Extraction of Active ingredients form tamarind fruit and incorporation it is due the develop of a personal care product.

By

M.D.S.P. Gunathilaka.

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DECLARATION.

The laboratory work described in this thesis was carried out by my self at the chemistry laboratory of Faculty of Applied Science. Under the supervision of Prof. D.B.M. Wickramaratne, Dean Faculty of Applied Science, during the Project period from 03.04 .2006 to 03. 08. 2006.

M.D.S.P. Gunathilaka.
Department of Physical Sciences
Faculty of Applied Science
Sabaragamuwa University of Sri Lanka

Signature
Date 15/09/2006

Certified by:

Porf, D.B.M. Wickramaratne
(Internal Supervisor)
Dean, Faculty of Applied Science
Sabaragamuwa University of Sri Lanka
Buttala.

Signature
Date 15/09/2006

Ms. Aasana Jurangpathy
(External Supervisor)
Quality Assurance Manager
Multichemi International Ltd
377/1, Welsirimawatha,
Hokandara south,
Hokandara.

Signature
Date 26.09.2006

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ABSTRCT

Tamarind (*Tamarindus indica*) a large to very large evergreen tree up to 30 m in height with dark grey bark having longitudinal Fissures and deep cracks, leaves paripinnate up to 15 cm long, rachis slender, channeled leaflets 10-20 pairs, fruits pods, brownish ash coloured .

Fruit contains tartaric acid, citric acid, malic acid and potassium bitartarate and traces of Oxalic acid, kernel, polysaccharides.

The fruits are sour, sweet, refrigerant, digestive, carminative, laxative, anti-scorbutic, anti- septic , ophthalmic and febrifuge tamarind is also used to the face for anti-freckle and anti-aging purposes, Currently, Tamarind extract is used as a ingredient in body cleansing products for adjusting skin acidity condition, and antiseptic anti-allergy and skin tightening purposes such as anti-aging ,anti-wrinkle and maintain the skin's elasticity due to the inhibition of proteolytic and hydrolytic enzymes that destroy the collagen , elastic and hyarulonic acid in the skin. which protect skin from sun damage. A cosmetic patch containing tamarind fruit extract was formulated and developed by blending tow types of natural polymers, chitosan with molecular weight of 100 000 and starch such as corn, potato or tapioca starch. The physicochemical characteristics, flexibility, colour, transparency, integrity, gloss, water sorption and bioadhesion property and the stability of the patch without tamarind content were investigated.

Nearly 50% of the tartaric acid is in the combined form mainly as potassium bitartrate and to a small extent as calcium tratrate, which is insoluble in cold water, Therefore, cold water extract of the pulp contains only a part of the available tartaric acid and hence hot water is to be used to extract the total acids in the pulp. About 2% of other acids (malic acid, citric acid, quinic acid, oxalic acid and succinic acid) are also present in the pulp.

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CHAPTER 01 INTRODUCTION

Tamarind is an arboreal Fruit of *tamarindus indica* Linn, which belongs to the family leguminosae or Caesalpiniaceae. The tree is indigenons to tropical Africa and Probably also to India and Sri Lanka. The tree can grow up to 20 m in height and stays evergreen almost throughout the year in regions without a dry season.

Tamarind is widely used as a condiment special in tropical Africa and probably also in Asian cuisine. Though every part of it is useful the fruit is the mot important part which is a population acidulant, used in the preparation of oriental dishes. It is used specially in cooking meat, In Sri Lanka there is a special preference among the Tamil communities for tamarind over other commodities like Goraka (*Goreina gambogia*) and lime (*citrus aurontitolia*)

The leaves ground into a paste with lime Juice and heartwood of *Acacia Chundra* Willd are applied on boils to prevent suppuration and inflammatory swellings. A decoction of the leaves is used as a fomentation on boils and abscesses. The teste of the seed mascerated with vinegar or lime juice is applied to the face to prevent formation of pimples. Internally, the leaves and pulp act as cholagogue laxatives and are often used in congestion of the liver, habitual constipation and heamorrhoids. The ripe fruit is regarded as a refrigerant digestive, carminative and laxative. The powder seed is used as a dressing on boils and the flower is given internally as a remedy for Jaundice, It is externally applied on eye diseases and ulcers.

This tamarind meat is also applied to the face for anti-freckle and anti-aging purposes. Currently tamarind extract is used as an ingredient in body cleansing products for adjusting skin acidity condition and antiseptic, anti-allergy, and skin tightening purposes.

Tartaric acid is in the combined form mainly as potassium bitartrate and to a small extend as calcium tartrate, which is insoluble in cold water. Therefore cold water extract of the pulp contains only a part of the available tartaric acid and hence hot water is to be used to extract the total acid in the pulp.

The present study focuses on the utilization of the above skin beneficial properties in formulating a facial application product from tamarind.

Multichemi International Ltd is a well known establishment involved in the manufacture and marketing of natural skin care products. The company intends to develop products based on tamarind extracts to their range of products as a means of providing their consumers with a totally natural solution for skin care problems.

1.2.1 Overall Objective:

Development of a cost-effective and a value – added product from tamarind in the form of a facial application in order to utilize its medicinal properties beneficial for skin conditioning thus enhancing its potential for non-food uses.

1.2.2 Specific Objective:

- Development of a natural tamarind extract with high proportions of Malic acid, citric acid, tartaric acid.
- Purification of the extracted active ingredients and incorporating it in a facial formulation with other ingredients.
- Qualitative testing for the retention of beneficial properties and application of other quality control procedures in finalizing the product formulation in a marketable format.

CHAPTER 02 LITRETURE REVIEVE

2:1Tamarind

2:1:1 Description:

Tamarind is an arboreal fruit of *Tamarindus indica* Linn. This belongs to the family Leguminosae or Caesalpiniaceae. Tamarind is a long living (80- 120 years) tree, a large to very large ever green tree up to 30 m in height with dark grey bark having longitudinal fissures and deep cracks, leaflets pafipinnate up to 15 cm long, rachis slender, channeled, leaflets 10 -12 pairs, subsessile, oblong, flowers yellow, striped with red in lax, few flowered racemes at the ends of the branchlets. (Viyoch, 2003).

Fig 2.1

2:1:2 Origin and distribution.

Native to tropical Africa, the tree grows wild throughout the Sudan and was so long ago introduced into and adopted in India that it has often been reported as indigenous there also, and it was apparently from this Asiatic country that it reached the Persians and the Arabs who called it *"tamar hindi"* (Indian date, from the date-like appearance of the dried pulp), giving rise to both its common and generic names. Unfortunately, the specific name, *"indica"*, also perpetuates the illusion of Indian origin. The fruit was well known to the ancient Egyptians and to the Greeks in the 4th Century B.C.

The tree has long been naturalized in the East Indies and the islands of the Pacific. One of the first tamarind trees in Hawaii was planted in 1797. The tamarind was certainly introduced into tropical America, Bermuda, the Bahamas, and the West Indies much earlier. In all tropical and near-tropical areas, including South Florida, it is grown as a shade and fruit tree, along roadsides and in dooryards and parks. Mexico has over 10,000 acres (4,440 ha) of tamarinds, mostly in the states of Chiapas, Colima, Guerrero, Jalisco, Oaxaca and Veracruz. In the lower Motagua Valley of Guatemala, there are so many large tamarind trees in one area that it is called "El Tamarindal". There are commercial plantings in Belize and other Central American

countries and in northern Brazil. In India there are extensive tamarind orchards producing 275,500 tons (250,000 MT) annually. The pulp is marketed in northern Malaya and to some extent wherever the tree is found even if there are no plantations. Tamarind (*Tamarindus indica*) is an economically important tree, which is found throughout the tropics and subtropics and has been naturalized at many places. The tree is Indigenous to tropical Africa.

It is highly cross- pollinated crop end hence wide variability is common in this species, plant breeding and selection programs need to be improving the quality, yield and earliness of fruiting.

Tamarind tree is found in the "dry" and arid Zones of Sri Lanka being the highest concentrated distribution in the northwestern province.(Agro forestry, 1993).

2:1:3 Varieties

In some regions the type with reddish flesh is distinguished from the ordinary brown-fleshed type and regarded as superior in quality. There are types of tamarinds that are sweeter than most. One in Thailand is known as **'Makham waan'.** One distributed by the United States Department of Agriculture's Subtropical Horticulture Research Unit, Miami, is known as **'Manila Sweet'.**

There are only a few varieties of tamarind grown in Sri Lanka .Some are less acidic (sweetish) and some are more acidic to taste and the colour of the pulp is usually brownish-red in the common variety and reddish in the so-called red variety. The red variety is not economically important, as it is not produced on a commercial scale. But, the red variety fetches better price and is preferred for making preserves. (Morton, J. 1987).

2:1:4 Season

Mexican studies reveal that the fruits begin to dehydrate 203 days after fruit-set, losing approximately 1/2 moisture up to the stage of full ripeness, about 245 days from fruit-set. In Florida, Central America, and the West Indies, the flowers appear in summer, the green fruits are found in December and January and ripening takes place from April through June. In Hawaii the fruits ripen in late summer and fall. (Morton, J. 1987).

2:1:5 Fruiting

A seedling tree will take 13-14 years for first flowering but a vegetatively propagated plant is precocious and come into bearing in 7 to 10 years. As tree grows in size and age productivity increase and continues to be productive for more than 60 years (Morton, J. 1987).

2:1:6 Yield

A mature tree may annually produce 330 to 500 lbs (150-225 kg) of fruits, of which the pulp may constitute 30 to 55%, the shells and fiber, 11 to 30 %, and the seeds, 33 to 40% (Morton, J. 1987).

