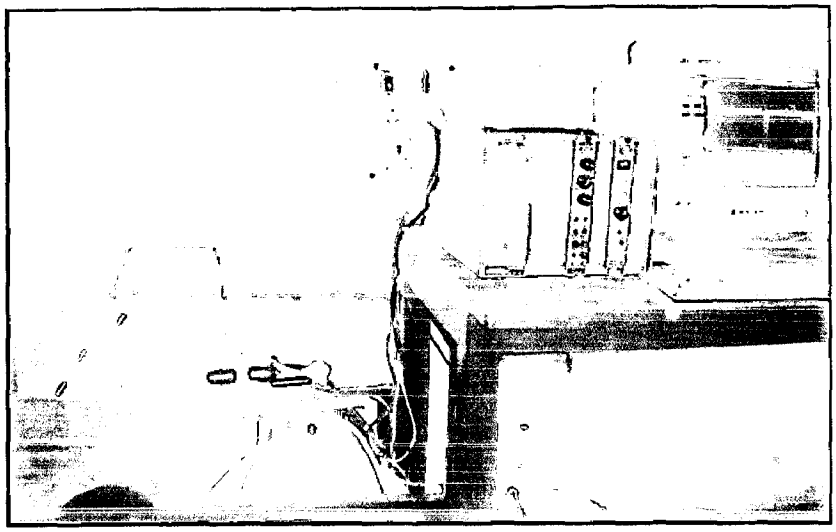


Fig. II.2 Detector assembly and counting set up at our laboratory

Irradiation, delay and counting schedule are given in Table II.1

Table.II.1 Irradiation, delay and counting schedule

Reactor (Irradiation time)	Delay time	Counting time	Radionuclides Identified
PCF DHRUVA Reactor (1min)	1 min	60 s	^{28}Al, ^{52}V
	5 min	100 s	^{49}Ca, ^{27}Mg
	15 min	300s	^{38}Cl, ^{56}Mn
	1 h	1000s	^{56}Mn
	2h	2000s	^{24}Na, ^{42}K, ^{82}Br, ^{76}As, ^{140}La, ^{153}Sm, ^{64}Cu
CIRUS (3d)	25d	2000s	^{32}P (β counting)
	20d	6h	^{51}Cr, ^{131}Ba, ^{203}Hg, ^{87}Rb, ^{232}Th,
	45d	12h	^{203}Hg, ^{87}Rb, ^{59}Fe, ^{124}Sb, ^{145}Ce
	3 months	24h	^{60}Co, ^{59}Fe, ^{46}Sc, ^{65}Zn, ^{124}Sb, ^{134}Cs, ^{152}Eu

Nuclear characteristics of various radionuclides identified and determined in this study are listed in Table II.2

Table II.2 Nuclear characteristics of radionuclides identified and determined

Target Element (Nuclide)	Isotopic Abundance (%)	Product Nuclide	Half Life ($t_{1/2}$)	Cross Section σ (b)	Energy E_γ (keV)
Al (^{27}Al)	100	^{28}Al	2.241m	0.23	1779
As (^{75}As)	100	^{76}As	26.3h	4.3	559, 657
Ba (^{130}Ba)	0.1	^{131}Ba	12d	8.8	373, 496
Br (^{81}Br)	49.5	^{82}Br	35.4h	3.31	554, 776
Ca (^{48}Ca)	0.187	^{49}Ca	8.718m	1.1	3084
Ce (^{140}Ce)	88.5	^{141}Ce	33d	0.6	145
Cl (^{37}Cl)	24.4	^{38}Cl	37.2m	0.43	1643, 2167
Co (^{59}Co)	100	^{60}Co	5.27y	37.2	1173, 1332
Cr (^{50}Cr)	4.35	^{51}Cr	27.8d	15.9	320
Cs (^{133}Cs)	100	^{134}Cs	2.06y	30	605,796
Eu (^{151}Eu)	47.8	^{152}Eu	13.5y	5900	244, 1408
Fe (^{58}Fe)	0.33	^{59}Fe	44.6d	1.2	1099, 1291
Hg (^{202}Hg)	29.7	^{203}Hg	46.6d	3.8	279
K (^{41}K)	6.88	^{42}K	12.5h	1.30	1524
La (^{139}La)	99.9	^{140}La	40h	8.93	487, 1596
Mg (^{26}Mg)	11.17	^{27}Mg	9.46m	0.038	1014
Na (^{23}Na)	100	^{24}Na	15h	0.5	1368
Mn (^{55}Mn)	100	^{56}Mn	2.58h	13.3	846
P (^{31}P)	100	^{32}P	14.3d	0.19	1708(β^-)
Rb (^{85}Rb)	72.1	^{86}Rb	18.7d	0.91	1077
Sb (^{123}Sb)	42.8	^{124}Sb	60d	3.3	603, 1691
Sc (^{45}Sc)	100	^{46}Sc	83.8d	27.2	889, 1120
Sm (^{152}Sm)	0.21	^{153}Sm	47 h	5800	103
Sr (^{84}Sr)	0.56	^{84}Sr	65.2d	1.4	514
Th (^{232}Th)	100	^{233}Th(^{233}Pa)	27d	7.4	312
V (^{51}V)	100	^{52}V	3.74m	4.9	1434
Zn (^{64}Zn)	48.6	^{65}Zn	244d	0.76	1115

II.4 CALCULATION OF CONCENTRATIONS

Concentrations of elements were calculated by using comparator method where RM with known and certified concentrations of various elements is simultaneously irradiated along with the sample under similar experimental conditions and counted in the same geometry [37]. For short lived nuclides where half life ($t_{1/2}$) and delay time (t_d) are comparable eqn (I.1) mentioned in Ch I was used. However, if $t_{1/2}$ of the radionuclide is long enough compared to delay and counting time then it can be further simplified to

$$\text{Concn. of element in sample } (S_a) = \text{Concn. of element in standard } (S_t) \times \frac{\text{Sp. Activity in sample}}{\text{Sp. Activity in standard}} \quad \ldots\ldots\ldots \text{(II.1)}$$

Thus all the elemental concentrations were calculated as discussed in Ch III.

II. 5 AAS DETERMINATION

The inorganic content of most medicinal herbs is only a minor constituent and interfered by major constituents. Therefore, first task in inorganic analysis is to remove the organic matter. Several methods have been reported in the literature [72, 73]. Organic matter is usually destroyed by dry oxidation i.e. burning in air and converting to ash. This requires heating at ~ 500° C where many volatile elements may be lost. Another method is by wet oxidation by acids, which has the advantage of being applicable to a wide variety of samples. It is fairly rapid and less prone to volatilization. Commonly employed acids include HCl, HNO_3 including aquaregia, H_2O_2 and $HClO_4$. For the analysis of medicinal herbs, a combination of above reagents is normally recommended.

(i) Dissolution of sample; About 2g of powdered sample was weighed accurately and taken in a 100mL beaker. 10 mL mixture of 5:1, nitric acid, perchloric acid was added and then heated on a hot plate [74]. After sometime slurry was formed which was cooled and then 10 mL of acid mixture was added drop wise. The mixture was continuously stirred till all the fumes vanished. All these operations were performed in a fume hood. It was further heated till a clear solution was obtained. On further heating a white gelatinous mass was observed. 10 mL of double distilled water was added. It was then heated to reduce the solution up to 2 mL. After further reducing it to slurry few drops of the acid mixture was added and heated. Water was added to see if all the organic matter has been oxidized. If not then 1 mL of 6 M HCl was added drop wise. Afterwards 5 mL double distilled water was added and the solution was filtered through Whatman filter paper no. 42 to remove any turbidity or suspended matter. The solution was made to 50 mL. All the solutions were stored in tightly capped polythene bottles. These were directly used for the determination of various elements by AAS and ICP-MS. All the common acids such as HCl, HNO_3 and H_2SO_4 were of AR grade. Double distilled water was used for preparing all the solutions..

(ii) Preparation of Standards for AAS and ICP-MS; 100 mL 0.1 M solutions of various metal ions were prepared by dissolving weighed amounts of their respective AR/High Purity grade salts with following specifications

1. *Molybdenum;* 12.359 g ammonium molybdate $(NH_4)_6MO_7O_{24}.4H_2O$, MW =1235.9, AR 99% Qualigens. Mumbai

2. *Nickel;* 3.95 g ammonium nickel (II) sulfate hex hydrate. $(NH_4)_2SO_4.NiSO_4.6\ H_2O$ MW=394.97.AR 98.5% E. Merck, Mumbai.
3. *Arsenic;* 1.97 g arsenous oxide As_2O_3, Mw = 197.82, A.R 99.8% BDH, Mumbai
4. *Antimony;* 2.28 g antimony trichloride $SbCl_3$ MW = 228.11 AR 99%, Sisco Research Laboratories. Mumbai
5. *Barium;* 2.44 g barium chloride $BaCl_2$ Mw = 244.28 AR = 99.5% , Merck Mumbai
6. *Calcium;* 2.36 g calcium nitrate tetra hydrate $Ca(NO_3)_2.4H_2O$, MW = 236.15, AR,99%., S.d. fine chemicals Mumbai
7. *Cadmium;* 3.08 g cadmium nitrate $Cd(NO_3)_24\ H_2O$, MW= 308.47, AR,99% Thomas Baker Mumbai.
8. *Cobalt;* 2.91 g cobalt nitrate hex hydrate $Co(NO_3)_2.6\ H_2O$ MW = 291.04, AR,99% Thomas Baker Mumbai
9. *Cesium;* 3.25 g cesium carbonate Cs_2CO_3, MW = 325.82 AR = 99%, Merck, Germany.
10. *Copper;* 1.704 g copper chloride, $CuCl_2.2\ H_2O$ MW= 170.48, AR = 99.5% Merck Mumbai.
11. *Chromium;* 4.00 g chromium nitrate, $Cr(NO_3)_3.9H_2O$ MW= 400.15, AR, 99% Merck Mumbai
12. *Iron;* 3.92 g ferrous ammonium sulfate, $(NH_4)_2SO_4.FeSO_4.6H_2O$ MW = 392.13 AR 99% , Thomas Baker (Chemicals) Mumbai.
13. *Mercury;* 2.71 g mercury (II) chloride. $HgCl_2$ MW= 271.50, AR 99%, E Merck, Mumbai
14. *Potassium;* 0.745 g potassium chloride KCl, MW= 74.55 AR 99.5 % S.d. Fine Chemicals, Mumbai.
15. *Lead;* 3.312 g lead nitrate $Pb(NO_3)_2$ MW = 331.21,AR,99% Samir Tech. Chem. Industry
16. *Zinc;* 2.19 g zinc acetate Extra pure $Zn(CH_3COO)_2.H_2O$ MW = 219.50, AR 99.9% Sisco Research Laboratories Mumbai

17. *Magnesium;* 2.46 g magnesium sulfate $MgSO_4$.$7H_2O$ MW= 246.48, AR 99% Merck Mumbai
18. *Manganese;* 1.97 g manganese (II) chloride $MnCl_2$. 6 H_2O MW= 197.9,AR 99%, Merck Mumbai
19. *Sodium;* 0.584 g sodium chloride NaCl MW = 58.44 AR, 99.9 % , Merck Mumbai.
20. *Strontium;* 2.66 g strontium chloride hex hydrate, $SrCl_2.6H_2O$, MW = 266.6, AR, 99%, Merck, Mumbai
21. *Tin;* 2.256 g stannous chloride dihydrate, $SnCl_2.2H_2O$, MW = 225.6, 99%, Merck, Mumbai

All solutions were prepared in doubly distilled water in standard Borosil glassware and stored in precleaned polythene bottles. These solutions were further diluted to desired concentration of ppm range.

(iii) Instrumentation; Atomic Absorption Spectrophotometer (GBC Avanta) was used at the Institute Instrumentation Centre (IIC). Oxy acetylene gas mixture was used and the burner height was adjusted to ignite the flame. The instrument was calibrated and its sensitivity was checked using standards. Wavelengths and the sensitivity for the measurement of 14 elements determined here are listed in Table. II.3. First calibration plots were drawn using standard solutions. Typical calibration plots for Pb and Cd are shown in Fig. II. 3a and b respectively.

