POSTHARVEST STUDIES IN *NEELAMARI*

(*Indigofera tinctoria* L.)

By

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2014-12-121

THESIS

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DECLARATION

I hereby declare that the thesis entitled "**POSTHARVEST STUDIES IN *NEELAMARI* (*Indigofera tinctoria* L.)**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that the thesis entitled **"POSTHARVEST STUDIES IN *NEELAMARI* (*Indigofera tinctoria* L.)"** is a record of research work done independently by **Ms. Arogyamary Supritha S.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

Introduction

1. INTRODUCTION

Traditional system of medicine with herbal plant remedies have become an important issue globally in recent decades. India is mainly known for its plant based medicine systems like *Ayurveda, Unani, Siddha,* Homeopathy and *Naturopathy*. Almost 80 per cent of the world population prefers different traditional systems of medicine which are herbal based due to their safety and efficacy. In the forests of Kerala, there are about 550 non-wood species yielding varied forest products. Among these, about 150 species are collected for the manufacturing of medicines, dyes, cosmetics etc. on a commercial basis. In Kerala, indigo plant is cultivated by several pharmaceutical entrepreneurs both in public and private sector. The National Bank for Agriculture and Rural Development has identified *Neelamari* to be a possible crop for commercial cultivation in Kerala (Sasidharan, 2000).

Even in modern system of medicines 25 per cent of raw material need is fulfilled by herbal source which implies their demand and efficacy (Uniyal *et al.,* 2006). Recent studies have revealed that nearly 7200 different herbal drugs are used by 7800 herbal pharmaceutical industries manufacturing 25000 medicinal plants based products (Ganesan *et al.,* 2017). Awareness about plant based medicinal formulations is creating market for herbal drug trade and expansion in pharmaceutical industries producing herbal formulations.

Indigofera tinctoria is a leguminous plant commonly called as *Neelamari* in Malayalam, referred to as 'Indian Indigo' in English and in Sanskrit known as *neelini /neelika/renjini*. India seems to be the birth place of this plant. The botanical name *I. tinctoria* got its roots from the Latin word "ferre" which means, "to bear". Thus the name '*Indigofera*' implicates "indigo bearing" plant whereas species name *tinctoria* originated from the word *tinctorious,* implicating "of dyes" or "belonging to dyes" (Marafioti, 1970; Simon *et al.,* 1984). *I. tinctoria* is a Fabaceous medicinal crop popular for its extensive use in dying and textile industry. Indigo- the blue dye extracted from *I. tinctoria* is the age old natural stuff used by mankind. There are reports of presence of Indigo dye in the

garments used to wrap the mummy of Egypt which is said to be more than 5000 years old.

The plant comprises of phytochemicals which are having hair nourishing property and the plant is being documented in *Ashtangahridaya* and *Charakasamhitha* for its array of medicinal applications. Leaf contains glycosides, steroids, tannins and flavanoids, quinones, saponins, etc. and phenols like gallic acid, quercetin and myrcetin. The blue dyestuff obtained from the indigo plant does not exist in readymade form, but is produced by fermentation of a chemical compound existing in the plant, known as indican. Indirubin is another component of the plant (Anon., 2002).

The leaves are also reported to have medicinal properties. The leaf extract of *I. tinctoria* is being used to relive the swelling caused by the sting or bite of venomous creatures like reptiles and insects. Infusion of roots is found to work against the heavy metal poisoning such as arsenic (Nadakarni, 1976). Han (1995) has studied the anti-cancerous property of *I. tinctoria* due to the presence of its bio-active compound indirubin. *I. tinctoria* has also got anti-tumorous property hence used as drug in such formulation. The presence of flavanoids, alkaloids, glycosides, and terpenoid in leaves was also reported (Verma and Suresh, 2002).

In any medicinal crop, harvesting stage plays a very important role as far as its quality and efficacy is concerned since early or late harvesting may cause deterioration of phytochemicals. In *I. tinctoria* the crop raised for leaf harvest should not be allowed to flower. Leaves are harvested after 3-4 months of planting and harvested leaves are marketed fresh. Cut leaves must be used within 8 hours (KAU, 2013).

The most important *Ayurvedic* preparation using *Neelamari* is *"Neelibhrngadi Taila"*. Fresh leaves of *Neelamari* are macerated and added to oil with extracts of other plants like *Amla, Eclipta, Cardiospermum helicacabum*, etc. in the preparation of "*Neelibhrngadi Taila*". The requirement for *Neelamari* leaf is more during January-March when *Amla* is available in plenty. Since the drug used is in the fresh form, the availability of fresh leaves at the required time and

quantity is a problem faced by the user industry. It is estimated that approximately 100-200 tonnes of fresh leaves are required annually in Kerala (Sasidharan and Muraleedharan, 2000). It is also one of the few drug plants which is obtained by cultivation in Kerala.

Hence with this background the present investigation was conducted with the objective of studying,

1. Effect of cutting stages and storage time on leaf quality
2. Effect of pre-treatments and drying techniques on indican content in leaves and oil quality

Review of literature

2. REVIEW OF LITERATURE

Neelamari, known as "*Neeli*" or "*Neelika*" in Sanskrit a plant reputed for its use as natural source of 'Indigo dye' is used as a major ingredient of "*Neelibhrngadi Taila*", "*Neeli thulasiadhi thailam*", and "*Neeli thulasiadhi kashayam*". Due to its antitoxic properties, it is also used as a good remedy against poisonous infections (Sivarajan and Balachandran, 1994). The plant is also used as stimulant, alterative, antiseptic and purgative (Singh and Panda, 2005).

The most important *Ayurvedic* preparation using *Neelamari* is "*Neelibhrngadi Taila*". Fresh leaves of *Neelamari* are macerated and added to oil with extracts of other plants like *Amla, Sida, Bacopa etc.* in the preparation of "*Neelibhrngadi Taila*". The medicinal property of oil is mainly due to the hair tonic effect and black colour given to the hair. Indican, the colour precursor present in the plant is the oxidized form of Luc-indigo or indigo-white, a chemical produced by the fermentation of leaves. The requirement for *Neelamari* leaf is more during January-March and the drug is used in the fresh form. The crop raised for leaf harvest should not be allowed to flower. Leaves are harvested after 3-4 months of planting and harvested leaves are marketed fresh. Cut leaves must be used within 8 hours (KAU, 2013). It is one of the few drug plants which are obtained by cultivation in Kerala. Approximately 100-200 tonnes of fresh leaves are required annually in Kerala (Sasidharan and Muraleedharan, 2000).

The possibility of extending the keeping quality of fresh leaves is one of the factors which can prolong the availability of the drug during the peak period of demand. Standardization of optimum cutting stage helps to improve the leaf quality. Similarly, studies on the effect of various post harvest factors on leaf quality remains unattended. The proposed study is taken up in this context.

The literature on related works hitherto carried out at difference locations is reviewed and cited here under the titles:

2.1 Effect of cutting stage and storage time on phytochemicals quality

2.2 Effect of pre-treatments and drying on sample constituents

2.3 Oil quality of herbal hair oils

2.1 EFFECT OF CUTTING STAGE AND STORAGE TIME ON PHYTOCHEMICALS QUALITY

2.1.1 Effect of maturity stage on the yield and quality of the crop

The herbs used for its pharmacological properties have different phytochemicals present in its various parts like leaf, stem, fruit, root etc. The maturity of the plant, determines the quality of phytochemicals present in the plant parts.

Badi *et al.* (2004) investigated the impact of harvesting time on herbage yield, quality and quantity of essential oil in Thyme (*Thymus vulgaris* L.) and among the three stages, on set of blooming has shown highest values for fresh herb yield of 19 t ha^{-1}, oil yield of 133 kg ha^{-1} and the contents of thymol (49%) and carvacrol (1%) in oil.

Seguin *et al.* (2004) reported that effect of harvesting stages on phyto estrogens (coumestrol, apigenin, luteolin and quercetin) which are non-nutritional but found to have significant effects on human health. They conducted study on cultivated alfalfa (*Medicago sativa* L.) where the differences between different plant parts and canopy segments, at three different physiological maturities stages and reported that early vegetative stage had highest concentration of all the phyto estrogens and only luteolin and quercetin concentrations were high during late flowering stage. Among the plant parts, flowers were found to have higher concentration of phyto estrogens than herbage and canopy segments.

In Lemon balm or sweet balm (*Melissa officinalis* L.) highest oil content with major bioactive compounds neral and geranial was during pre-flowering stage at harvesting time of 6 am in an area, where as in another geographical area it was during post flowering stage of crop harvested at 7 pm which had highest amount oil and active principles (Ayanoglu, 2005).

Effect of harvesting period and keeping time were investigated in Pepper mint (*Mentha piperita*) by Rohloff *et al.* (2005) and they observed that Peppermint with higher oil yield and flavor impact compounds like menthol (54%) and menthone (30%) is at highest level in essential oil at full bloom stage. The essential oil content was found to be decreasing from 7.7-1.5 per cent of total herb weight and biomass and herb yield was found to be increasing with the stages of maturity.

In European dill (*Anethum graveolens* L.) the early maturity stage had higher oil yield with considerably higher level of active principles like carvone and α-phellandrene. Thus the harvest date and plant maturity levels have their significance on quality and quantity of oil (Callan *et al.,* 2006).

In Greek Oregano (*Origanum vulgare sp. hirtum*) among the active principles like volatile terpenes, flavonoids and phenolic acids, flavonoids recorded the highest concentration (7.7 mg g^{-1} dry matter) in aerial parts at the stage of full flowering whereas at the stage of 10-20 per cent of open flowers phenolic acids (10.1 mg g^{-1} dry matter) content was highest (Grevense *et al.,* 2009).

Sellami *et al.* (2009) investigated the effect of four various phenological stages *viz.,* early vegetative, late-vegetative, budding and full flowering on the essential oil yield and quality of sweet marjoram (*Origanum majorana* L.) and its constituents. Full flowering stage was recorded with highest value for oil yield (0.09%) and flavour imparting compounds of essential oils.

Dri *et al.* (2010) conducted studies on the effect of different growth phases on the bioactive principle of *Solanum indicum* L. berries and they concluded that the polyphenols, seeral phenolic acids, flavonoids, p-coumaric acid and feruloylquinic acids were present in all maturity stages of berries. Ascorbic acid content was same in both green and yellow berries but lesser in red berries, where as red berries recorded a higher accumulation of carotenoids than green and yellow berries. Also the presence of caffeoylquinic acid, flavonol glycosides and

naringenin were highest in the red stage of the berries. Anti oxidant capacity was found to increase with the maturity.

The highest oil content recorded in *Mentha arvensis* cultivars Kosi and Kalka was 1.2 per cent from the leaves harvested at 120 and 150 days and the menthol content in oil varied from 74.24- 82.18%. The crop harvested at 150-180 days recorded higher oil content of 0.37-0.60% in different cultivars of *M. piperita*. But menthol content (42-22%) and piperitone (0.5-2.2%) was highest at the stage of 120-180 days in cultivars Kukrail, CM-Madhurus and CIM- Indus (Verma *et al*,. 2010).

Gupta *et al.* (2011) studied the effect of different growth stages of Buck wheat (*Fagopyrum sp.*) a major source for a medicinally important secondary metabolite rutin which is a flavonoid and they opinioned that rutin content exponentially increased from seedling stage to complete maturity stage and maximum at the inflorescence stage.

Fritillaria cirrhosa, an important medicinal plant of traditional Chinese medicine system has isosteroidal alkaloids as the bioactive principle in the tubers. The alkaloid content in tubers was highest at the early fruiting stage which declined with maturation and lowest at fruit maturation stage (Konchar *et al.* 2011).

Tajidin *et al.* (2012) investigated the effect of stage of maturity on the oil composition and citral content in lemongrass (*Cymbopogon citratus*) and they concluded that citral content which is the most important parameter that determines the quality of lemongrass oil was highest in the crop harvested at 6-7 months, the optimum stage for harvesting.

Significance of date of leaf picking in Senna (*Cassia angustifolia* Vahl.) on Sennoside content was studied by Upadhyaya *et al.* (2011). Both Sennoside A (0.126%) and Sennoside B (0.069%) were highest in the crop harvested at 90 days after sowing than in the crops of 130 and 110 DAS.

Cosmos caudatus harvested at the age of 8 weeks during dry season recorded with highest amount of bioactive compounds like quercetin 3-O-rhamnoside, quercetin 3-O-glucoside, rutin, quercetin 3-O-arabinofuranoside, quercetin 3-O-galactoside and chlorogenic acid (Mediani *et al.* 2012).

Stevia rebaudiana used as alternate source for sugar due to its anti-hyperglycemic and antihypertensive properties was harvested in 60 days, 90 days and 120 days after planting and glycosides content of plants harvested at 120 days recorded highest value for rebaudioside A (398.80 kg ha^{-1}) and stevioside (512.21 kg ha^{-1}) even with single harvest than that of multiple harvests of other two stages (Moraes *et al.* 2013).

Omezzine and Haouala (2013) reported that vegetative phase recorded the highest values for total phenolics, flavonoids, flavonols and flavones, precipitable alkaloids and pro-anthocyanidins in fenugreek.

Farhat *et al.* (2014) revealed the impact of harvesting time on the anti oxidant potential of the aerial parts of *Salvia officinalis* and they stated that flowering stage of the crop have best antioxidant activity with the higher content of phenolic diterpenes in essential oil (20055.17 µg g^{-1} dry weight), but rosmarinic acid content was highest in vegetative phase (10768.55 µgg^{-1} dry weight) and total phenols recorded the highest value (142.72 mg gallic acid equivalent g^{-1} dry weight) at fruiting stage.

Singh *et al.* (2014) examined the effect of harvesting periods of lemon balm (*Melissa officinalis* L.) on yield and quality of oil. Study revealed that aerial parts of the crop harvested at the age of 160 days had highest values for herbage as well as oil yield. It also had good geranial (24.53%), neral (18.80%) and trans-caryophyllene (7.70%).

Raya *et al.* (2015) studied the effect of keeping time and type of plant part used for the extraction of phytochemicals from *Clinacanthus nutans* (Burm. f.) a medicinal herb which is a source of numerous phytochemicals mainly phenolic

and flavonoid components. The highest content of phytochemicals was found in the young leaves stored upto to 24 hours. The study also revealed that foliage of *C. nutans* at early stages used at the earliest after harvesting recorded concentration of phytochemicals.

Avci and Giachino (2016) reported that in lemon balm the flowering stage of the plant recorded highest yield of both herbage (1076.80 kg/ ha) and essential oil (0.36%) with highest concentration of flavour yielding compound E-Citral (37.74%).

2.1.2 Phytochemicals in *Indigofera tinctoria*

Glycosides are a major biochemical group consisting of a number of secondary metabolites such as indican. Indican (indoxyl-β-D-glycoside), a glycoside compound which is colourless which further gets converted to indigotin, a pigmenting agent. Schunk in 1855 coined the term indican by referring to indigo precoursor, which yields indoxyl and glucose upon hydrolysis. Indigotin the colour yielding substance of 'Indigo dye' cannot be seen as it is in *Indigofera*.

Subsequent oxidation of indoxyl by means of atmosphere will result in blue precipitated particles of indigotin Trans-indirubin (isoindigotin) is also present in *Indigofera* in addition to indican. Ceratin impurities such as cis-indigo, indigo brown, indigo yellow, cis-indirubin, traces of flavonoids and indigo gluten are some other important secondary metabolites present. All these compounds result in natural, intensive blue tinge by means of plant source than synthetic one (Perkin, 1907).

Different species of *Indigofera* had varied range of indigotin which varied according to their age and atmospheric factors too (CSIR, 1959). Indigotin ($C_{16}H_{10}O_2N_2$) and indican ($C_{14}H_{17}O_6N$), the coloring principles of *Indigofera tinctoria* are concentrated in aerial parts more in leaf lamina and midribs with lesser quantity. Finar (1967) reported that indigotin is the official name of indigo,

which are present in many plants as natural sources but genus *Indigofera* was major among them. Indican is the available form in plants which further converted into indigotin by the process of hydrolysis.

Sugar synthesis and accumulation of glycoside in the dye form in the leaves of *I. tinctoria* was studied by Zavatskaya and Mashanova (1978) and they are of the opinion that, full bloom stage had relatively highest sugar synthesis and dye formation in leaves, where as organic acid was found to be stable along with growth of plant. When leaves get crushed with hydrochloric acid or enzymes, indoxyl will be hydrolysed and get exposed to atmospheric oxygen causing oxidation of indoxyl resulting in indigotin (Kochhar, 1981)

Species *tinctoria* has got more indican content than other members of *Indigofera* genus (Khazhakyan, 1986). Study on hydroponically grown *Indigofera tinctoria* plants was done by Khazhakyan and Egibyan in 1988 and reported the indican content ranged between 5 to 15 mg g^{-1}. Kamal and Mangala (1987) investigated the retinoid content in *Indigofera tinctoria* at different parts like leaves (0.60%), stem (0.32%), fruits (0.34%), seeds (0.42%) and root (0.51%) and found decreased retinol content with increase in age. Maximum retinol was in leaves and stem had least content.

Ya and Hua (1989) determined the indirubin and indigo content of *Indigofera tinctoria* by first derivative spectrophotometry and recorded the levels of indirubin (0.2-0.47%) and indigo (2.21-6.90%).

Preliminary phytochemical screening of *Indigofera tinctoria* done by Nair *et al.,* (1991) revealed the presence of carbohydrates in leaves there by confirmed the presence of glycoside. Leaf extracts obtained by means of water, alcohol and chloroform gave positive result for steroids but carbohydrates and proteins were present only in alcohol and water extracts. Saponins were reported to be present only in aqueous extract. Thomas (2000) reported that *Indigofera tinctoria* is source of indican which is the precursor of the indigo dye and it is a yellow amorphous substance readily soluble in water, ether and alcohol.

Indigofera tinctoria is reported to have antibacterial, antioxidant and cytotoxic effect on lung cancer cell. Flavonoids, saponins, tannins, terpenoids, quinone and phenols were identified through Thin Layer Chromatography technique. Leaf extract of Indigo behaved as potential antioxidant which is significantly superior over standard ascorbic acid (Renukadevi and Sultana, 2011).

Shanthi and Gowri (2012) did the analysis of bio active compounds in *Neelamari* and found that Indican is the colour precursor and mom-insitol is responsible for hair tonic effect.

2.1.3 Therapeutic properties of *Indigofera tinctoria*

Indigo being a rich source of phytochemicals is responsible for the curative properties of the plant. Scripts of ancient systems of medicine like *Siddha* and *Ayurveda* have evidence, showing the importance of *Indigofera tinctoria* in different formulations (Nadakarni, 2002).

Sreepriya and Devaki (2001) investigated the action and found that extract of *Indigofera tinctoria* have hepatoprotective effect against the endotoxicity caused by D-galactosamine. Anti oxidant enzymes present in Indigo extract scavenged the free harmful species of oxygen formed during the process and hence provided hepato-protection.

Anti dyslipidemic activity of *Indigofera tinctoria* was investigated by Puri *et al.* (2007). Indigofera extract at the rate of 250mg kg^{-1} of body weight found to decrease the levels of plasma triglycerides, total cholesterol, glycerol and free fatty acids which were also coupled with increase in high density lipoproteins.

Warrier *et al.* (2007) have stated that the leaves, stem and roots of *Neelamari* are useful for promoting growth of hair in addition to other pharmacological properties.

Jain *et al.* (2010) evaluated the phytochemical quality of *Indigofera* by using shade dried leaf powder extracts with methanol and petroleum ether. Preliminary

screening revealed the therapeutically potential group of phytochemicals such as alkaloids, flavonoids, carbohydrate glycosides, tannins, terpenoids and phenols.

2.1.4 Indigo as a dye

Kochhar (1981) reported the method of extracting dye from *Indigofera* species. Freshly cut plants, after crushing, were steeped in water for 10-15 hours in specially designed indigo-vats, during which the glycoside was converted into glucose and indoxyl by the activity of indimulsin, an enzyme naturally present in the plant. The yellow supernatant liquid was passed through beating or oxidizing vats equipped with paddle wheels, which facilitated aeration of the solution. The liquid was continuously agitated to bring about oxidation and the operation was stopped, by heating as soon as the blue colour developed. The blue dye formed a fine bluish mud at the bottom of the vat. The spent liquor was then drawn off. The bluish mass was treated with boiling water or lime water sometimes to aid further granulation. The filtered sludge, after pressing, was cut into small cubes of roughly 7 cm size, which after air drying was graded into various types.

Francis (1984) reported that most of the *Indigofera* species, about 40 species yield dyes and are useful for hair and cloth dyeing.

Nayar *et al.* (1999) reported a simplified method in which fresh leaves of *I. tinctoria* are expressed with water to make juice. The juice is sieved through a piece of cotton and kept for a long time for the sediment to form. Supernatant liquid is decanted and sediment is dried to a powder that serves as the blue colour.

2.2 EFFECT OF PRE-TREATMENTS AND DRYING ON SAMPLE CONSTITUENTS

2.2.1 Effect of pre-treatments on bio active compounds

Blanching is an essential operation after harvest, as far as enzymatic degradation of plant part is concerned. As aerial parts like leaves are highly

fragile, they are prone to enzymatic degradation thereby causing undesirable changes and affecting phytochemical content. The research work done on influence of blanching and different drying methods revealed that drying techniques had a significant effect on active ingredients of medicinal plants. Drying not only improves the storability of the commodity but also make it available for pharmaceutical industries round the clock.

Effect of type of water, steam and microwave blanching on physio-chemical properties of spinach leaves was investigated by Quenzer and Burns (1981). Higher carotene content of leaves (32.32mg $100g^{-1}$ of leaves) was found in steam blanched samples where as microwave sampled leaves gave highest values for ascorbic acid (309.98 mg $100g^{-1}$ DW) and α- tocopherol (2.67 $100g^{-1}$ of leaves). The organoleptic characters like colour, odour, flavour, texture and appearance of both steam and microwave blanched samples gave best results in comparison to control.

Muftugil (1985) is of the opinion that among various methods of blanching done in green beans least time required for the maximum destruction of peroxidase enzyme activity was in microwave blanching and also with highest amount of ascorbic acid and chlorophyll retention. In other treatments ascorbic acid was higher in steam blanched samples than water blanched and least in oven blanched samples.