2:1:7 Harvesting

Tamarinds may be left on the tree for as long as 6 months after maturity so that the moisture content will be reduced to 20% or lower. Fruits for immediate processing are often harvested by pulling the pod away from the stalk which is left with the long, longitudinal fibers attached. In India, harvesters may merely shake the branches to cause mature fruits to fall and they leave the remainder to fall naturally when ripe. To keep the fruit intact for marketing fresh, the stalks must be clipped from the branches so as not to damage the shell, (Shankaracharya, N.B., (1998).

2:1:8 Keeping quality

To preserve tamarinds for future use, they may be merely shelled, layered with sugar in boxes or pressed into tight balls and covered with cloth and kept in a cool, dry place. For shipment to processors, tamarinds may be shelled, layered with sugar in barrels and covered with boiling syrap. East Indians shell the fruits and sprinkle them lightly with salt as a preservative. In Java, the salted pulp is rolled into balls, steamed and sun-dried, then exposed to dew for a week before being packed in stone jars. In India, the pulp, with or without seeds and fibers may be mixed with salt (10%), pounded into blocks, wrapped in palmleaf matting, and packed in burlap sacks for marketing. To store for long periods, the blocks of pulp may be first steamed or sun-dried for several days. (Shankaracharya, N.B., 1998).

2:2 Descriptions of leaves and flower

2:2:1 Leaves: The leaves are eaten by cattle and goats, and furnish fodder for silkworms– *Anaphe sp.* in India, *Hypsoides vuilletii* in West Africa. The fine silk is considered superior for embroidery.

Tamarind leaves and flowers are useful as mordents in dyeing. A yellow dye derived from the leaves colors wool red and turns indigo-dyed silk to green. Tamarind leaves in boiling water are employed to bleach the leaves of the buri palm *(Corypha elata* Roxb.) to prepare them for hat-making. The foliage is common much for tobacco plantings. Shankaracharya, N.B., 1998).

2:2:2 Flowers: The flowers are rated as a good source of nectar for honeybees in South India. The honey is golden-yellow and slightly acid in flavor.

2:2:3Chemistry of Leaves and flower (Table 2.1).

Contend	*Leaves (young)/100g*	*Flowers/100g*
Moisture	70.5 g	80 g
Protein	5.8 g	0.45 g
Fat	2.1 g	1.54 g
Fiber	1.9 g	1.5 g
Carbohydrates	18.2 g	--
Ash	1.5 g	0.72 g
Calcium	101 mg	35.5 mg
Magnesium	71 mg	--
Phosphorus	140 mg	45.6 mg
Iron	5.2 mg	1.5 mg
Copper	2.09 mg	--
Chlorine	94 mg	--
Sulfur	63 mg	--
Vitamin A	250 mcg	0.31 mg
Thiamine	0.24 mg	0.072 mg
Riboflavin	0.17 mg	0.148 mg
Niacin	4.1 mg	1.14 mg
Ascorbic Acid	3.0 mg	13.8 mg
Oxalic Acid	196 mg	--
Tartaric Acid	--	--
Oxalic Acid	--	--

Source: Bhattacharya et al (1993); Ishola et al (1990); Marangoni et al (1988)

2:2:4 Medicinal values of Leaves and Flower

Tamarind leaves and flowers, dried or boiled, are used as poultices for swollen joints, sprains and boils. Lotions and extracts made from them are used in treating conjunctivitis, as antiseptics, as vermifuges, treatments for dysentery, jaundice, erysipelas and hemorrhoids and various other ailments.

Leaf extracts exhibit anti-oxidant activity in the liver, and are a common ingredient in cardiac and blood sugar reducing medicines. Young leaves may be used in fomentation for rheumatism, applied to sores and wounds, or administered as a poultice for inflammation of joints to reduce swelling and relieve pain. A sweetened decoction of the leaves is good against throat infection, cough, fever, and even intestinal worms. Filtered hot juice of young leaves and a poultice of the flowers are used for conjunctivitis. Shankaracharya, N.B., 1998).

2:3 Descriptions of tamarind seed

Tamarind seed is the raw material used in the manufacture of tamarind kernel powder (TKP), polysaccharide (jellose), adhesive and tannin. Also, the seed is gaining importance as an alternative source of proteins, rich in some essential amino acid. Hence, a lot of interest is shown by the chemists, technologists and nutritionists on the chemical aspects of tamarind seed.

Fig 2.2

The seeds form about 30% of the whole fruit and 30% of the seed is testa (seed coat) and 70 % is endosperm or seed kernel. The chemical composition of whole seed, seed (cotyledons), and hull (testa) is given in Table 2. The whole seed and the kernel are rich in proteins (13 – 20 %) and the seed coat is rich in fiber (20%) and tannins (20- 24 %). The mineral contents of the seeds, kernels and testa (seed coat) .The kernel is rich in potassium and magnesium. (Basu, B.D., Kirtikar, K.R., 1993).

2:3:1 Composition of Tamarind seed and Seed Kernel (Table 2.2).

constituent	Whole seed	Seed kernel
Moisture %	9.4-11.3	11.4-22.7
Proteins 5	13.3-26.9	15.0-20.9
Fat / oil %	4.5-16.2	3.9-8.0
Crude fibre %	7.4-8.8	2.5-8.2
Carbohydrates %	50.0-57.0	65.1-72.2
Total ash %	1.60-4.2	2.4-4.2
Nitrogen-free	59.0	--
Yield of TKP %	50.0-60.0	--
Calories /100g	340.3	--
Total sugars %	340.3	--
Reducing sugars %	11.3-25.3	--
Starch %	33.1	--

Source: Bhattacharya et al (1993); Ishola et al (1990); Marangoni et al (1988)

Mineral contents of Tamarind seed and kernel (Table 2.3).

Mineral, mg/ 100g	Seed	Kernel
Calcium	9.3-786.0	120.0
Phosphorus	68.4-165.0	--
Magnesium	17.5-118.3	180.0
Potassium	272.8-610.0	1020.0
Sodium	19.2-28.8	210.0
Copper	1.60	--
Iron	6.5	80.0
Zinc	2.8	100.0
Manganese	0.9	--
Nickel	--	--

Source: Bhattachatya et al (1993); Isholaa et al (1990); Marangoni et al (1988)

The tamarind kernel powder (TKP) develops rancid smell and turns brown on storage , whereas the deoiled meal (0.5% oil) remains white and odorless. This indicates the desirability of oil extraction of TKP to maintain its quality for use as sizing material and to recover the fatty oil that is going as waste. Solvent extraction of TKP removes oil, containing very long chain fatty acids. The TKP contains a polysaccharide (jellose) 60%, proteins, fibre, fat, inorganic salts, some free sugars and tannins. The polysaccharide (jellose) consists of D-glucose, D- xylose, D- galactose and L- arabinose in the molar ratio of 8: 4: 2 . The polysaccharide can be used as a substitute for starch and pectin, although it is structurally different from them.

Albumins and globulins constitute the bulk of seed proteins. Linoleic acid followed by palmitic acid and oleic acid constitute the predominant fatty acids. The seeds are high in

saturated fatty acids, constituting 65- 75 % of total lipids. They contain recommended levels of all essential amino acids except threonine and tryptophan. They contain only moderate amounts of antinutritional factors (tannins, phytic acid , hydrogen cyanide , trypsin inhibitor activity, phytohaemagglutinating activity). Therefore, they can be adopted as cheap, alternate protein source to alleviate protein malnutri- tion in developing countries. The results of experiments on roasting have shown that antitryptic activities of the seeds decrease by 83 % and antichymotryptic activity was absent in the seed.

Reymond et al (1980) used a rapid potentiometric method for determining alkaline earth ions (calcium and magnesium) in solution of natural hydrocolloids (e.g., aqueous solutions of gum Arabic and tamarind meal). A study of the fatty acids extracted with ether from the gums of guar, locust bean (carob) and tamarind was carried out to facilitate differentiation of their colloids using walbeca's chromatographic method of isolating sterols. Pasting and flow properties of cooked solution of TKP, its carboxyl methyl and hydroxyl propyl derivatives were examined by prabhajan and Zakiuddin Ali (1995) with respect to their application in the food industry.

Enzymatic degradation of tamarind kernel powder (TKP) was studied by kooiman (1957) and srivastava et al (1970) , A method for purification of tamarind gum has been patented by Jones (1978). Ari classification of finely ground crude tamarind gum provides a purified tamarind seed polysaccharide. The crude gum may also be admixed with finely divided siliceous matter or may be defatted prior to air classification to increase the degree of purification. A process for preparing tamarind oligosaccharides has been patented by Whistler and Barkalow (1995) and another process for separating polysaccharides from tamarind seeds has been patented by Teraoka (1990).

Table 2.4.

Amino acids and soluble sugars of tamarind seeds.