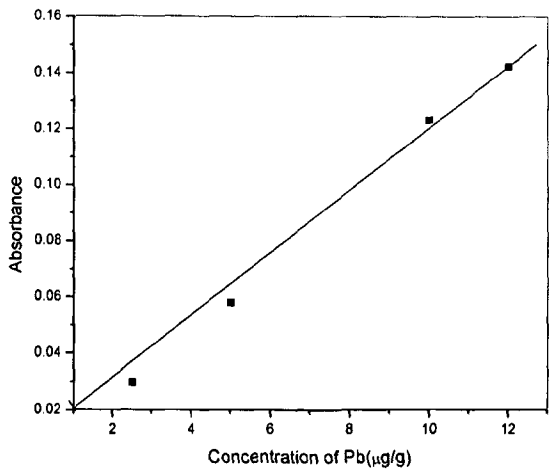


Fig. II.3(a): Calibration plot of Pb

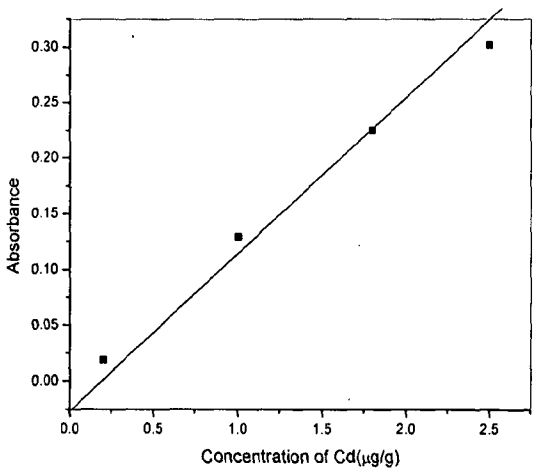


Fig. II.3(b): Calibration plot of Cd

Table II.3 Wavelength, working range and Detection limits of elements analyzed by AAS.

Element	Wavelength (nm)	Working Range (ppm)	Sensitivity (ppb)
As	193.7	30-190	640
Ca	422.7	1 - 4	2
Cd	228.8	0.2 - 1.8	9
Co	240.7	2.5 - 9	50
Cr	357.9	2 - 15	50
Cu	324.5	1 - 5	25
Fe	248.3	2 - 9	50
Hg	253.7	73 - 290	1600
Mn	279.5	1 – 3.6	20
Mg	285.2	0.1 – 0.4	3
Mo	313.3	5 - 20	110
Ni	232.0	1.8 - 8	40
Pb	217.0	3 - 11	6
Zn	213.9	0.4 - 15	8

II. 6 ICP-MS DETERMINATION

Four standards of 10-100ppb (10, 50, 100 ppb) were prepared of 22 elements and they were then analyzed by the instrument, Perkin Elmer, Elan DRC-e with following accessories at IIC. Liquid sample introduction system, Plasma ion source, Ion extraction interface, Dynamic Reaction Cell, Quadrupole Mass Analyzer and Electron Multiplier Ion Detector. Specifications; RF generator: Frequency 27.12MHz Air Cooled,

Frequency stability > 0.5%,

Power o/p: 1000-2000Watt., Interface: Water-cooled.

Nebulizer; Cross flow, Direct injection,

Spray chamber: Peltier Cooled.

II.7 SEPARATION OF ORGANIC CONSTITUENTS

25g *Terminalia Arjuna* bark powder was extracted repeatedly with methanol using Soxhlet apparatus for 6 h. The solvent was then distilled off to obtain 6.5 g materials. 6 g of extract was taken in a 1.5X45 cm^2 glass column packed with 200g Silica Gel-G (60-120 mesh) from Merck, Mumbai. A series of solvents were used as eluants in order of their increasing polarity. In all seven fractions were collected;

Fraction-1 in Petroleum ether/CCl_4/benzene (60:30:5) shows a single spot with R_f=0.79. The solvent was distilled off and a reddish compound (**PG I**) was recrystallized in acetone (yield, 321 mg).

Fractions -2 to 7 were impure and mixed together and the solvent was removed to get ~5 mL residue. The mixture was subjected to preparative TLC (20x20cm^2) on 1 mm thick layer Silica Gel (SRL-Mumbai) using CCl_4 /CH_2Cl_2/ MeOH (3:3:1) five times. The bands were allowed to develop in an iodine chamber. The bands were then scrapped out and dissolved in dichloromethane. It was then filtered using cotton wad and the solvent was distilled off. Two compounds with R_f values of 0.63 in $CHCl_3$/ diethyl ether (10:1) and 0.72 in $CHCl_3$ / MeOH (20:1) were obtained. The first compound was obtained as yellowish oil (**PG II**) while second one on recrystallizaion in methanol yielded sharp white crystals (**PG III**) with yield of 79 mg. The compounds **PG I** and **PG III** showed m.pt of 199 °C and 212 °C. PGIII also gave acid test in litmus paper.

Whole scheme of separation is shown in Fig. II.4. All the three organic compounds were characterized by elemental analysis, IR and GC-MS.

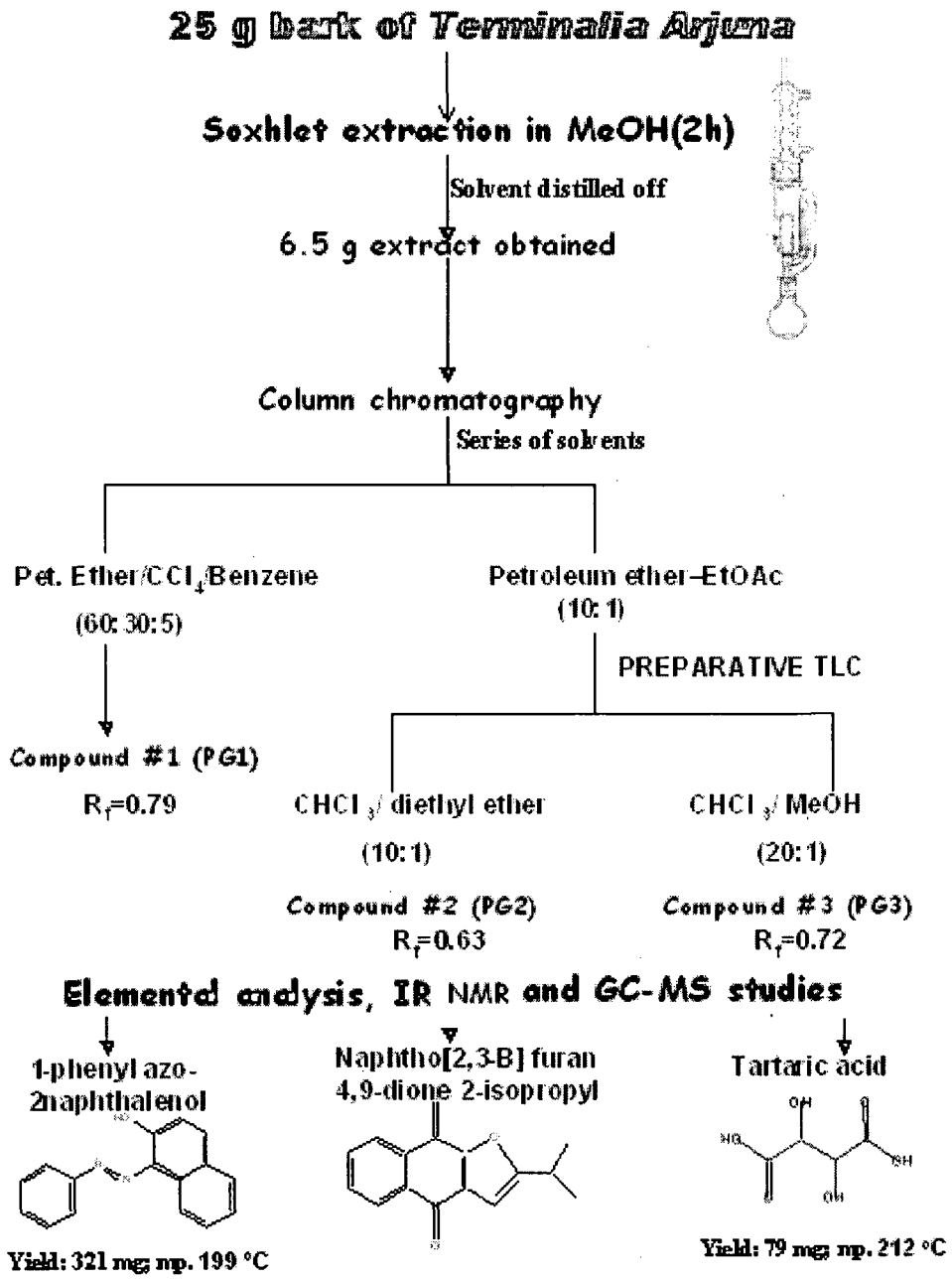


Fig. II.4. Flowsheet illustrating the separation of organic constituents from *Arjuna* bark

II.8 INSTRUMENTATION

The identity the separated compounds and to study carbonaceous matter Elementar Vario-EL III (Germany) was used to carry out C, H, N and S analysis. Infrared spectra were recorded in KBr using Thermo Nicolet (Nexus, USA) FT-IR Spectrophotometer in the range 400-4000 cm^{-1}. ^1H NMR was recorded in $CDCl_3$ at the Regional Sophisticated Instrumentation Centre (RSIC), Chandigarh using 200 MHz av Bruker spectrometer through the courtesy of Prof. Alok Srivastava.

A Perkin-Elmer Clarus-500, gas chromatograph coupled with a mass spectrometer was used. The compound mixture was separated on a fused silica capillary column (HP-5MS) (5% phenyl methyl siloxane), 30 mm x 0.32 mm, 0.25 μm film thickness, in a temperature program from 50 (2 min hold) to 250 °C (10 min hold) at a heating rate of 8 °C/min. The injector temperature was 250 °C, and the flow rate of 1mL/min He gas. The interface, which kept the capillary column end into the ion source block, was at 280 °C.

Thermograms were recorded on a Perkin Elmer (Pyris Diamond) Thermo Gravimetric Analyzer in air and nitrogen at a heating rate of 10°C min^{-1} using alumina as a standard. Typical Thermograms of *Arjuna* bark (Himalaya Drug Co., Bangalore) in N_2 and air are shown in Fig. II. 5(a) and (b)

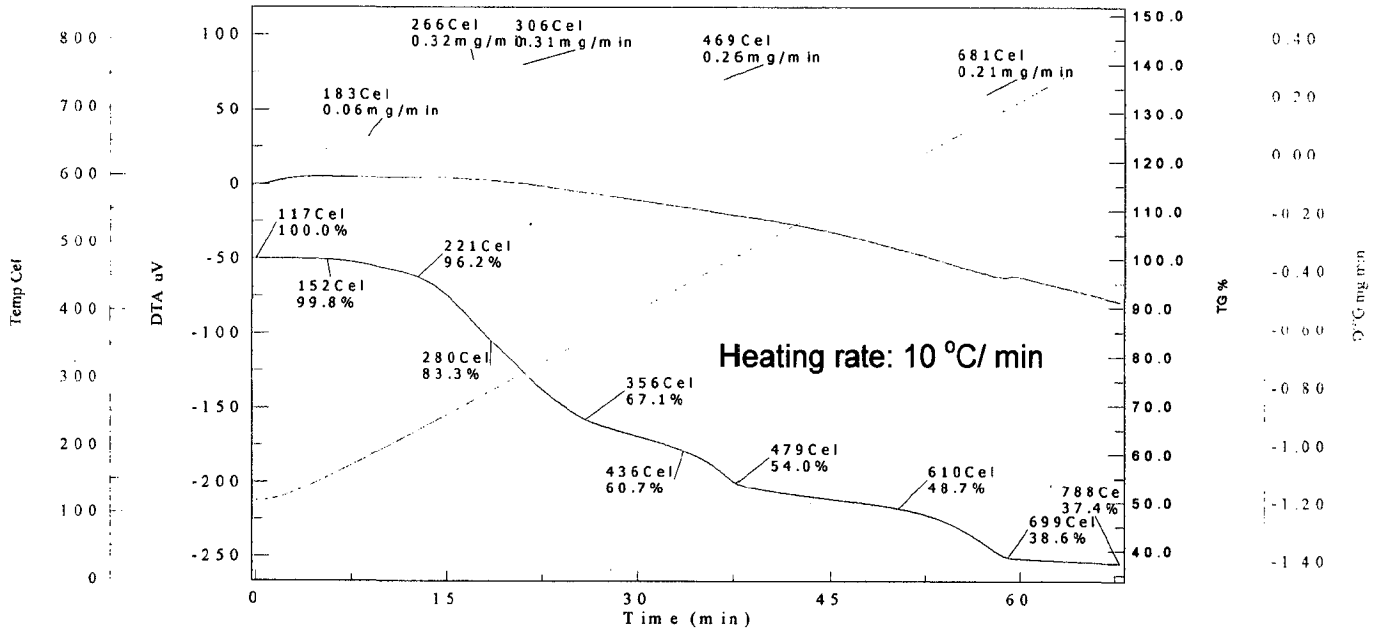


Fig III.5 (a) Thermogram of *Terminalia Arjuna* bark powder in N_2.