Investigation conducted by Maharaj and Sankat (1996) on the quality of dasheen leaves (*Colocasia esculenta* L.) by giving the pre-treatments like water, steam and chemical blanching (0.06% of magnesium carbonate) showed that treatments altered the quality. Steam blanching gave best result with better retention of ascorbic acid, chlorophyll and pheophytin (695 mg $100g^{-1}$ dry matter)

Study on influence of blanching and drying techniques on the retention of ascorbic acid, β-carotene and chlorophyll was carried out on leaves of savoy beet (*Beta vulgaris* var *bengalensis*), amaranth (*Amaranthus tricolor*) and fenugreek (*Trigonella foenum graecum*) by Negi and Roy (2000). Hot water blanching at

95±3°C followed by dip in KMS (5 g L^{-1} in water) solution coupled with freeze drying was found to have significantly superior effect on all the parameters in all four crop samples.

Osinboyejo *et al.* (2003) investigated the influence of hot water and microwave blanching on turnip greens (*Brasica rapa*), a widely used leafy vegetable and the study revealed that microwave blanched samples had significantly lesser loss of thiamine, riboflavin, folic acid and ascorbic acid contents than hot water blanched samples.

Oboh (2004) opined that blanching had its effect on antioxidant activity of selected tropical leafy vegetables viz., *Structium sparejanophora, Amarantus cruentus, Telfairia occidentalis, Baselia alba, Solanum macrocarpon, Corchorus olitorus, Vernonia amygdalina,* and *Ocimum gratissimum.* Blanching also found to decrease the reducing property and free radical scavenging ability of the samples significantly.

Olivera *et al.* (2007) reported the effect of blanching on the organoleptic property of the Brussels sprout (*Brassica oleracea L. gemmifera* DC). There was better total chlorophyll retention in the samples blanched by microwave method but after 8 months of storage highest retention of chlorophyll was for frozen storage after hot water blanching. The highest ascorbic acid and radical scavenging assay percentage was found to be high in microwave blanched samples soon after blanching as well as after storage of 8 months.

Dwivedy and Rayaguru (2013) studied the blanching adequacy on the phytochemical quality of Indian borage (*Coleus aromaticus*) leaves also known as medicinal coleus and blanching done at 360 and 180W gave best results with maximum destruction of peroxidase enzyme, highest retention of total phenols and anti oxidant properties.

Microwave blanching done in *Centella asiatica* leaves for 30seconds resulted in best values for total phenols of 9.2 mg g^{-1} (Trirattanapikul and Phoungchandang, 2014).

Galoburda *et al.* (2015) investigated the influence of hot water blanching for 3 minutes done at different temperatures of 70°C, 80°C, 90°C and 100°C on *Cantharellus cibarius*, an edible mushroom. Among the treatments, blanching done at 70°C gave best results for colour and hyphal thickness with least weight loss after blanching.

2.2.2 Effect of drying on raw herbal drugs

The keeping quality of herb increases with the loss of moisture. Medicinal plants used for preparation of drugs also require drying without the loss of particular phytochemicals determining the pharmacological property of that herb. Drug industry needs products with phytochemicals of good quality, mainly influenced by the method of drying. Medicinal plants can be dried in numerous ways like shaded open air, direct sun light, in electrical / solar dryers, lyophilization and micro wave or by infra red waves. But care should be taken to manage the temperature and humidity at desired levels in order to avoid loss of active chemical constituents (WHO, 2003). Drying is the most common and fundamental technique of extending the shelf life of medicinal raw drugs as it allows the quick conservation of therapeutic values of the herbal drug in an uncomplicated manner (Muller and Heindal, 2006). Due to expense of operation and influence on phytochemicals, drying plays a crucial role in raw medicinal drugs trading which is also depends on investment and energy requirement.

Herbs entre trade in several forms but crude dried herbs are most commonly traded and preferred in international markets. After drying medicinal plants will be stored and sold in different forms like crushed, powdered, chopped or as a whole in case of aerial parts like leaves and flower heads (Atal and Kapur, 1982).

Initially *Hyoscyamus* was sun dried for 2-3 days followed by shade drying with regular racking for 6-7 days was found to lose characteristic odour upon drying but dried leaves sample had more pronounced bitter taste (Anupkumar *et al.* 1984).

Moyler (1994) stated that the foliage of aromatic and medicinal plants is dried to reduce water content in leaf tissues which results in better extraction. Drying process results in accumulation of phytochemicals at leaf surface as moisture loss occurs due to evaporation.

According to Muller and Heindl (2006), solar dryer is ideal for drying medicinal plants. Cost effectiveness, drying capacity and energy requirement should also be considered while choosing the method (Heindl and Muller, 1997).

Yousif *et al.* (1999) conducted studies in *Ocimum basilicum* and found that vacuum microwave dehydrated leaf samples yielded approximately 2.5 times the linalool and 1.5 times the methyl chavicol than that of air dried samples.

Negi and Roy (2001) reported the effect of drying methods on the quality of selected savoy beet and amaranth leaves by studying the parameters like retention of β–carotene and ascorbic acid in solar and cabinet drying methods. Cabinet dried samples were having higher retention of ascorbic acids, β–carotene and chlorophyll content as compared to solar dried samples.

Dehydration of herbs by shade drying at 27-34°C can be considered as preferable method of drying in case of medicinal drug plant parts as bioactive compounds and sensory attributes of colour, odour, taste and other quality parameters can be maintained to the level of acceptance at a commercial viable scale (Meisheri, 2001).

According to Lorenzi and Matos (2002) drying of medicinal plants will meet the demand of pharmaceutical industries throughout the year in case of formulations which does not demand fresh herbal drugs.

Sujatha (2002) conducted studies in *Adathoda,* and found that vasicine the medicinally active compound in the plant, was retained in higher quantity when dried under sun. Blanching reduced the enzymatic action inside the cells thereby decreasing the chemical degradation.

Chinese chive subjected to different methods of drying consisting of conventional hot air drying, freeze drying and microwave-vacuum drying to investigate the significance of drying methods over pigment retention showed that microwave coupled with vacuum was the best as retention of chlorophyll in Chinese chives leaves was highest, and it implied added effect of blanching due to microwave (Cui *et al.,* 2004).

Omidbaigi *et al.* (2004) reported that methods of drying have significant effect on oil recovery from the flowersof *Chamaemelum nobile* L. However, shade drying reported higher values for oil recovery and higher proportion of phytochemicals in oil, when compared to shade and cabinet drying.

Lim and Murtijaya (2007) stated that medicinal plant parts like leaves with perishable nature will be deteriorated soon after harvest or in shorter time span. So drying is the easiest possible way of preserving such commodities with all the desired phytochemicals.

Comparison of hot air drying, fluidized bed drying, and microwave drying on Indian Borage leaves was done by Dwivedy *et al.* (2012). They concluded that microwave dried samples were the best with highest values for total phenols (692mg/100g), antioxidant activity (377μM g^{-1}) and sensory parameters including colour (8.5), shape (8.0), aroma (8.5)and overall acceptability (8.5) shown highest values.

Galoburda *et al.* (2015) have reported that, microwave vacuum dried product resulted in significantly superior samples of *Cantharellus cibarius*an edible mushroom, with highest total phenols, protein content, better hyphal length

and width, best organoleptic characters and highest keeping quality than other methods of drying.

Potisate and Phoungchandang (2015) opined that microwave drying is the best method having significant difference in quality of whole *Moringa olerifera* leaves with their different power levels. Among the power levels ranged from 150 to 900 W, microwave drying done at 900 W was the best one as it resulted in best quality end product with highest bioactive compound as quercetin , kaempferol and also higher radical scavenging capacity.

2.2.2.1 *Effect of drying on bioactive compounds:*

Drying techniques also have a significant effect on active ingredients of medicinal plants. Drying not only improves the storability of the commodity but also make it available for pharmaceutical industries round the clock.

For *Digitalis lanata,* source of cardiac glycoside was found to have deleterious effect on active constituents due to sun drying (Monteverde and Ordovaskaria, 1928). For drying *Digitalis* foliage, shade drying and artificial drying (at 30-40°C) proved as most suited methods of drying (Silva and Constantinescus, 1986).On a large scale *D. lanata* will be dried in hot air oven. Lanotoside 'C', a major active ingredient was found to be highest in samples dried at 40°C. But, condenolide, another important bioactive principle present in *D. lanata* was found to be highest in samples dried at 80°C but had deleterious effect on Lanotoside 'C' at that drying temperature (Elbanowska and Kaczmarick, 1966).

Influence of air drying at room temperature and lyophilization was studied in *Drosera medagascariensis, D. pellata* and *D. rotundifolia* by Krenn *et al.* (1998). They studied naphthoquinones content which is a quality marker of crude drug dried with different methods of drying. HPLC analyses of all the species revealed that 7-methyl juglone was the main compound in *Drosera medagascariensis* and *D. rotundifolia* and lyophilization had significantly

superior effect over air drying. But *D. pellata* reported with plumbagin as main naphthaquinone had highest content in air dried sample.

In *Camptotheca acuminate,* there was a difference of 27% in Camptothecin content between the freeze dried and hot air oven dried sample. Oven drying found to be deleterious on the retention of Camptothecin (Lui Zhi Jun *et al.,* 1998).

Leaves of *Mikania glomerata* dried in a green house at ambient temperature (at 35°C for 15 days) reported the coumarin content of 4.02 mg g^{-1} dry weight but oven dried sample with circulating air system (at 50°C for 24 hours) had the coumarin content of 7.31 and coumarin content of 6.73 mg g^{-1} dry weight was reported without circulating air flow (at 50°C for 24 hours). While analyzing the sample dried under air conditioned room (at 25°C for 7 days) coumarin content was reported as 7.32 mg g^{-1} dry weight (Pereria *et al.,* 2000).

Meisheri (2001) suggested that drying the medicinal plants at room temperature with proper ventilation is the best eco-friendly method of dehydration as it retains the active principles and organoleptic characters to the maximum possible extent.

Four medicinal crops were dried using different drying techniques and evaluated for physico-chemical properties after drying. Leaves of *Houttuynia cordata* dried at 30°C reported highest quercetin content with vivid yellowish green colour. Shade dried leaves of *Geranium thunberagi* reported with reduced geraniin content decreased by half and colour of leaves after shade drying was unacceptable due to browning. In case of freeze drying the spike of *Prunella vulgaris* var lilacina reported the highest content of rosmarinic acid. In *Gardenia jasminoides* freeze drying, artificial drying at 30°C and flow drying at 50°C caused fruits to turn into black and had deleterious effect on geniposide (Yamaguchi *et al.,* 2001).

Saponin content of safed musli (*Chlorophytum brovillianum*) was more in sun dried samples than the samples dried in cabinet drier (Anon., 2002).

Mehta *et al.* (2005) conducted a comparative study by considering sun drying solar drying, shade drying and tray drying at 60°C techniques for drying liquorice (*Glycyrrhiza glabra*) and reported that shade dried samples had higher glycyrrhizin content of 9.81 % followed by tray dried sample with 8.76% but sun dried sample had least glycyrrhizin content of 8 %.

According to Lim and Murtijaya (2007), aqueous extract of microwave dried sample of *Phyllanthus amarus* exhibited the significantly stronger anti-oxidant activity and higher total phenolics content due to greater solubility of compounds break down of cellular constituents and also hydrolysis of tannins will occur.

Yuan and Zhehi (2007) reported that in *Glechoma longituba* the content of germacerene D, an important bio active compound varied with the method of drying and the highest germacerene content was reported in shade dried sample (19%).

Tinosporine a major alkaloid of *Tinospora cardifolia* was significantly varied with different drying techniques mechanically dried samples at 40°C reported the highest content of tinosporine of 0.045% where as only 0.033% of tinosporine was found in sun dried samples (Padmapriya *et al.*,2009).

Balakumbhan *et al.* (2010) opinioned that alkaloid content of *Gloriosa superba* was much higher in shade dried samples than samples dried in other method of drying. Though the drying took more time, it was significantly superior over other methods.

Sejali and Anuar (2010) studied the influence of shade drying and oven drying at 45°C and 70°C on leaves of *Azardirachta indica* and found that moisture content, colour, crispness and phenolic contents had significant difference. Total phenols were found to be higher in shade dried samples.

2.2.2.2 *Advances in drying technology*

In *Alismatis rhizoma* while drying in hot air dryer at 45-60°C, tri-terpenes like alisol-A and alisol-A monoacetate were formed artificially which are the major phytochemicals of therapeutic value (Yoshikawa *et al.,* 1994).

Reynolds (1998) conducted a study on roots and rhizomes of *Panax quimquefolius* and reported the influence of drying on concentration of major carbohydrates and ginsenoside. Results revealed that varying the drying temperature from 32-44°C caused darkening of roots and reduced concentrations of the malonyl ginsenoside mRb1, mRb2, mRc and mRd and the neutral ginsenoside Re and Ro but a higher concentration of gypenoside XVII. Drying temperature had no effect on ginsenoside Rb1, Rc, Rd and Rgl contents. Fall in drying temperature from 38 to 32°C caused the increased content of sucrose and Ro but decreased starch levels. The ideal temperature for drying *P. quimquefolius* was suggested to be 38°C.

Investigation on drying time after microwave blanching (300-1200W) was done by Von and Kartnig (1999) on *Althaea sp.* and *Crataegus sp.* Results revealed that microwave reduced the drying time thus contributing to the improved appearance and product quality with causing much damage to phyto chemical profile of the commodities.

Comparission of effects of air drying and vacuum drying on the leaves of *Ocimum basilicum* was studied by Yousif *et al.* (1999). While comparing they found that vacuum dried samples yielded 2.5 time linalool and 1.5 times methyl chavicol content more than that of hot air dried samples and the chemical reactions occurred during drying yielded more volatiles than that of fresh herb. Vacuum microwave dried leaves had better green hue than hot air dried leaves and higher potential for rehydration too. Scanning in electron microscope revealed that hot air drying caused the dramatic and pronounced collapse of cell structure and other attributes of samples in hot air dried herb.

Drying methods *viz.*, freeze drying, vacuum microwave drying and air drying were applied on fresh roots and leaves of *Echinancea purpurea* and studied their influence on alkamides content which revealed that each method significantly influenced the alkamides content in both roots and leaves. Freeze drying reported with highest alkamide contents and found to be best method of drying but vacuum microwave drying was reported to be superior than air drying at 70°C for drying roots while leaves were preferably dried by air drying at 50°C (Hyunock *et al.*, 2000).

Effect of hot drying and combine microwave hot air drying was studied on roots of *Panax ginseng* by Ren and Chen (2000). Lesser deleterious effect on ginsenoside content was reported by microwave- hot air drying then hot air drying alone.

According to Cesare *et al.* (2001) blanching followed by drying in *Ocimum basilicum* caused drastic decrease in all volatile compounds. Hot air drying and freeze drying had a greater influence on *O. basilicum* and *Salviaofficinalis*. While comparing the bioactive compounds, it was revealed that *S. officinalis* was least affected by drying, than *O. basilicum*. Blanching followed by freeze drying resulted in good product with better aroma and quality retention. Volatile principles in *O. basilicum* like eucalyptol, linalool and eugenol had concentration on par with control samples in freeze drying while decrease in eugenol and linalool content was reported in microwave dried samples.

2.3 OIL QUALITY

Hair oil prepared using herbal drugs are called as hair tonics or Herbal hair oils. Different formulations of ingredients extracted by oil base usually by coconut or sesame oil are preferred. These poly herbal formulations are used for the ailment of different hair care issues and disorders such as baldness, aggression of hair, discoloring of hair, hair falling, and dryness of hair, *etc.* (Nema, 2009).

2.3.1 Refractive Index

Kumar *et al.* (2006) studied the preparation of *Nirgundi* (*Vitex negundo*) oil followed by heating and prepared *Moorchhit oil* at the end with *Tila Taila* as base. Refractive index of this oil was examined as raw oil (1.480), after heating (1.480) and finally *Moorchhit oil* (1.481) of poly herbal mixture.

An ayurvedic formulation called *Yashtimadhuka Taila* was examined for the detection of refractive index (at 40°) where 1.466 was the refractive index of the sample and 1.464 was refractive index of *Tila taila* alone which was used as base of the formulation (Shailajan *et al.*,2012).

Refractive Index of *Grahanimihira tailam* with different concentrations of herbal drugs in it was assessed by Joshi *et al.* (2013). It ranged from 1.469-1.470 with different concentration of samples.

Joshi *et al.* (2013) conducted the physico-chemical analysis of *Ksheer Bala Taila* a formulation containing *Sida cardifolia* and Cow's milk with *Tila Taila* as base. Formulation was having a refractive index of 1.471 at 40°.

Raut (2014) studied a herbal formulation *Baalashwagandha Taila* and analysed the properties of the formulation and the refractive index of the oil was revealed as 1.45-1.46 which had *moorchhita tila taila* as its base.

Jain *et al.* (2015) carried out a comparison study between the poly herbal formulations and base oil and refractive index of these oils *Varnashodhanahara taila* (1.468) and *Doorvadi tail* (1.471) in comparison with *Tila Taila* (1.469) and recorded the values.

Kumar and Krishna (2015) stated that coconut oil is widely used and preferred oil due to its pharmaceutical uses and physico-chemical properties.

Padmakiran *et al.* (2015) revealed that refractive index of oil prepared using *Vitex negundo* with *Tila Taila* as base detected at 40° was 1.471.

Jain *et al.* (2016) reported that poly herbal oil prepared using *Emblica officinalis, Bacopa monnieri* and *Cyprus rotundus* as ingredients at different proportions with coconut oil as base had refractive index ranging from 1.498-1.361. Increase in the herbal ingredients concentration was associated with lowering of refractive index.

Shailajan *et al.* (2017) studied and compared the refractive index of three different herbal hair oils with *Tila Taila* as the base, refractive index was found to be 1.468, 1.475 and 1.463 in *Krishna Tila Taila, Murcchita Tila Taila* and *Shadbindhu,* respectively

.2.3.2 Acid value

Hepsibah *et al.* (1998) conducted a study on *Karpooradi Tailam* containing *Trachyspermum ammi* and *Cinnamomum camphorum* in comparison to its base coconut oil and estimated acid value as 2.95 and 1.67 mg KOH g^{-1} of formulation and base oil, respectively.

Herbal hair oil formulation containing oil of *Vitex negundo* followed by the process of *Moorchhit* was studied by Kumar *et al.* (2006). While analyzing the acid value of the sample they noticed that 4.5, 3.9 and 4.1mg KOH g^{-1} were the result for raw sesame oil, after heating the oil and *Moorchhit* oil, respectively.

Gautham *et al.* (2012) evaluated the physico-chemical quality of an ayurvedic preparation containing 12 different herbal ingredients and base as a mixture of both *Tila Taila* and coconut oil. The formulation upon analysis recorded the acid value of 4.5 mg KOH g^{-1}.

Shailajan *et al.* (2012) reported that a herbal preparation called *Yashtimadhuka Taila* which contains *Tila Tail,* Glycyrrhiza glabra, cow's milk and *E. officinalis* as constituents had a acid value of 0.5613 mg KOH/g where as *Tila Taila* alone had an acid value of 0.4433 mg KOH g^{-1}.

Joshi *et al.* (2013) experimented on *Grahanimihira tailam*, a medicated hair oil and analysed its physico-chemical property. Acid value of the oil was ranged from 3.9 – 4.5 mg KOH g^{-1} depending on various concentrations.

Hewageegana *et al.* (2013) conducted a study on *Vipadikahara Ghrita Taila* with *Leptadenia reticulate*, *Rubia cordifolia*, *Berberis aristata*, *Mallotus phillipinensis*, Cow's milk, Bee's wax, Resin of *Shorea robusta* with *Sesamum indicum* oil as base with cow's ghee. While studying the quality of the formulation acid value was found to be 3.63 mg KOH g^{-1}.

Ksheer bala taila, ayurvedic hair oil was titrated against KOH to find out the acid value of the sample. Result revealed that 3.548 mg KOH g^{-1} was the acid value of that sample prepared using sesame oil, *S. cardifolia* and cow's milk (Joshi *et al.*, 2013).

Lahorkar *et al.* (2009) studied the different formulations of poly herbal mixtures prepared with *Tila Taila* as base oil with ancient technique called *Taila pak vidhi* and analysed the physico-chemical parameters and while reporting the acid values they revealed that acid value of the base oil was about 3.12 mg KOH g^{-1} and acid value of the 14 different formulations varied from 2.54-4.99 mg KOH g^{-1} based on their composition.

An analytical study on *Baalashwadhi Taila*, a poly herbal preparation using *Tila Taila* was conducted by Raut (2014) and found out the various physico-chemical properties of oil using standard analytical procedures. Acid value of the oil ranged from 4-5.5 mg KOH g^{-1} depending on different samples of same formulation.

Jain *et al.* (2015) investigated on the physico-chemical properties of two different ayurvedic oil formulations *Varnashodhanahara taila* and *Doorvadi taila* in comparison with *Tila Taila* which is base and revealed the acid value as 6.69 mg KOH g^{-1}, 1.443 mg KOH g^{-1} and 1.255 mg KOH g^{-1}, respectively.

Kamal (2015) conducted a study on commercially available ayurvedic formulations of hair and reported their acid values as 0.8 (Himani Navaratna oil) and 0.9 mgKOH g^{-1} (Dabur amla oil), both the formulations had coconut oil as their base.

Acid value of *Nirgundi Tail* having *Tila Taila* as base was revealed as 1.198 mgKOH g^{-1} while assessing the physico-chemical quality of the formulation (Padmakiran *et al.*,2015)

Hewageegana and Arawwawala (2016) conducted a study on base material which is widely used in numerous ayurvedic preparations. A mixture of ghee and sesame oil was taken in same proportion and subjected for determination of acid value which was found to be 2.000 mg KOH g^{-1}.

Herbal hair oil prepared with coconut oil consisting of *E. officinalis*, *B. monneri* and *C. rotundus* were was subjected to the analysis of acid value of oil and found that it varied from 2.76-1.42 mg KOH g^{-1} where acid value was inversely proportionate to concentration of herbal drugs in oil (Jain *et al.*, 2016).

Rakesh *et al.* (2016) studied the acid value of a herbal hair oil formulation containing coconut oil as base and fenugreek, neem and *Semecarpus anacardium* as ingredients and reported acid value as 3.2, 2.5 and 2.12 mg KOH g^{-1} by examining formulation with different concentrations of herbal drugs.

Joshi and Dyawarkonda (2017) stated that a hair oil formulation prepared using coconut oil with ingredients like aonla, *Bhringaraja*, hibiscus, milk, aloe, *Triphala*, Indian liquorice and grated coconut along with coconut water indicated an acid value of 2.97mg KOH g^{-1}.