Amino acid	g / 16gN	Soluble sugars	Per cent of total sugars
Aspartic acid	11.59-11.82	Arabinose	1.54
Glutamic acid	16.91-18.53	Ribose	10.89
Serine	4.74-7.71	Xylose	6.89
Glycine	4.62-912	Mannose	17.35
llistidine	2.01-2.68	Fructose	6.16
Arginine	4.20-9.18	Galactose	4.75
proline	6.19-8.70	Glucose	11.80
Alanine	4.99-6.96	Inositol	7.27
Cystine Methionine	0.63-1.04	Sucrose	5.23
Thrconine	3.78-3.90	Maltose	1.84
Tyrosine phenylalanine	6.32-8.32	Raffinose	3.25
Valine	4.60-6.03	Unidentified	0.10
Isoleucine	4.12-4.19	Raffinose	traces
Leucine	7.93-8.12	stachyose	traces
lysine	5.96-6.49		

Source: Lumen et al (1986); Marangoni et al (1988); sone and sato (1994)

Marangoni et al (1988) found that seed oil (lipid) contained a relatively large proportion of unsaturated fatty acids (75%) with linoleic acid (56.10 %) as the predominant fatty acid. Calcium, magnesium and potassium were low in comparison to cultivated legumes. Alkali extraction of the seeds showed that 70 % of the proteins were extractable. The protein isolated was relatively high in lysine (406 mg /g N), phenylalanine and tyrosine (520 mg /g N) and leucine (496 mg /g N). The results of these studies showed that tamarind seeds could be potentially useful as a source of food proteins .protein qualities of tamarind and African locust bean seed meals were studied by Kapu et al (1990) According to Lumen-Bo-de et al (1986), however, tamarind seeds are rich in proteins (18%) and methionine and cysteine (3.5%) and the seeds have a very favorable amino acid balance. (Shankaracharya, N.B., 1998).

2:3:2 Medicinal and nutritional values of Tamarind Seed

2:3:2:1 Nutritional values

The powder made from tamarind kernels has been adopted by the Indian textile industry as 300% more efficient and more economical than cornstarch for sizing and finishing cotton, jute and spun viscose, as well as having other technical advantages. It is commonly used for dressing homemade blankets. Other industrial uses include employment in color printing of textiles, paper sizing, leather treating, the manufacture of a structural plastic, glue for wood, a stabilizer in bricks, a binder in sawdust briquettes, and a thickener in some explosives. It is exported to Japan, the United States, Canada and the United Kingdom.

2:3:2:2 Medicinal values

The powdered seeds are made into a paste for drawing boils and, with or without cumin seeds and palm sugar, are prescribed for chronic diarrhea and dysentery. The seedcoat, too, is astringent, and it, also, is specified for the latter disorders. An infusion of the roots is believed to have curative value in chest complaints and is an ingredient in prescriptions for leprosy. The testa of the seed mascerated with vinegar or lime juice is applied on the face to prevent formation of pimples. The seed are astringent, cooling, aphrodisiac, stomachic, constipating and tonic. The are useful in dipsia, burning sensation, haematuria, giddiness, vertigo, hepatopathy, inflammations, chronic ulcers, abscess, haemorrhoids, vaginopathy, metroptosis, diabetes and general debility.

2:4 Descriptions of Tamarind Fruit.

The fruits are most important part of plant, They are straight or curved ,brown 5-18 cm long and 1.5-2.25 cm wide, They are somewhat Flattened ,constricted at Intervals, with a thin brittle shell, containing a soft brownish or red pulp. The pods contain 3-12 seeds, which are ovate – oblong (1.5 x 0.8 cm), glossy and smooth, Flattened, brownish-ash in colour.

2:4:1 Chemistry of Tamarind Pulp.

The ripe frit contains 63-69% pulp due to the presence of high moisture in it and the pulp content come down to 45-55% after removal of the shell and drying. The fruit pulp contains mainly tartaric acid, reducing sugars, pectin, tannin, fiber and cellulosic material. The edible potion of the ripe pod, before harvest, contains moisture 63.3-68.6%; tartaric acid 8.4-12.4%, sugars 23-30% and pH around 3.15.As the ripened pods are high in moisture content, it is necessary to allow them to dry up on the tree itself. The colour and flavour of the pulp of the ripened fruit are very attractive and the mature pulp of tamarind pod is probably one of the most acidic natural products.

The dried pulp of commerce contains moisture 15-30%, tartaric acids 8-18%, and reducing (invert) sugars 25-45% .Of the reducing sugars present, about 70% is glucose and 30% is fructose. Table 2.5 shows the proximate composition of the dried pulp of tamarind. Nearly 50% of the tartaric acid is in the combined form mainly as potassium bitartrate and to a small extend as calcium tartrate, which is insoluble in cold water. Therefore, cold water extract of the pulp contains only a part of the avsilable tartaric acid and hence hot water is to be used to extract the total acids in the pulp. About 2% of other acids (malic, oxalic, succinic, citric and quinic) are also present in the pulp. Malic acid being predominant (The Wealth of India 1976; Lewis and Neelakantan 1964).

The chief amino acid present are praline and pipecolinic acid. The pulp is fairly rich in minerals especially calcium, potassium and phosphorus. (Table 2.5) The tamarind pulp does not contain any detectable amount of phytic acid, but the seed contains 47 mg per 100g, which should have a minimal effect on its nutritive value. Trypsin inhibitor activity is higher in the pulp than in the seed, but is heatlabile in both. Although the pulp is relatively poor in protein and fat, the seed is a good source of both. Small amounts of vitamins, carotene, thiamine and nicotinic acid are found in the pulp.

Table 2.5.
Mineral contents of Tamarind pulp.

Mineral, mg/100g	pulp
Calcium	81-466
Phosphorus	86-190
Magnesium	72.03
Potassium	62-570
Sodium	3.0-79.7
Copper	21.83
Iron	1.3-10.9
Zinc	1.06
Manganese	--
Nickel	0.52

Source: Bhattacharya et al (1993); Ishola et al (1990); Marangoni et al (1988)

Table 2.6.
Composition of edible portion of ripe fruit of Tamarind.

Constituent	Percentage
Moisture	62.50-69.20
Proteins	1.40-3.30
Fat / oil	0.27-.081
Sugars, total	21.40-30.85
Cellulose	1.80-3.20
Ash	1.20-1.72
Tartaric acid ,total	8.40-12.40
Malic acid	
Citric acid	
Ascorbic acid	
Total acidity ,as tartaric acid	17.10-18.40
PH	3.15
Pentoses	4.20-4.80

The tender fruits contain most of the tartaric acid in free form (upto 16%), which can be easily extracted with water. The acid and sugar contents vary within a narrow range among the varieties. Unlike in other fruits, ripening in the tamarind fruit is not accompanied by a decrease in acid content. The formation and breakdown of starch in a short period during the process of ripening result in the accumulation of 30-40 % reducing sugars in the harvested fruit, giving it a sweet taste.

2:4:2 Biosynthesis of Tartaric acid

Tartaric acid is an unusual plant acid, which perhaps is formed from the primary carbohydrate products of photosynthesis and once formed cannot be used further in the plant because of the absence of necessary enzymes. The titrable acidity is low in young leaves, but increases with age and again comes down in old leaves. The total tartaric acid content in leaves decreases from 28 to 12% from May to December and the free tartaric acid disappears after the first three months and the alkalinity of the ash increases rapidly due to adsorption of calcium and potassium, which neutralizes the acid .Simultaneously, there is a shift in the pH of the leave's sap from 2.3 to 4.1%

Since oxaloacetate is the only tricarboxylic acid cycle intermediate found to be slowly oxidized by young tamarind leave in respiratory studies, Ramakrishnan and Joshi (1960) have suggested that sugar gets converted to oxaloacetate through the operation of the tricarboxylic acid cycle enzymes and that oxaloacetate gets converted to tartrate via dihydroxy fumarate. In old leaves, the rate of oxidation for all intermediates is low, which suggests a general slow down of metabolism. Both young and old fruits oxidize all the intermediates, suggesting that the site of tartaric acid formation is in the leaves and the acid gets translocated to the fruits. Tartaric dehydrogenize is active only towards 1-tartaric acid and not dextro and meso forms. The inability of dehydrogenize to attack dextro-tartaric acid is considered to be responsible for the accumulation of dextro-tartaric acid in fruits and leaves. (Lewis and Neelakantan 1959, Lewis et al. 1957).

2:4:3 Acidity of Tamarind.

The so-called red variety of tamarind is sweeter than the common brown variety, evidently because it has a lower contet of free acid. In the red varirty, The acid is present in the combined form mostly as potassium bitartrate and to a small extent as calcium tartrate. The common variety has high proportion of free acid and less pectin content as compared to the red variety. An analysis of the pulp from red and common varieties showed, respectivelt, the following values; moisture 20.1, 18.2 ; tartaric acid (free) 6.6, 9.8; tartaric acid (combined) 11.4, 6.7; invert sugars 36.4, 38.2 and pectin 4.4, 2.4% .

2:4:4 Colour of the tamarind pulp

During storage, the brownish-red coloured pulp becomes darker and after about a year, is almost black. This is perhaps due to the onset of Maillard reaction, since free amino acids and reducing sugars are present in the pulp. The pulp also becomes soft and sticky as pectolytic degradation takes place and moisture is absorbed, especially in humid weather.

The anthocyanic pigment, chrysanthemin, is responsible for the colour of the pulp in the red variety of tamarind and the common variety contains leucocyanidin. The anthoxanthin pigments lutein and apigenin are present to the extent of about 2% in the tamarind leaves. The fruits have low anthoxanthin content, while the flowers contain only xanthophylls. The seed testa contains leucoanthocyanidin.

2:4:5 Flavour of tamarind pulp.