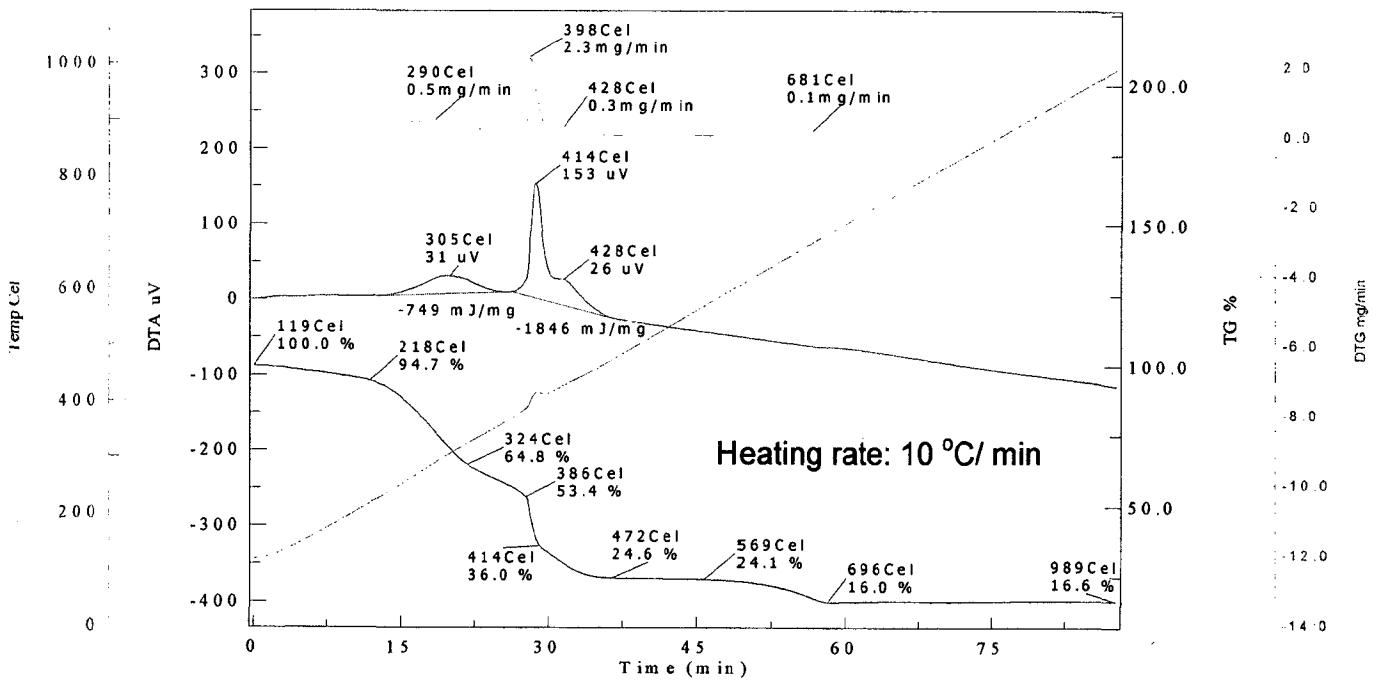


Fig. III.5 (b) Thermogram of *Terminalia Arjuna* bark powder in air

Ch III

RESULTS & DISCUSSION

III.1. RESULTS

Elemental contents in RMs as calculated on the basis of other RM as comparator standard are listed in Table III.1. Also included are certified values [68-71] for comparison. Since irradiations were carried out in different batches of short and long durations, at least Peach Leaves (SRM 1547) was common in both irradiations and for this reason maximum number of 27 elements could be

determined in this case whereas in other cases only a few elements are reported. Elemental concentrations in five different samples of *Arjuna* bark powder including ranges and mean ± SD are listed in Table III.2. 14 and 22 elements were also determined by AAS and ICP-MS for comparison and data validation. The data obtained by the two techniques are listed in Tables III.3 and 4 respectively. In order to validate data Z-score plot for Peach leaves was drawn and the same is shown in Fig III.1. Typical γ-ray spectra for short and long-lived nuclides produced in *Arjuna* bark after 1min and 3d irradiation are shown in Figs III.2 and 3 respectively. Also shown are the identified photo peaks corresponding to various radionuclides. A comparison of minor, trace and toxic elemental concentrations in five brands is shown as bar plots in Figs. III.4, 6 and 8 respectively. Variation in minor and trace element concentrations in five brands is shown in Fig III.5 and 7 respectively. Comparison of our data with those reported in literature is listed in Table III.5. Bar plots of elemental ratios of K/P and Ca/P are shown in Fig. III. 9 and 10 respectively. Infra red spectra, GC-MS and fragmentation pattern of the three organic compounds 1-phenylazo 2-naphthalenol, 2-isopropyl naphtha [2,3-b] furan 4,9-dione and tartaric acid are shown in Figs III.11, 12 and 13 respectively.

III.2 DATA VALIDATION

A comparison of our data with those of certified values in Table III.1 shows that most values are in good agreement within ±10% except in few cases. Though all the data are not available for all four RMs analyzed in this study because different sets of SRMs were used in different irradiation batches. Typical γ-ray spectra for short and long-lived radionuclides are shown in Fig. III.1 and 2 respectively. The

radionuclides were identified from the calibration plot before each measurement. For determining short lived nuclides, Peach leaves (SRM-1547) and Apple leaves (SRM-1515) from the NIST, USA were used whereas for long lived nuclides, Peach leaves and Pine needles (SRM-1575a) from NIST, USA and MPH-2 from INCT Poland were used. Hence, %RSD was calculated for Peach leaves as listed in Table III.1. It is observed that %RSD for most elements, are below 5% except for Cs, Cr, Hg, Na, Rb, Sb and Th. Also, z-score plot for all the 27 elements determined by NAA is shown in Fig. III.1. A cursory look at the data and Fig. III.1 shows that except for As, Cl, Fe, Mn, Eu and Th, z-scores of majority of elements lie between +3 to –3 suggesting that our values are within 95 % confidence limit. On the basis of this comparison it may be concluded that most elemental concentrations in samples should also be reliable within 95% confidence limit whereas for others it may be within 90% confidence.

III.3 VARIATION IN ELEMENTAL CONTENTS

Elemental contents in 5 different brands of *T. Arjuna* bark as determined by NAA are listed in Table III.2. For the sake of simplification we have classified all the elements as essential (for those found at mg/g level), trace (for those found at μg/g and ng/g level) and toxic elements as mentioned in *Ch. I*, Table I.2. since large variations are observed in all the elemental contents in five samples, these are discussed in following lines.

Essential Minor Constituents; It is observed that Al, Na, Mg, Fe, P, Cl, K and Ca, found at mg/g level, are all well known essential constituents in plant species and their functions as required for various biochemical processes are mentioned in Table

I.3. Variation in elemental contents amongst individual brands is quite marked as evident from the SD values mentioned in last column of Table III.2 and bar plot shown in Fig III.4. Not only Ca content is highest in all the brands but also its variation is within a small range of 18.0-46.6 mg/g, by a factor of 3. Similar is the case with K which varies in the range of 2.88-8.07 mg/g and Mg (2.46-7.18 mg/g) and P (0.19-0.62 mg/g) which all vary by a factor 3 to 4. On the other hand Na (0.06-0.40 mg/g) and Cl (1.44-6.29 mg/g) show much larger variations by a factor of 4 to 5. Incidentally Himalaya brand shows quite high concentrations of Na and Cl suggesting that probably some common salt might have been added to suppress its bitterness and enhance its shelf life. Fe content (0.14-4.84 mg/g), however, varies by more than an order of magnitude. It is observed that in powdered samples collected from Mumbai, Roorkee and Nagpur, Ca, Cl, P and K contents are lower compared to those from Vyas Pharmacy (Indore) and Himalaya Drugs (Bangalore). Incidentally Vyas Pharmacy sample shows the highest Ca (46.6±1.6 mg/g), K (8.07±0.20 mg/g) and P (0.62±0.04 mg/g) content. These variations may be attributed to variation in geographical locations and climatic conditions in places from where the samples were collected [75-77]. It may be noted that all the five samples were collected from a large area covering from Roorkee in North to Bangalore in South where soil conditions are widely varying. In order to further see the variation in elemental concentrations a plot has been drawn in Fig. III.5. It is observed that basic elemental profiles are same for all the samples. Ca and Mg are in higher amounts and their ratio should have remained same. However, it is found to vary in a wide range of

2.56 to 18.9 with a mean value of 8.1±6.5, lowest for Roorkee sample and highest for the sample from Vyas Pharmacy (Indore).

Tun and Nguyen [78] observed strong correlation between Ca deficiency and the pathogenesis of hypertension due to cardiovascular disfunctioning in chicks during embryonic development. Slotki [79] observed patients with severe cardiac failure and anemia to benefit from iron supplementation. Thus, high Ca and Fe contents besides other elements in the *Arjuna* bark could explain its use in acute conditions of cardiac failure and dropsy

Essential Trace Elements; As pointed out in *Ch I* trace elements play vital role in biochemical and enzymatic processes. However, their concentrations also vary in a wide range depending on geo-environmental factors. Variation in concentrations of essential trace elements in different brands is bar plotted in Fig. III.6. It is observed that Cu (2.27-4.00 μg/g), Mn (38.0-101μg/g), Rb (5.54-15.4 μg/g) and V (0.18-0.54 μg/g) vary by a factor of 2-3 while Co (0.09-0.86 μg/g), Zn (2.48-21.6 μg/g) and Cr (0.25-10.5 μg/g) vary by an order of magnitude and more. It is observed that mean elemental contents of Cu (4.00± 0.10μg/g), Mn (101±1.0 μg/g) and V (0.54±0.03 μg/g) are very high in sample from Himalaya Drugs while that from the Vyas Pharmacy has the lowest Co (0.09±0.02 μg/g) and Cr (0.25±0.05 μg/g). Powdered sample from Mumbai has excessively high Co (0.86±0.19 μg/g), Cr (10.5±2.0 μg/g) and Zn (20.7±0.2 μg/g) content while Rb content (15.4±3.0 μg/g) is highest in Roorkee sample. Sample from Nagpur showed lowest Mn (38.0±0.6 μg/g), Rb (5.54±1.08 μg/g) and Zn (2.48 0.18 μg/g) contents. In order to see the variation in trace elemental concentrations a plot has been drawn in Fig. III.7. It is observed that

not only Mn and Zn contents in all the samples are high but also it varies in a wide range.

It is reported that Mn deficiency affects the capacity to scavenge endogenously produced reactive O species in streptozotocin-diabetic Sprague-Dawley rats [80]. Thus high Mn content in the bark could explain the antioxidant property of *Arjuna* bark [14]. Overall, we observe quite a difference in elemental contents between raw powdered and Pharmaceutical brands. Vyas pharmacy in particular has the lowest Na, Fe, Mg, Co and Cr but rich in Ca, P and K contents. Sample from Himalaya Drugs (Bangalore) has the highest Cl, Cu, V and Mn content.

Toxic elements: In recent years there has been an increasing controversy in western countries regarding the safety aspects of herbal products because of toxic metal contents [81-84]. In order to see the safety aspects of *Arjuna* bark we determined As, Br, Sb, Hg and Th by NAA and additionally Cd and Pb by AAS. All these elements are environmentally toxic [19] and may originate from industrial emissions as mentioned in *Ch I*, Table I.2. It is observed that As, Sb, Hg and Th are all found in <100 ng/g amounts whereas Br, Cd and Pb are found in < 10 μg/g amounts. All the elements are environmental contaminants and no choice in sample collection seems to have been followed. Amongst the 5 toxic elements, As, Br, Hg, Sb and Th, not much variation is observed for As and Br (Fig. III.8). Surprisingly Roorkee, a much smaller and pollution free town compared to Mumbai and Nagpur has exceedingly high Hg (108±5 ng/g), Sb (95.5±17.8 ng/g) and Th (758 ± 170 ng/g). Further Cd and Pb contents in μg/g amounts seem to be quite high and it is a

matter of concern. This is essentially due to not following good agricultural practice as advocated by some workers [85]

III.4 ELEMENTAL RATIOS

Many a times elemental ratios rather than its contents also play an important role. Variation in K/P and Ca/P ratio in five brands is shown in Fig. III.9 and 10 respectively. K/P ratio varies in a small range of 10-23 while Ca/P ratio varies in a much higher range of 45-180 in five brands. We observed that the sample from Nagpur has the highest ratio for both. Fe and Zn are physiologically important elements in our body system [18] and their ratio Fe/Zn also varies in a wide range of 0.03 to 1.6 with a mean value of 0.48±0.66, lowest being for Vyas Pharmacy, Indore and highest for Nagpur. Thus it is interesting to observe that in Indore sample, Ca/Mg is highest whereas Fe/Zn is lowest. Therefore, Ca and Fe contents seem to be inversely correlated. Our studies have clearly shown that in spite of variation in elemental contents in samples from different geographical locations, elemental profile remain same and *Arjuna* bark is enriched in some essential nutrients in bioavailable form.