Acid value in *Tila Taila* based formulations namely *Krishna Tila Taila*, *Murcchita Tila Taila* and *Shadbindhu*, various medicated oils were reported as 0.545, 0.670 and 0.658 mg KOH g^{-1} respectively (Shailajan *et al.*, 2017).

2.3.3 Thin Layer Chromatography (TLC)

Hepsibah *et al.* (1998) reported the TLC profile of *Karpooradi Tailam* with *Trachyspermum ammi* and *Cinnamomum camphorum* as ingredients and coconut oil base. Thus the presence or absence of phytochemicals was possibly detected in *Karpooradi taila* using T.L.C and the quantitative estimation of thymol and camphor were done which were the intended bioactive compounds which in turn correspond to the amount of *T. ammi*, and *C. camphorum*.

Hewageegana *et al.* (2013) carried out the TLC analysis of *Vipadikahara Ghrita Taila* with different raw herbal drugs, cow's milk, bee's wax and mixture of *Sesamum indicum* oil and cow's ghee as base. Phytochemical fingerprint obtained was viewed under UV of 366nm and also after spraying vanillin sulphuric acid and Rf values were recorded. Bands obtained from the formulation matched to the standard mixture of plant ingredients.

Lahorkar *et al.* (2009) studied 14 different oil formulations containing Neem, *Manjishta* and *Mulethi* as major ingredients with varied proportions of *kalka* ingredients and *khwatas*. Oil formulations had modifications against the procedures and ingredients mentioned in *Charaka samhita* and TLC was conducted in order to compare the phytochemical profile of the oil. The pattern of modified formulations had similarities with actual formulation profile even though there was drastic change in the proportion and form of herbal as well as other ingredients used for the preparation.

Padmakiran *et al.* (2015) carried out TLC for assessing the quality of *Nirgundi Taila* and reported the results. They opined that, TLC helped in separating the closely related compound from complex mixture of *Nirgundi* oil which made assessment of quality easier. Four spots, two spots and seven spots of formulation were observed at 254nm, 366nm and after derivatisation with vanillin-sulphuric acid respectively on TLC plate, which assured the quality of oil.

Varna shodhana Taila and *Doorvadi Taila* were prepared based on ayurvedic literature standards and TLC was conducted to check the quality

standard. The unsaponifiable matter of base oil in comparison with medicated oils were subjected to TLC to ascertain whether any phytochemical have been transferred into the medicated oil or not.TLC plates were visualized for sports by naked eye, under UV light (at 254 nm) and exposing to iodine vapours at the end of the process. The spot of the medicated oil was found to be identical with those of sesame oil indicating no undesired changes in quality (Jain *et al.*, 2015).

Rai (2015) stated that to analyse a complex mixture like herbal formulations TLC is a simple, quick and less expensive method that gives an idea about the number of bioactive ingredients got extracted to the base used from the herbal drugs of the composition. By comparing the Rf values obtained we can also possibly detect the active principle by comparing against known standards which is a retention factor depending on the travel of the compound on TLC plate with specified mobile phase.

TLC of mixture containing *Tila Tilam* and *Ghritam* which are widely used in ayurvedic formulations like *Dashamooladi Ghrita, Chitrakadi Ghrita, Baladi Ghrita, Ashwandha Ghrita, Vipadikahara Ghrita, Moolaka Taila, Sacharadi Taila, Swandranstha Taila* and other preparations as base was done by Hewageegana and Arawwawala (2016). TLC fingerprints of dichloromethane and ethyl acetate fractions were developed and Rf values for ghee and sesame oil mixture using two solvent systems were recorded. In dichloromethane fraction found two spots, one spot and two spots and for acetate fraction detected three spots, three spots and five spots respectively at 254nm, 366nm and after spraying reagents, respectively.

Materials and methods

3. MATERIALS AND METHODS

The present investigation on the "Postharvest studies in *Neelamari* (*Indigofera tinctoria* L.)" was carried out at the Department of Processing Technology, College of Horticulture, Vellanikkara during 2014 - 2016.

In this study an attempt has been made to reveal the best cutting stage and impact of storage time on phytochemical quality of leaves. As drying is an essential operation for a medicinal herb for storage and also for making various formulations, drying method was also standardized after giving pre-treatments.

Indian indigo (*Indigofera tinctoria*) being a major ingredient in the formulation of *Neelabhringadhi thailam* is available in abundance throughout the year. According to the specifications given in literature related to *Ayurveda* it should be used at particular maturity stage as soon as possible after cutting to have greater efficacy. Thus the need for studying the correct stage of cutting gains importance. The other ingredients of the oil are gooseberry (*Emblica officinalis*), *bhringaraja* (*Eclipta alba*) and uzhinja (*Cardiospermum halicacabum*) which are used in the form of *svarasa* and they are season specific. Hence a suitable pretreatment and drying technique needed to be investigated in order to store the fresh leave round the year for ensuring availability of *Indigofera* leaves with better quality for preparing *Neelibhrngadi Taila* by retaining the overall quality of the product and indican content, a quality marker along with one other important bio-active compound.

The whole programme was divided into two major experiments

1. Studies on the effect of cutting stages and storage time on leaf quality

Herbage yield of *Indigofera* was observed at three different stages of maturity Relative Water Content (RWC) and presence of phytochemicals along with indican, total sugar and tannin, *etc.* was also assessed.

2. Effect of pre-treatment and drying on the leaf and *Neelamari*-coconut oil quality.

Evaluation of the quality of *Neelibhrngadi Taila* prepared using pre-treated and dried *Neelamari* leaves was done in this experiment.

3.1 STUDIES ON THE EFFECT OF CUTTING STAGES AND STORAGE TIME ON LEAF QUALITY

3.1 .1 Cutting stage and storage time

Leaves of *I. tinctoria* were collected from All India Co-ordinated Research Project on Medicinal and aromatic plants and Betel, Vellanikkara at 70 days after transplanting (Plate 1), 115 days after transplanting (Plate 2) and 160 days after transplanting (Plate 3). Harvested parts were shifted to laboratory of Department of Processing Technology at the earliest. Later the leaflets were separated from hard shoots and spread over the clean floor in the laboratory as it is stored in processing industries.

Leaves harvested were assessed for different biochemical and physiological parameters in order to study the best cutting stage with significantly higher leaf quality. For analysis separated leaflets were apportioned and samples prepared by pooling the lot using quartering method

3.1.2 Storage time after harvest

The leaves were stored for different time interval and assessed for quality in order to study deleterious effect on bio-active compounds by storage. Leaves which were freshly harvested and kept for 4 hours, 8 hours, 24 hours and 48 hours after harvest were five different time intervals studied.

3.1. 3 Treatments

T_1: Seventy days after transplanting and freshly harvested

T_2: Harvested at 70 DAT and kept for 4 hours after harvest

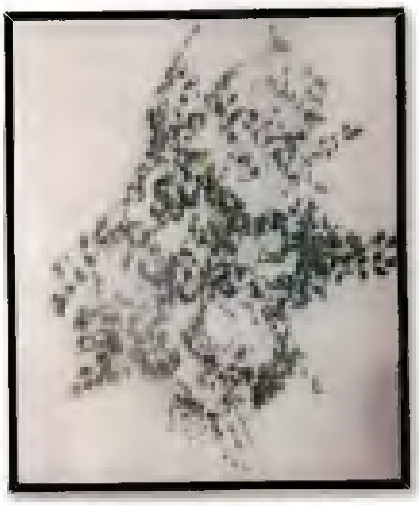
T_3: Harvested at 70 DAT and kept for 8 hours after harvest

T_4: Harvested at 70 DAT and kept for 24 hours after harvest

Freshly harvested leaves

After 4 hours

After 8 hours



After 24 hours

After 48 hours

Plate1. Treatments of C_1 (70 DAT)

Freshly harvested leaves

After 4 hours

After 8 hours

After 24 hours

After 48 hours

Plate2. Treatments of C_2 (115 DAT)

Freshly harvested leaves

After 4 hours

After 8 hours

After 24 hours

After 48 hours

Plate3. Treatments of C_3 (160 DAT)

T_5: Harvested at 70 DAT and kept for 48 hours after harvest

T_6: One hundred and fifteen days after transplanting and freshly harvested

T_7: Harvested at 115 DAT and kept for 4 hours after harvest

T_8: Harvested at 115 DAT and kept for 8 hours after harvest

T_9: Harvested at 115 DAT and kept for 24 hours after harvest

T_{10}: Harvested at 115 DAT and kept for 48 hours after harvest

T_{11}: One hundred and sixty days after transplanting and freshly harvested

T_{12}: Harvested at 160 DAT and kept for 4 hours after harvest

T_{13}: Harvested at 160 DAT and kept for 8 hours after harvest

T_{14}: Harvested at 160 DAT and kept for 24 hours after harvest

T_{15}: Harvested at 160 DAT and kept for 48 hours after harvest

Best stage of cutting and storing time were assessed using different parameters.

3.1.4 Layout

The experiment was laid out in a Completely Randomised Design (CRD) with two replications of desired weights.

3.1.5 Observations

Observations on yield, physiological and phytochemical parameters were documented as described below.

3.1.5.1. *Yield of fresh leaves*

Aerial parts of 30 plants were harvested, at 70 days after transplanting (DAT), 115 DAT and 160 DAT. Average yields of those plants were considered

as yield of fresh leaves for that particular cutting stage and yield of fresh was expressed as g plant^{-1}.

3.1.5.2 *Relative Water Content (RWC)*

Leaves removed from leaflets were pooled and leaf discs were prepared out of them and recorded the fresh weight (FW). Petridish were filled with distilled water, leaf discs were allowed to float in petridish and left for one hour to attain the full turgidity. After wiping those leaf discs gently, turgid weight (TW) was immediately recorded. Later those leaf discs were dried in hot air oven at 80 °C for hours, till they attained constant dry weight and recorded the dry weight (DW) and RWC was expressed in percentage. (Catsky, 1960).

$$RWC = \frac{FW - DW}{TW - DW} X100$$

3.1.5.3 *Indican content*

The quality of the leaves as affected by cutting stage and storing time after harvest was studied in terms of indican content.

Estimation of indican from fresh leaves of *Indigofera* was done by the method given by Maier *et al.,* (1990). 50 mg of fresh leaf sample was taken, crushed with 80% methanol in a pestle and mortar and volume made up to 15 ml in a test tube. Indican standard solution (Indoxyl β-D- glucoside) was prepared by dissolving 10mg of standard in 10ml of 80% methanol.

Indican standard (Indoxyl β-D-glucoside) for the estimation of indican content was purchased from Sigma-Aldrich, Bengaluru.

Working standards were prepared from the stock by pipetting out 0.5, 1.0, 1.5 2.0 and 2.5ml of the stock solution into different test tubes and made up

volume to 15ml with 80% methanol later. A test tube containing 15 ml of 80% methanol alone was taken as blank.

All these test tubes were heated at 70°C for 5 minutes later sample containing tubes were centrifuged and filtered to get supernatant. Clear supernatants were read at 280nm in UV- spectrophotometer along with blank and standards.

With help of standard graph later the concentration of indican was calculated by plotting standard concentration on X-axis and absorbance on Y-axis of the graph.

3.1.5.4 *Total sugars*

Estimation of total sugars by anthrone method reported by Cerning-Beroard (1975) was used to estimate the total sugars in *Neelamari* fresh leaves.

Fresh leaves of *Neelamari* were taken and 100mg weighed into a boiling tube, added 5ml of 2.5N HCl and kept in water bath for three hours to hydrolyze the sample completely. After cooling it to the room temperature sodium carbonate was used to neutralize the solution where end point will be attained with the ceasing of effervescence upon adding sodium carbonate with the help of spatula and later volume was made up to 100ml with distilled water. Supernatant collected after filtering was taken in two different test tubes as 0.5ml and 1ml aliquots. Standards of 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standards were taken in different test tubes and volume was made up to 1ml in all the test tubes including sample. '0' served as blank with distilled water alone.

Four ml of anthrone reagent was added to all the test tubes, heated for 8 minutes in boiling water bath and cooled rapidly followed by reading in UV-Spectrophotometer at 630nm. Concentration of total sugars in samples was calculated by plotting concentration of standards on X- axis and absorbance at 630nm on Y-axis in the graph.

$$\text{Amount of total sugars present in 100 mg of leaves} = \frac{\text{mg of total sugars}}{\text{Volume of test}} \times 100$$

3.1.5.5 *Tannin*

Tannin content of *Neelamari* leaves was estimated by the Folin-Dennis method (Sadasivam and Manickam, 1996).

Weighed 0.5 g finely chopped leaf sample and transferred it to a 250 ml conical flask with 75 ml distilled water and the flask was heated gently for 30 minutes. Aliquot was centrifuged at 2000 rpm for 20 minutes. The supernatant was collected in 100 ml volumetric flask and volume made up. One ml aliquot was transferred into 100 ml volumetric flask containing 75 ml water, 5 ml of Folin-Denis reagent and 10 ml sodium carbonate was added and made up to 100 ml. Optical density was recorded at 700 nm in a spectrophotometer after 30 minutes. Blank was prepared with water.

A standard graph was drawn using serial dilution of tannic acid solution and from the graph tannin content of leaves was estimated. Tannin was expressed as mg g^{-1}.

3.1.5.6 *Preliminary phytochemical screening*

The *Neelamari* leaves were air dried under shade and ground to obtain a fine powder. About 25 g of fine powdered sample was soaked in methanol for 48 hours with occasional shaking. The filtrate was concentrated to 35ml and further used for analysis. The preliminary screening of the three extracts from three different stages of harvest of *Neelamari* leaf sample was carried out.

3.1.5.6.1 *Test for carbohydrate*

Fehling's test: Dissolved 2 ml of extract in 4 ml distilled water and heated with 2 ml of Fehling's reagent (A and B). Reddish brown colour indicated the presence of carbohydrates.

3.1.5.6.2 ***Test for flavonoids***

Lead acetate test: To 5 ml of extract, added 1 ml of lead acetate. Flocculent white precipitate indicated the presence of flavonoids.

To 1 ml of the extract, added 1 ml of sulphuric acid along the sides of the test tubes. Orange color formation indicated the presence of flavonoids.

3.1.5.6.3 ***Test for tannins***

Braemer's test: To 3 ml of the extract added 3 ml of 10 % alcoholic ferric chloride solution. Dark blue or greenish colouration of the solution indicated the presence of tannins in the sample.

3.1.5.6. 4. ***Test for alkaloids***

Wagner's test: To 3 ml of the extract added 2 ml of Wagner's reagent. Reddish brown precipitate indicated the presence of alkaloids.

3.1.5.6.5. ***Test for saponins***

Foam test: Diluted 1 ml of the extract with 20 ml distilled water and shaken in a graduated cylinder for 15 minutes. One centimeter layer of foam indicated the presence of saponins.

3.1.5.6.6 ***Test for fixed oils and fats***

A small quantity of the extract was pressed between two filter papers. Oil stains were not developed on the paper which indicated the absence of fixed oils.

3.1.5.6.7 ***Test for phenols***

To 1 ml of the extract, added 2ml of 5 % ferric chloride solution along the sides of the test tube. A dark green colour indicated the presence of phenolic compound.

3.1.5.6.8 ***Test for terpenoids***

To 1 ml of the extract, added 1 ml of chloroform followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate was not produced immediately indicating the absence of terpenoids.

3.1.5.6.9 ***Test for steroids***

To 1 ml of the extract added 1 ml acetic acid, 1 ml of chloroform followed by 0.5 ml sulphuric acid. Violet to blue green formation denoted the absence of steroids.

3.1.5.6.10 ***Test for quinones***

One milliliter of the extract was treated with 5 ml of concentrated hydrochloric acid. Formation of yellow coloured precipitate denoted the presence of quinone.

3.1.5.6.11 ***Test for coumarin***

Two ml of the extract was treated with 3 ml of 10 % sodium hydrochloride in a test tube. As solution did not turn to yellow colour it denoted the absence of coumarin.

3.2 EFFECT OF PRE-TREATMENT AND DRYING ON THE LEAF AND *NEELAMARI*-COCONUT OIL QUALITY.

Combination of various blanching and drying methods were tried to treat the samples in order to retain maximum quality. Leaves of *Indigofera* harvested at 160 days after transplanting was collected as that stage was found to be the best based on the results of Experiment-I. Aerial part of the plants were harvested and shifted to lab at the earliest. Later the leaflets were separated from hard shoots and spread over the clean floor. For 12 treatments and two replications each with 250g were apportioned. For each treatment samples were prepared by pooling the lot using quartering method. Approximately 40kg of bulk of *Neelamari* aerial part

were harvested which later yielded only 10-15 per cent of leaves after separating from shoots, later used for the treatments.

For drying the samples under vacuum, vacuum drier from Kelappaji College of Agricultural Engineering and Technology (Vacuum tray drier, GMP, MILK-TECH ENGINEERS), Thavanur was used.

Coconut oil required for the preparation of *Neelamari*-Coconut oil (*Neelibhrngadi Taila*) was procured from farm maintained by central nursery of Kerala Agricultural University, herbal drugs and other necessary ingredients for oil preparation was procured from Thrissur *Ayurvedics*, Thrissur, a whole-seller of ayurvedic raw drugs. Milks of Cow, Buffalo and Goat were procured from Diary plant of Kerala Veterinary and Animal Sciences University, Mannuthy.

3.2.1 Treatments

T_1: Unblanched and dried under shade

T_2: Unblanched and dried in cabinet drier (50±5°C)

T_3: Unblanched and dried in vacuum drier (35±5°C)

T_4: Steam blanched (1 min.) and dried under shade

T_5: Steam blanched (1 min.) and dried in cabinet drier (50±5°C)

T_6: Steam blanched (1 min.) and dried in vacuum drier (35±5°C)

T_7: Hot water blanched (90 °C for 1 min.) and dried under shade

T_8: Hot water blanched (90 °C for 1 min.) and dried in cabinet drier (50±5°C)

T_9: Hot water blanched (90 °C for 1 min.) and dried in vacuum drier (35±5°C)

T_{10}: Microwave blanched (45 sec.) and dried under shade

T_{11}: Microwave blanched (45 sec.) and dried in cabinet drier (50±5°C)

T_{12}: Microwave blanched (45 sec.) and dried in vacuum drier (35±5°C)

3.2.2 Pre-treatment methods

Samples were subjected to different blanching methods as pre-treatments before drying the samples (Plate 4).

3.2.2.1 *Hot water blanching*

The temperature for blanching was fixed by a preliminary test at 70, 80 and 90 °C for one minute. Blanching done at 90°C was fixed as blanching temperature as it was found to have highest indican (Table 3.1) when compared to other two temperature levels.

3.2.2.2 *Steam blanching*

Steam was generated using steam cooker and samples were subjected to steam blanching for one minute by covering the lid of cooker after placing the sample.

3.2.2.3 *Microwave blanching*

The duration for microwave blanching of the samples were fixed after preliminarily test using Samsung Micro Wave Oven for 30, 45 and 60 seconds. Samples were taken in microwaveable container and lightly sprinkled with distilled water to avoid excess moisture loss during the course of blanching and blanched samples were immediately cooled (Muftugil, 1985). Blanching duration was fixed as 45 seconds due to the highest leaf quality as indicated by indican content (Table 3.2).

3.2.3 Drying

In combination with pre-treated and unblanched samples, three different drying methods were combined and leaf quality assessed for preparing *Neelamari*-coconut oil out.

Plate4. Pre-treatments

3.2.3.1 *Shade drying*

Samples after blanching treatment along with control were dried in a room till constant weight was attained. Temperature during the drying ranged between 23-31°C.

Table 3.1 Effect of hot water blanching temperature on indican content (%) *Neelamari* leave

Blanching temperature	Shade dried	Cabinet dried	Vacuum dried
70^0C	2.325	2.145	2.231
80^0C	2.410	2.190	2.384
90^0C	2.550	2.300	2.578
CD (5%)	0.075	0.036	0.074

Table 3.2 Effect of microwave blanching duration on indican content (%) *Neelamari* leaves

Blanching duration	Shade dried	Cabinet dried	Vacuum dried
30 sec	2.645	2.661	2.868
45 sec	2.806	2.905	3.091
60 sec	2.719	2.799	2.960
CD (5%)	0.041	0.022	0.033

3.2.3.2 *Cabinet drying*

Samples after blanching were dried mechanically using a cabinet dryer at 50±5°C. Cabinet drier with inner dimension as 0.9 X 1 X 0.61 X m with 2.5 K W heating capacity was used for drying.

3.2.3.3 *Vacuum drying*

Vacuum drying was done at Kelppaji College of Agricultural Engineering and Technology, Thavanur using Vacuum Tray Dryer, with 4 drying trays of dimension 600m x 600 m and equipped with water ring vacuum pump of the




Plate5. Vacuum tray drier, KCAET, Thavanur

capacity 2.2 Kw (3hp). Samples were dried at (35±5°C) for 4.5 to 6.0 hours which varied with samples (Plate 5).

3.2.4 Oil preparation

The samples were subjected to different combinations of blanching with drying and completely dried samples were powdered and used for the preparation of *Neelibhrngadi Taila* (*Neelamari*-coconut oil). It is an ayurvedic formulation containing fresh *I. tinctoria* leaves as its major ingredient. This experiment is to assess the effect of blanching and drying of *Neelamari* leaves on oil quality.

Oil was prepared based on the procedure given in *Ayurvedic* Pharmacopeia of India. *Neelibhrngadi Taila* is a medicated oil preparation made with the ingredients in the standard formulation with coconut oil as base. All the ingredients with API standard were taken where liquid ingredients were taken at the quantity of 240ml each and solid powdered ingredients as 10g.

Fresh Bhringaraja (*Eclipta alba*), Kakatika (*Cardiospermum halicacabum*) and Amalaki (*Emblica officinalis*) were thoroughly washed and *svarasas* extracted without adding water using a juicer (Plate 6). Dried leaf samples from each treatment were powdered finely and 60g of powder was taken in two replications and 960ml of water added and decoction prepared by boiling and reducing the volume to 240ml finally, which is of $1/4^{th}$ initial volume. In the standard procedure of API expressed juice of Nili fresh leaves was used. Instead in this experiment leaf decoction of dried samples was used. Sample prepared with fresh leaf juice was referred as absolute control with 12 other sampled prepared with leaf decoction. All these juices and decoctions were filtered using muslin cloth to avoid sediments.