The chemical composition of tamarind pulp flavour depends on the method of extraction, raw pulp used and the method of analysis. This is the reason of the flavour composition.
The volatile constituents of the pulp were investigated by the combined technique of GLC-MS with 61 constituents identified as artifacts appeared to originate from the vacuum steam distillation apparatus during isolation of the volatiles. The results of the study suggested that the overall aroma of tamarind consisted of citrus notes and warm spice-like flavours with some roasted character. The major constituents identified were: hexanol, cis-3-hexen-1-ol, trans-2-hexaen-1-ol, trans and cis –linalool oxides, 2-acetyl furan,benzaldehyde, linalool,4-terpineol, phenylacetal-dehyde, a-terpineol, 2-phenyl ethyl alcohol, dibutyl phthalate and geraniol. The volatile compounds of the pulp were extracted with pentane / diethyl ether (1:1), separated by column chromatography and analysed by GLC and 35 volatile were identified, including 10 hydrocabons and 25 polar compounds. Monoterpenes, furan derivatives, benzaldehyde derivatives and methyl pyrazines were the important constituents.
Zhang and Ho (1990) isolated the volatiles of tamarind pulp by simultaneous-steam distillation / solvent extraction and analysed by GC-MS. A total of 28 compounds were identified with furfural (123 ppm); 5-methyl-2 (3H) furanone (10.61 ppm), phenyl (8.60 ppm) accounting for 88.74 of the total volatiles.

Supercritical fluid extraction of the tamarind pulp followed by GC and GLC-MS analysis showed that 16 compounds accounted for 97.5 % 0f the extract and the major compound was aroma-dendrone (90%) (Sagrero et al .1994). The non-volatile flavour components in extract of tamarind pulp were analyzed by HPLC and the major components were glucose (37.5 %) fructose (18.4 %) and alanine (14.2%) (Khurana and Ho 1989).

2:4:6 Tamarind pulp Preservation

The pods (fruits) are gathered when fully ripe and the brittle and hard pod shell is separated either manually or mechanically. The fruit pulp is separated from the seed and fibrous material and dried in the sun for a few days to reduce the moisture content. Then, the dried pulp is packed in leaf mats, polythene or jute bags or bamboo or wooden boxes and stored in a cool and dry place. In some places, the salted (10%) pulp is trodden into a mass and made into balls and exposed to the sun or steamed for a short time and then exposed to the sun and dew for about a week. (The Wealth of India 1976).

Various method of prolonging the storage life of whole and pulped tamarind were investigated by Chumsai-Silvanich et al (1991). For whole tamarind, steaming for 5 min followed by drying in a hot air oven at 80 ^{0}C for 2 h proved to be the most suitable method and the resultant fruit could be stored in plastic bags at room temperature for 4 months without affecting quality and acceptability. For pulped tamarind, after removal of peel, veins and any unwanted part, steaming for 20 min, followed by drying at 60 ^{0}C for 2.5 h, cooling and packaging in clear plastic bags gave the best results. The product could be stored at room temperature for 3 months with satisfactory quality and acceptability.

An improved procedure for extracting and preserving tamarind pulp is outlined by Benero et al (1972).Tamarind pulp cannot be separated from the fruit by mechanical means alone, dilution being necessary. A 1:2 fruit: water ratio produced the highest yields of soluble and total solids. Pulps obtained at this dilution had about 13.2^{0} Brix with excellent fruit flavour. The proportions of weights of pulp, seed and shells in ripe tamarind fruit for processing were found to be 30, 40 and 30 %, respectively. This mechanical extraction method for unpeeled tamarind fruit produced high quality tamarind fruit pulps with prolonged shelf life. A water alcohol extraction of tamarind having a P^{H} of 2.0-3.5 and 80% solids has replaced the whole, shelled fruit formerly shipped in 500 lb wooden barrels (Anon 1969)

2:4:7 Food Value

Analyses of the pulp are many and varied. Roughly, they show the pulp to be rich in calcium, phosphorus, iron, thiamine and riboflavin and a good source of niacin. Ascorbic acid content is low except in the peel of young green fruits.

Food Value Per 100 g of Edible Portion (Table 2.7)

Compound	*Pulp (ripe)* *
Calories	115
Moisture	28.2-52 g
Protein	3.10 g
Fat	0.1 g
Fiber	5.6 g
Carbohydrates	67.4 g
Invert Sugars	30-41 g
(70% glucose; 30% fructose)	
Ash	2.9 g
Calcium	35-170 mg
Magnesium	
Phosphorus	54-110 mg
Iron	1.3-10.9 mg
Sulfur	
Sodium	24 mg
Potassium	375 mg
Vitamin A	15 I.U.
Thiamine	0.16 mg
Riboflavin	0.07 mg
Niacin	0.6-0.7 mg
Ascorbic Acid	0.7-3.0 mg
Oxalic Acid	
Tartaric Acid	8-23.8 mg
Oxalic Acid	trace only

*The pulp is considered a promising source of tartaric acid, alcohol (12% yield) and pectin (2 1/2% yield). The red pulp of some types contains the pigment, chrysanthemin.

2:4:8 Food Use of Tamarind Pulp.

Tamarind fruit has been used as a raw material for the manufacture of several products like Tamarind juice Concentrate (TJC), Tamarind pulp powder (TPP) , Tamarind Kernel Powder (TKP) , tartaric acid, pectin, tartrates and alcohol. The following section will deal with technology of some of the important products.

Tamarind Juice Concentrate (TJC)
Tamarind Pulp Powder (TPP)
Tamarind kernel Powder (TKP)
Tartaric acid (TA)
Pectin
Tartrates
Alcohol

Pectin, tartrates, tartaric acid and ethanol Studies have been carried out for the isolation of tartaric acid and fermentation of sugars for useful by products like ethanol, lactic acid and citric acid .Since the pulp also contains pectin, an integrated process has been worked out for the production of pectin. Tartrates and ethanol from it. The pulp is repeatedly extracted with boiling water and the filtered extract is cooled to separate potassium bitartrate. The supernatant is concentrated under vacuum and the pectin is separated by the addition of alcohol. The filtrate after recovering alcohol is treated with lime to precipitate calcium tartrate. The remaining sugars are fermented with yeast and alcohol is recovered. The recovery of about 2.5% pectin in addition to 12% tartaric acid and 12% alcohol from tamarind pulp makes the process attractive. For the isolation of tartaric acid, the use of unripe green pods has been suggested, as they contain most of the acid in the free form. Krishna (1995) patented a process for the extraction of tartrates with acidified ethanol and subsequent extraction of pectin. After removal of alcohol, the residual syrup can be used for edible purposes.

2:4:8:1 Tamarind juice concentrate.

The tamarind concentrate or tamarind juice concentrate (TJC) is a convenient product and it is easy to disperse and reconstitute well in hot water. The concentrate is hygienic and can be stored well for longer periods.

The process for the manufacture of tamarind concentrate has been developed by the Central Food Technological Research Institute, (CFTRI), Mysore and several firms are producing the concentrate on commercial scale based on this process. For the preparation of concentrate, the cleaned pulp is extracted with boiling water using counter current principle, where dilute extracts are used for extracting fresh batches of the pulp, an extract containing about 20% soluble solids is then obtained. The extract is separated from the pulp, using suitable sieves and concentrated under vacuum in a forced- circulation evaporator. When the concentration of the soluble solids reaches 68%, the material is removed and directly filled in cans or

bottles. It sets like a Jam on cooling. The yield of the concentrate will be about 75% of the pulp used. For getting a good product, it is necessary to use freshly harvested fruit pulp, which is free from insect infestation and rodent contamination (Lewis et al. 1970).

A process for extraction, concentration and preservation of sour principles from the fruit of tamarind was patented by pillai (1973). The sour principle is extracted with hot water, filtered, clarified by bleaching with SO_2 and then evaporated in vacuum to required consistency and treated with vinegar or acetic acid (2%) to provide a storage stable extract.

Tender green fruits of tamarind dared to prepare tamarind pickles, as they are very acidic. Tender fruits can be taken as a row material for the manufacture of natural tartaric acid. The ripened fruit pulp is used in the manufacture of mixed fruit jams, sauces, beverage and tamarind paste (Goce et al. 1993). The tamarind pulp is also canned for long use. The tamarind juice is concentrated after mixing with spices, sugar and salt and converted into a preserve. Tamarind juice extract is also used in certain confectionery products like tamarind candy (Sadasivam et al. 1979; Girdharilal et al. 1958; Gowramma et al. 1968).

2:4:9 Medicinal Uses of Tamarind pulp

Medicinal uses of the tamarind are uncountable. The pulp has been official in the British and American and most other pharmacopoeias and some 200,000 lbs (90,000 kg) of the shelled fruits have been annually imported into the United States for the drug trade, primarily from the Lesser Antilles and Mexico. The European supply has come largely from Calcutta, Egypt and the Greater Antilles. Tamarind preparations are universally recognized as refrigerants in fevers and as laxatives and carminatives. Alone, or in combination with lime juice, honey, milk, dates, spices or camphor, the pulp is considered effective as a digestive, even for elephants, and as a remedy for biliousness and bile disorders, and as an anti-scorbutic. In native practice, the pulp is applied on inflammations, is used in a gargle for sore throat and, mixed with salt, as a liniment for rheumatism. It is, further, administered to alleviate sunstroke, *Datura* poisoning, and alcoholic intoxication. In Southeast Asia, the fruit is prescribed to counteract the ill effects of overdoses of false chaulmoogra, *Hydnocarpus anthelmintica* Pierre, given in leprosy. The pulp is said to aid the restoration of sensation in cases of paralysis. In Colombia, an ointment made of tamarind pulp, butter, and other ingredients is used to rid domestic animals of vermin.

The fruits are sour, sweet, refrigerant digestive, anti-scorbutic, anti-septic, ophthalmic and febrifuge. They are useful in gastropathy, bilious vomiting, Datura poisoning, alcoholic intoxication dipsia, scabies, pharyngitis, pharyngodynia, stomatitis, constipation, heamorrhoids and ophthalmopathy.