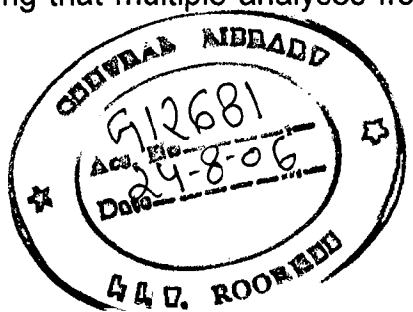
III.5 COMPARISON OF NAA WITH AAS AND ICP-MS

In order to validate our data several elements such as Cu, Fe, Zn, Mn, Cr etc were determined by AAS and ICP-MS. 14 elements were determined by AAS and 22 by ICP-MS. A comparison of our NAA data with those of AAS and ICP-MS shows that As, Ca, Co, Cr, Cu and Hg match within ~10%. Considering the fact that the

samples had to be dissolved while using the other two techniques, it is well within reasonable limits. It may be noted that sample dissolution is not only a tedious task especially for plant matrices, but it is likely to add errors in measurements. Therefore, this amount of variation is expected. For Cu, no variation is observed. This is an interesting observation as for determining Cu by NAA, we used 511 keV photo peak which is also the annihilation peak. This agreement suggests that our delay time and counting schedule for determining Cu was right on the button. Toxic elements Pb, Cd and Ni that could not be determined by NAA were determined by AAS. Bar plots of the variation in Pb and Cd contents in different brands does not show much variation (Fig.III.8). Not surprisingly, Mumbai a mega city has the highest Pb, Cd and Ni contents. Variations in samples from two pharmaceutical brands are not much. In case of ICP-MS data we have observed as little as 1-3% variation in the results obtained by the two methods. However for Cd, Mo, Ni and Pb, which were also determined by AAS, variation of an order of magnitude exists. Hence, ICP-MS is a superior technique compared to AAS, which is quite obvious.

III.6 COMPARISON WITH LITERATURE DATA

In order to see the validity of our data, an attempt has been made to compare some of our data with those reported in literature [47,48]. A comparison is shown in Table III.5 where of course not all the elements are reported. It is observed that the mean elemental concentration of Ba, Br, Cl, K, P, Rb and Th in the present study matches well with those reported by Kumar et al. [48] while only the Rb concentration agrees with that reported by Singh and Garg [47]. It is noteworthy mentioning that multiple analyses from various brands were not carried in previous



works. Hence, mean contents of Al, Co, Cr, Cs, Fe, Hg, La, Na, Sc and Zn reported by Kumar et al. [48] is in the range reported by us in Table III. 2. However, a look at Table III.5 shows that Mg and Mn content in this study is high while V and Ca content is much lower.

III.7 IDENTIFICATION OF ORGANIC CONSTITUENTS

As already pointed out we have been able to separate and identify three new organic compounds by elemental analysis, spectral and GC-MS studies. These details are discussed in following lines.

***(i) 1-phenylazo 2-naphthalenol* (PG I):** It is obtained as a reddish crystalline solid, yield: 321 mg, m. pt 199 °C, (lit. m.pt. 205 °C), R_f=0.79 in Petroleum ether/CCl_4/benzene (60:30:5), Elemental content (%): C, 77.9 (77.4); H, 5.06 (4.83); N, 10.98 (11.3). This corresponds to the molecular formula $C_{16}H_{12}N_2O$.

IR (cm^{-1}) in KBr

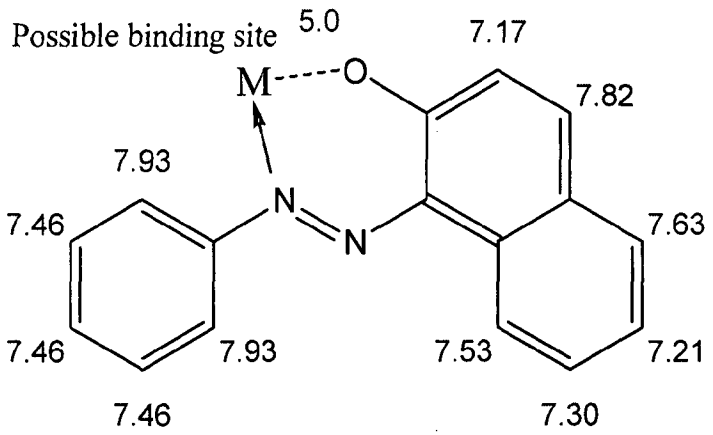
3469	ν_{O-H}
2917, 2843	ν_{C-H} aromatic
1637	$\nu_{N=N,}$

MS at R_t=9.58 min., m/z (rel. int.)

248 [66]	$C_{16}H_{12}N_2O$
171 (19)	$C_{10}H_7N_2{}^+O$
143 (71)	$C_{10}{}^+H_7O$
115 (100)	$^+C_9H_7$
105 (9.2)	$C_6H_5N_2{}^+$
77 (59.9)	$^+C_6H_5$
51 (36.2)	$^+C_4H_3$

These assignments match well with that for 1-phenylazo 2-naphthalenol [86, 87] IR spectrum, mass spectrum of 1-phenylazo 2-naphthalenol and the possible

fragmentation pattern is shown in Fig. III.11. The compound is an azo dye and is commonly known as Sudan Red I, probably because of the interest of the British Empire in Sudan at the time it was developed in the late 19th century [88]. This compound is not reported in Terminalia Arjuna. Amenduni et al [89] developed a rapid and reliable method to determine this dye from red chili using HPLC with diode array detection. Vanker et al [90] have reported special treatment for dyeing cotton with *T. Arjuna*. It all fastness parameters, it showed good results. Though no efforts were made to identify the dye, the compound isolate here may be responsible for dyeing characteristics. Further, it has two binding sites, a coordinate bond through N and a covalent bond through O to form a six membered ring with the transition metal ions [91]. Possible binding site along with the ^{1}H NMR δ values are depicted here.



1-phenylazo 2-naphthalenol

(ii) 2-isopropyl naphtha [2,3-b] furan 4,9-dione (PG II): It is obtained as a yellowi sh oil, R_f=0.63 in $CHCl_3$/ diethyl ether (10:1), Elemental content (%): C, 75.84 (75.0); H, 5.20 (5.0). This corresponds to the molecular formula $C_{15}H_{12}O_3$

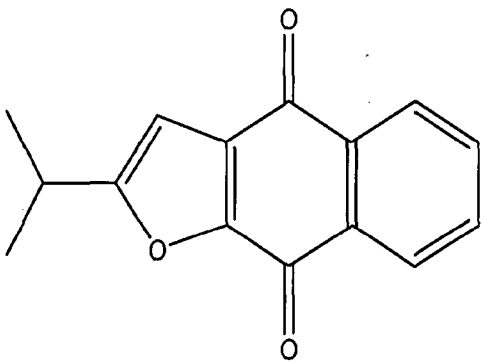
IR (cm^{-1}) in KBr

2935	ν_{C-H} aromatic
2835	ν_{C-H} aliphatic
1626	$\nu_{C=O}$
1508	$\nu_{C=C}$
1206	ν_{C-O} asymmetric
1025	ν_{C-O} symmetric

MS at R_t=9.58 min., m/z (rel. int.)

240 (59.3)	$C_{15}H_{12}O_3$
225 (100),	$^+C_{14}H_9O_3$
197 (16.2),	$^+C_{12}H_5O_3$
77 (24.1),	$^+C_6H_5$
69 (34.6)	$^+C_4H_5O$

These assignments match well with that for 2-isopropyl naphtha [2,3-b] furan 4,9-dione [86]. IR spectrum, mass spectrum and the possible fragmentation pattern is shown in Fig. III.12.



2-isopropyl naphtho [2,3-b] furan 4,9-dione

De Nys et al. [92] observed that furanone derivatives inhibit bacterial colonization through interference with a key bacterial quorum-sensing pathway hereby explaining the role of Arjuna bark as an antibacterial agent [93]

(iii) Tartaric acid (PG III): It is obtained as a white crystal, yield: 79 mg, m. pt 212 °C, (lit. mpt. 205 °), R_f=0.72 in $CHCl_3$ / MeOH (20:1), Elemental content (%): C, 31.68 (32.0); H, 4.20 (4.0). This corresponds to the molecular formula $C_4H_6O_6$

IR (cm^{-1}) in KBr

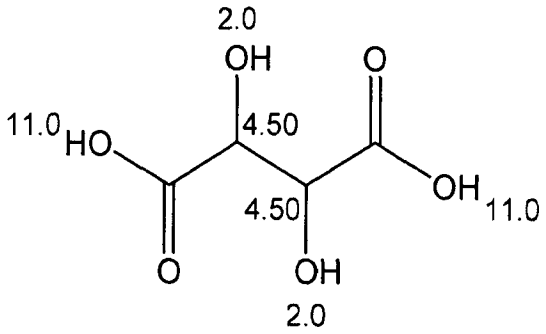
3437	b, ν_{O-H}
2604	ν_{C-H} aliphatic
1734	$\nu_{C=O}$
1129	ν_{C-O} asymmetric
1078	ν_{C-O} symmetric

MS at R_t=9.58 min., m/z (rel. int.)

150 (1.71),	$C_4H_6O_6$
105 (28.3),	$^+C_3H_5O_4$
104 (1.62),	$C_3H_4O_4$
76 (100),	$C_2H_4O_3$
59 (47.3),	$^+C_2H_3O_2$
58 (77.6),	$C_2H_2O_2$

These assignments match well with that for tartaric acid [86,87]. IR spectrum, mass spectrum of tartaric acid and the possible fragmentation pattern is shown in Fig. III.13. Tartaric acid is widely distributed in natural products and acts as a refrigerant, antiseptic and antiscorbutic agent [94]. Chen et al. [95] developed a rapid, simple and sensitive HPLC method combined with a novel double cell quartz

crystal (DCQC) detector for the determination of divalent carboxylic acids including tartaric acid in traditional Chinese herbal and patented medicines. Structure of tartaric acid along with ^1H NMR δ values are shown here.



Tartaric acid

III.8 THERMOGRAVIMETRIC ANALYSIS

In recent years thermal decomposition of medicinal herbs has attracted attention because of use of DTA for taxonomical investigations. In view of these literature reports, we had investigated thermal decomposition behavior of all the samples of Arjuna bark powder in air and in N_2 atmosphere. Typical thermo grams of Himalaya brand in air and N_2 atmospheres are shown in Fig.II.5. In order to avoid the effect of moisture content, the starting temperature was maintained at ~120°C. Earlier we had observed that if starting temperature was 25°C then a weight loss of ~5% had occurred at 120°C. In both cases decomposition occurs in three stages, though it is much sharp in air atmosphere compared to that in N_2 atmosphere. Another major difference in air is that residual wt obtained at 990°C is ~16%, whereas in N_2 atmosphere, it is ~37% at 790°C.

In general, decomposition in air is fast compared to that in N_2 atmosphere as evident from large difference in weight loss in two media. Also stage I is the fastest

process whereas stage III is slowest. Temperature ranges of three stages of decomposition are same though weight losses in stages I and II are different in air and N_2 atmosphere.

Stages	*Temp. Range*	*Percent wt Loss*	
	(°C)	*Air*	*N_2*
Stage I	220-350	~40	~ 30
Stage II	350-400	~30	~ 15
Stage III	600-700	~12	~ 12
	Total wt Loss	82	57

The major difference between decomposition occurring in two media is that in air, residual weight is 16% at ~700°C whereas at the same temperature in N_2 atmosphere it is ~40% in N_2.The extra loss clearly indicates oxidation leading to the formation of volatile oxides in air. DTA plot in air shows two exotherms at ~300°C and 415°C but no such enthalpy change (ΔH) is observed in N_2 atmosphere. Actually DTA plot in N_2 atmosphere is convex in nature. Wesolowski and Konieczynski [96,97] observed a similar behaviour while studying thermal decomposition of several Polish medicinal plants and concluded that the elemental composition reflected by the results of their thermal decomposition supported its chemotaxonomy.