Coconut milk (*Nalikera Kshira*) was obtained by squeezing grated coconut through muslin cloth. *Ajaka Kshira*, *Mahisi Kshira* and *Dhenudbhava* were also strained through muslin cloth.

Plate6. Ingredients of *Neelibhrngadi Taila*

Cleaned and powdered *kalka dravya* consisting of roots of *Yastyahva* (*Glycyrrhiza glabra*) and *Gunja* (*Abrus precatorious*) along with *Anjana* and later sieved to get fine powder. By adding the 10 g each of all these powders with little water *kalka/ kalkam* was prepared by homogenizing.

Coconut oil (240ml) was taken in a copper bottomed stainless steel vessel and heated gently. *Kalkam* was added and stirred while adding *svarasas* and leaf decoction (240ml) of *Nili*. The mixture was heated for 3 hours with constant stirring and by maintaining the temperature between 50 and 90 °C during the first hour of heating. Later heating was stopped and allowed to stand overnight.

Next day, *Narikela Kshira*, *Ajadugdha*, *Mahisidugdha* and *Godugdha* were added and continued the process of heating intermittently over a period of three days. Consistency of *kalka* was checked constantly by rolling between fingers. Heating was stopped when the *kalka* broke down into pieces on attempting to form a *varti (khara paka laksana*) and the appearance of froth (phenodgama) over the oil. The *kalka* and oil was exposed to flame and confirmed the absence of moisture. Hot oil with a temperature of about 80°C was filtered and allowed to cool (Plate 7). *Neelibhrngadi Taila* obtained was stored in glass container and packed air-tight to protect from light and moisture (Fig 1). Later oil quality of all the samples was assessed by Thin Layer Chromatography (TLC) and determining the refractive index and acid value (API Part I, 8:26).

3.2.5. Treatments

There were twelve treatments with different pre-treatments and drying methods. One treatment was with fresh leaves.

T_1: Unblanched and dried under shade

T_2: Unblanched and dried in cabinet drier (50±5°C)

T_3: Unblanched and dried in vacuum drier (35±5°C)

T_4: Steam blanched (1 min.) and dried under shade

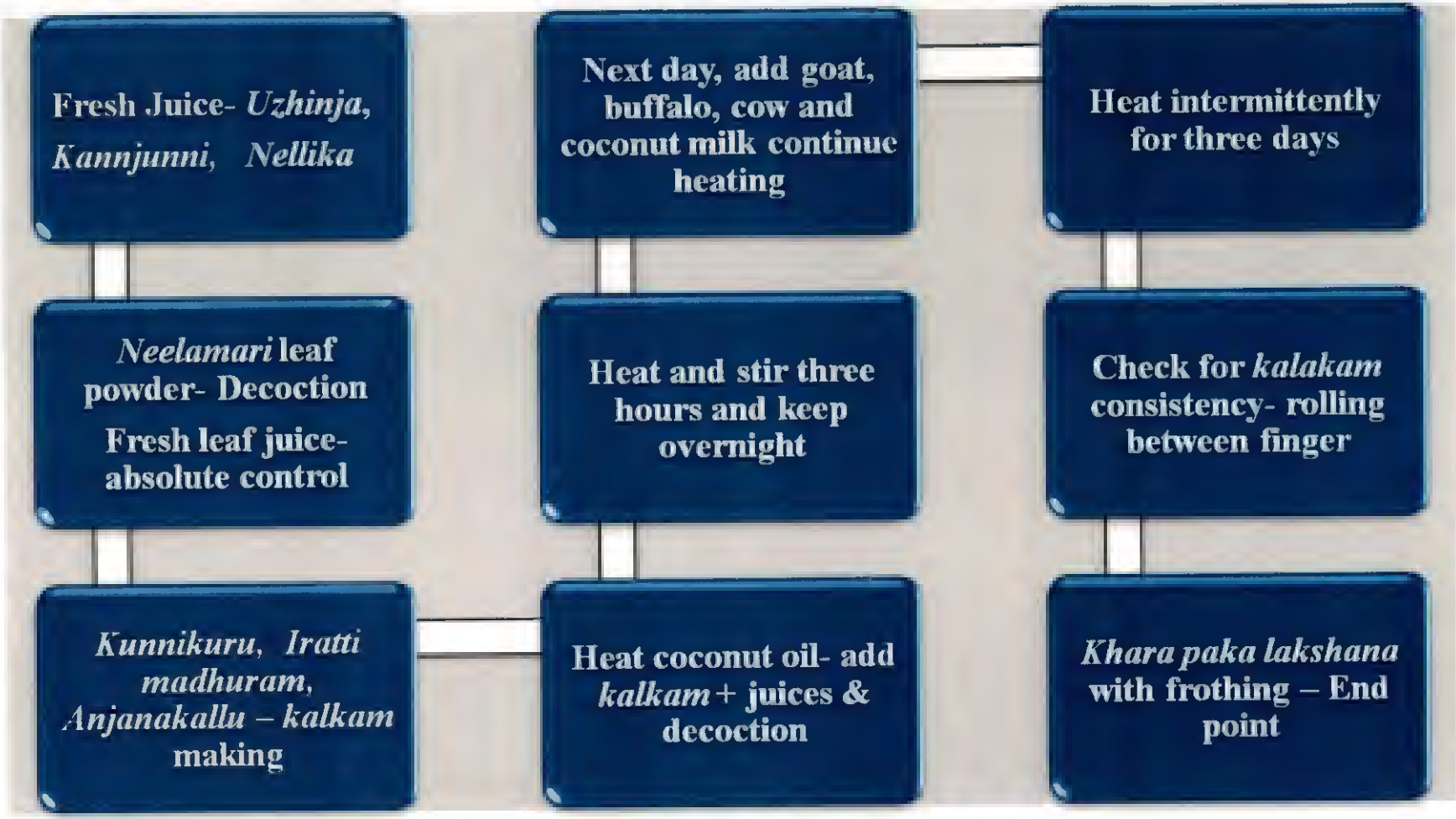


Fig. 1 Steps in preparation of *Neelibhrngadi Taila*

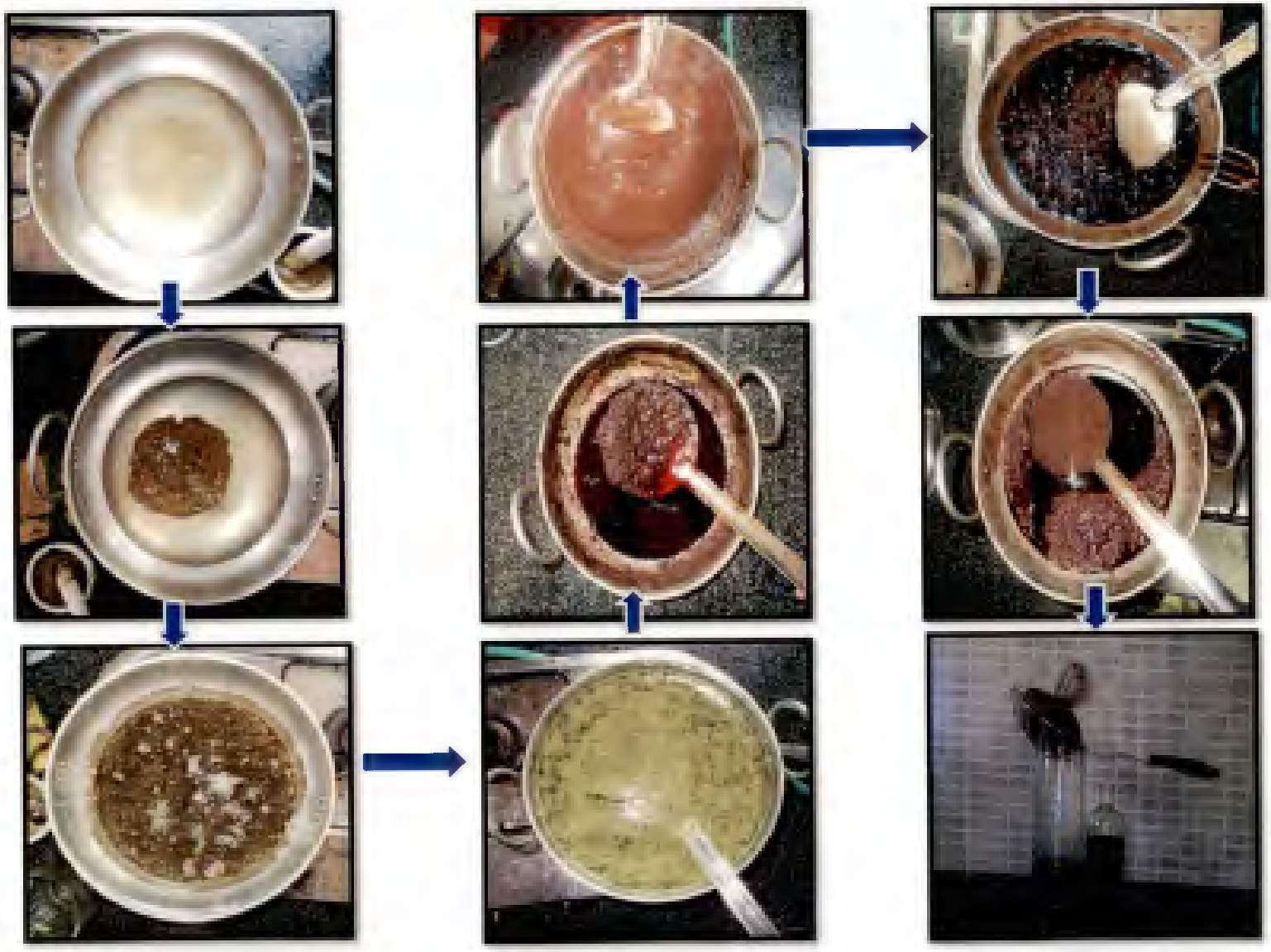


Plate7. Steps involved in preparation of *Neelibhrngadi Taila*

T_5: Steam blanched (1 min.) and dried in cabinet drier (50±5°C)

T_6: Steam blanched (1 min.) and dried in vacuum drier (35±5°C)

T_7: Hot water blanched (90 °C for 1 min.) and dried under shade

T_8: Hot water blanched (90 °C for 1 min.) and dried in cabinet drier (50±5°C)

T_9: Hot water blanched (90 °C for 1 min.) and dried in vacuum drier (35±5°C)

T_{10}: Microwave blanched (45 sec.) and dried under shade

T_{11}: Microwave blanched (45 sec.) and dried in cabinet drier (50±5°C)

T_{12}: Microwave blanched (45 sec.) and dried in vacuum drier (35±5°C)

3.2.6 Layout

The experiment was laid out in a Completely Randomized Design (CRD) with two replications of 250g each for all the blanching and drying treatments.

3.2.7 Observations

3.2.7.1 *Indican content*

Indican content from dried leaf sample were analysed by using the same method as described in 3.1.5.3using dried leaf powder instead of fresh leaves.

3.2.7.2 *Oil quality*

Even though there are numerous test and analysis that can be done for assessing the quality of oil formulation was done by doing TLC, and finding out refractive index at 40° and acid value of the oil as suggested in API.

The oil quality was found for thirteen samples consisting of twelve treatments and one absolute control.

3.2.7.2.1 *Thin Layer Chromatography (TLC)*

TLC was done in reference to API protocol which is the standard procedure. The samples were analysed based on number of the peaks observed. Bands were obtained for all the 13 samples with different Rf values which were furthered compared with the standard Rf values of API in order to confer their quality. Thirteen samples, including an absolute control were analysed. TLC analysis was done at CARe Keralam, Koratty.

Along with 20ml alcohol 2ml of the sample was agitated for three hours and allowed them to form separate layers. Sample layer was then separated and concentrated to 5ml which was used to perform TLC. Samples were applied on TLC plate at the rate of 10µl and developed a plate to a distance of 8 cm using toluene: ethyle acetate: n-hexane (6:3:1) as mobile phase by placing in a Twin Trough Chamber of 20x20cm.After getting bands plate was air dried at 60° C temperature for 5 minutes. Later the plate was sprayed with ethanol-sulphuric acid reagent followed by heating at 105°C for about 10 minutes. It showed the major spots as brown spots in visible light.

3.2.7.3 *Refractive index*

Refractive index of oil samples was examined using Abbeys Refractor meter at Oushadhi, Thrissur using the procedure described in API (Part I, 8:26). Prism surface was ensured of thorough cleaning by soft rag wetted with neutral solvents (spirit). Samples to be tested were carried to measuring surface by means of rounded glass rod in such a way that after the prism was closed the entire measuring plane was covered with sample liquid.

Upper prism was displaced down and pressed against the measuring plane. The illuminating window of the upper prism was directed towards the most intensive light source. In course of measuring in transmitted light, the mirror should cover the opening of the refractometric prism. While revolving the knobs a

sharp, distinct and colourless demarcation line between the bright and the dark background in the field of view of the eye piece is observed. Knob was adjusted in order to bring the demarcation line exactly on the central point of the cross hair in the upper window of the eye piece. The vertical line of the lower window of the eye piece was observed to record the refractive index of the sample. Whole process was carried out at the temperature of 20±0.5°C with the help of reference plate attached to the refractometer.

3.2.7.4 *Acid value*

Acid value a measure of free fatty acids of the oil samples was determined by the method suggested by (Sadasivam and Manickam, 2008).

Neutral solvent (250ml) was taken in a conical flask along with sample (5g) and added few drops of phenolphthalein. The aliquot mixture was titrated against 0.1N potassium hydroxide. End point was determined by the appearance of pink colour. Acid value was calculated with the formula given below.

$$\text{Acid value (mg KOH g}^{-1}) = \frac{\text{Titrate value X Normality of KOH X 56.1}}{\text{Weight of the sample (g)}}$$

Results

4. RESULTS

The current investigation was carried out in the Department of Processing Technology, College of Horticulture, Vellanikkara during 2014-16 to postulate the suitable stage of harvest and the most efficient pre-treatment and drying method for the preparation of *Neelibhrngadi Taila* according to *Ayurvedic* Pharmacopeia of India. The findings of the study titled "Postharvest studies in *Neelamari* (*Indigofera tinctoria* L.)" are presented in this chapter under the following headings.

1. Effect of cutting stage and storage time on leaf quality
2. Effect of drying techniques on leaf quality

4.1 EFFECT OF CUTTING STAGE AND STORAGE TIME ON LEAF QUALITY

The experiment was conducted with the objective of standardizing the cutting stage of *Neelamari*. Effect of cutting stages on yield of fresh leaves and phytochemical quality was observed. Indican content, total sugars, tannin and relative water content of leaves with respect to cutting stages were also recorded. Keeping quality of leaves with respect to storage times was evaluated. Preliminary screening for phytochemicals was also carried out.

4.1.1 Effect of cutting stages on quality of leaves

Neelamari (*Indigofera tinctoria*) leaves were harvested at C_1 (70 days after transplanting), C_2 (115 days after transplanting) and C_3 (160 days after transplanting) and the fresh yield was recorded indican content, total sugars, tannin content of *Neelamari* leaves and the observation on fresh yield and Relative Water Content (RWC) were made with the objective of selecting the best stage of cutting. The results are presented in the tables below.

4.1.1.1 *Yield*

Harvesting the aerial parts of 30 plants was done and yield was recorded at 70 days after transplanting and two consecutive harvestings with an interval of 45 days i.e. at 115 days and 160 days after transplanting. The yield was statistically significant at three different stages of harvest. Highest yield was recorded during last cutting stage at 45 days after second cutting (48.05g/plant) and least yield was recorded in harvest done at 70 days after transplanting (22.75g/plant). The last stage of cutting recorded significantly higher yield hence selected as the best stage for further studies (Table 4.1).

4.1.1.2 *Indican content*

The amount of indican, a glucoside compound which is responsible for pigmentation and with numerous other therapeutic values was analysed in the leaf samples harvested at three different stages of maturity. There was significant difference in indican content among harvesting stages which is revealed in Table 4.1.

Fresh crop harvested after 160 days after transplanting recorded the significantly highest indican of 1.23 per cent whereas the least content of indican (0.625%) was found in the leaves harvested at 70 days after transplanting.

4.1.1.3 *Total sugar*

Total sugar content was estimated in the leaf samples of different stage of cutting. Cutting stages C_1, C_2 and C_3 recorded the significantly different total sugar content. C_1 stage had least level of 2.55 per cent and highest level of 4.62 per cent was noticed in C_3 stage of harvest, which was the best stage for cutting.

4.1.1.4 *Tannin*

Tannin is an essential bioactive compound as far as herbal preparations are concerned and the leaves are being utilized in dying industries. It can also consider as a key to measure antioxidant activity of the substances.

Highest concentration was recorded at C_3 stage (1.78mg 100 g^{-1}) and least was (0.94 mg $100g^{-1}$) C_1 stage. As shown in the table 4.1 the plants with highest maturity (C_3) is best stage with respect to presence of tannins.

4.1.1.5 *Phytochemical screening*

Leaves of *Neelamari,* the commercially important part of the plant used in herbal hair oil was extracted in aqueous as well as in ethanolic medium to study the therapeutic value by detecting the group of secondary metabolites present which are responsible for the final quality of the products. All the three stages of crop harvested were subjected to phytochemical screening process using both the extracts. It is understood that all the stages of the crop denoted the presence of carbohydrates, flavonoids, tannins, alkaloids, saponins, phenols, terpenoids and quinone for both the extracts but fixed oils and steroids were absent in all the stages of crop and in both aqueous and ethanolic extracts (Table 4.2).

4.1.1.6 *Relative water content*

Relative water content in the leaves was assessed in all the three stages where the highest value recorded was in C_3 (67.95%) stage and least was in C_1 (21.80%) stage. C_3 has high moisture there by contributing to a physiological condition resulting in better accumulation of secondary metabolites and higher yield.

The experiment I, showed that, cutting neelamari plants for herbage is best at 160 days after transplanting as it had significantly superior yield, indican, total sugar

Table 4.1 Effect of cutting stage on yield and quality of leaves

Treatment	Yield plant^{-1} (g)	Indican (%)	Tannins (mg 100g^{-1})	Total sugars (%)	RWC (%)
C_1	22.750	0.625	0.948	2.550	21.800
C_2	34.050	0.860	1.154	3.540	46.155
C_3	48.050	1.230	1.744	4.620	67.950
SE	0.395	0.002	0.016	0.062	0.401
CD (5%)	1.840	0.005	0.048	0.190	1.870

C_1- Leaves harvested at 70 days after transplanting

C_2- Leaves harvested at 115 days after transplanting

C_3- Leaves harvested at 160 days after transplanting

Table 4.2 Effect of cutting stages on the presence of phytochemicals

Cutting Stages / Phyto-chemicals	C_1(70 DAT)		C_2(115 DAT)		C_3(160 DAT)	
	Aqueous extract	Methonolic extract	Aqueous extract	Methonolic extract	Aqueous extract	Methonolic extract
Carbohydrates	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Fixed oils	-	-	-	-	-	-
Phenols	+	+	+	+	+	+
Terpenoids	-	-	-	-	-	-
Steroids	-	-	-	-	-	-
Quinone	+	+	+	+	+	+
Coumarin	-	-	-	-	-	-

DAT – Days After Transplanting

C_1- Leaves harvested at 70 days after transplanting

C_2- Leaves harvested at 115 days after transplanting

C_3- Leaves harvested at 160 days after transplanting

and tannins content and with better relative water content. Hence the leaves from C_3 was considered as best and used for second experiment.

4.1.2 Effect of keeping time on quality of *Neelamari* (*Indigofera tinctoria*) leaves

In many pharmaceutical industries, there is considerable delay in processing the leaves collected for oil production. The leaves which are the basic material, in *Neelibhrngadi Taila,* will be procured and kept for a long period after harvest according to the convenience. Hence to find out the effect of keeping time, on leaf quality the investigation on quality of leaves based on chemical parameters was undertaken after cutting the crop at three different physiological stages. Freshly harvested leaves as well as leaves harvested and kept for 4 hours, 8 hours, 24 hours and 48 hours were studied in all the cutting stages and drawn inference.

Indican content, a key parameter along with total sugars and tannins were studied and tabulated in Table 4.3.

4.1.2.1 *Indican*

The samples harvested at three different stages and kept for different time intervals including freshly harvested leaf samples were analysed for indican content. Significant difference was observed in the indican content between keeping time (Table 4.3).

Freshly harvested leaves had the highest content of indican (0.913%) and the least content (0.897%) was recorded in the leaves kept for 48 hours after harvesting.

4.1.2.2 *Total sugar content*

Total sugar content in leaf samples was not found influenced by keeping time. Since there was no significant variation in total sugars among the different treatments, even if the leaves were stored for 48 hours after harvest showed no

significant loss in total sugar content was observed. Total sugar content varied from 3.46-3.70% in leaves (Table 4.3).

4.1.2.3 *Tannin content*

The change in tannin content of leaves due to the storing was also studied. There was no significant loss in tannin content even after storing upto 48 hours after harvest. The tannin ranged from 1.267-1.307mg $100g^{-1}$ of leaves as shown in Table 4.3.

The interaction of keeping time and cutting stages had significant effects on indican content but not on total sugars and tannins. Freshly harvested crop had highest indican content in all the cutting stages and at C_3 stage recorded the highest content of indican of 1.24per cent in freshly harvested leaves (Table 4.4).

The results showed that crop harvested at C_3 stage and freshly used (Plate 8) is the best than rest of the harvesting stages and keeping time. This stage can be carried forwarded to conduct the pre-treatment and drying study followed by preparing the *Neelamari*-coconut oil.

4.2 EFFECT OF DRYING TECHNIQUES ON LEAF QUALITY

This experiment was conducted with the aim of identifying the best suited pre-treatment and drying method with respect to the retention of indican. Preliminary tests were conducted to fix the blanching temperature and duration of hot water blanching and microwave blanching (Plate 9).

4.2.1 Change in leaf quality due to blanching and drying treatments

After treating the leaf samples with various pre-treatments and drying methods were used for estimating the indican content. The indican content in dry leaf samples was significantly influenced by pre-treatments and drying and varied among the different treatments.