2:4:10 Other uses of Tamarind

Feed: The leaves and foliage of tamarind can be used as forage for cattle. The foliage has a high forage value, though it is rarely lopped for this purpose because it affects fruit yields.

Fuel: Provides good firewood with calorific value of 4850 kcal/kg, it also produces an excellent charcoal.

Pesticide: Extracts from the fruit pulp have shown some molluscicidal activity and has been reported to have potent fungicidal and bactericidal properties. Extracts from the plant also have an inhibitory effect on plant viruses.

Tannin or dyestuff: Both leaves and bark are rich in tannin. The bark tannins can be used in ink or for fixing dyes. Leaves yield a red dye, which is used to give a yellow tint to clothe previously dyed with indigo. Ashes from the wood are used in removing hair from animal hides.

Lac: The tamarind tree is a host for the lac insect, *Kerria lacca,* that deposits a resin on the twigs. The lac may be harvested and sold as stick-lac for the production of lacquers and varnish. If it is not seen as a useful byproduct, tamarind growers trim off the resinous twigs and discard them.

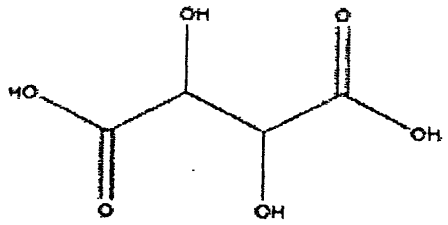
2:5 Active Ingredient of Tamarind Extract.

Tamarind meat is also applied to the face for anti-freckle and anti-aging purposes. Currently, Tamarind extract is used as an ingredient in body cleansing products for adjusting skin acidity condition, and antiseptic, anti- allergy, and skin tightening purposes.

- Tartaric acid
- Citric acid
- Malic acid

2:5:1 Tartaric acid

Tartaric acid is a white crystalline organic acid. It occurs naturally in many plants, particularly grapes and tamarinds, and is one of the main acids found in wine. It is added to other foods to give a sour taste, and is used as an antioxidant. Salts of tartaric acid are known as tartrates. It is a dihydroxy derivative of dicarboxylic acid.



2,3dihydroxybutanedioic acid

Physical and Chemical Properties

Appearance: White crystals

Odor: Odorless.

Solubility: ca. 133 g/100 g of water.

Density: 1.76

Boiling Point: Not applicable.

Melting Point: 206C (403F)

Other name 2,3-dihydroxysuccinic acid , threaric acid , racemic acid , uvic acid

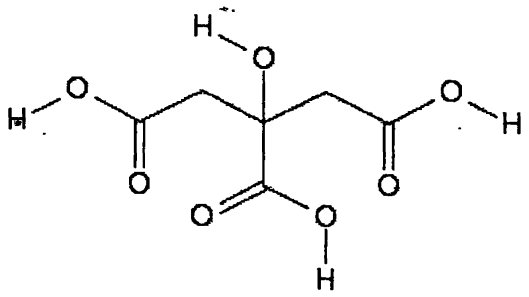
Molecular formula $C_4H_6O_6$

Molar mass C(C(C(=O)O)O)(C(=O)O)O

Appearance white powder

2:5:2 Citric acids

Citric acid is a weak organic acid found in citrus fruits. It is a good, natural preservative and is also used to add an acidic (sour) taste to foods and soft drinks. In biochemistry, it is important as an intermediate in the citric acid cycle and therefore occurs in the metabolism of almost all living things. It also serves as an environmentally benign cleaning agent and acts as an antioxidant.



2-hydroxypropane-1,2,3-tricarboxylic acid

Acid base propertis

pKa1 3.15 , pKa2 4.77 , PkA3 6.40,

pH 2.1

Chemical formula $CH_2(COOH) \cdot COH(COOH) \cdot CH_2(COOH)$

Melting point 426 K (153 °C)

Citric acid analysis

Principle

In the reaction catalyzed by the enzyme citrate lyase (CL) citric acid (citrate) is converted to oxaloacetate and acetate

$$\text{Citrate} \xrightarrow{\text{CL}} \text{Oxaloacetate} + \text{Acetate}$$

In the presence of the enzymes malate dehydrogenase (MDH) and lactate dehydrogenase (LDH), oxaloacetate and its decarboxylation product pyruvate are reduced to L- malic respectively, by reduced nicotinamide-adenin dinucleotide (NADH)

$$\text{Oxaloacetate} + \text{NADH} + \text{H} \xrightarrow{\text{MDH}} \text{L-malate} + \text{NAD}^+$$

The amounts of NADH oxidized in reaction and are stoichiometric with the amount of citrate. It is NADH which is measured at a wavelength of 334, 340 or 365 nm.

Calculation

According to the general formula for calculating the concentration, the equation is:

$$C = \frac{V \times MW}{\varepsilon \times d \times v \times 10000} \times \Delta A \ [g/l]$$

Where V = final volume (ml)

v = sample volume (ml)

MW = molecular weight of the substance to be assayed

d = light path (cm)

ε = absorption coefficient of NADH at
340 nm = 6.3 [1x $mmol^{-1}$ x cm^{-1}]

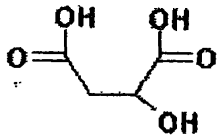
It follows for L- malic acid

$$C = \frac{3.02 \times 210.1}{\varepsilon \times 1 \times 0.2 \times 1000} \times \Delta = 3.173 \times \frac{\Delta A}{\varepsilon}$$

g { L-malic acid / l sample solution]

2:5:3 Malic acid

Malic acid is a tart-tasting organic acid that plays a role in many sour or tart foods. also known as apple acid, hydroxybutanedioic acid and hydroxysuccinic acid, is a chiral molecule. The naturally occurring stereoisomer is the L-form. The L-form is also the biologically active one. There is some preliminary evidence that malic acid, in combination with magnesium, may be helpful for some with fibromyalgia. Malic acid sold as a supplement is mainly derived from apples and, therefore, is the L-form. L-malic acid has the following chemical structure



Synonyms: Butanedioic acid, hydroxy-; hydroxysuccinic acid; hydroxybutanedioic acid

Molecular Weight: 134.09
Chemical Formula: C4H6O5
Physical and Chemical Properties
Appearance: White or colorless crystals.
Odor: Odorless.
Solubility: Soluble in water.
Specific Gravity: 1.601
Boiling Point: 150C (302F) Decomposes
Chemical formula $C_4H_6O_5$Molecular mass 134.09 g/mol
Melting point 128°C to 130°C
Density 1.609 g/cm^3

Analysis of Malic acid

Principle

In the presence of L- malate dehydrogenase (L- MDH), L- malic acid (L- malate) is oxidized by nicotinamide-adenine dinucleotide (NAD) to oxaloacetate. The equilibrium of this reaction lies for on the side of malate. Removal of equilibrium in favour of oxaloacetate. In the reaction catalyzed by the enzyme glutamate-oxaloacetate transaminase (GOT) , oxaloacetate is converted to L- aspartate in the presence of L-glutamate.

$$\text{L-malate} + NAD^+ \xrightarrow{\text{L-MDH}} \text{oxaloacetate} + NADH + H^+$$

$$\text{Oxaloacetate} + \text{L} - \text{glutamate} \xrightarrow{\text{GOT}} \text{L-aspartate} + \text{ketoglutarate}$$

The amount of NADH formed is stoichiometric with the concentration of L- malate. It is NADH which is measured at a wavelength of 334, nm.

Calculation

According to the general formula for calculating the concentration, the equation is:

$$C = \frac{V \times MW}{\varepsilon \times d \times v \times 10000} \times \Delta A \text{ [g/l]}$$

Where V = final volume (ml)

v = sample volume (ml)

MW = molecular weight of the substance to be assayed

d = light path (cm)

ε = absorption coefficient of NADH at

340 nm = 6.3 [1x $mmol^{-1}$ x cm^{-1}]

It follows for L- malic acid

$$C = \frac{2.22 \times 134.09}{\varepsilon \times 1 \times o.1 \times 1000} \times \Delta = 2.977 \times \frac{\Delta A}{\varepsilon}$$

g { L-malic acid / l sample solution]

Determination of Fat using Solvent extraction.

The fact that lipids are soluble in organic solvents, but insoluble in water, provides the food analyst with a convenient method of separating the lipid components in foods from water soluble components, such as proteins, carbohydrates and minerals. In fact, solvents extraction techniques are one of the most commonly used methods of isolating lipids from foods and of determining the total lipid content of foods. The total lipid content determined by solvent extraction depends on the nature of the organic solvent used to carry out the extraction. The total lipid content determined using one solvent may be different from that determined using another solvent. In addition to the above considerations, a solvent should also be inexpensive, have a relatively low boiling point, be non toxic and be nonflammable. It is difficult to fined a single solvent which meets all of these requirements. Ether and petroleum ether are the most commonly used solvents.

2.6 Ultrasound-assisted extractions (UAE) of tartaric, citric, malic Acids from Tamarind fruit.

Introduction

Ultrasound-assisted extraction (UAE) can be used for extraction methods with liquid solvents applied to analyses in solid matrices. This extraction process is fast in comparison with the traditional methods, because of the contact surface area between solid and liquid phase is much greater, due to particle disruption taking place

The application of UAE to plants has produced very interesting results to the extent that industrial processing has been proposed for obtaining compounds with pharmacological properties

These techniques were used because several of the extraction variables must be optimized and such techniques allow the most significant variables to be determined easily

Tartaric, citric, and malic acids in grapes as well as isoflavones in soy beans were analyzed by HPLC after the extraction.

Experimental

Samples

Around 10 g of Tamarind pulp were used in the extractions. All samples were Freeze-dried before the extraction in order to increase sensitivity of the analysis and because of different tamarind pulp could have different moisture.