III.9 CARBONACEOUS MATTER

In order to check the carbonaceous matter in *Arjuna* bark powder, elemental analysis of all the powder samples was performed. The data are given in Table III.6. No sulfur content could be detected in any of the samples though no N content was

found in at least Nagpur sample. A perusal of data shows that similar to mineral elemental contents, C, H, and N contents also vary in a wide range. Mean C, H and N contents are found to be 34.93±3.27, 4.00±0.51 and 0.11±0.07% respectively. Thus, total of all C, H, N contents is found to be 39.01±3.76 which means that the total carbonaceous matter should be ~40%. However, it is observed from the thermogram in N_2 atmosphere (Fig. II.5a) that ~39% residual wt. remains compared to ~17% in air atmosphere. Assuming that all the carbonaceous matter is oxidized in air, we observed a residual wt.=15.4±2.7% compared to that of 33.6±3.0 % in N_2 atmosphere. It means that even after heating in air at ~900° C ~16% wt is left which must non-volatile metal oxides suggesting that *Arjuna* bark is rich in mineral content. Large difference in residual wt of *Arjuna* bark powder in air and N_2 atmosphere by ~20% is indicative of the combustible material.

Table III.1 Elemental concentrations in SRMs used for data validation in NAA.

Element	Mixed Polish Herbs (MPH-2)	Peach Leaves (SRM 1547)		Pine Needles (SRM 1575 a)	Apple Leaves (SRM 1515)
		Certified	RSD (%)		
Al (µg/g)		265±6 (249±7)	2.3		269±6 (286±9)
As (ng/g)		67.1±0.5 (60±18)	0.7		187±2 (191±23)
Ba (µg/g)	31.6±3.8 (32.5±2.5)	107± 1.8 (124±4)	1.7		
Br (µg/g)		11.8±0.1 [11]	0.8		7.18±0.01 (7.17±0.61)
Ca (mg/g)		14.3±0.2 (15.6±0.2)	1.4		16.6±0.2 (15.3±0.15)
Cl (mg/g)		0.39±0.01 (0.36±0.02)	2.6		0.54±0.01 (0.58±0.02)
Co (ng/g)	193±3 (210±25)	83±2 [70]	2.2		
Cr (µg/g)	1.27 (1.69±0.13)	1.33 [1]	7.0	1.57±0.11 (1.33±0.11)	
Cs (ng/g)	66.4±14.6 (76±7)	78.4±15.8 (84.2±1.2)	20.2	199±7 -	
Cu (µg/g)		3.89±0.09 (3.7±0.4)	2.3		8.16±0.23 (7.77±0.53)
Eu (ng/g)	16.7±2.9 (15.7±1.8)	163±1 [170]	0.6	4.28±0.17 -	
Fe (µg/g)	465±30 [460]	200±7 (218±14)	3.5	52±6 (46±20)	
Hg (ng/g)	15.6±2.0 (17.6±1.6)	29.2±2.9 (31±7)	9.9	37.3±2.2 [35.1]	
K (mg/g)		25.4±0.1 (24.3±0.3)	0.4		18.3±0.1 (19.1±1.2)
La (µg/g)		7.96±0.33 [9]	4.1		0.63±0.01 (0.57±0.05)
Mg (mg/g)		4.51±0.12 [4.32]	2.7		2.35±0.11 (2.71±0.08)
Mn (µg/g)		95.5±0.5 (98±3)	0.5		55.4±0.3 (54±3)
Na (µg/g)		27.5±2.2 (24±2)	8.0		363±31 [350]
P (mg/g)	2.46±0.218 [2.5]	1.25±0.069 (1.37±0.07)	5.5	1.19±0.04 (1.07±0.08)	
Rb (µg/g)	12.1±2.9 (10.7±0.7)	18.9±2.3 (19.7±1.2)	12.2	16.1±1.8 (16.4±0.4)	
Sb (ng/g)	59.3±5.6 (65.5±9.1)	23.1±4.5 [20]	17.2	29.2±5.4 (33±6)	
Sc (ng/g)	119±2 (123±9)	41.4±0.9 [40]	2.2	91.8±1.9 (101±3)	
Sm (ng/g)		1065±6 [1000]	0.6		88.8±0.3 (94.4±8.2)
Sr (µg/g)	36.1±2.2 (37.6±2.7)	56.1±1.6 (53±4)	2.8	82.7±12.9 -	
Th (ng/g)	143± 1 (154±13)	31.6±2.4 [50]	7.6	169±8 (205±26)	
V (µg/g)		0.33±0.01 (0.37±0.03)	3.0		0.30±0.01 (0.26±0.03)
Zn (µg/g)	30.3±0. (33.5±2.1)	19.7±0.6 (17.9±0.4)	3.0	41.7±3.6 (38±2)	

Table III.2 Elemental concentrations in *Arjuna* bark of different brands by NAA

Element	Powdered Sample			Vyas Pharmacy, Indore	Himalaya Drugs, Bangalore	Range	Mean±SD
	Mumbai	Roorkee	Nagpur				
Al (mg/g)	0.38±0.01	0.47±0.01	0.23±0.01	0.75±0.03	0.59±0.02	0.23-0.5	0.48±0.19
As (ng/g)	54±2.5	63±2.8	97.8±4.5	98±4.4	80.5±3.4	54-98	78.6±19.9
Ba (µg/g)	62±10.7	70±13.4	33.0±5.7	55.2±9.6	55.0±9.5	33–70	55.0±13.8
Br (µg/g)	10.4±0.4	10.8±0.4	8.27±0.31	14.7±0.5	16±0.6	8.27-16	12.0±3.3
Ca (mg/g)	28±1.5	18.0±0.9	34.1±1.8	46.6±1.6	43.8±1.6	18.0±46.6	34.1±11.6
Cl (mg/g)	1.44±0.06	4.80±0.20	2.0±0.1	5.54±0.13	6.3±0.2	1.44-6.3	4.0±2.2
Co (ng/g)	857±189	747±165	300±66	92±19	358±80	92-857	471±321
Cr (µg/g)	10.5±2	6.35±1.3	8.29±1.7	0.25±0.05	9.0±1.8	0.25-10.5	6.9±4.0
Cs (ng/g)	501±4	602±9	38.3±4.7	62.5±1.8	48.2±10.3	38.3-602	250±277
Cu (µg/g)	3.5±0.09	2.3±0.06	2.51±0.06	2.74±0.06	4.0±0.10	2.3-4.00	3.0±0.72
Eu (ng/g)	88±10.7	82±10	<1.0	4.75±0.59	1.37±0.17	1.37-88.1	44±47.4
Fe (mg/g)	2.91±0.15	3.00±0.17	4.00±0.21	0.14±0.01	4.84±0.25	0.14-4.84	2.99±1.77
Hg (ng/g)	80±4	108±5	31±1	105±5	22±1	22-108	69±41
K (mg/g)	2.9±0.07	6.24±0.15	4.17±0.14	8.07±0.20	7.46±0.18	2.9-8.07	5.87±2.18
La (µg/g)	6.64±0.40	3.31±0.20	10.6±0.63	6.18±0.37	4.17±0.25	3.31-10.6	6.18±2.33
Mg (mg/g)	7.18±0.63	7±0.61	4.76±0.42	2.46±0.30	5.64±0.68	2.46-7.18	5.41±1.93
Mn (µg/g)	67.7±1.1	77±1.2	38.0±0.6	93.7±1.0	101±1.0	38.0-101	75.5±24.8
Na (mg/g)	0.94±0.06	0.14±0.01	0.14±0.01	0.06±0.01	0.78±48	0.06-0.94	0.41±0.41
P (mg/g)	0.27±0.02	0.37±0.03	0.2±0.01	0.62±0.04	0.38±0.03	0.2-0.62	0.37±0.16
Rb (µg/g)	13.7±2.7	15.4±3	5.54±1.08	8.38±1.64	8±1.56	5.54-15.4	10.2±4.2
Sb (ng/g)	91.5±17	95.5±17.8	9.8±1.8	6.59±1.2	10.1±1.9	6.59-95.5	42.7±46.4
Sc (ng/g)	882±24	1064±33	66.7±5.8	38±1.1	85.1±5.2	38-1064	427±503
Sm (ng/g)	81±2.4	88±2.5	114±4	78±2.3	86±2.5	78-114	89.3±14.3
Sr (µg/g)	120±19	128±20	68±11	217±34	36±7	36-217	114±69
Th (ng/g)	654±209	758±170	6.44±1.5	41.8±9.4	31.6±2.4	6.44-654	298±374
V (µg/g)	0.18±0.01	0.35±0.02	0.29±0.02	0.31±0.02	0.54±0.03	0.18-0.54	0.34±0.13
Zn (µg/g)	20.7±0.2	21.6±1.5	2.48±0.18	4.65±0.33	10.3±0.7	2.48-21.6	11.9±8.9

Table III.3 Elemental concentrations in *Arjuna* by AAS.

Element	Powdered Samples			Vyas Pharmacy Indore	Himalaya Drugs, Bangalore	Mean ±SD	SRMs	
	Mumbai	Roorkee	Nagpur				Mixed Polish Herbs MPH-2	Peach Leaves SRM 1547
As (ng/g)	56	65.7	31.8	102	90.7	69.2±28	-	-
Ca (mg/g)	30.4	17.6	35	48	46.11	35.4±12.4	12.2 (10.8±0.7)	15.8 (15.6±0.2)
Cd (µg/g)	21.7	2.5	1.12	1.1	1.4	5.60±9.04	0.22 (0.20±0.02)	1.6 (2.6±0.3)
Co (ng/g)	775	656	271	90.6	356	429±281	-	-
Cr (µg/g)	9.70	6.60	8.50	0.30	9.6	6.94±3.91	1.70 (1.69±0.13)	1.60 [1]
Cu (µg/g)	3.3	2.3	2.52	2.7	4.03	2.96±0.69	7.90 (7.77±0.53)	4.25 (3.7±0.7)
Fe (mg/g)	2.7	3.62	4.7	0.15	4.90	3.21±1.92	0.45 [0.460]	0.20 (0.21±0.01)
Hg (ng/g)	76	105	31.8	102	90.7	81±30	-	-
Mn (µg/g)	67	76.7	37.8	104	104	78±28	194 (191±12)	94 (98±3)
Mo (ng/g)	5.7	0.85	0.63	1.70	0.90	1.95±2.13	-	-
Ni (µg/g)	10	0.90	0.64	2.30	2.28	3.22±3.87	1.33 (1.57±0.16)	1.02 (0.69±0.09)
Pb (µg/g)	11.2	2.93	3.67	2.93	4.28	5.0±3.5	4.0 (2.16±0.23)	-
Zn (µg/g)	21.40	21.45	2.30	4.81	10.63	12.1±9.0	32.0 (33.5±2.1)	20.44 (17.9±0.4)

Table III.4 Elemental concentrations in *Arjuna* bark and RMs by ICP-MS

Elem.	Powdered Sample			Vyas Pharmacy Indore	Himalaya Drugs, Bangalore	Mean±SD	SRMs	
	Mumbai	Roorkee	Nagpur				Mixed Polish Herbs MPH-2	Peach Leaves SRM 1547
Al (µg/g)	388	464	229	751	590	484±198	610 (670±111)	275 (249±7.5)
As (ng/g)	2.1	72.6	96.8	87.0	86.2	80.9±13.6	177 (191±23)	61.5 (60±18)
Ba (µg/g)	63.0	70.5	32.3	52.9	52.2	54.2±14.4	35 (32.5±2.5)	107 (124±4)
Ca (mg/g)	26.0	17.0	35.1	43.6	48.8	34.1±13.0	8.80 (10.8±0.7)	15.4 (15.6±0.2)
Cd (µg/g)	21.4	2.01	1.15	1.17	1.24	5.4±8.9	0.23 (0.20±0.02)	0.16 (2.6±0.3)
Co (ng/g)	856	750	301	91.9	360	472±320	202 (210±25)	78.5 [70]
Cr (µg/g)	10.3	6.2	8.1	0.28	9.0	6.69±3.90	1.75 (1.69±0.13)	1.56 [1]
Cs (ng/g)	503	607	40.4	65.8	9.25	245±286	71.9 (76±7)	71.7 (84.2±1.2)
Cu (µg/g)	3.48	2.28	2.50	2.75	3.99	3.0±0.71	7.77 (7.77±0.53)	4.01 (3.7±0.4)
Fe (mg/g)	2.72	3.14	4.16	0.15	4.48	2.93±1.71	0.44 [0.460]	0.24 (0.2±0.014)
Hg (ng/g)	79.6	104	28.2	103	23.3	67.5±39.6	15.5 (17.6±1.6)	29.1 (31±7)
Mg (mg/g)	6.96	7.18	5.13	2.36	5.85	5.5±1.9	2.81 (-)	3.2 [4.32]
Mn (µg/g)	67.3	77.1	38.1	92.6	101	75.2±24.6	200 (191±12)	97.8 (98±3)
Mo (ng/g)	5.80	0.85	0.75	1.80	0.89	2.0±2.2	560 (520)	60.3 (60±8)
Na (µg/g)	963	143	141	61.4	792	420±423	327 (350)	25.7 (24±2)
Ni (µg/g)	10.7	0.92	0.61	2.48	2.25	3.4±4.3	1.54 (1.57±0.16)	1.1 (0.69±0.09)
Pb (µg/g)	12.0	3.2	3.73	3.4	4.4	5.4±3.8	2.1 (2.16±0.23)	0.64 (0.87±0.09)
Rb (µg/g)	13.3	15.2	5.40	7.78	9.73	10.3±3.8	12.4 (10.7±0.7)	19.8 (19.7±1.2)
Sr (µg/g)	134	127	71.1	219	42.5	118±68	36.2 (37.6±2.7)	57.2 (53±4)
Sn (µg/g)	251	6.42	3.04	1.95	10.2	54.5±109	4.65 (-)	0.34 [0.2]
V (µg/g)	0.17	0.36	0.25	0.34	0.56	0.34±0.15	0.83 (0.95±0.16)	0.35 (0.37±0.03)
Zn (µg/g)	20	21.4	2.46	4.49	10.3	11.7±8.6	36.3 (33.5±2.1)	16.3 (17.9±0.4)