Table 4.3 Effect of storage time on leaf quality

Treatment	Indican (%)	Tannins mg $100g^{-1}$	Total sugars (%)
T_1	0.913	1.307	3.700
T_2	0.908	1.290	3.600
T_3	0.905	1.280	3.550
T_4	0.902	1.267	3.533
T_5	0.897	1.267	3.467
SE	0.002	0.020	0.081
CD (5%)	0.006	NS	NS

T_1- Freshly harvested

T_2- Harvested and kept for 4 hours after harvest

T_3- Harvested and kept for 8 hours after harvest

T_4- Harvested and kept for 24 hours after harvest

T_5- Harvested and kept for 48 hours after harvest

Table 4.4 Effect of cutting stage and storage time on quality of *Neelamari* leaves

Cutting stage	Treatment	Indican (%)	Tannin (mg $100g^{-1}$)	Total sugars (%)
C_1 (70 DAT)	T_1	0.63^{h}	0.960^{c}	2.800^{c}
	T_2	0.625^{hi}	0.950^{c}	2.550^{c}
	T_3	0.625^{hi}	0.950^{c}	2.500^{c}
	T_4	0.625^{hi}	0.940^{c}	2.500^{c}
	T_5	0.620^{i}	0.940^{c}	2.400^{c}
C_2 (115 DAT)	T_1	0.870^{d}	1.180^{b}	3.600^{b}
	T_2	0.865^{de}	1.160^{b}	3.600^{b}
	T_3	0.860^{ef}	1.150^{b}	3.550^{b}
	T_4	0.855^{fg}	1.140^{b}	3.500^{b}
	T_5	0.850^{g}	1.140^{b}	3.450^{b}
C_3 (160 DAT)	T_1	1.240^{a}	1.780^{a}	4.700^{a}
	T_2	1.235^{ab}	1.760^{a}	4.650^{a}
	T_3	1.230^{b}	1.740^{a}	4.600^{a}
	T_4	1.225^{bc}	1.720^{a}	4.600^{a}
	T_5	1.220^{c}	1.720^{a}	4.550^{a}
	SE	0.003	0.035	0.140
	CD (5%)	0.010	0.108	0.425

T_1-Freshlyharvested

T_2- Four hours after harvest

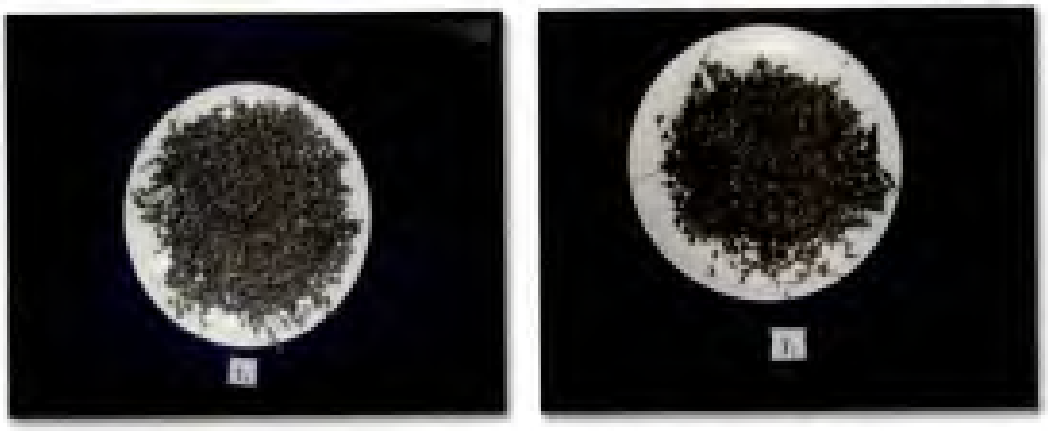
T_3- Eight hours after harvest

T_4- Twenty four hours after harvest

T_5- Fourty eight hours after harvest

DAT- Days After Transplanting

Plate8. Best cutting stage (C_3) and storage time (fresh leaves)



T_1 - Unblanched + Shade dried

T_2 – Steam blanched + Shade dried

T_3 – Hot water blanched + Shade dried

T_4 – Microwave blanched + Shade dried

T_5 - Unblanched + Dried in Cabinet drier

T_6 – Steam blanched + Dried in Cabinet drier

Plate9. Methods of blanching and drying

T_7 – Hot water blanched + Dried in Cabinet drier

T_8 – Micro wave blanched + Dried in Cabinet drier

T_9– Unblanched + Vacuum dried

T_{10}– Steam blanched + Vacuum dried

T_{11}– Hot water blanched + Vacuum dried

T_{12}– Micro wave blanched + Vacuum dried

Plate9. Methods of blanching and drying (Contd…)

4.2.1.1 *Indican*

Indican content in powdered samples after blanching and drying was analysed and tabulated in Table 4.5. Indican content varied significantly with each treatment. Sample which was microwave blanched and dried in the vacuum drier had the highest content of 3.045 per cent indican and least indican content of 2.328 per cent in hot water blanched and dried in cabinet drier. Hence microwave blanching coupled with vacuum drying had superior effect on retention of indican in leaves whereas hot water blanching coupled with cabinet drying was deleterious, resulting in lowest indican content in leaves.

4.2.2 Effect of blanching and drying on oil quality

After treating the leaf samples with various pre-treatments and drying methods were used for preparing *Neelamari*-coconut oil (*Neelibhrngadi Taila*) was prepared according the protocol described in *Ayurvedic* Pharmacopeia of India (API). Decoctions of dried samples were used. The quality of oil influenced by pre-treatments and drying was assessed by estimating the acid value and refractive index (RI).The oil samples were also subjected to Thin Layer Chromatography (TLC) method to assure the quality as given by API (Plate 10a and 10b).

4.2.2.1 *Acid value*

Effect of blanching and drying methods had significant effect on acid values (Table 4.6). Highest value was recorded in the oil prepared by using unblanched sample dried under shade (2.260 mg KOH g^{-1}) and also dried in cabinet drier (2.230 mg KOH g^{-1}) where as lowest acid value (1.460 mg KOH g^{-1}) was observed in the oil sample prepared by using microwave blanched and vacuum dried sample revealing the best treatment contributing to the better oil quality. Hence blanching and drying methods had significant effect on oil quality of *Neelamari*- coconut oil will be good if acid value does not exceeds 5. All the

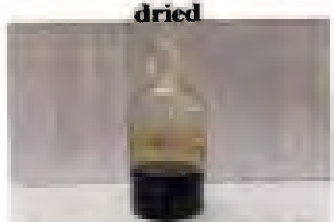
Absolute Control

T_1 -Unblanched+ Shade dried

T_2 -Steam blanched + Shade dried

T_3 – Hot water blanched + Shade dried

T_4 - Microwave blanched + Shade dried



T_5 -Unblanched+ Dried in cabinet drier

T_6 - Steam blanched+ Dried in cabinet drier

T_7-Hot water blanched+ Dried in cabinet drier

T_8 -Microwave blanched+ Dried in cabinet drier

T_9 - Unblanched+ Dried in vacuum drier

T_{10} - Steam blanched+ Dried in vacuum drier

T_{11} - Hot water blanched+ Dried in vacuum drier

T_{12} – Microwave blanched+ Dried in vacuum drier

Plate10a. Different treatments of *Neelibhrngadi Taila*

10b. Different tratments of *Neelibhrngadi Taila*

samples had acid value below 5, and it ranged from 1.460- 2.260 mg KOH g^{-1} (Table 4.6).

4.2.2.2 *Refractive index*

Refractive index of all the 12 samples were determined at 40° and looked for the difference caused by blanching and drying treatments. It is suggested by API that R.I. ranging from 1.451 to 1.462 is the indication of better quality *Neelamari*- Coconut oil. There was no significant difference between the treatments on R.I. of the oil, R.I. was found to range from 1.451- 1.455. So all the oil samples prepared, contained the described range of R.I. proving their quality.

4.2.2.3 *Thin Layer Chromatography (TLC)*

Another major parameter for assessing the oil quality was TLC which was performed for all the 12 samples by keeping an absolute control as standard, which was the *Neelamari*-coconut oil prepared by using fresh leaf juice against the leaf decoction prepared and used in rest of the samples. The results obtained are represented in the form of bands with Rf values. Each band in the plates represents the different phytochemical compounds which are unknown. All the oil samples except the oils prepared by using the steam blanched and vacuum dried leaves (T_{10}) and hot water blanched and vacuum dried leaves (T_{11}) conferred the quality as they shown the standard Rf value given by API (Plate 11).

For better understanding, visualization and documentation HPTLC was also performed using all the 13 samples and got the phytochemicals present in the oil samples in terms of peaks. Number of peaks obtained representing the number of phytochemical compounds present in oil samples where the compounds were unknown. Absolute control sample prepared using fresh leaves showed 16 peaks where as the oil sample prepared out of microwave blanched and vacuum dried leaves recorded the highest number of peaks (17). Least number of 13 peaks was

Table 4.5 Effect of drying with pre-treatment on indican content (%)

Treatments	Indican (%)
T_1- Unblanched+ Shade dried	2.837^{c}
T_2- Steam blanched + Shade dried	2.607^{de}
T_3- Hot water blanched+ Shade dried	2.546^{e}
T_4- Microwave blanched + Shade dried	2.888^{bc}
T_5- Unblanched+ Dried in cabinet drier	2.546^{e}
T_6- Steam blanched+ Dried in cabinet drier	2.607^{de}
T_7- Hot water blanched+ Dried in cabinet drier	2.328^{f}
T_8- Microwave blanched+ Dried in cabinet drier	2.918^{bc}
T_9- Unblanched+ Dried in vacuum drier	2.948^{ab}
T_{10}- Steam blanched+ Dried in vacuum drier	2.694^{d}
T_{11}- Hot water blanched+ Dried in vacuum drier	2.585^{e}
T_{12}- Microwave blanched+ Dried in vacuum drier	3.045^{a}
SE	0.048
CD (5%)	0.107

Table 4.6 Effect of drying with pre-treatments on oil quality parameters

Treatment	Acid value mg KOH g^{-1}	Refractive Index
T_1- Unblanched+ Shade dried	2.260^{a}	1.451
T_2- Steam blanched + Shade dried	1.680^{bcd}	1.455
T_3- Hot water blanched+ Shade dried	1.740^{bc}	1.455
T_4- Microwave blanched + Shade dried	1.570^{cde}	1.452
T_5- Unblanched+ Dried in cabinet drier	2.230^{a}	1.455
T_6- Steam blanched+ Dried in cabinet drier	1.790^{b}	1.454
T_7-Hot water blanched+ Dried in cabinet drier	1.830^{b}	1.452
T_8-Microwave blanched+ Dried in cabinet drier	1.740^{bc}	1.452
T_9- Unblanched+ Dried in vacuum drier	1.855^{b}	1.454
T_{10}- Steam blanched+ Dried in vacuum drier	1.730^{bcd}	1.455
T_{11}- Hot water blanched+ Dried in vacuum drier	1.515^{de}	1.452
T_{12}- Microwave blanched+ Dried in vacuum drier	1.460^{e}	1.452
SE	0.099	0.002
CD (5%)	0.218	NS

Plate11. TLC Plate of *Neelibhrngadi Taila*

observed in hot water blanched and shade dried sample. Number peaks obtained from all the samples ranged from 13-17. While ranking the samples, oil samples with same number of peaks were ranked considering the area of peaks obtained. Here also each peak represented particular phyto chemical which was unknown (Table 4.7).

Choosing the best treatment by inferring the TLC and HPTLC was not possible as the identity and quantity of the phytochemicals were unknown. Still the all the oil samples conferred the quality in terms of TLC by showing standard Rf value range given by API except the samples prepared by using the steam blanched and vacuum dried leaves (T_{10}) and hot water blanched and vacuum dried leaves (T_{11}). Range of standard Rf values considered were 0.15-0.19, 0.21-0.25, 0.34-0.35, 0.44-0.46 and 0.76-0.82.

By considering all these parameters it can be concluded that microwave blanching was the best among other pre-treatments where as vacuum drying was found to be best over shade and cabinet drying (Table 4.5 and 4.6) and oil prepared using all the samples conferred the quality in terms of acid value and R.I. Least acid value which was a desirable character was observed in the oil sample prepared out of leaves which are microwave blanched and vacuum dried. In case of TLC, all the oil samples met the standards of API in terms of Rf values except for the sample prepared by using the steam blanched and vacuum dried leaves (T_{10}) and hot water blanched and vacuum dried leaves (T_{11}).

Table 4.7 High Performance Thin Layer Chromatography (HPTLC) peaks obtained for oil samples

Treatment	No. of peaks obtained	Oil Sample
Absolute Control	16	Sample 3
T1 -Unblanched+ Shade dried	15	Sample 1
T2 -Steam blanched + Shade dried	15	Sample 11
T3-Hot water blanched+ Shade dried	13	Sample 2
T4- Microwave blanched + Shade dried	16	Sample 10
T5-Unblanched+ Dried in cabinet drier	15	Sample 9
T6- Steam blanched+ Dried in cabinet drier	15	Sample7
T7-Hot water blanched+ Dried in cabinet drier	15	Sample12
T8 -Microwave blanched+ Dried in cabinet drier	15	Sample 6
T9- Unblanched+ Dried in vacuum drier	16	Sample 13
T10- Steam blanched+ Dried in vacuum drier	15	Sample 4
T11- Hot water blanched+ Dried in vacuum drier	14	Sample 5
T12- Microwave blanched+ Dried in vacuum drier	17	Sample 8

Discussion

5. DISCUSSION

In all the ancient systems of medicines, herbal drugs are the major constituents with their wide range of curative properties with less or no ill effects treating wide range of disorders and diseases. Usage of such medicinal plants started thousands of years ago and since then mankind started improvising throughout the course of civilization. *Neelamari* (*Indigofera tinctoria*) is one such plant known to mankind as a natural source of indigo dye loaded with numerous medicinal properties where hair tonic property of the leaves is one among them which is of our interest in the present study.

Neelamari, known as "*Neeli*" or "*Neelika*" in Sanskrit a plant reputed for its use as 'Indigo dye' also used as a major ingredient of "*Neelibhrngadi Taila*", "*Neeli thulasiadhi tailam*", and "*Neeli thulasiadhi kashayam*" as per the *Ayurvedic* system of medicine. Due to its antitoxic properties, it is also used as a good remedy against poisonous infections (Sivarajan and Balachandran, 1994). The plant is also used as stimulant, alterative, antiseptic and purgative (Singh and Panda, 2005).

Neelamari leaves used in the formulation of '*Neelibhrngadi Taila*' for which it is to be harvested at a particular stage at which it possesses maximum content of phytochemicals and at the same time it should be profitable to growers with good yield. After harvesting it is to be used at the earliest to minimize the deleterious effect on bioactive compounds profile in leaves. Availability of the raw drug is a problem in January- March which is the season for *Amla* production. So to ensure the availability of *I. tinctoria* during *Amla* season, there is a need to store the leaves in a condition, with good pharmaceutical properties.

Thus an attempt has been made to tackle the problem by investigating the influence of cutting stage and storage time on phytochemical profile of *I. tinctoria* leaves and also making the foliage available in a suitable form throughout the year.

5.1 EFFECT OF CUTTING STAGE AND STORAGE TIME ON LEAF QUALITY

Leaves of *Neelamari* are used in the ayurvedic formulation of *Neelibhrngadi Taila* have to be harvested after 3-4 months of planting before flowering and harvested leaves are to be marketed fresh. Cut leaves must be used within 8 hours (KAU, 2013). Hence the standardization for cutting stage and maximum possible storage time of *Neelamari* was studied to ensure the better quality of the end product with maximum retention and accumulation of phytochemicals. In the present study leaves of *I. tinctoria* were harvested at three different stages of maturity like C_1-70 days after transplanting, C_2-115 days after transplanting and C_3-160 days after transplanting and observed the physico-chemical properties of harvested leaves. The effect of storage was studied by keeping the harvested leaves for 4, 8, 24 and 48 hours and fresh leaves by observing phytochemical quality.

5.1.1 Effect of cutting stage on physico-chemical parameters of leaves in *Indigofera tinctoria*

Best stage of cutting was fixed by considering physiological as well as content of bioactive compounds.

5.1.1.1 *Yield of fresh leaves*

Highest yield (48.05g plant $^{-1}$) was found when harvesting was done at 160 days transplanting (Fig. 2) Similar findings were reported by *Badi et al.* (2004) that harvesting stage had its impact on herbage yield in Thyme (*Thymus vulgaris* L.) and among the three stages, on set of blooming shown highest values for fresh herb yield of 19 t ha^{-1}, oil yield of 133 kg ha^{-1}. Sellami *et al.* (2009) investigated the effect of four various phenological stages viz., early vegetative, late-vegetative and budding and full flowering on the yield and quality of sweet marjoram (*Origanum majorana* L.) where full flowering stage was recorded with highest value for herbage and oil yield.

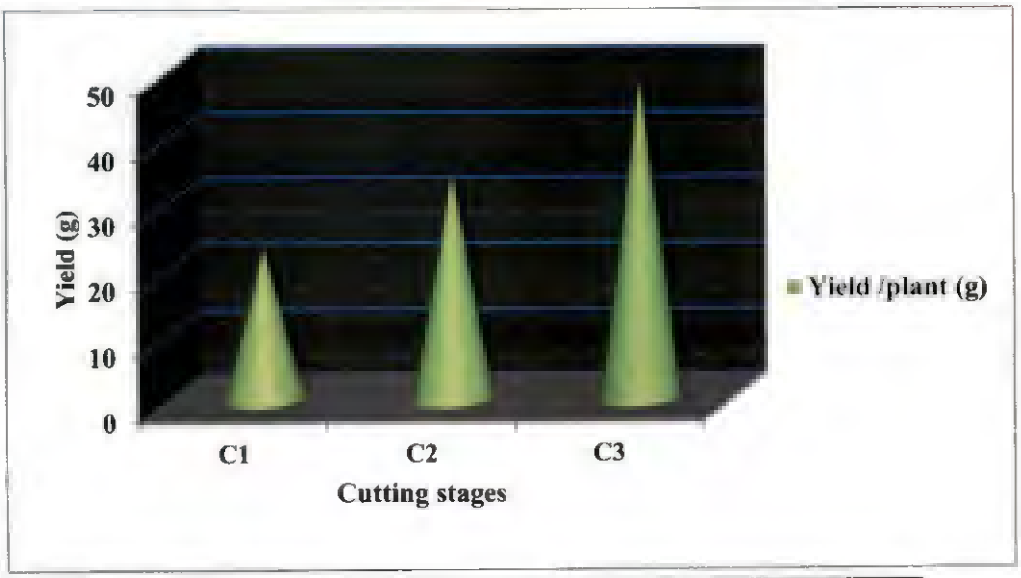


Fig2. Effect of cutting stages on yield of *Neelamari* leaves

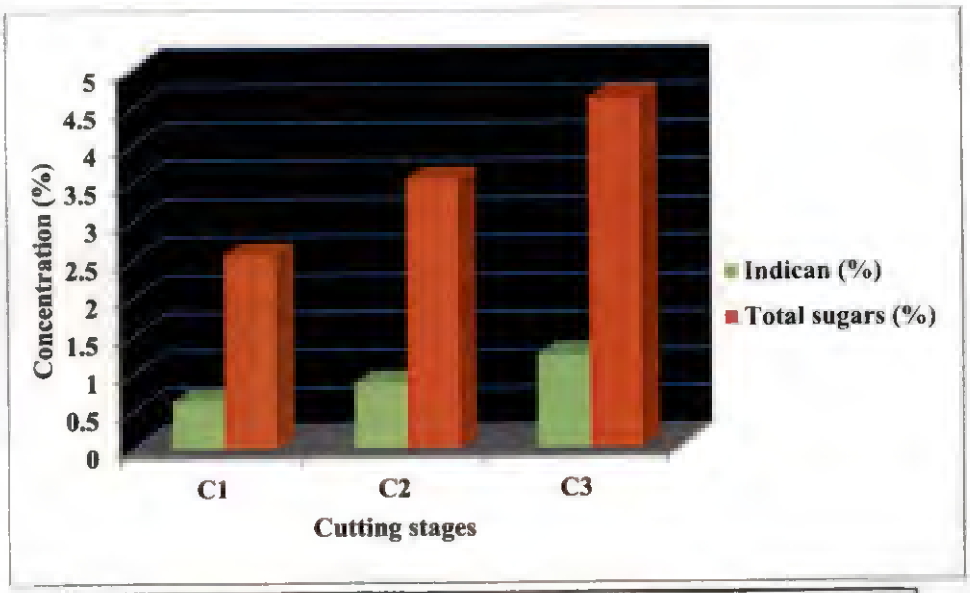


Fig2a. Effect of cutting stage on quality of *Neelamari* leaves

C_1- Leaves harvested at 70 days after transplanting

C_2- Leaves harvested at 115 days after transplanting

C_3- Leaves harvested at 160 days after transplanting

Increase in yield with advanced cutting stages is due to the increase in leaf and canopy area with age of the plant which will result in increased photosynthesis and better allocation of metabolite in herbage.

5.1.1.2 *Indican content*

Indican is a glycoside and a major phytochemical present in leaves of *Indigofera* which is a marker for determining the leaf quality. It is also an important constituent imparting the therapeutic property to *Neelibhrngadi Taila.* The last cutting stage of 160 days after transplanting recorded the highest indican content of 1.23 per cent (Fig. 2a). The plant constituents like metabolic waste and toxic products for defense are secondary metabolites accumulated in the cell structures like cell vacuoles, cytosol and cell wall (Luckner, 1990). Minami *et al.* (2000) have also observed similar results in *Polygonum tinctoria* commonly known as indigo plant and they reported that the secondary metabolite present in the plant is a colourless glucoside called indican (indoxyl-β-D-glucoside) which is mainly accumulated in vacuoles of leaf tissues and not in other tissues of that plant. Indican was reported to have increasing concentration along with increase in growth and age of aerial parts. Indican content per gram of leaf tissue was more in first and second leaves of the mature stage of the plant than basal leaves. As leaf area increased, indican content also increased with age of plant. Indican, a secondary metabolite is an indigo precursor which yields indoxyl and glucose upon hydrolysis and it gives the intensive blue tinge to the plants. In younger leaves indigo precursor is present at a higher level than older ones, but, the plant prevents the formation of indigo by glycosylation. But in older leaves oxidation occurs readily by yielding indigo more easily (Maugard *et al.*, 2001).

Moraes *et al.* (2013) reported that *Stevia rebaudianan* harvested in 60 days, 90 days and 120 days and glycosides content of plants harvested at 120 days recorded highest value for the glycosides, rebaudioside A (398.80 kg ha^{-1}) and stevioside (512.21 kg ha^{-1}).Significance of date of leaf picking in Senna (*Cassia angustifolia* Vahl.) on Sennoside content was studied by Upadhyaya *et al.* (2011).