Extraction

A high intensity probe ultrasound generation system of 200 W, 24 kHz was used. The instrument was a model UP 200S from dr.Hielscher GmbH (Teltow, Germany). Its amplitude controller allows the ultrasonic vibrations at the probe microtip to be set at any desired level in the 10-100% range of the nominal power. Also the cycle controller allows the duration of the application of the ultrasound to be set, to a fraction of a second in the 0.1-1.0 range.

Extraction variables

A fractional factorial experimental design was carried out in order to determine the more significant variables for the extraction process. All the concentrations are shown relative to the amount found using the most effective conditions (100%).

Analyzing the main effect plots, it can be concluded that the more significant variables for the Extraction process are temperature and the solvent used as extracting liquid.

Table2:8 Extraction conditions in the fractional factorial experimental design.

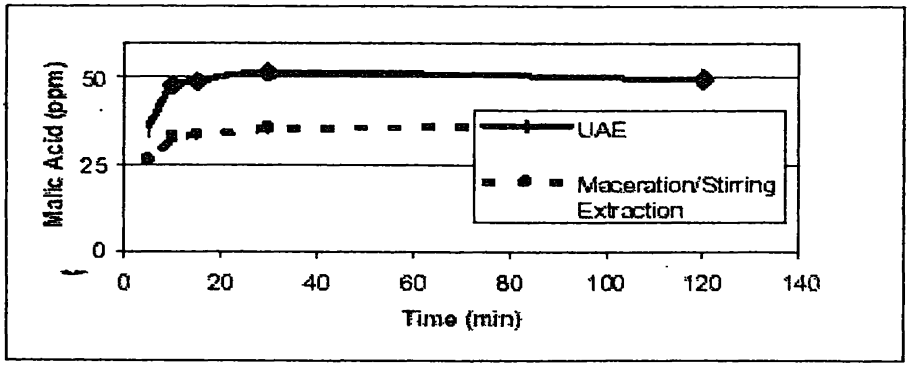


Fig 2.3

Table 2:8
Extraction condition in the fractional experimental design.

Experiment	Temperature °C	solvent	Volume (ml)	Time (min)	Tartaric acid	Citric acid	Malic acid
1	20	methanol	25	5	×	×	×
2	50	water	25	5	×	×	×
3	20	methanol	100	5	×	×	×
4	50	water	100	5	×	×	×
5	20	methanol	25	15	×	×	×
6	50	water	25	15	×	×	×
7	20	methanol	100	15	×	×	×
8	50	water	100	15	×	×	×
9	20	methanol	25	5	×	×	×
10	50	water	25	5	×	×	×
11	20	methanol	100	5	×	×	×
12	50	water	100	5	×	×	×
13	20	methanol	25	15	×	×	×
14	50	water	25	15	×	×	×
15	20	methanol	100	15	×	×	×
16	50	water	100	15	×	×	×

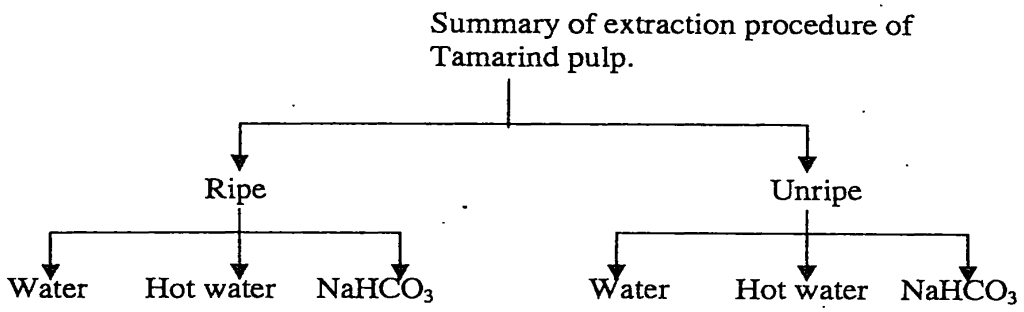
Optimization of extraction time

The extraction time must be adjusted to obtain quantitative recoveries of organic acids. To determine the time needed, different extractions were done using increasing extraction times to establish the kinetic of the extraction.

CHAPTER 03 METHODOKOGY

The organic acid extraction (Summary)

Ripe and unripe tamarind fresh fruit samples were taken and three types of extraction procedures were followed, extraction of organic acid using sodium bicarbonate, hot water and cold water. Active ingredient of tamarind fruit (Tartaric, citric and malic acid) were purified and analyzed. Active ingredients of tamarind seed were analyzed by using TLC



3:1:1 preparation of Tamarind extract using sodium bicarbonate ($NaHCO_3$).

Tamarind pulp was ground using electric grinder then sodium bicarbonate was added to tamarind pulp step by step while shaking well until of all Co_2 gas is removed stirred using for 24 hours magnetic stirrer. All soluble matter in sodium bicarbonate (all organic acid dissolved in sodium bicarbonate mainly tartaric , citric , malic and oxalic acid) was filtered using a vacuum filter. Sodium bicarbonate was evaporated at 40 ^0C temperature using a Rotary evaporator. Finally extracts were analyzed and purified.

Materials and equipments

Fresh Tamarind fruit.
Electric balance
Grinder
Beaker
Pipette
Separating funnel
Measuring cylinder
Watch glass
Magnetic stirrer
Rotary evaporator

P^H meter

Ethanol

Distil water

Sodium bicarbonate.

Sodium Hydrochloride

Preparation of Tamarind extract using sodium bicarbonate ($NaHCO_3$).

Fresh Tamarind fruit

↓

Tamarind pulp (without seed.)

↓

Ground tamarind pulp. (50 g)

↓

Extraction of organic acid, using of $NaHCO_3$
(24 hours and using magnetic stirrer)
Until all CO_2 was remove from Tamarind pulp.

↓

Filtration of tamarind extract.

↓

Extracts were neutralized with 5 N sodium hydroxide solutions free of carbonates

↓

Separate organic acid and ethanol.
(Evaporation using of Rotary evaporator)

↓

Analysis of organic acid
(Tartaric, citric, malic acid)

↓

Purification (Column chromatography)

3:1:2 preparation of Tamarind extraction using cold water.

Fresh Tamarind fruits were taken for Organic acid extraction using cold water. Fresh tamarind fruits were taken for organic acid extraction. Kernel of tamarind was removed and separated from tamarind pulp and seed. 50g of tamarind pulp were weighed and ground using electric grinder. Tamarind juice (all organic acid and inverted sugars) was extracted using 250 ml of coldwater by keeping in it for 24 hours. Tamarind extract was filtered using vacuum filter. Organic acid was extracted by solvent extraction using ethanol. Solvent extraction was carried out 3 times in order to remove all organic acid from water phase to ethanol phase. Organic acid and ethanol were separated using Rotary evaporator. Finally tartaric, citric, malic acid extract were analyzed and purified by using column chromatography.

Materials and equipments

Fresh Tamarind fruit.
Electric balance
Grinder
Beaker
Pipette
Vacuum filter
Separating funnel
Measuring cylinder
Watch glass
Magnetic stirrer
Rotary evaporator
P^H meter
Ethanol
Distil water
Sodium bicarbonate.
Sodium Hydrochloride

Preparation of Tamarind extraction using cold water.

Fresh Tamarind fruit

↓

Tamarind pulp (without seed.)

↓

Ground tamarind pulp. (50 g)

↓

Tamarind Juice Extraction of organic acid, using water.
(Stirrer for 24 hours)

↓

Filtration of tamarind extract.

↓

Solvent extraction.
(Remove all organic acid from water to ethanol solvent, 3 potion .)

↓

Extracts were neutralized with 5 N sodium hydroxide solutions free of carbonates

↓

Separate organic acid and ethanol.
(Evaporation using Rotary evaporator)

↓

Analysis of organic acid
(Tartaric, citric, malic acid)

↓

Purification method.
(Column chromatography)

3:1:3 preparation of Tamarind extraction using hot water (40 ^{0}C).

Fresh Tamarind fruits were taken for Organic acid extraction using hot water. Fresh tamarind fruits were taken for organic acid extraction. Kernel of tamarind was removed and separated from to tamarind pulp and seed. 50g of tamarind pulp were weighed and ground using electric grinder. Tamarind juice (all organic acid and inverted sugars) was extracted using 250 ml of hot water by keeping in it for 24 hours. Tamarind extract was filtered using vacuum filter. Organic acid was extracted by solvent extraction using ethanol Solvent extraction was carried out 3 times in order to remove all organic acid from water phase to ethanol phase. Organic acid and ethanol were separated using Rotary evaporator. Finally tartaric, citric, malic acid extract were analyzed and purified using column chromatography.

Materials and equipments

Fresh Tamarind fruit.
Electric balance
Grinder
Beaker
Pipette
Vacuum filter
Separating funnel
Measuring cylinder
Watch glass
Magnetic stirrer
Rotary evaporator
P^H meter
Ethanol
Distil water
Sodium bicarbonate.
Sodium Hydrochloride

Preparation of Tamarind extraction using hot water (40 ^{0}C).

Fresh Tamarind fruit

↓

Tamarind pulp (without seed.)

↓

Ground tamarind pulp. (50 g)

↓

Tamarind Juice Extraction of organic acid, using water.
(Stirred for 24 hours)

↓

Filtration of tamarind extract.

↓

Solvent extraction.
(Removing all organic acids from water to ethanol solvent, 3 potions.)