Table III.5 Comparison of our data with those reported in the literature

Element	*This work*	*Kumar et al. Ref. 48*	*Singh and Garg Ref. 47*
Al (µg/g)	480±198	380±20	-
Ba (µg/g)	55.0±13.8	55.2±11.0	-
Br (µg/g)	12.1±3.3	13.6±1.5	64.3
Ca (mg/g)	34.1±10.5	102±4	-
Cl (mg/g)	4.02±2.16	3.81±0.41	1.50
Co (ng/g)	471±321	130±10	23.0
Cr (µg/g)	7.01±4.06	1.10±0.21	0.60
Cs (ng/g)	250±277	85.4±8.0	-
Fe (mg/g)	2.99±1.77	0.20±0.03	0.07
Hg (ng/g)	69±41	49±6	-
K (mg/g)	5.87±2.11	6.86±0.52	2.51
La (µg/g)	6.18±2.33	2.65±0.22	-
Mg (mg/g)	5.41±1.93	1.97±0.24	-
Mn (µg/g)	75.5±24.8	7.45±0.50	3.28
Na (µg/g)	412±412	290±90	200
P (mg/g)	0.37±0.16	0.55±0.07	2.83
Rb (µg/g)	10.2±4.2	11.8±0.5	13.5
Sb (ng/g)	42.7±46.4	-	16.0
Sc (ng/g)	427±503	39.8±2.1	8.0
Th (ng/g)	298±374	253±44	-
V (µg/g)	0.34±0.13	0.97±0.02	-
Zn (µg/g)	11.9±8.9	26.5±0.1	45.9

Table III.6 Elemental Analysis of *Arjuna* bark powder

Sample	*%C*	*%H*	*%N*	*%S*	*Total*
Mumbai	34.53	3.99	0.13	0.00	38.65
Roorkee	35.71	4.22	0.11	0.00	40.04
Nagpur	30.46	3.10	0.00	0.00	33.56
Vyas Pharmacy	33.48	4.06	0.21	0.00	37.75
Himalaya Drugs	40.45	4.65	0.09	0.00	45.19
Range	30.46-40.85	3.10-4.65	0.00-0.21	-	33.56-45.19
Mean±SD	34.93±3.27	4.00±0.51	0.11±0.07	-	39.01±3.76

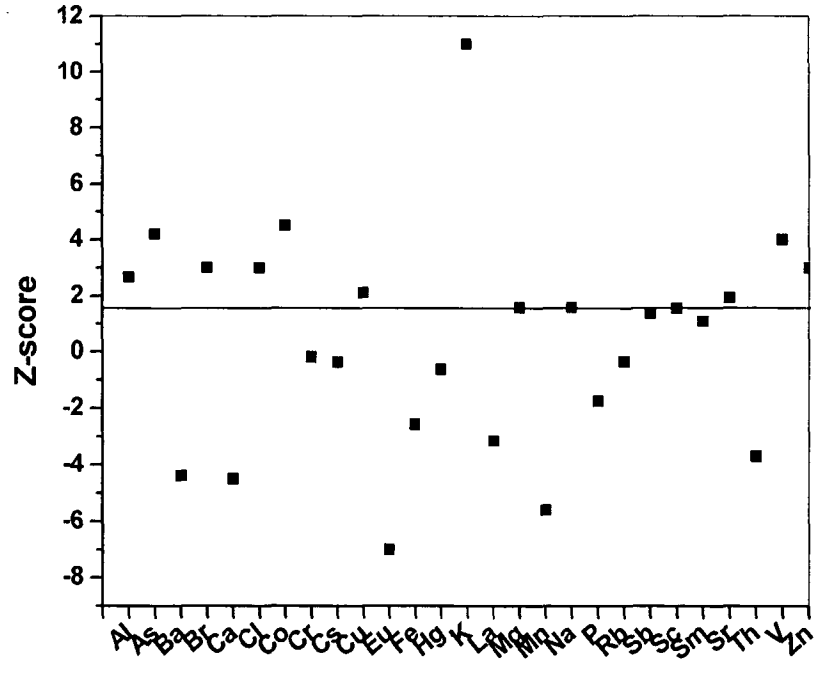


Fig III.1 Z-score plot for elements in Peach Leaves (SRM-1547)

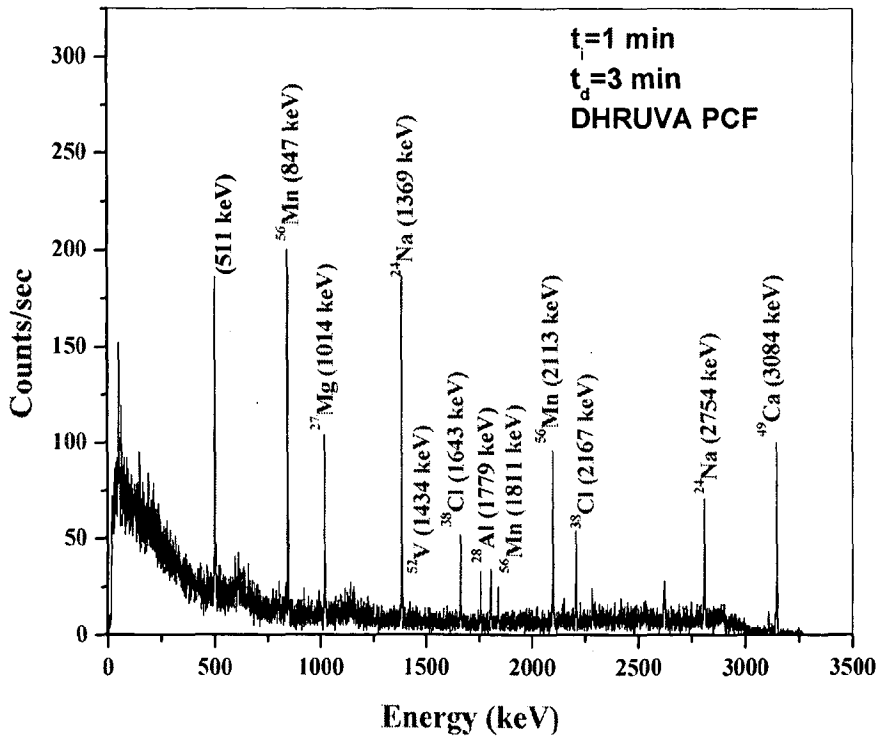


Fig III.2 A typical γ–ray spectrum of short lived nuclides in *Arjuna* bark

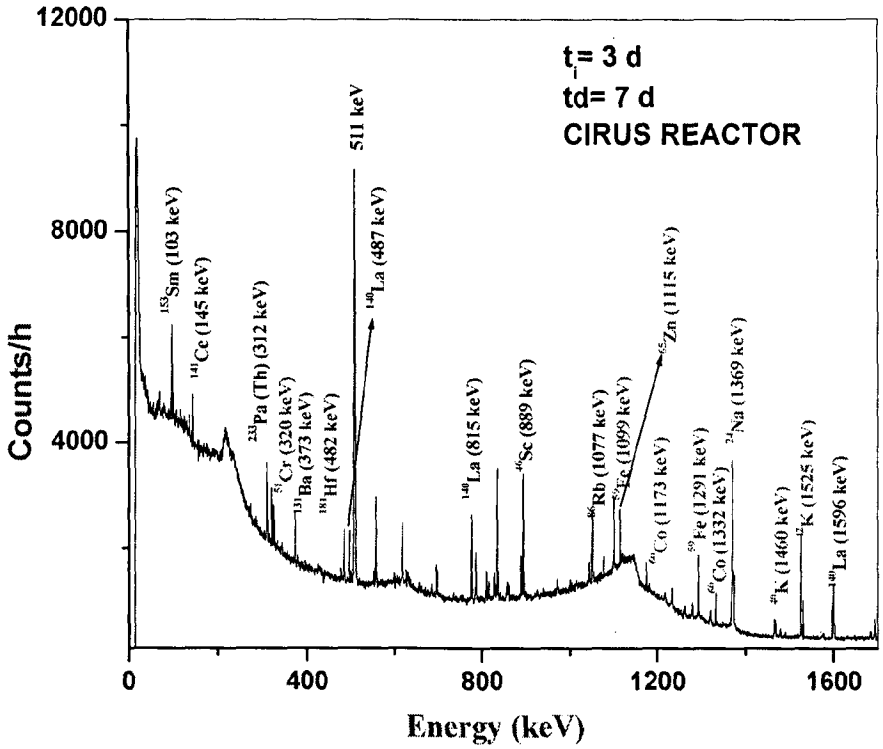


Fig. III.3 A typical γ-ray specrum of long lived nuclides in *Arjuna* bark

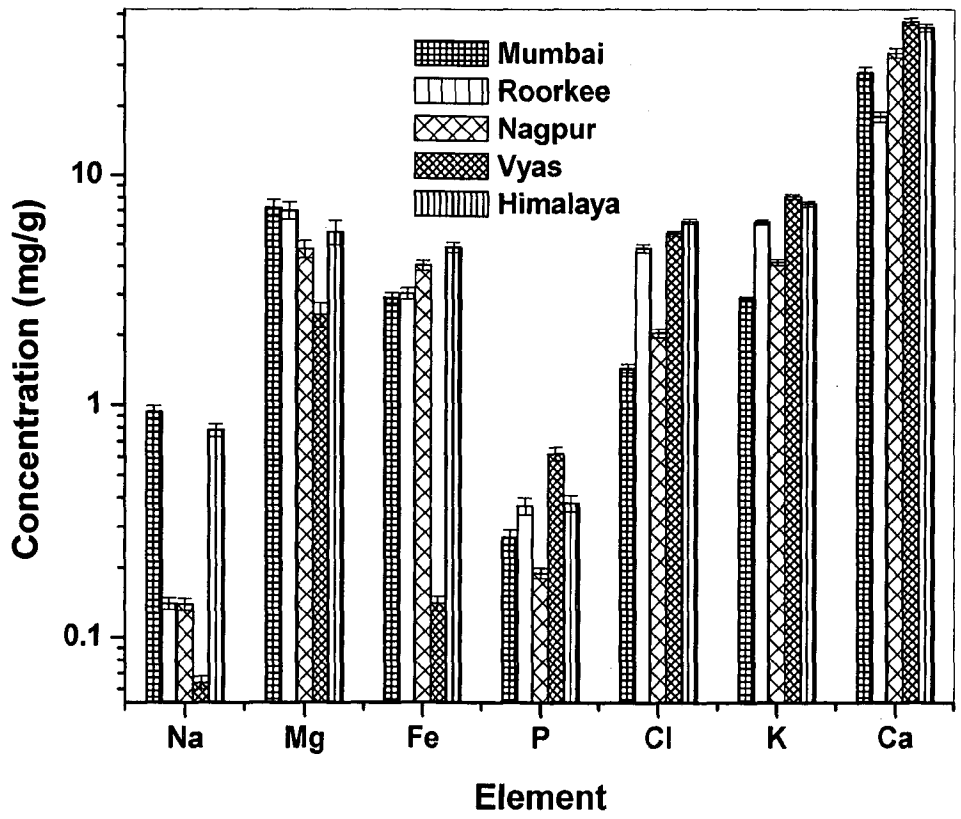


Fig. III.4 Variation in concentration of minor elements in five brands of *Arjuna* bark