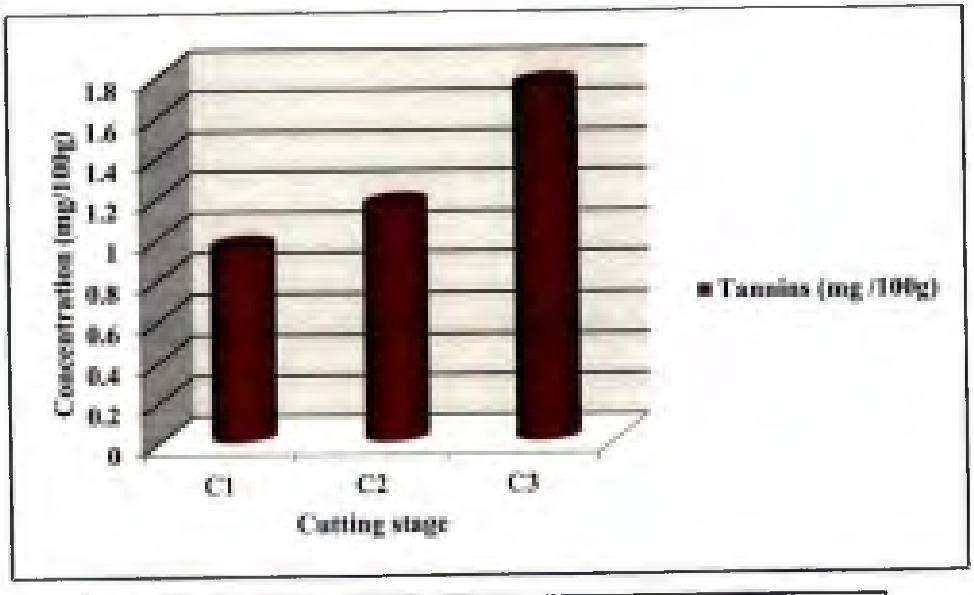


Fig2b. Effect of cutting stage on quality of *Neelamari* leaves

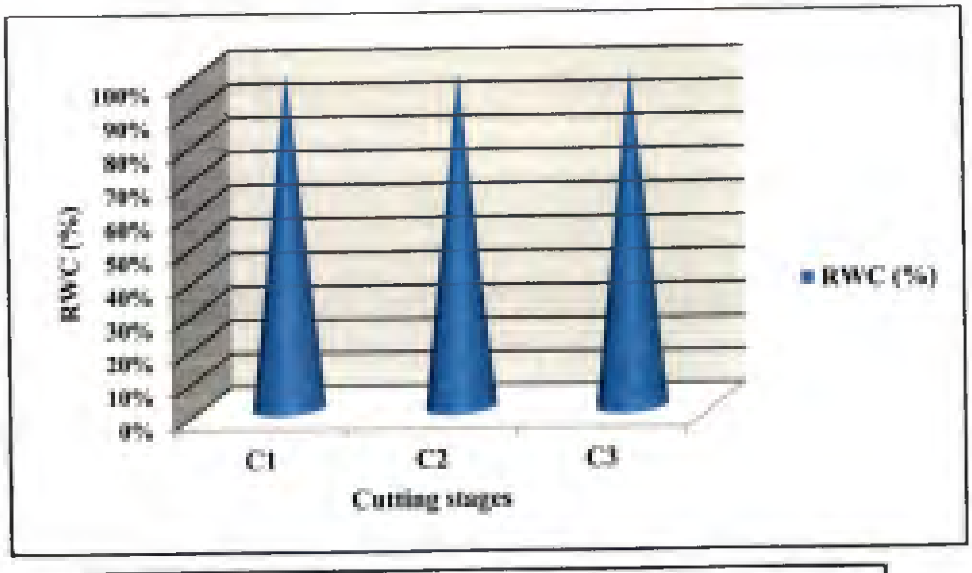


Fig2c. Effect of cutting stage on quality of *Neelamari* leaves

C_1- Leaves harvested at 70 days after transplanting

C_2- Leaves harvested at 115 days after transplanting

C_3- Leaves harvested at 160 days after transplanting

Both Sennoside A (0.126%) and Sennoside B (0.069%) were highest in the crop harvested at 90 days after sowing than in the crops of 130 and 110 DAS.

5.1.1.3 *Total sugar*

Total sugar content was studied in relation with phytochemical property of the leaves. Leaves harvested at 160 days after transplanting recorded significantly higher sugar content of 4.62 percent. Sugar synthesis increases with the age of the plant due to the accumulation of carbohydrates and enhanced enzyme production (Fig. 2a).

Sugar synthesis and accumulation of glycoside in the leaves of *I. tinctoria* was studied by Zavatskaya and Mashanova (1978) and reported that full bloom stage had relatively highest sugar synthesis and dye formation in leaves, where as organic acid found to be stable along with growth of plant. Fruiting stage reported with higher volatile acids but lower non-volatile acids. In *Cosmos caudatus* harvested at the age of 8 weeks during dry season recorded with highest contents of bioactive compounds, where the concentration increased with maturity (Mediani *et al.*, 2012).

5.1.1.4 *Tannin*

Tannin is also an important secondary metabolite with curative and dyeing properties. The observations in tannin content of *Indigofera* leaves harvested at different stage of maturity, crop harvested at 160 days after transplanting had significantly high amount of tannin (1.78mg $100g^{-1}$ of fresh leaves). Being a secondary metabolite the accumulation may increase with the age (Fig 2b). This finding was on par with the study conducted by Mutalib (2015) where he reported that tannin content in *Plantago major* leaves was highest at matured stage (0.12mg g^{-1} of dry weight) than younger one. Seguin *et al.*, (2004) also reported that coumestrol, apigenin, luteolin and quercetin content in *Medicago sativa* L. was high in early vegetative stage and only luteolin and quercetin concentrations were high during late flowering stage.

5.1.1.5 *Phytochemical screening*

To study the efficacy of any herbal drug phytochemical screening is a preliminary tool. Leaf samples harvested from all the cutting stages were assessed for their phytochemical efficiency. It gave positive result for the presence of carbohydrates, flavonoids, tannins, alkaloids, saponins, phenols, terpenoids and quinone. But there was no difference in the profile of active constituents in the samples at three cutting stages. Similar findings were reported by Verma and Suresh (2002) after conducting phytochemical investigations of *I. tinctoria* L. leaves and the qualitative analysis of the extracts where it showed the presence of flavonoids, alkaloids, glycosides, and terpenoids. Renukadevi and Sultana (2011) reported that leaf extract of *I. tinctoria* was identified with secondary metabolites of therapeutic importance like flavonoids, saponins, tannins, terpenoids, quinone and phenols. The therapeutic potential of *Indigofera* is thus confirmed.

5.1.1.6 *Relative water content (RWC)*

Relative water content (RWC) is an integrative index of plant water status which is used to evaluate the tolerance capacity of plants to water stress. It is related to root water absorption and loss of water through transpiration. R W C of the leaves from all the cutting stage was assessed. It was found to be highest (Fig. 2c) in 160 days after transplanting (67.95%) and least was in the crop harvested at 70 days after transplanting (21.80%). Maintenance of higher RWC helps in sustaining the photosynthetic capacity of plants which ultimately contributes to better yield (Sinclair and Ludlow, 1986). Difference in RWC may be due to the differences in their ability to accumulate and adjust osmotically to maintain tissue turgor and hence physiological activities (Almeselmani, 2011). Thus it is evident that growth stage of the plant has its influence on the phytochemical composition of the herbal drugs. Plant bioactive constituents either increase or decrease according to maturity stage of plant, and hence harvesting at the right period for better quality of the leaves is very important to obtain desired therapeutic property for the herbal formulation (Fig. 2c).

5.1.2 Effect of storage time on quality of *Neelamari* leaves

After harvesting, storage time also play a crucial role in assuring the quality of the harvested leaves. In most of the medicinal crops bioactive constituents will increase or decrease with storage time. Fresh sample soon after harvesting, kept for 4, 8, 24 and 48 hours were studied to make out the influence of storing time on the major phytochemicals in the leaves of *I. tinctoria*

5.1.2.1 *Indican content in leaves*

Indican which is a quality marker of *I. tinctoria* leaves were analysed at different time interval and it showed significant difference during postharvest storage. Indican content was found to decrease with prolonged storage time and highest indican content (0.913%) was found in the freshly harvested leaves and least (0.897%) in the leaves kept for 48 hours after harvest (Fig. 3a).When the leaves are exposed to air for longer period of time, there are chances for oxidation and hence reduction in the quantity of indican with prolonged storage.

5.1.2.2 *Total sugar*

Total sugar content of *Neelamari* leaves was declined with the storage time but did not have significant effect by storage time. Total sugar content in leaves varied from 3.467- 3.700 per cent (Fig. 3a). The sugar synthesis and accumulation occur only during active growth period of a plant, and further deterioration of sugar occurs during fruiting stage. Since it acts as source, on storage there may not be any changes. It is supported by a study in *Stevia rebaudiana* where the content of rebaudioside and stevioside had no significant difference upon storage after harvest (Moraes *et al.*, 2013).

5.1.2.3 *Tannin content*

Tannin content in leaves was also a quality marker as far as leaf quality and end product quality is concerned. While conducting the study to find out the effect of storage time on leaf tannin content of *Neelamari* leaves there was no significant variation in tannin content. However tannin content in leaves ranged

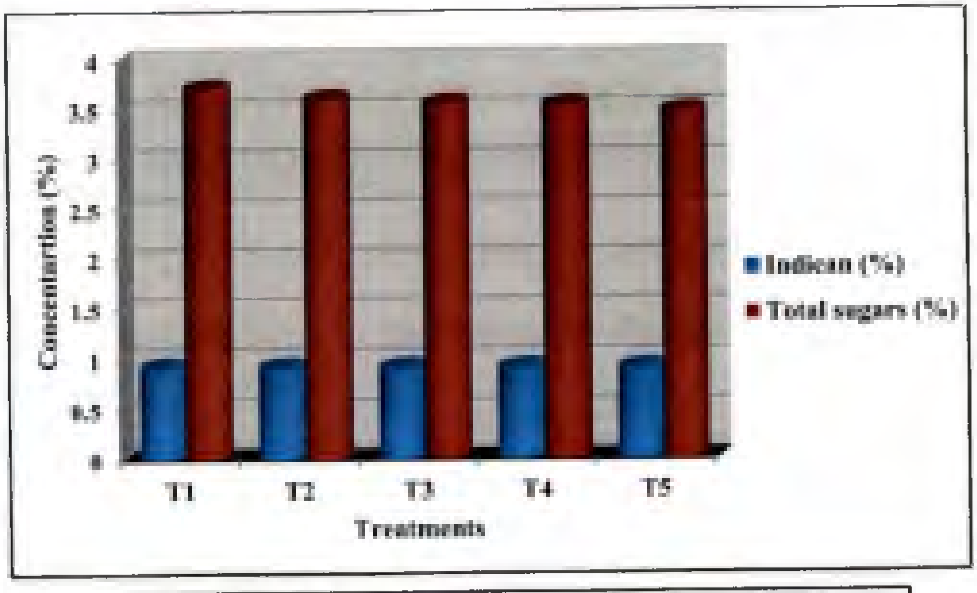


Fig3. Effect of storage time on *Neelamari* leaf quality

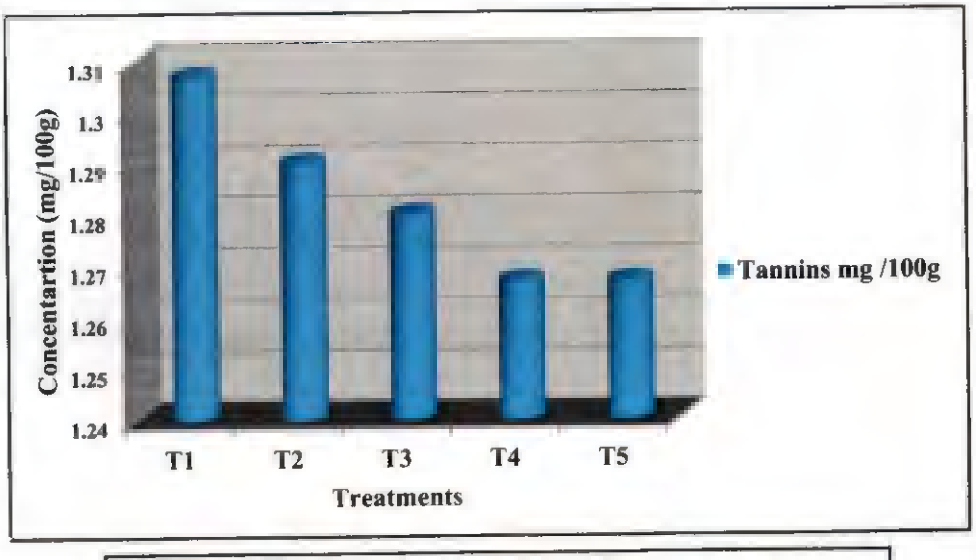


Fig3a. Effect of storage time on *Neelamari* leaf quality

T_1: Freshly harvested

T_2: Harvested and kept for 4 hours after harvest

T_3: Harvested and kept for 8 hours after harvest

T_4: Harvested and kept for 24 hours after harvest

T_5: Harvested and kept for 48 hours after harvest

from 1.267-1.307 mg $100g^{-1}$ of leaves (Fig. 3a). The tannin which is a secondary metabolite having its role in plant defense get accumulated during active growth phase. The result was contradictory to the findings of Raya *et al.* (2015) who studied the effect of keeping time on *Clinacanthus nutans* a medicinal herb which is a source of phenolic and flavonoid components. The highest content of phytochemicals was found in the young leaves stored upto to 24 hours.

The interaction of keeping time and cutting stages had significant effects on indican content but not on total sugars and tannins. Freshly harvested crop had highest indican content in all the cutting stages stage and at C_3 stage recorded the highest content of indican of 1.24per cent in freshly harvested leaves (Fig. 4 and Fig. 4a)

According to the parameters considered in his study, leaves harvested 160 days after transplanting without post harvest storage was chosen for further investigations as they recorded the best result for indican (1.240%), total sugar (4.700%) and tannin (1.780mg $100g^{-1}$) along with high fresh yield (48.05g $plant^{-1}$) and RWC (67.95%).

5.2 EFFECT OF DRYING TECHNIQUE ON LEAF QUALITY

The most important Ayurvedic preparation using *Neelamari* is *"Neelibhrngadi Taila"*. Leaves of *Neelamari* are macerated and added to oil with extracts of other plants like *Amla, Eclipta, Cardiospermum, etc.* in the preparation of "*Neelibhrngadi Taila*". Herbage of medicinal plants is dried to reduce water content in leaf tissues which results in better extraction. Drying process results in accumulation of phytochemicals at leaf surface as moisture loss occurs due to evaporation Moyler (1994).Storing *Neelamari* leaves in dried form with maximum possible retention of phytochemicals can be a solution for ensuring the availability of *Neelamari* in the season of demand. Hence three different drying methods coupled with pre-treatments with the aim of conserving phytochemicals at its best were applied on *Neelamari* fresh leaves and conducted the study.

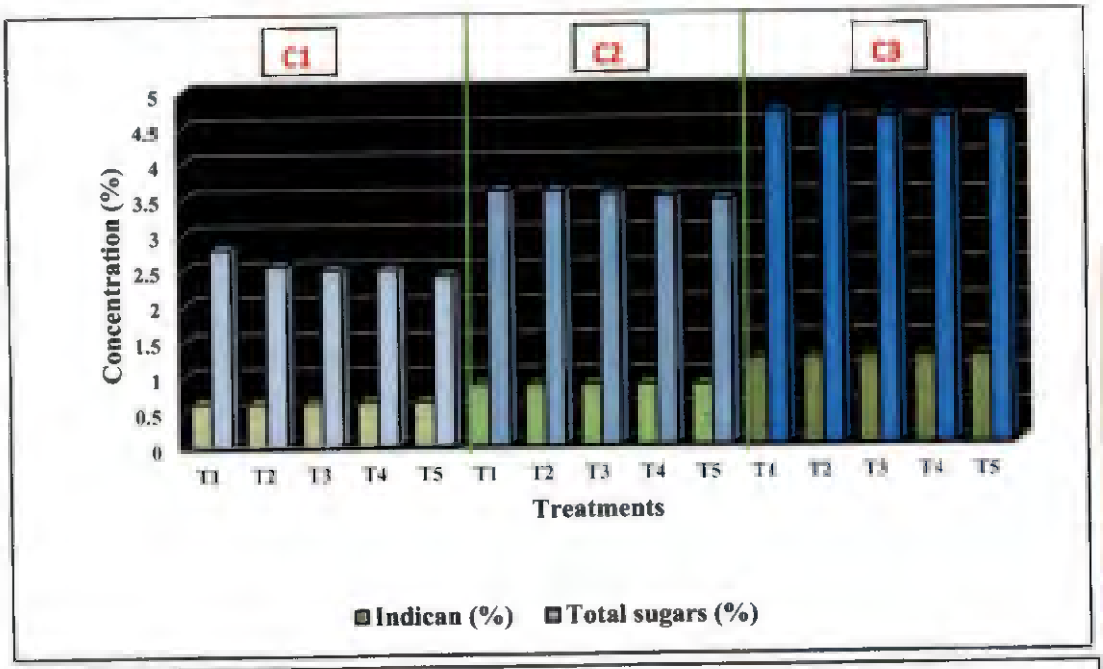


Fig4. Effect of cutting stage and storage time on quality of *Neelamari* leaves

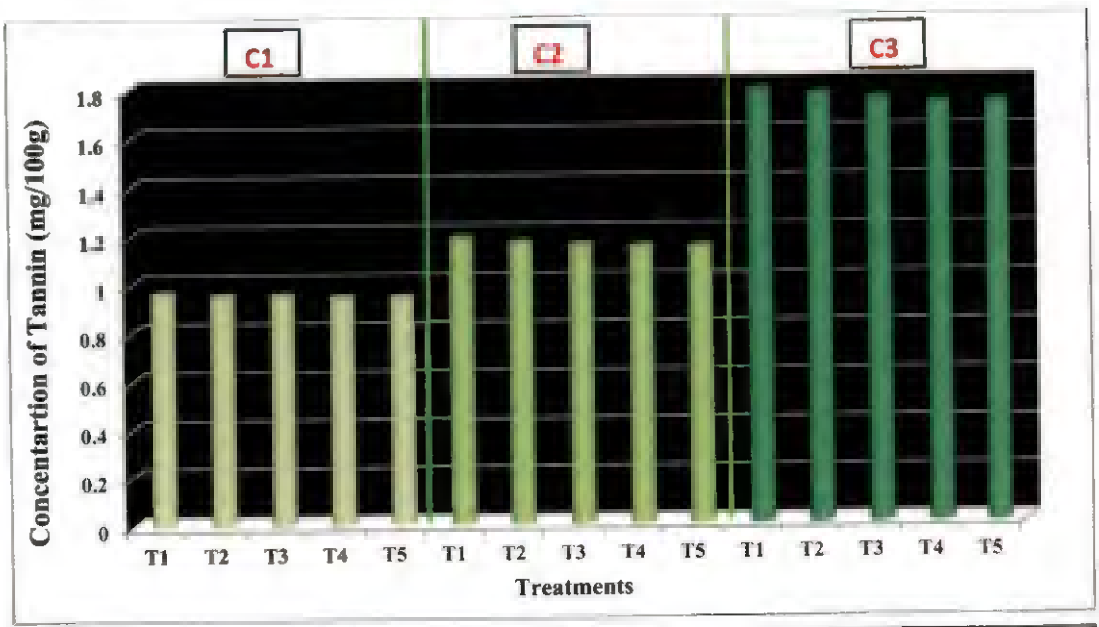


Fig4a. Effect of cutting stage and storage time on quality of *Neelamari*

C_1- Leaves harvested at 70 days after transplanting

C_2- Leaves harvested at 115 days after transplanting

C_3- Leaves harvested at 160 days after transplanting

T_1: Freshly harvested

T_2: Harvested and kept for 4 hours after harvest

T_3: Harvested and kept for 8 hours after harvest

T_4: Harvested and kept for 24 hours after harvest

T_5: Harvested and kept for 48 hours after harvest

5.2.1 Change in indican content of leaf due to blanching and drying treatments

A combination of steam, hot water and microwave blanching coupled with shade, cabinet and vacuum drying was done and leaf samples were analysed for indican content in order to find the best treatment. Blanching reduces the enzymatic action inside the cells thereby decreasing the chemical degradation. The result showed significant difference between the treatments. The leaf sample blanched with microwave and dried in vacuum dryer had highest (3.045%) indican content and least (2.328%) was in sample dried in cabinet drier after hot water blanching (Fig. 5). The highest indican content in microwave blanched and vacuum dried sample is due to the blanching and drying occurring at lower temperature and shorter duration. In case of microwave blanching done for 45 seconds indican content had lesser deleterious effect. In vacuum drier, drying occurred at a lower temperature due to high pressure which retained the indican content comparatively more than other drying treatments.