↓

Extracts were neutralized with 5 N sodium hydroxide solutions free of carbonates

↓

Separated organic acid and ethanol.
(Evaporator for using of Rotary evaporator)

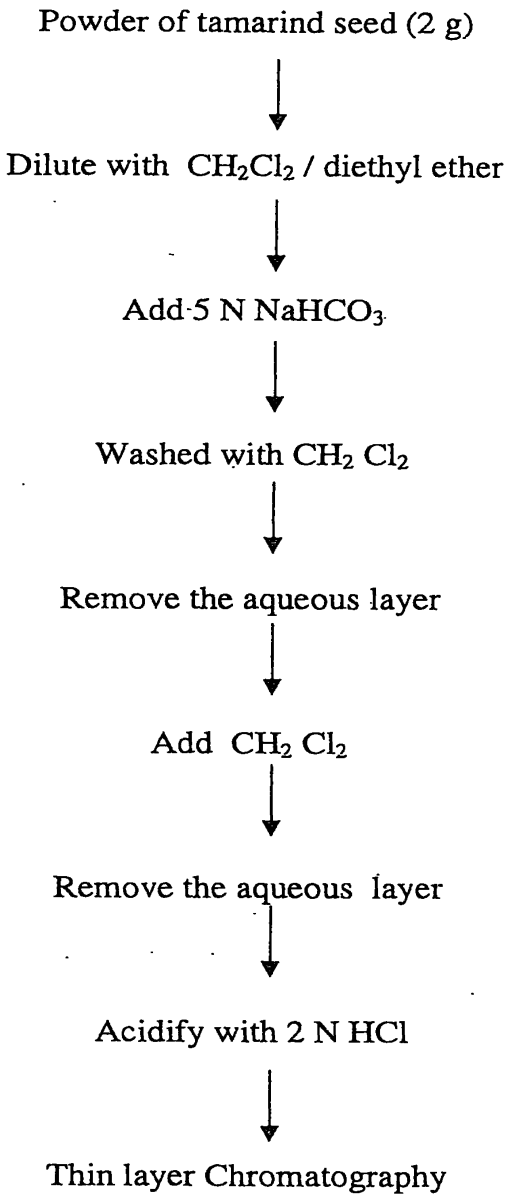
↓

Analysis of organic acid
(Tartaric, citric, malic acid)

↓

Purification (Column chromatography)

3:1:4 Analysis of organic acids in tamarind seed.



3:2:1 Tartaric acid: Metavanadate Spectrometric (VIS) Analysis.

The metavanadate method used in this study involves a colorimetric reaction between sodium (or ammonium) meravanadate and tartaric acid in acetic acid solution.

1 Equipment

125- ml volumetric flasks

Electric hot plate

Volumetric pipettes (2, 4, 25 ml)

Graduated cylinder (100 ml)

Spectrophotometer (Visible)

Matched cuvettes

Whatman # 1 filter paper

Glass funnels

Wash bottles

2 Reagents

Tartaric acid standard solution (10 g/l): In a 100-ml volumetric flask dissolve complete grade tartaric acid in deionized water. After dissolution is complete, bring to volume with deionized water.

Metavanadate solution (5% wt/ vol): In a100-ml volumetric flask, dissolve 5g of KVO_3 in hot (less than 70 ^{0}C) deionized water. Bring to volume so filter the solution, using Whatman # 2 papers, before use

Decolorizing carbon

Boiling chips

Preparation of standard solution of tartaric acid from 10g/ l stock.

Standard solution (ml)	Volume H_2O (ml)	Final concentration H_2T (g/l)
0	20	0.00
2	18	1.00
4	16	2.00
6	14	3.00
8	12	4.00

Dilution procedures for preparation of working solutions from stock hydrochloric acid.

Desired approximate normality	mL of stock HCl per 1.0 L
0.01	0.89
0.02	1.78
0.10	8.90
0.50	44.50
1.00	89.00
2.00	178.00

Procedure

(a) Standard Curve

(b) Using 125-ml Erlenmeyer flasks, a series of standards were prepared.

(1) With these 20-ml standards, proceeded with steps 4- 11 below, concentration (g H2T/L) vs. absorbance was plotted at 520 nm.

(c) Tamarind sample

(3) 125-ml Erlenmeyer flask was cleaned, 20 ml of tamarind Extract was transferred volumetrically.

(2) It was added with 2 ml N HCL, 30 ml, deionized water, 0.50 g (accurately weighed) decolorizing carbon and several boiling chips.

(3) The solutions were boiled on hot plate 2-3 min, and cooled to room temperature.

(4) The 100ml solution was filtered into 100 ml volumetric flasks using Whatman # 1 filter paper.

(5) Clarity was examined; Erlenmeyer flask and filter paper were thoroughly rinsed with deionized water. Multiple rinses using small amounts of water were carried out

(6) The solution was brought to 100-ml volume with deionized water.

(7) Transfer 25 ml decolorized solution to another 100 ml volumetric flask. Add 2 ml concentrated acetic acid, 10 ml filtered metavanadate. solution, and bring to volume.

(8) Allow 30 min for color development. Record absorbance on spectrometer at 520 nm.

(9) With reference to a standard curve, determine tartaric acid content of the sample.

3:2:2 Citric acid analysis

Each Test – combination was prepared according to the following procedure

1 Three bottles 1 each with ca. 1.4 g of lyophilisate, composed of glycylglycine buffer P^H 7.8.

Malate dehydrogenase

Lactate dehydrogenase

NASH 6.0 mg

Stabilizers

2 Tree bottles 2 each with 50 mg of lyophilized citrate lyase

Preparation of solutions for 10 Determinations

1 Dissolve contents of one bottle 1 in 12 ml of redist water,

2 Dissolve contents of one bottle 2 in 0.3 ml of redist water,

Stabilization of Solutions

Solution 1 was stabilized for 2 Weeks when by storing at + 4 ^{0}C or 4 weeks by storing at -20 ^{0}C

Solution 2 was stabilized by storing for2 Weeks when stored at + 4 ^{0}C or 4 weeks by sttoring at -20 ^{0}C

Procedure

Wavelength: 340 nm, Hg nm or 334 nm

Glass cuvette: 1 cm light path

Temperature: 20-25 ^{0}C

Read against air (without a cuvette in the light path) or against water.

Sample solution 2-35 g of L-malic acid / cuvette.

Pipette into cuvettes	blank	Sample
Solution 1	10.0 ml	10.0 ml
Solution 2	2 .0 ml	2 ml
Redist. water	10.0 ml	9 ml
Suspension 3	0.1 ml	0.1 ml
Sample solution	--	10.0 ml
Mix, rad absorbances of the solutions (A1) after approx. 3 min, see also further instructions 2.1. Start reaction by addition of		
Solution 4	0.1 ml	0.1 ml
Mix; on completion of the reaction (approx. 5- 10 min) rad absorbances of solutions (A2)		

Calculate the absorbance differences (A2- A1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample.

$$\Delta A = \Delta As - \Delta A$$

3:2:3 Analysis of Malic acid

Each Test- combination contains

1 Bottle 1 with Ca 30 ml of solution consisting of
Glycylglycine buffer – p^H 10.
L- glutamic acid 440 mg
Stabilization

2 Bottle 2 with Ca 210 mg of NAD lyophilisate

3 Bottle 3 with 0.4 ml of glutamate-oxaloacetate transaminase

4 Bottle 4 with 0.4 ml L- malate dehydrogenase

Preparation of solutions

1 contents of bottles 1, 3 and 4 undiluted was used

2 Dissolve contents of bottle 2 in 6 ml redist. Water.

Stabilization of Solutions

The contents of bottles 1,3 and 4 were stabilized for 1 week by storing at + 4 ^{0} C , while solution 2 was stabilized for 3 weeks at + 4 ^{0}C and for 1 week at -20 ^{0} C.

Procedure

Wavelength used : 340 nm , Hg nm or 334 nm

Glass cuvette: 1 cm light path

Temperature: 20-25 ^{0}C

pipette into cuvettes	blank	Sample
Solution 1	10.0 ml	10.0 ml
Solution 2	2 .0 ml	2 ml
Redist. water	10.0 ml	9 ml
Suspension 3	0.1 ml	0.1 ml
Sample solution	--	10.0 ml
Mix, rad absorbances of the solutions (A1) after approx. 3 min, see also further instructions 2.1. Start reaction by addition of		
Solution 4	0.1 ml	0.1 ml
Mix; on completion of the reaction (approx. 5- 10 min) rad absorbances of solutions (A2)		

Calculate the absorbance differences (A2- A1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample.

$$\Delta A = \Delta A_S - \Delta A_B$$

Determination of free fat using solvent extraction method.

2.0g of finely ground Tamarind pulp (Extract) sample was placed in 50 ml beaker, 2 ml of 95 % ethanol and 10 ml of HCl were added , which was prepared, by adding 25 ml of con HCl and 1 ml water. Then the contents were mixed thoroughly. The beaker was placed in (70 ^{0}C 80 ^{0}C) water bath and stirred for 30-50 minutes frequently. Then beaker was removed from the water bath and cooled in the atmosphere. 10 ml of ethanol was added to it and transfered the mixture in to majoinner flask. Beaker was washed with 25 ml of ether in 3 portion and the washing was added it to the flask. The flask was coppered with a cork and shook vigorously for about few minutes and added 25 ml of pet ether and shooke again .The flask was allowed to stand until a clear layer of pet ether was appeared. The upper ether layer was taken in to a clean previously weighed dried flask. It was dried in a water bath (90 ^{0}C) until the constant weight was obtained.

3:3 The separation and Estimation of Acid by chromatography on columns of Anion Exchange Resins.