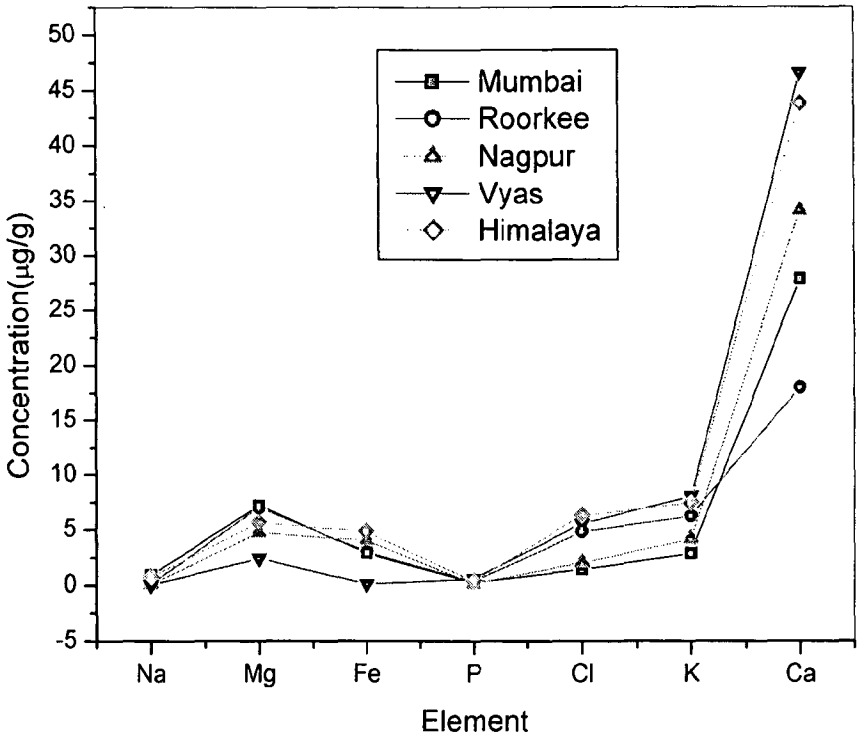


Fig. III.5 Variation of Essential elemental Concentrations in *Arjuna* bark

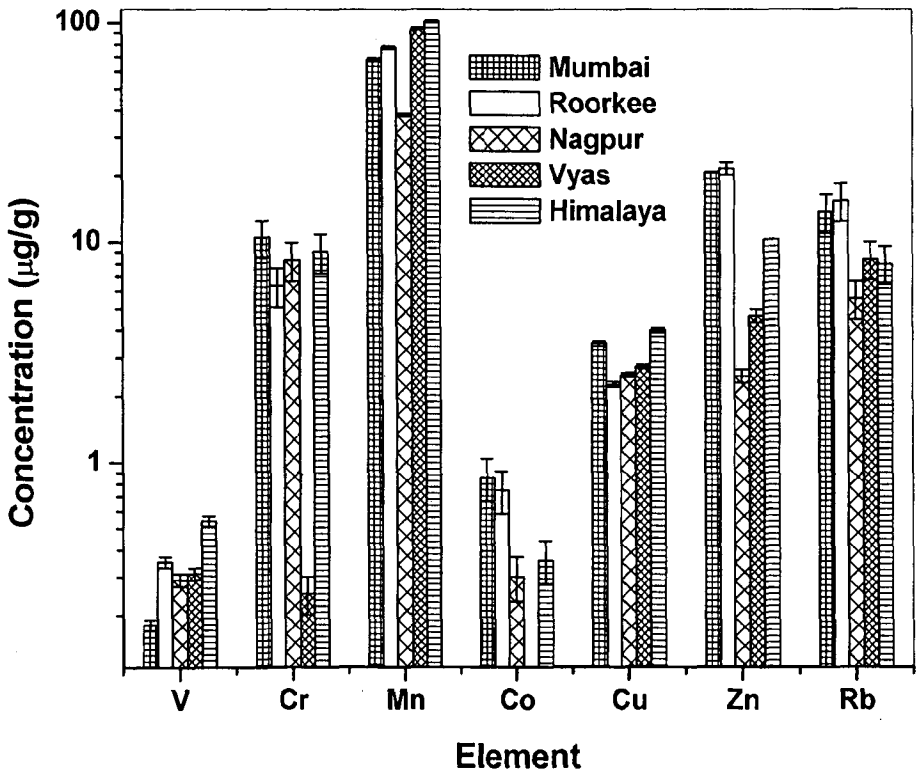


Fig. III.6 Variation in concentration of trace elements in five brands of *Arjuna* bark

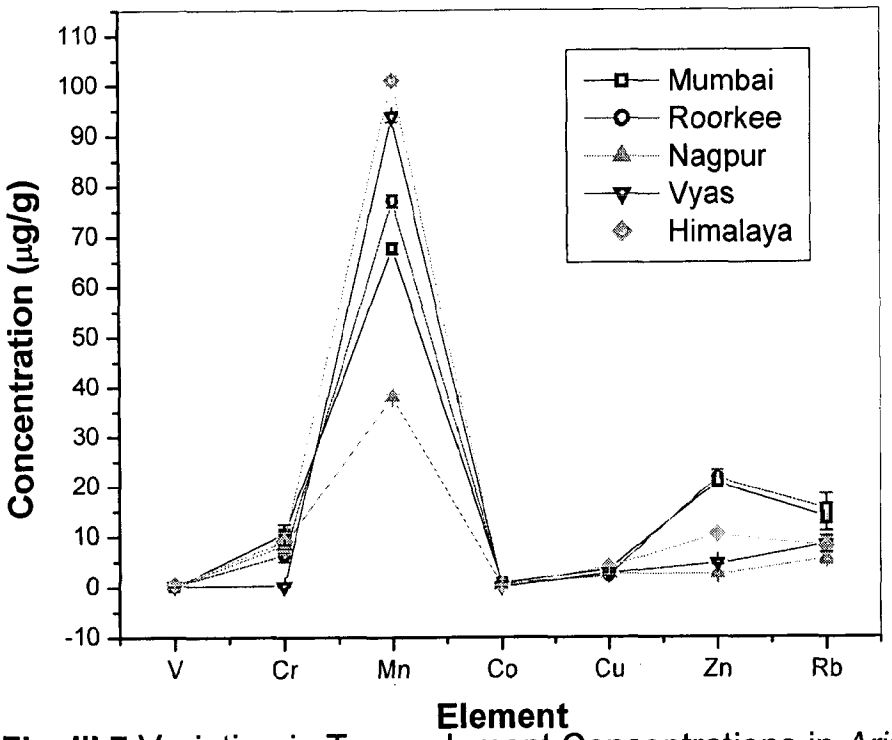


Fig. III.7 Variation in Trace element Concentrations in *Arjuna* bark

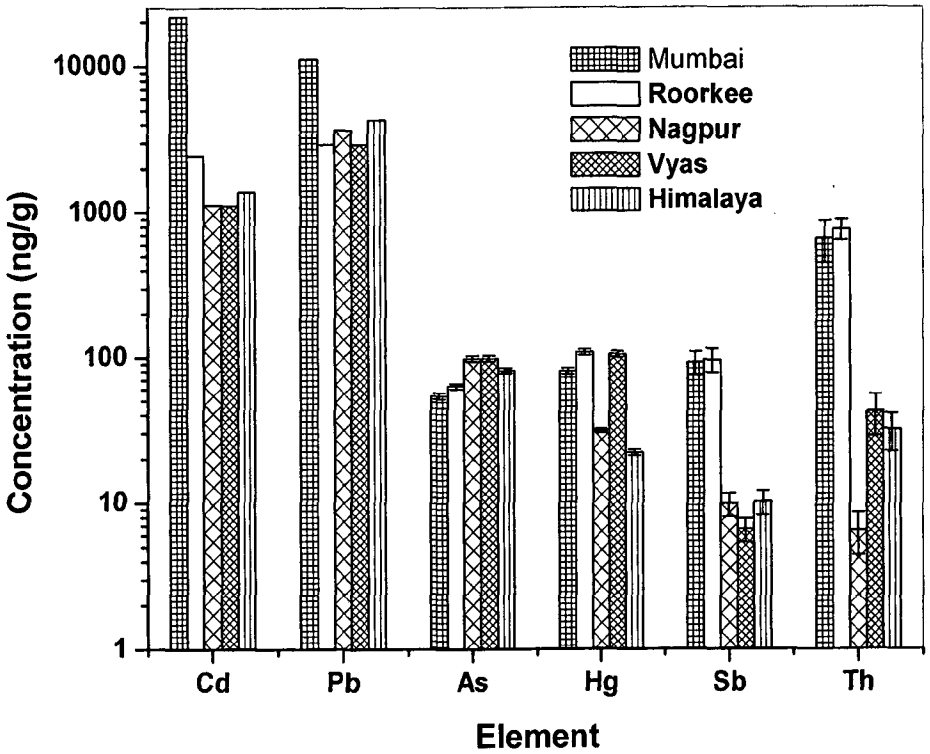


Fig. III.8 Variation in toxic elemental concentrations in Arjuna Bark.