Investigation on drying time after microwave blanching (300-1200W) was done by Von and Kartnig (1999) on *Althaea sp.* and *Crataegus sp.* Results revealed that microwave reduced the drying time thus contributing to the improved appearance and product quality with causing much damage to phyto-chemical profile of the commodities. Comparison of effects of air drying and vacuum drying on the leaves of *Ocimum basilicum* was studied by Yousif *et al.* (1999). While comparing they found that vacuum dried samples yielded 2.5 time linalool and 1.5 times methyl chavicol content more than that of hot air dried samples and the chemical reactions occurred during drying yielded more volatiles than that of fresh herb. Vacuum microwave dried leaves had better green hue than hot air dried leaves and higher potential for rehydration too. Scanning in electron microscope revealed that hot air drying caused the dramatic and pronounced collapse of cell structure and other attributes of samples in hot air dried herb. Vacuum microwave drying was reported to be superior to air drying at 70°C for

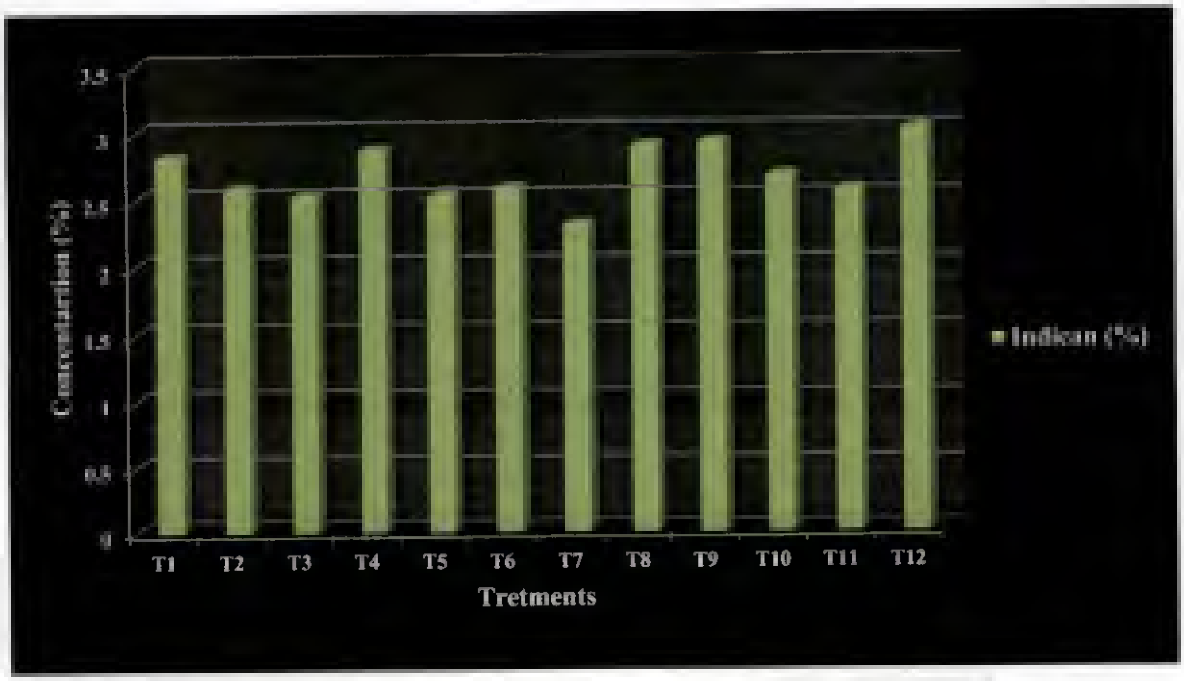


Fig5. Effect of drying with pre-treatment on indican

T_1- Unblanched+ Shade dried

T_2- Steam blanched + Shade dried

T_3- Hot water blanched+ Shade dried

T_4- Microwave blanched + Shade dried

T_5- Unblanched+ Dried in cabinet drier

T_6- Steam blanched+ Dried in cabinet drier

T_7- Hot water blanched+ Dried in cabinet drier

T_8- Microwave blanched+ Dried in cabinet drier

T_9- Unblanched+ Dried in vacuum drier

T_{10}- Steam blanched+ Dried in vacuum drier

T_{11}- Hot water blanched+ Dried in vacuum drier

T_{12}- Microwave blanched+ Dried in vacuum drier

drying roots of *Echinancea purpurea* while its leaves were preferably dried by air drying at 50°C (Hyunock *et al.*, 2000).

Sujatha (2002) conducted studies in *Adathoda*, and found that vasicine the medically active compound in the plant, was retained in higher quantity when dried under sun. Microwave blanching is more effective in retaining the selected water-soluble vitamins with the exception of ascorbic acid in vegetables (Osinboyego *et al.*, 2003). Turkmen *et al.* (2005) found that in both water-blanched and steam-blanched samples, of selected green vegetables disruption of tissues was noted but no clear difference between the methods could be seen. The fractures took place mainly inter-cellularly, meaning that individual cells remained intact

According to Lim and Murtijaya (2007) aqueous extract of microwave dried sample of *Phyllanthus amarus* exhibited the significantly stronger anti-oxidant activity and higher total phenolics content due to greater solubility of compounds break down of cellular constituents and also hydrolysis of tannins will occur. Galoburda *et al.* (2015) have reported that, microwave vacuum dried product resulted in significantly superior samples of *Cantharellus cibarius* with highest total phenols, protein content, better hyphal length and width, best organoleptic characters and highest keeping quality than other methods.

It was inferred that blanching *Coleus* leaves at 540W enabled the leaves to retain maximum therapeutic as well as sensory characteristics (Dwivedy and Rayaguru, 2012). Microwave blanching may be a better option for pre-treatment before drying of leaves as it adds flatness to the shape of the dried leaves. Microwave blanching has shorter blanching time requirement. Further, it is a dry blanching method because of which the handling of leaves becomes easy during subsequent processing operations.

5.2.2 Effect of blanching and drying on oil quality

As *"Neelibhrngadi Taila"* is an important *Ayurvedic* formulation containing *Indigofera* as a major ingredient, influence of pre-treatment and drying can be studied by preparing the oil formulations out of all the dried samples made into decoction according to the description given in *Ayurvedic* Pharmacopeia of India (API). Cooking with *qwatha* the decoction, showed increase in the amount of herbal components dissolved in oils as compared to cooking with *svarasas* and *kalka* alone (Lahorkar *et al.*, 2009). After preparing oils out of 12 dried sample oil quality parameters were analysed and check the quality standards by referring API.

5.2.2.1 *Acid value*

Acid value otherwise known as "neutralization number", "acid number" or "acidity" is the mass of potassium hydroxide (KOH) in milligrams which is required to neutralize one gram of oil sample. It is a measure of the amount of carboxylic acid groups in an oil formulation such as a fatty acid or oil-fats which causes rancidity. Increase in acid number is due to the conversion of triglycerides into fatty acids and glycerol (Rai, 2015).

Acid value is a factor describing the quality of *Ayurvedic* formulation which will also be influenced by base oil. In this study coconut oil was used as base and samples were tested for acid value. The least acid value of 1.460 mg KOH g^{-1} and highest acid value of 2.260 mg KOH g^{-1} were in oil prepared out of microwave blanched vacuum dried and hot water blanched cabinet dried samples, respectively (Fig. 6). Difference in the acid value is due to the presence of free fatty acids at different levels which decides the shelf life oil as it is the factor causing rancidity. So higher the acid value more will be the chances of rancidity (Padmakiran *et al.*, 2013)

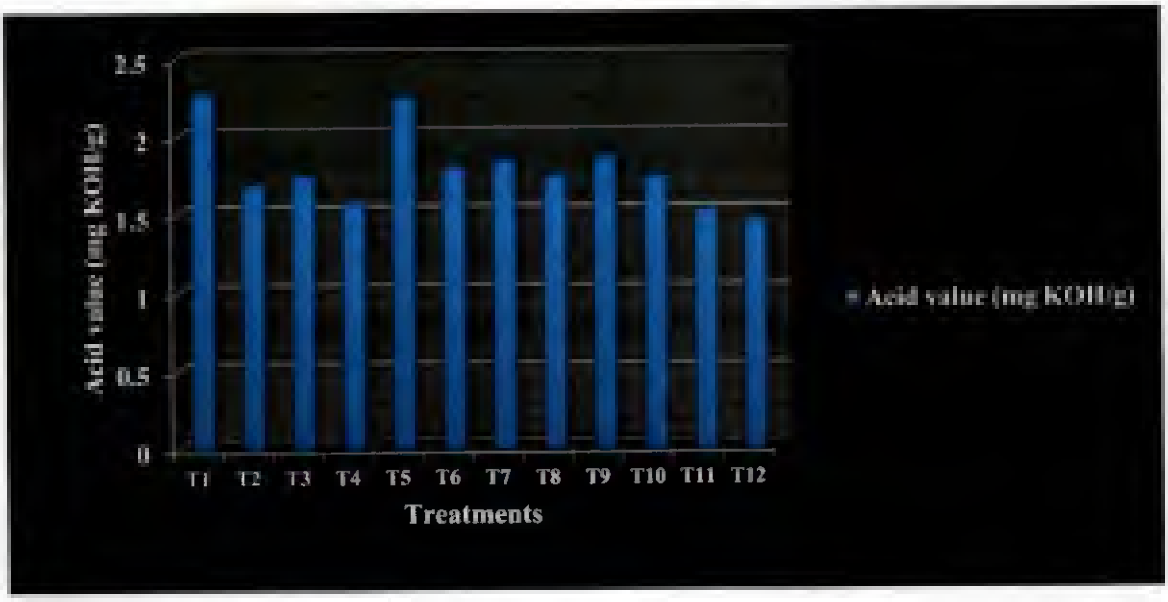


Fig6. Effect of drying with pre-treatments on acid value of the *Neelibhrngadi* oil

T_1-Unblanched+ Shade dried

T_2: Steam blanched + Shade dried

T_3: Hot water blanched+ Shade dried

T_4: Microwave blanched + Shade dried

T_5: Unblanched+ Dried in cabinet drier

T_6: Steam blanched+ Dried in cabinet drier

T_7: Hot water blanched+ Dried in cabinet drier

T_8: Microwave blanched+ Dried in cabinet drier

T_9: Unblanched+ Dried in vacuum drier

T_{10}: Steam blanched+ Dried in vacuum drier

T_{11}: Hot water blanched+ Dried in vacuum drier

T_{12}: Microwave blanched+ Dried in vacuum drier

Hepsibah *et al.* (1998) conducted a study on *Karpooradi Tailam* containing *Trachyspermum ammi* and *Cinnamomum camphora* in comparison to its base coconut oil and estimated acid value as 2.95 and 1.67 mg KOH g^{-1} of formulation and base oil respectively. Joshi and Dyawarkonda (2017) stated that a hair oil formulation prepared using coconut oil with ingredients like *Aonla, Bhringaraja, Hibiscus,* milk, Aloe, *Triphala,* Indian liquorice and grated coconut along with coconut water indicated an acid value of 2.97mg KOH g^{-1}. Acid value in *Tila Taila* based formulations namely *Krishna Tila Taila, Murcchita Tila Taila* and *Shadbindhu,* various medicated oils were reported as 0.545, 0.670 and 0.658 mg KOH g^{-1} respectively (Shailajan *et al.,* 2017).

5.2.2.2 *Refractive index*

Refractive index of a medium is a measure for how much the speed of light is reduced inside the medium, determined by the ratio of the velocity of light in a vacuum to its velocity in the sample taken. Refractive index is a physical property which confirms the purity of oil, by measuring its concentration. In any oil formulation, the refractive index can be used to determine its constituents (Rai, 2015).

Analysis of oil samples revealed that there was no significant difference between the samples in refractive index. Refractive index of the samples varied from 1.451-1.455 which conforms to the standard specified by API.

Jain *et al.* (2015) carried out a comparative study between the poly herbal formulations and base oil and they found that refractive index of *Varnashodhanahara taila* was 1.468 and *Doorvadi taila* was 1.471 in comparison with *Tila Taila* which was 1.469.

Jain *et al.* (2016) reported that poly herbal oil prepared using *Emblica officinalis, Bacopa monnieri* and *Cyprus rotundus* as ingredients at different proportions with coconut oil as base reported refractive index ranging from 1.498-1.361. Increase in the herbal ingredients concentration was associated with lowering of refractive index.

5.2.2.3 *Thin Layer Chromatography (TLC)*

TLC is a simple and less expensive procedure that gives information on the components in a mixture. It is also used to support the identity of a compound in a mixture by comparing the Rf of the compound with the Rf of a known compound. All oil samples showed standard Rf value specified by API, except samples from treatments T_{10} (Steam blanched and vacuum dried) and T_{11} (Hot water blanching and vacuum dried).

HPTLC of all the 12 treatments along with the treatment using fresh expressed juice of actual *Neelamari* leaves treated as absolute control was also done. Absolute control was found to have 16 peaks and the sample prepared with microwave blanched and vacuum dried leaf decoction had highest number of peaks (17). The quality of the poly herbal oils has been investigated using these technologies by various other research workers (Fig. 7-7m).

Varnashodhana taila and *Doorvadi Taila* were prepared based on *Ayurvedic* literatures standards and TLC was conducted to check the quality standard. The Unsaponifiable matter of base oil in comparison with medicated oils were subjected to TLC to ascertain whether any phytochemical have been transferred into the medicated oil or not.TLC plates were visualized for bands by naked eye, under UV light (254 nm) and exposing to iodine vapors at the end of the process. The spot of the medicated oil were found to be identical with those of sesame oil indicating no undesired changes in quality (Jain *et al.*, 2015).

Rai (2015) opinioned that to analyse a complex mixture like herbal formulations TLC is a simple, quick and less expensive method that gives an idea about the number of bioactive ingredients got extracted to the base used from the herbal drugs of the composition. By comparing the Rf values obtained we can also possibly detect the active principle by comparing against known standards which is a retention factor depends on the travel of the compound on TLC plate with specified mobile phase.

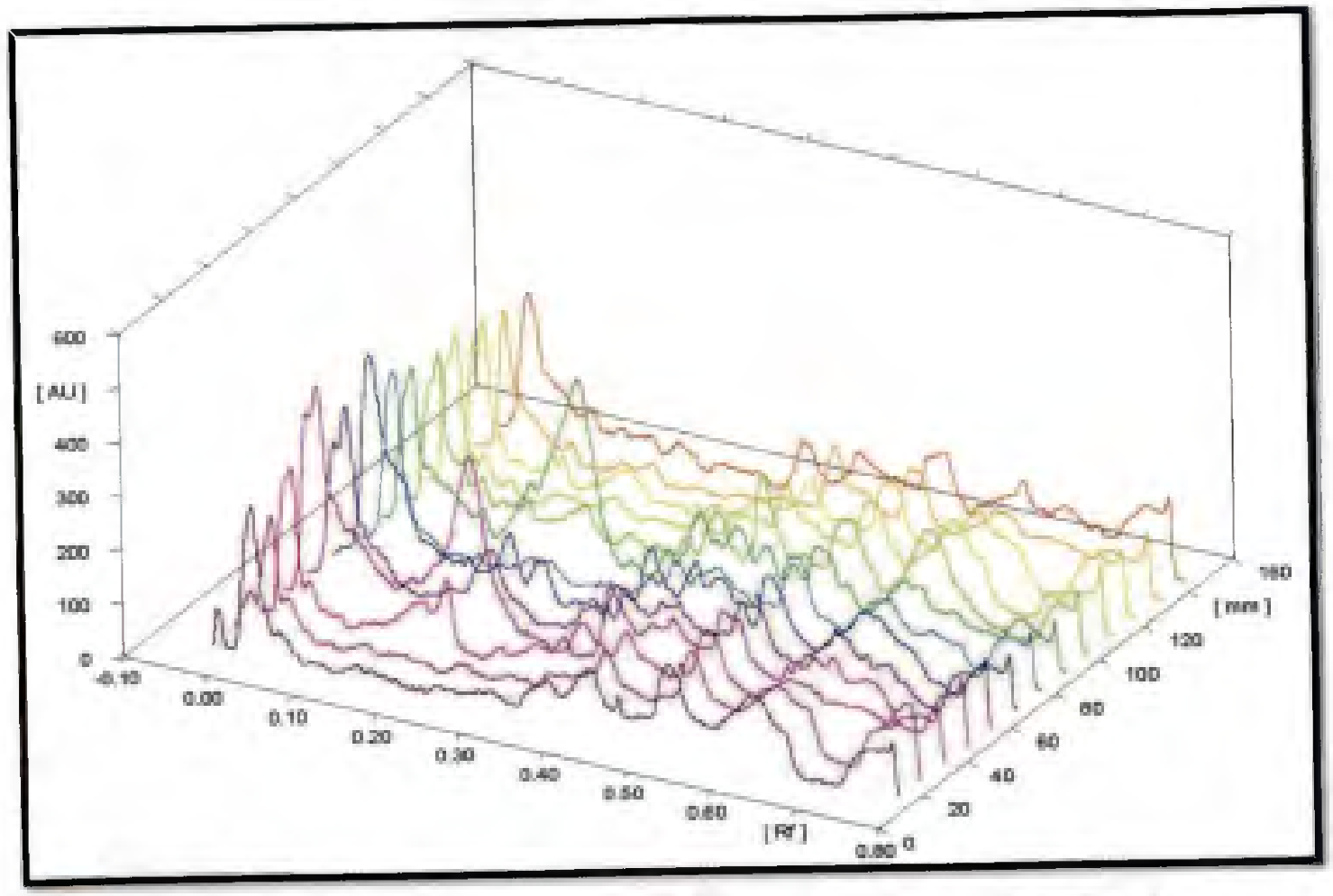
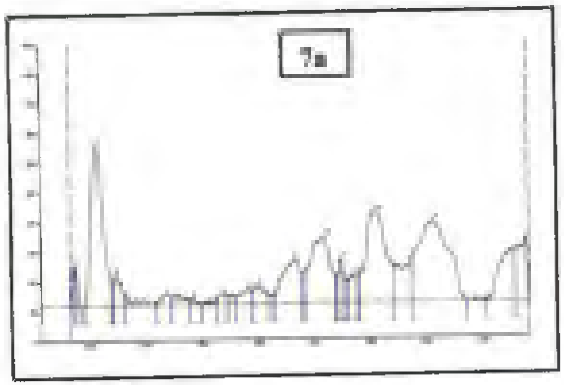
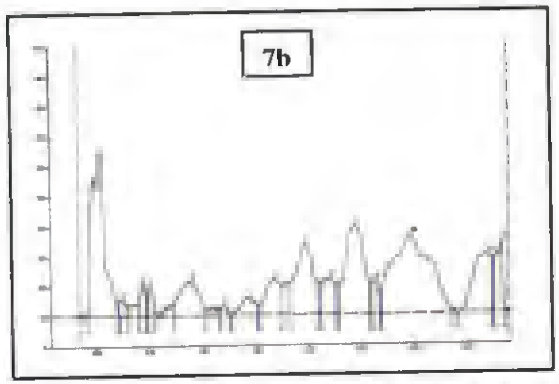


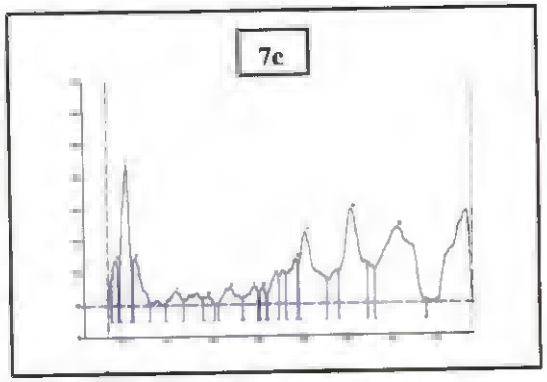
Fig7- 7m. HPTLC peaks of *Neelibhrngadi Taila*



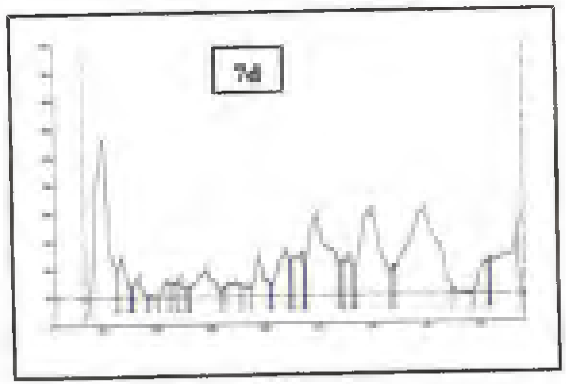
T_1 –Unblanched + Shade dried



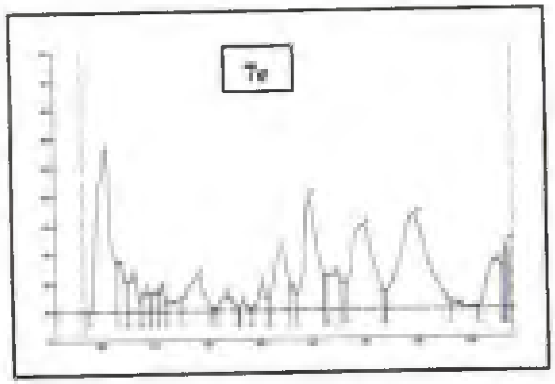
T_2–Steam blanched + Shade dried



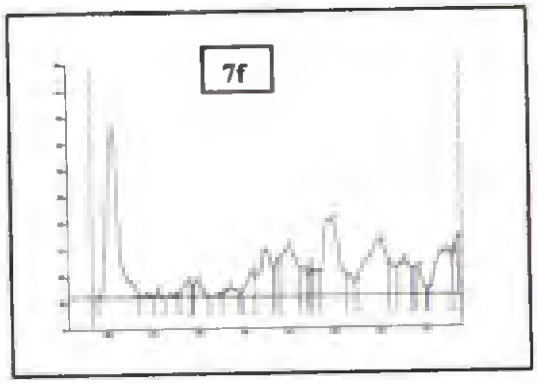
T_3–Hot water blanched + Shade dried



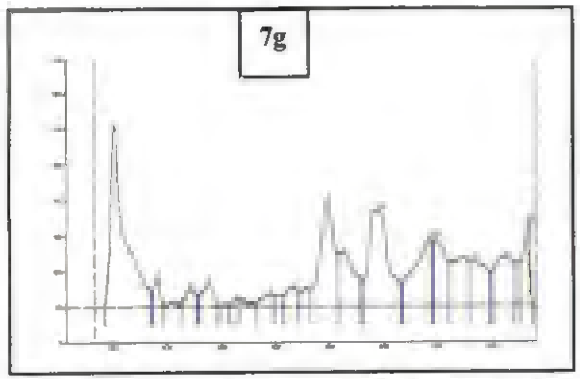
T_4–Microwave blanched + Shade dried



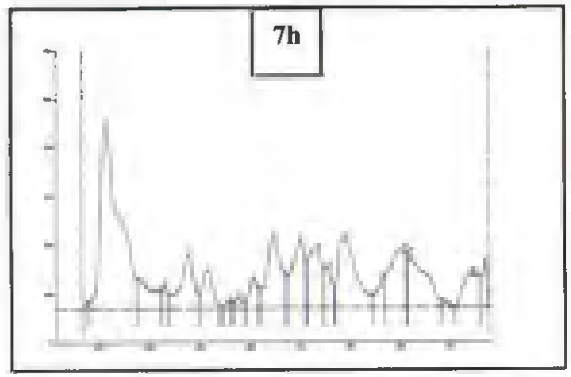
T_5–Unblanched + Dried in cabinet drier



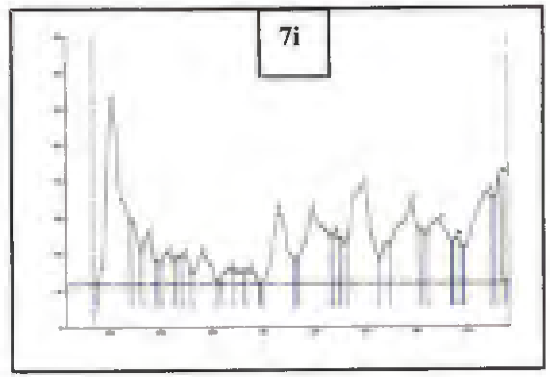
T_6 – Steam blanched + Dried in cabinet drier