The round bottomed flask, containing 6 N formic acid.(Flask B) capacity 200 ml, was fitted to a flask with a stirrer which contains 200ml. of water. The flasks were connected together and to the resin column. Air were forced into flask, a transferring formic acid to flask, B,

where it was mixed with water. The diluted formic acid was displaced to the resin column, C, The air pressure was adjusted to give an elution rate of 10-12 drops per minute.

Preparation of resin and resin column.

The resin, Dowex 1 (300-500 mesh), was usually purchased in the chloride form. To convert it to the formation form the procedure was as, Place a batch of the resin in a glass tube fitted with a sintered glass disc, or with a wad of glass wool supported at a constriction near its base. The resin should occupy less than half of the tube, backwash the resin with an upward flow of water. When the resin was freed from the fine particles disconnect the water supply and pass 1 M sodium formate solution through the resin in the downward direction at a slow rate until the effluent was free from chloride when tested with acidified silver nitrate. Approximately 1 liter of sodium formate is required for the generation of each 25 ml. of the resin. When the generation was complete wash thoroughly with water and store the resin in water until required.

For the chromatography column a glass tube, 20 cm, to 30 cm, in length and 1 cm, diameter, fitted with a filter disc at its base and with filter paper disc pressed firmly in the tube above the plate is required. Add enough of the prepared resin to form a column about 11.5 cm in length when the resin has settled down; Cover the top of the resin with a thin wad of glass wool.

Column Chromatography.

Transfer the acid mixture to be analysed to the resin column in a few ml,. of water and rinse with more water. Add a further 15 ml, of water and connect the column immediately to the remainder of the air inlet to give the required flow rate of 10 to 12 drops per minute and collect the eluate in 2 ml . Fractions. The concentration of formic acid in the eluate should be 0.1 N in fraction- on 15, 1.0 N in fraction 25, 3 N in fraction 65, and 5N in fraction 140.

Recovery of acids.

Place the fraction in a desiccator over a mixture of 2 parts calcium chloride and 1 part solid sodium hydroxide and dry at reduced pressure (25 to 30 mm. Hg). The drying may be sepeeded by slight heating from infrared lamps placed above the desiccator.

Purification of tamarind extract

A glass chromatography tube, 5.2 cm. diameter, X 20 cm. fitted with a disc at its base and a disc of filter paper, wetted with the n butanol- chloroform solvent, pressed firmly over the disc. An equivalent , 50 % (v/v) n butanol of sugar as the tamarind extract and adjusted to P^H 2 with H_2SO_4 and NaOH.

A glass chromatography tube, 1.3 cm. diameter X 25. fitted with a filter plate and filter paper disc. 50% (v/v) tert- amyl alcohol- chloroform equilibrated against M Na_2 SO_4. Thoroughly mixed 3g gel with 1 ml water and 0.15 ml. of concentrated H2SO4. scurried the mixture with 50 ml. of chloroform, pour into a glass tube , and pack the column by passage of a further 200 ml, of chloroform. Applied the acid mixture to the column dissolved in 1ml. of 4% (V/V) tert-amyl alcohol chloroform. Developed the chromatogram by the successive addition of 50 ml, chloroform, 50 ml, of 4% tert –amyl alcohol-chloroform, 50 ml 10 %, and 50 ml, of 12% tert-amyl alcohol-chloroform. Collected the eluate in 2 ml , fractions, to each fraction add 2 ml, of eather and phenolphthalein indicator, and titrate with 0.05 N NaOH.

4:2 Discussion

- Out come amount of organic acid is change as the following consequences, hot water > cold water > sodium bicarbonate
- Therefore dissolving power of tartaric acid (rapidly) is higher in hot water, than in cold water for Tamarind pulp.
- When hot water was used tamarind seed for the extraction and pulp were separated automatically.
- Therefore soluble of water (hot and cold) sugar, carbohydrate, pectin substance, which are soluble in water (hot and cold) observed as impurities.
- For the separation of organic acid and impurities (sugar and carbohydrate) column chromatography, could be used but too complex for industrial level.
- A significantly high level of fungal growth was associated with the cold water extracts and therefore preservatives were required to control the growth of microorganisms.
- Water (hot and cold) extraction process created crystal form of organic acid.
- Tamarind extract using sodium bicarbonate, sodium bicarbonate and tamarind pulp showed a tendency to out come large foam, there fore we needed large vessels.
- A pungent smell was sensed for sodium bicarbonate extract.
- Appearance of the Sodium extract is a liquid brown colure liquid. (Can't convert to powder by using Rotary evaporator.
- Therefore a colouring agent is present in the sodium bicarbonate extract (colouring agent fruit gets of tamarind dissolved in $NaHCO_3$)

4:3 Conclusion

It can be conducted that the hot water extraction has a higher efficiency than the cold water extraction process and extraction with sodium bicarbonate and cold water is not efficient in extracting tartaric acid from the pulp. The organic acid content varies between ripe and unripe tamarind fruits where a higher level is found in the ripe fruit. The most suitable method for the purification of tamarind extract is column chromatography.

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CHAPTER 04 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Extract of $NaHCO_3$ (Table 4.1)

Active ingredients	Ripe % (w/w)	Unripe (w/w)
Tartaric acid	20.1	17.1
Citric acid	7.5	5.5
Malic acid	3.4	2.1
Fat	1.3	1.1

4.1.2 Extract of Hot water. (Table 4.2)

Active ingredients	Ripe (w/w)	Unripe (w/w)
Tartaric acid	44.7	38.2
Citric acid	15.6	11.1
Malic acid	4.7	3.9
Fat	2.4	3.0

4.2.3 Extract of water. (Table 4.3)

Active ingredients	Ripe (w/w)	Unripe (w/w)
Tartaric acid	32.3	30.1
Citric acid	12.4	9.8
Malic acid	4.7	3.9
Fat	1.1	0.9

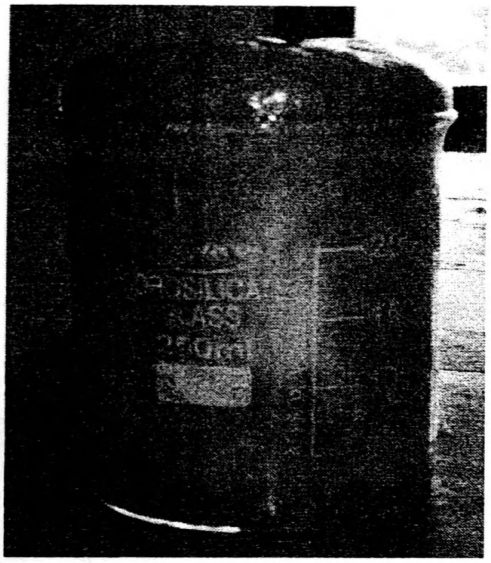
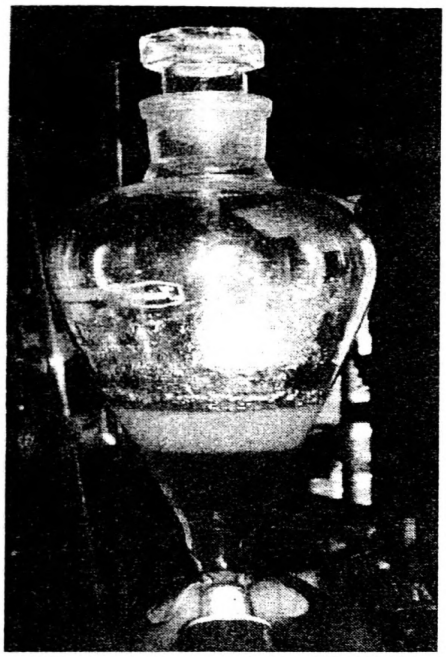


Fig 4.1 , Reaction of the $NaHCO_3$ and Tamarind pulp, Remove form CO_2,

3 1 2

FIF 4.2 ,Out come of the differences of the colour of Extraction

Solvent of (1) Extract of NaHCO3, (2) water Extract (3) Hot water Extract after filtration



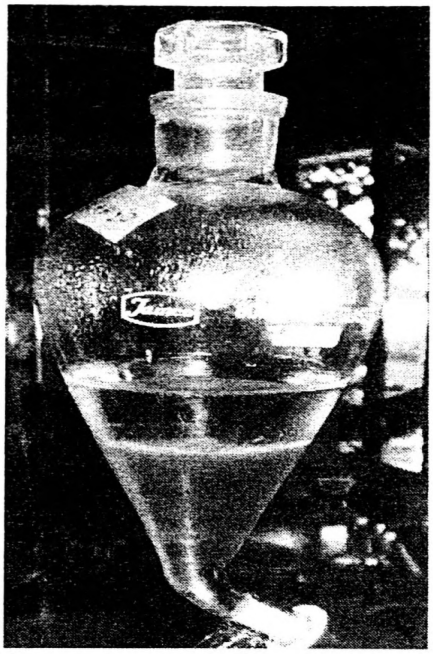


Fig (a) Fig (b)

Fig 4.3 , Out come in the organic acid in organic layer, Solvent Extraction, Remove organic acid to ethanol solvent

(a) Before and (b) after extraction of 3 portion.

Fig 4.4 , Crystallize in the using hot and clod water Extract of organic acid in tamarind fruit.

- Difference layer combined of mobile phase, out come is not efficiently by column chromatography, and out put is not pure (Negative result).

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Mrs. T.N. NEIGHSOOREI
(MSSc.PGD.ASLA,BA)
Librarian
Sabaragamuwa University of Sri Lanka
P.O.Box 02 Belihuloya,Sri Lanka
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