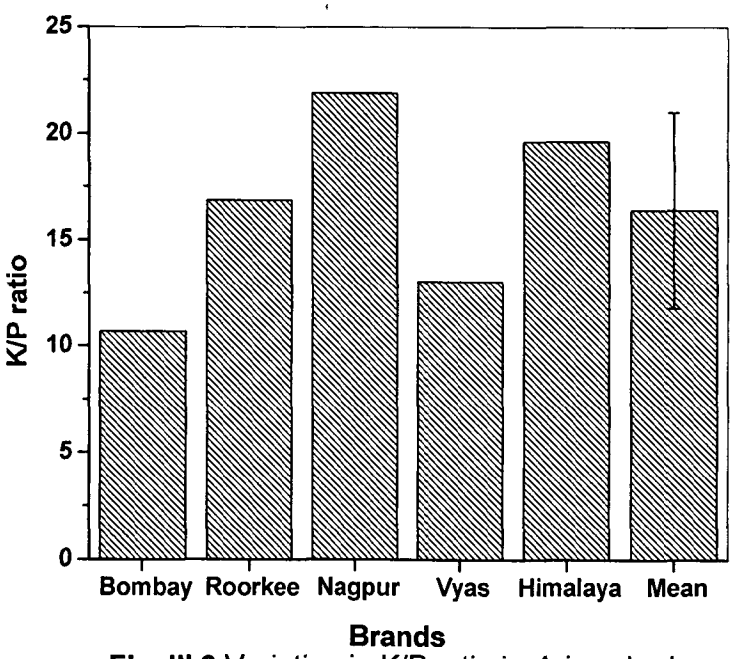


Fig. III.9 Variation in K/P ratio in *Arjuna* bark

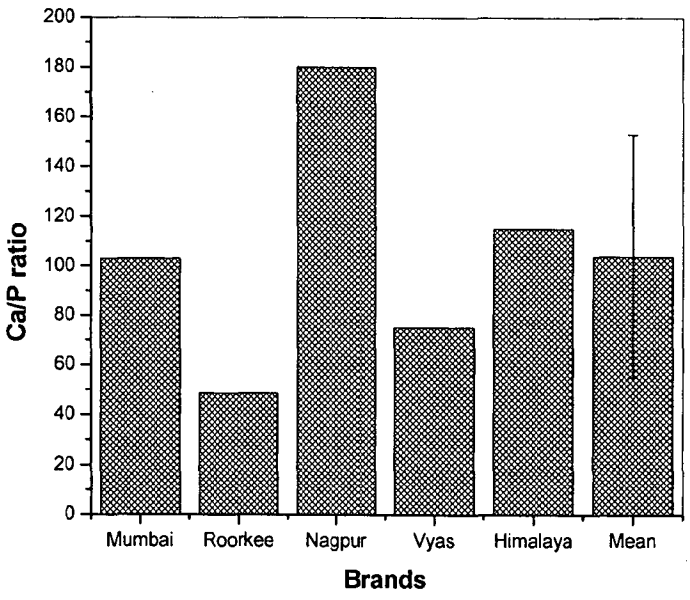


Fig. III. 10 Variation in Ca/P ratio in *Arjuna* bark

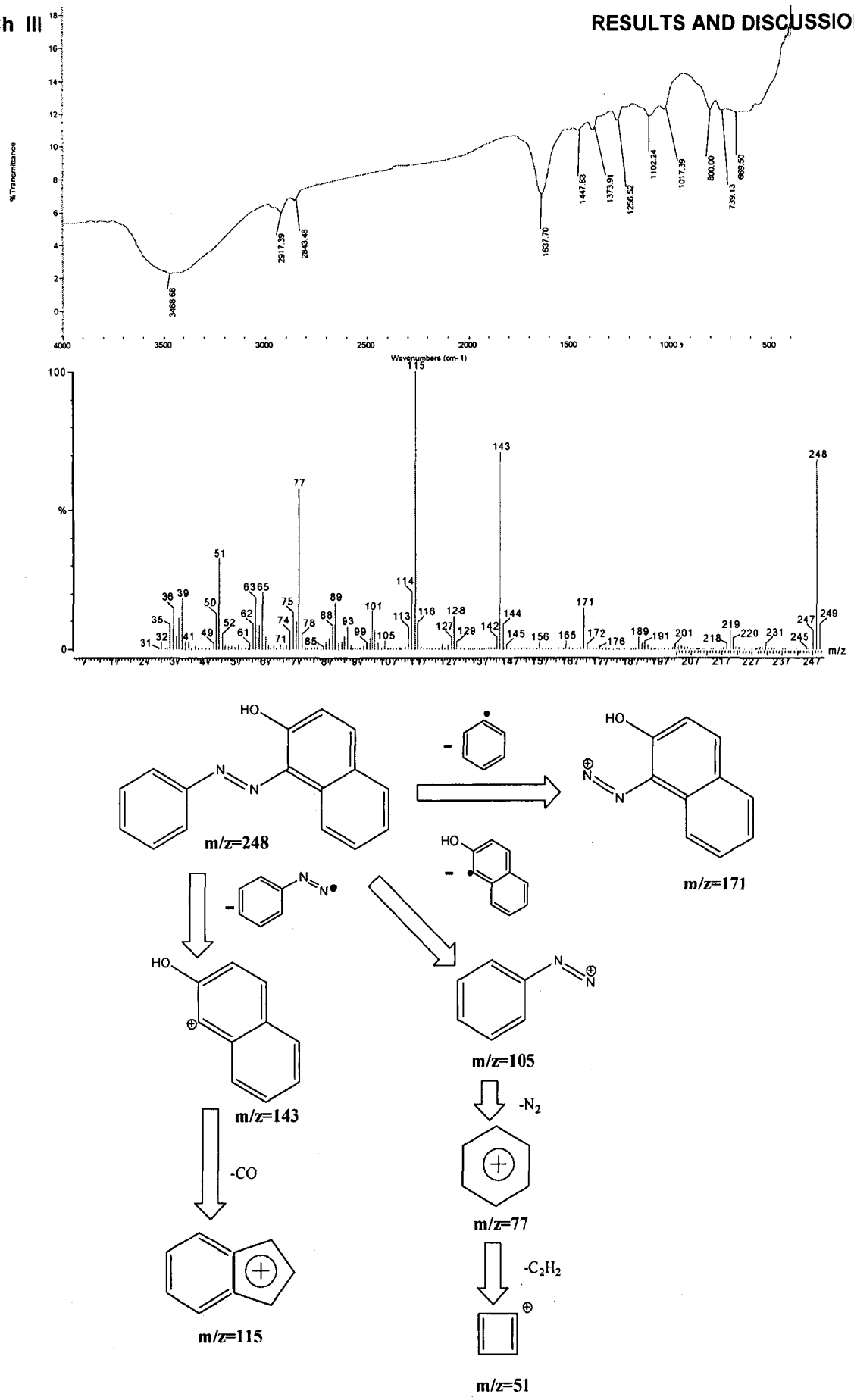


Fig. III.11 IR spectrum, mass chromatogram and fragmentation pattern of 1-phenylazo 2-naphthalenol

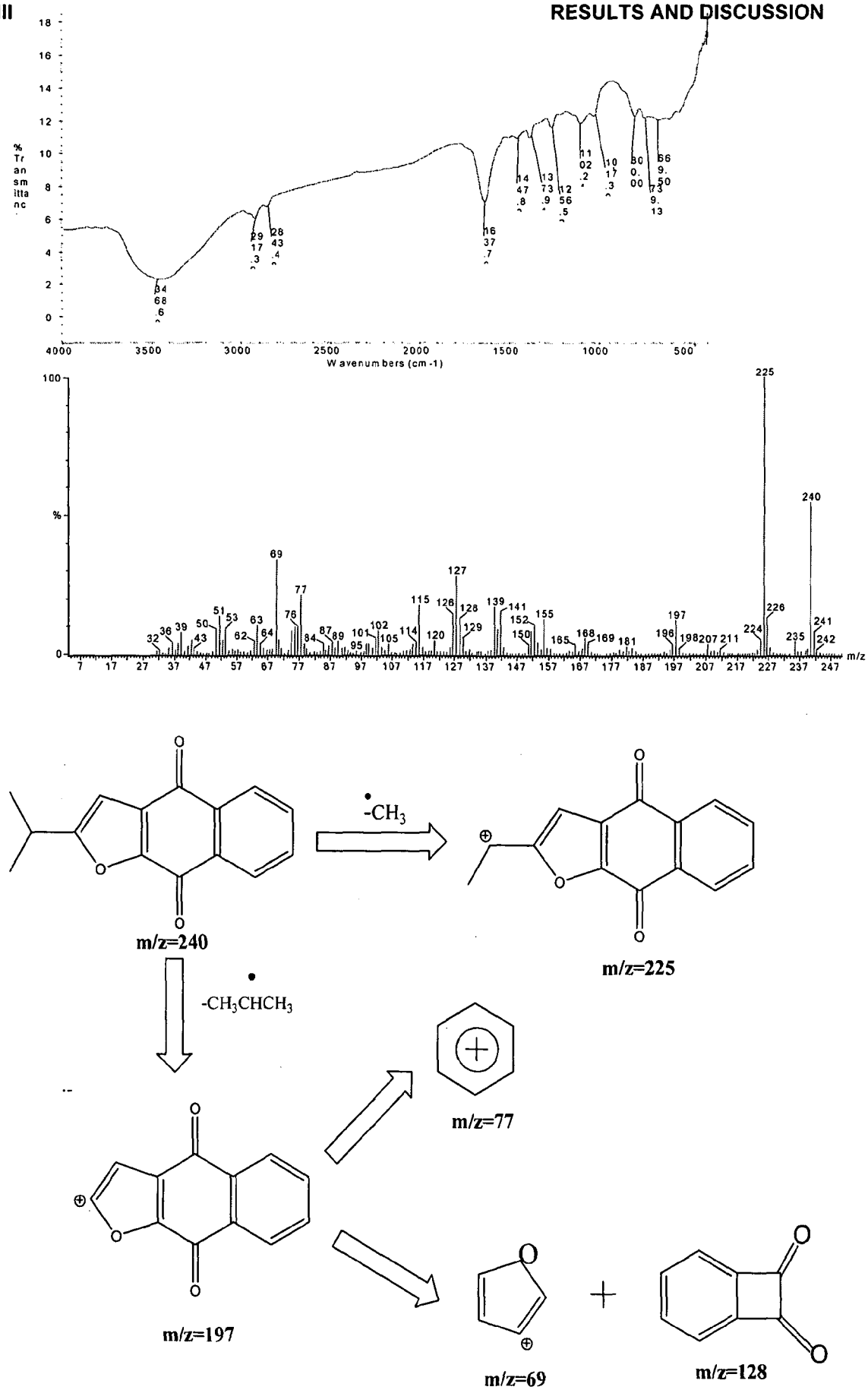


Fig. III.12 IR spectrum, mass chromatogram and fragmentation pattern of 2-isopropyl naphtha [2,3-b] furan 4,9-dione

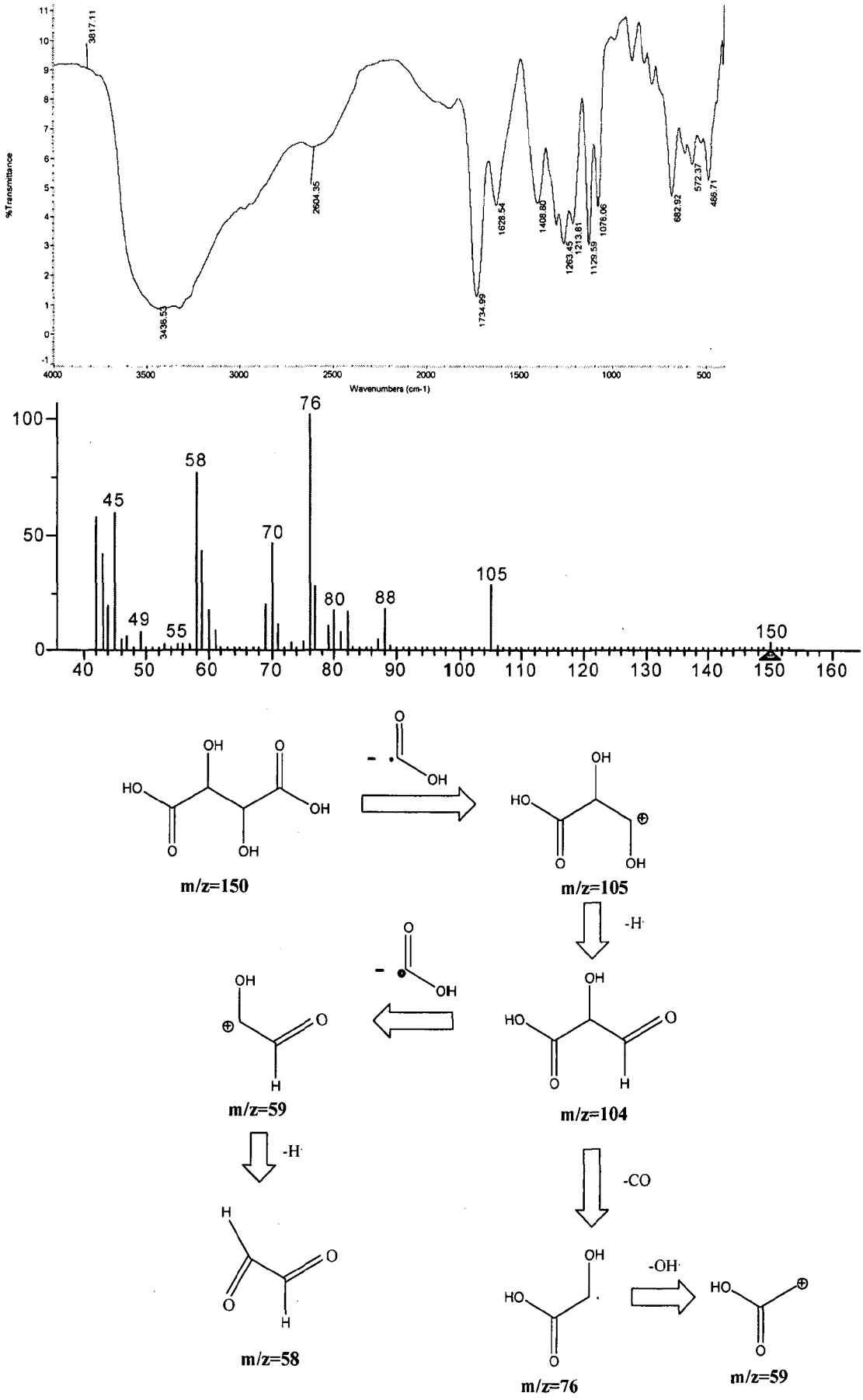


Fig. III.13 IR spectrum, mass chromatogram and fragmentation pattern of tartaric acid

CONCLUSION

Five samples of *Arjuna* bark powders including two commercial brands were analyzed for 8 minor (Al. K, Na, Ca, Mg, Cl, P and Fe), 14 trace (Ba, Ce, Co, Cr, Cs, Cu, Mn, Rb, Eu, La, Sm, Sr, V, Zn) and 5 toxic (As, Br, Hg, Sb, Th) elements by instrumental neutron activation analysis (INAA). Also 14 elements including Ni, Pb, Cd and Mo were determined by AAS. Further, 22 elements were including Sn were determined by ICP-MS. In each case Standard Reference Materials (SRMs) were analyzed for quality control and data validation. Most elemental concentrations vary in a wide range, by a factor of 5 except Fe, Cs, Na, Sb, Eu, Zn which vary by an order of magnitude. This may be attributed to local soil characteristics and environmental factors of the region from where the samples were derived.. Bark powder is enriched in K, Ca, Mn, Fe and Zn which are all essential and play vital role in biochemical processes. Thin layer and column chromatography were used to separate three new organic compounds an azo dye, a furanone and tartaric acid which were further confirmed by infrared and NMR spectra and GC-MS. Essential elements seem to remain complexed with organic ligands so as to make them bioavailable in the body system. Thermogravimetric studies have suggest three stage decomposition with ~15% non-volatile material due to metal oxides.

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