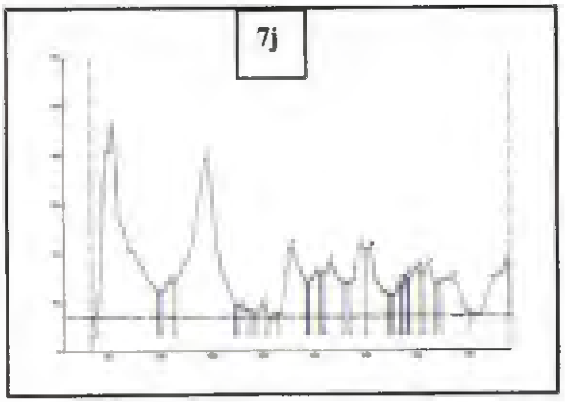
T_7 –Hot water blanched + Dried in cabinet drier



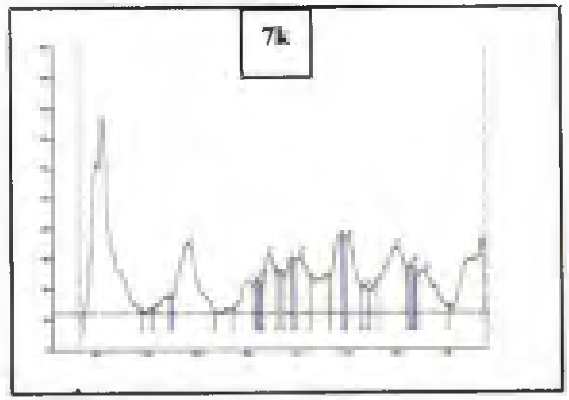
T_8 –Micro wave blanched + Dried in cabinet drier



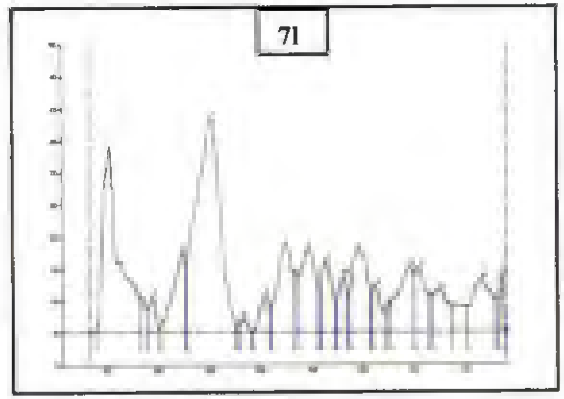
T_9 – Unblanched + Vacuum dried



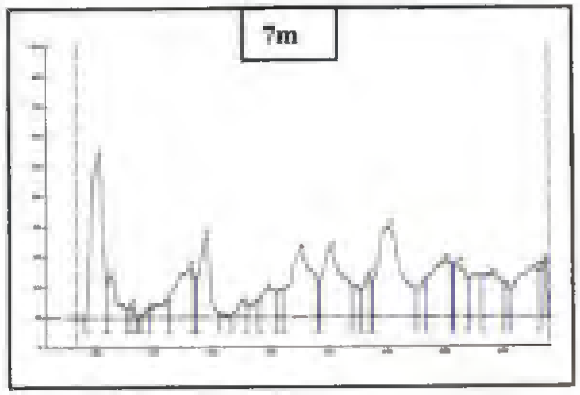
T_{10} – Steam blanched + Vacuum dried



T_{11} – Hot water blanched + Vacuum dried



T_{12} –Microwave blanched + Vacuum dried



Absolute Control

Summary

6. SUMMARY

Postharvest studies in *Neelamari* (*Indigofera tinctoria* L.) was carried out in the Department of Processing Technology, College of Horticulture, Vellanikkara, during 2014-2016. The main objectives were to standardize the best stage of cutting and storage time and to study the effect of pre treatment and drying methods on the quality of leaves. *Neelibhrngadhi Taila* prepared by using dried leaf samples and oil quality was also assessed.

Cutting stage at 160 days after transplanting (C_3) was found to be best as it recorded significantly higher fresh yield (48.050g plant^{-1}), relative water content (67.95%), indican (1.230%), total sugar (4.620%) and tannin content (1.744 mg g^{-1}). The study conducted on storage time revealed that there was no significant difference between fresh leaves and leaves kept for 4, 8, 24 and 48 hours after harvest. But indican content was significantly superior in freshly harvested leaves. Studies on interaction between cutting stage and storing time recorded the highest content of phytochemicals harvested fresh at 160 days after transplanting.

The best stage (C_3) chosen was subjected to various pre treatments and drying methods. Leaf sample treated with microwave blanching and vacuum drying reported best quality with highest indican content (3.045%). *Neelibhrngadi Taila* prepared out of these samples along with absolute control were analysed for acid value and refractive index. Least acid value (1.460 mg KOH g^{-1}) was recorded in the oil sample prepared using microwave blanching and vacuum dried leaves but there was no significant difference between refractive indices of the oil samples. All the samples prepared were conforming to Ayurvedic Pharmacopeia of India (API) standards for acid value and refractive index. TLC was also performed for all these samples and Rf values obtained were compared with the standard Rf values prescribed by API.

HPTLC was performed for better documentation of results. The number of peaks obtained for the oil samples prepared from various treatments through HPTLC was also comparable to the peaks for oil sample prepared by using standard procedure. TLC showed that oil prepared from leaf samples giving various treatments showed standard Rf value specified by API, except samples from treatments T_{10} (Steam blanched and vacuum dried) and T_{11} (Hot water blanched and vacuum dried).

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Appendices

APPENDIX I

NĪLĪBHṚṄGĀDI TAILA

(AFI Part I, 8:26)

Definition:

Nīlībhṛṅgādi Taila is a medicated oil preparation made with the ingredients in the Formulation Composition given below with mūrcchita Tila taila as the basic ingredient.

Formulation Composition:

1	Nīlī Rasa (Nīlī API)	*Indigofera tinctoria*	Lf. juice	768 ml
2	Bhṛṅga Rasa (Bhṛṅgarāja API)	*Eclipta alba*	Pl. juice	768 ml
3	Śatakratu latā Rasa (Kākatiktā API)	*Cardiospermum halicacabum*	Pl. juice	768 ml
4	Dhātrī Phala Rasa (Āmalakī API)	*Emblica officinalis*	P. juice	768 ml
5	Ājaka Kṣīra (Ajādugdha (API))	Goat milk		768 ml
6	Nālikera Kṣīra (Nārikela (API))	*Cocos nucifera*	Milk from End.	768 ml
7	Mahiṣī Kṣīra (Mahiṣīdugdha (API))	Buffalo milk	–	768 ml
8	Dhenūdbhava (Godugdha (API))	Cow milk	–	768 ml
9	Taila (Tila Taila API)	*Sesamum indicum*	Sd. oil	768 ml
10	Yaṣṭyāhva (Yaṣṭī API)	*Glycyrrhiza glabra*	Rt.	32 g
11	Guñjā API	*Abrus precatorius*	Rt.	32 g
12	Añjana (Rasāñjana (API))	*Berberis sps.*	Ext.	32 g

Method of preparation:

- Take all ingredients of pharmacopoeial quality.
- Treat *Tila taila* to prepare *mūrcchita Tila taila* (Appendix 6.2.9.4).
- Take fresh *Nīlī, Bhṛṅgarāja, Kākatiktā* and *Āmalakī* and wash thoroughly with water. Grind and filter separately through *muslin cloth* to obtain *svarasa*.
- Wash and clean the cut pieces of Nārikela, grind and squeeze through *muslin cloth* to obtain *Nārikela Kṣīra*.
- Strain the *Ajādugdha, Mahiṣīdugdha* and *Godugdha* separately through *muslin cloth*.
- Wash, clean, dry the ingredients numbered 10 to 12 *(kalka dravya)* of the Formulation Composition, powder separately and pass through 180 μm IS Sieve (sieve number 85) to obtain fine powder. Transfer the powdered ingredients to wet grinder and grind with sufficient quantity of water to prepare homogeneous blend *(kalka)*.
- Take *mūrcchita Tila taila* in a Stainless steel vessel and heat it.
- Add increments of *kalka*, stir thoroughly while adding *svarasa*.
- Heat for 3 h with constant stirring, maintaining the temperature between 50^0 and 90^0 during the first hour of heating. Stop heating and allow to stand overnight.
- Next day, add *Nārikela Kṣīra, Ajādugdha, Mahiṣīdugdha* and *Godugdha* and continue the process of heating intermittently over a period of three days. Constantly check the *kalka* by rolling between the fingers.

- Stop the heating when the *kalka* breaks down into pieces on attempting to form a *varti* (*khara pāka lakṣaṇa*) and at the appearance of froth (*phenodgama*) over the oil. Expose the *kalka* and oil to flame and confirm the absence of crackling sound indicating absence of moisture.
- Filter while hot (about 80°) through a *muslin cloth* and allow to cool.
- Store it in glass containers and pack them air-tight to protect from light and moisture.

Description:

Medicated oil, dark brown in colour, odour characteristic of sesame oil

Identification:

Thin layer chromatography:

Shake 2 ml of the formulation with 20 ml of *alcohol* for 3 h. Allow the two layers to separate. Separate the alcohol layer, filter and concentrate to 5 ml and carry out the thin layer chromatography. Apply 10 µl of the extract on TLC plate and develop the plate to a distance of 8 cm using *toluene*: *ethyl acetate*: *n-hexane* (6: 3: 1) as mobile phase. After development, allow the plate to dry in air. Spray the plate with *ethanol-sulphuric acid reagent* followed by heating at 105° for about 10 min. It shows major spots at R_f 0.18, 0.23, 0.35, 0.45 and 0.82 (all brown) in visible light.

Physico-chemical parameters:

Refractive index at 40°:	1.4510 to 1.4620,	Appendix 3.1
Specific gravity at 40°:	0.905 to 0.928,	Appendix 3.2
Saponification value:	194 to 212,	Appendix 3.7
Iodine value:	80 to 90,	Appendix 3.8
Acid value:	Not more than 5,	Appendix 3.9
Peroxide value:	Not more than 4,	Appendix 3.10

Other requirements:

Microbial limits:	Complies with Appendix 2.4
Aflatoxins:	Complies with Appendix 2.6
Mineral oil:	Absent, Appendix 3.12

Storage:

Store in a cool place in tightly closed containers, protected from light and moisture.

Therapeutic uses:

Keśapāta (hair fall) and Palita (premature greying of hair)

Dose:

External application for Śiro-abhyaṅga

APPENDIX II

Rf VALUES OF *NEELIBHRNGADI TAILA* SAMPLES

Sample 1- Unblanched and shade dried

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.03	69.1	-0.03	69.2	5.79	-0.02	0.3	410.5	1.21	unknown *
2	-0.01	1.0	0.01	274.0	22.93	0.04	42.5	6051.2	17.90	unknown *
3	0.04	43.5	0.05	52.9	4.42	0.06	17.5	784.5	2.32	unknown *
4	0.12	5.3	0.14	21.2	1.78	0.15	11.7	348.5	1.03	unknown *
5	0.18	5.8	0.18	12.3	1.03	0.20	1.0	103.5	0.31	unknown *
6	0.22	7.9	0.24	17.2	1.44	0.24	13.1	270.1	0.80	unknown *
7	0.26	12.1	0.28	27.4	2.29	0.29	22.7	575.3	1.70	unknown *
8	0.29	23.8	0.30	31.3	2.62	0.32	15.6	682.3	2.02	unknown *
9	0.33	16.9	0.37	76.3	6.39	0.38	51.7	2496.9	7.39	unknown *
10	0.38	50.9	0.42	113.7	9.51	0.44	47.2	4358.0	12.89	unknown *
11	0.44	47.3	0.45	74.5	6.24	0.46	39.4	713.7	2.11	unknown *
12	0.46	37.8	0.47	48.8	4.08	0.48	48.0	752.1	2.23	unknown *
13	0.48	47.7	0.51	153.2	12.82	0.54	62.1	5915.6	17.50	unknown *
14	0.58	75.8	0.61	137.6	11.52	0.67	0.1	7844.6	23.21	unknown *
15	0.71	1.1	0.76	85.4	7.15	0.76	84.6	2492.0	7.37	unknown *

Sample 2- Hot water blanched and shade dried

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.03	38.7	-0.02	70.7	7.28	-0.01	51.0	978.0	3.40	unknown *
2	-0.01	52.0	0.01	221.2	22.80	0.02	65.6	3891.2	13.54	unknown *
3	0.02	65.7	0.03	74.0	7.63	0.06	1.0	1427.8	4.97	unknown *
4	0.09	0.6	0.12	21.3	2.20	0.14	7.5	486.1	1.69	unknown *
5	0.18	10.7	0.19	12.6	1.30	0.21	0.0	222.6	0.77	unknown *
6	0.21	0.6	0.24	25.8	2.66	0.27	9.2	768.7	2.68	unknown *
7	0.27	9.3	0.29	26.8	2.76	0.30	20.0	630.6	2.19	unknown *
8	0.30	19.9	0.31	26.2	2.70	0.32	14.5	325.0	1.13	unknown *
9	0.32	14.8	0.34	45.1	4.65	0.35	43.7	856.4	2.98	unknown *
10	0.36	45.9	0.39	70.1	7.23	0.39	66.1	1423.3	4.95	unknown *
11	0.39	64.5	0.41	111.9	11.53	0.45	37.7	3907.6	13.60	unknown *
12	0.48	50.5	0.51	146.9	15.14	0.55	60.1	5629.1	19.59	unknown *
13	0.56	52.3	0.61	117.7	12.13	0.68	3.4	8187.6	28.49	unknown *

Sample 3- Absolute control

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	1.5	0.01	273.7	18.90	0.02	55.8	5739.5	13.78	unknown *
2	0.02	56.4	0.03	69.3	4.79	0.05	15.2	1172.6	2.81	unknown *
3	0.06	15.5	0.06	21.2	1.47	0.07	0.3	193.8	0.47	unknown *
4	0.08	0.6	0.09	17.0	1.17	0.09	16.7	164.8	0.40	unknown *
5	0.13	31.3	0.16	83.9	5.79	0.17	61.8	2678.7	6.43	unknown *
6	0.17	62.1	0.19	133.0	9.19	0.21	5.2	2572.1	6.17	unknown *
7	0.23	0.5	0.25	30.3	2.09	0.26	19.9	475.8	1.14	unknown *
8	0.28	28.7	0.29	47.2	3.26	0.31	42.8	1175.7	2.82	unknown *
9	0.32	47.2	0.35	108.8	7.51	0.38	59.6	4430.4	10.63	unknown *
10	0.38	60.1	0.40	111.3	7.69	0.44	53.4	4195.0	10.07	unknown *
11	0.45	43.5	0.47	72.7	5.02	0.47	56.5	1074.3	2.58	unknown *
12	0.48	58.6	0.50	149.1	10.30	0.55	47.2	6612.4	15.87	unknown *
13	0.57	62.0	0.60	92.3	6.37	0.62	85.3	3565.8	8.56	unknown *
14	0.62	85.4	0.62	88.3	6.10	0.64	63.8	1937.9	4.65	unknown *
15	0.67	66.5	0.68	69.6	4.80	0.70	57.2	2423.8	5.82	unknown *
16	0.71	44.2	0.76	80.2	5.54	0.77	74.7	3252.4	7.81	unknown *

Sample 4- Steam blanched and dried in vacuum drier

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.03	1.3	0.01	392.1	20.28	0.10	48.9	18487.8	28.93	unknown *
2	0.10	49.3	0.12	76.1	3.93	0.13	67.7	1685.6	2.64	unknown *
3	0.13	68.3	0.19	325.6	16.84	0.25	21.4	16666.5	26.08	unknown *
4	0.25	21.3	0.26	26.3	1.36	0.27	11.3	420.9	0.66	unknown *
5	0.29	0.1	0.30	32.2	1.66	0.31	1.0	343.9	0.54	unknown *
6	0.33	6.3	0.36	145.6	7.53	0.39	66.2	4562.9	7.14	unknown *
7	0.39	66.4	0.41	92.0	4.76	0.41	86.8	1979.8	3.10	unknown *
8	0.42	86.9	0.43	111.3	5.76	0.46	66.0	3868.8	6.05	unknown *
9	0.47	70.3	0.49	148.2	7.67	0.50	135.3	3436.4	5.38	unknown *
10	0.50	135.5	0.51	139.7	7.23	0.55	45.7	3435.6	5.38	unknown *
11	0.55	43.6	0.57	73.3	3.79	0.57	60.5	957.1	1.50	unknown *
12	0.57	61.0	0.58	85.6	4.43	0.58	72.6	882.0	1.38	unknown *
13	0.59	73.8	0.60	99.8	5.16	0.61	85.3	2127.7	3.33	unknown *
14	0.61	85.7	0.62	106.8	5.52	0.64	66.7	2243.8	3.51	unknown *
15	0.65	70.4	0.67	78.6	4.07	0.70	8.7	2802.6	4.39	unknown *

Sample 5- Hot water blanched and dried in vacuum drier

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.03	0.8	0.01	322.9	22.29	0.09	3.9	11760.9	28.80	unknown *
2	0.11	6.9	0.15	28.0	1.93	0.15	25.0	689.8	1.69	unknown *
3	0.15	25.6	0.19	117.7	8.13	0.24	0.2	4635.2	11.35	unknown *
4	0.27	4.7	0.31	56.7	3.91	0.32	43.6	1571.8	3.85	unknown *
5	0.32	44.5	0.32	61.4	4.24	0.33	40.4	546.6	1.34	unknown *
6	0.33	41.6	0.34	102.0	7.04	0.36	69.5	2338.8	5.73	unknown *
7	0.37	61.5	0.39	92.9	6.42	0.39	85.8	1613.2	3.95	unknown *
8	0.39	86.3	0.41	102.7	7.09	0.43	59.9	2730.1	6.69	unknown *
9	0.47	61.2	0.49	127.1	8.78	0.49	125.9	2767.4	6.78	unknown *
10	0.50	126.0	0.50	126.7	8.75	0.53	42.6	3117.0	7.63	unknown *
11	0.53	42.9	0.54	45.3	3.13	0.55	34.5	537.2	1.32	unknown *
12	0.56	54.8	0.60	111.0	7.66	0.63	70.7	4974.9	12.18	unknown *
13	0.63	71.4	0.63	81.8	5.65	0.64	57.9	865.6	2.12	unknown *
14	0.64	58.2	0.66	72.1	4.98	0.70	11.3	2688.2	6.58	unknown *

Sample 6- Microwave blanched and dried in cabinet drier

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	14.3	0.01	386.0	23.25	0.08	61.6	15399.8	30.30	unknown *
2	0.13	33.3	0.13	50.6	3.05	0.14	25.7	580.4	1.14	unknown *
3	0.14	26.0	0.18	111.5	6.72	0.20	24.9	3054.1	6.01	unknown *
4	0.20	27.0	0.22	78.3	4.72	0.24	0.3	1781.7	3.51	unknown *
5	0.24	1.0	0.26	13.8	0.83	0.26	8.5	153.3	0.30	unknown *
6	0.27	15.1	0.28	24.6	1.48	0.29	10.8	353.5	0.70	unknown *
7	0.29	11.4	0.31	61.3	3.69	0.32	45.2	1071.2	2.11	unknown *
8	0.32	38.1	0.35	145.2	8.74	0.37	74.8	4043.4	7.96	unknown *
9	0.38	64.9	0.40	139.8	8.42	0.41	96.2	3606.0	7.10	unknown *
10	0.41	96.6	0.43	118.3	7.13	0.45	81.0	3490.3	6.87	unknown *
11	0.45	81.4	0.45	84.3	5.08	0.47	44.1	1329.8	2.62	unknown *
12	0.47	44.8	0.49	144.1	8.68	0.55	24.8	5470.8	10.77	unknown *
13	0.57	63.7	0.60	119.1	7.18	0.61	107.7	3876.0	7.63	unknown *
14	0.62	108.4	0.62	112.3	6.77	0.68	16.2	4301.1	8.46	unknown *
15	0.71	0.4	0.74	70.9	4.27	0.76	60.4	2307.5	4.54	unknown *

Sample 7- Steam blanched and dried in cabinet drier

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	0.2	0.01	321 3	25.24	0 07	3.4	8402.0	24.28	unknown *
2	0.10	0.7	0.11	16.4	1.29	0.13	1.2	128.9	0.37	unknown *
3	0.16	8.5	0.18	30.0	2.36	0 19	22.9	590.2	1.71	unknown *
4	0 19	23.2	0.20	35.5	2 78	0.22	0.0	679.7	1.96	unknown *
5	0.25	4.3	0.27	19.3	1.52	0.29	9.4	481.5	1.39	unknown *
6	0.29	9.4	0.32	47.3	3.72	0.32	34.3	857.4	2.48	unknown *
7	0.33	35.1	0.35	87.5	6.88	0.37	51.2	2412.8	6.97	unknown *
8	0.38	69.9	0.40	94.9	7 45	0 42	56.6	3270.8	9.45	unknown *
9	0.44	38.8	0.45	60.7	4.76	0.45	42.6	539 3	1.56	unknown *
10	0 47	46.5	0.49	145 6	11.43	0.53	42.3	5355.9	15.47	unknown *
11	0.55	24.6	0.60	107 6	8.45	0.62	65.4	5072.7	14.66	unknown *
12	0.64	55.0	0 65	66.9	5.26	0.67	51.1	1822.3	5.26	unknown *
13	0.68	52.0	0.68	52 1	4 09	0.71	12.6	937.3	2.71	unknown *
14	0.71	13.2	0.75	84 0	6.60	0.76	79.1	3036.8	8.77	unknown *
15	0.76	79.4	0.77	104.0	8.17	0.78	0.0	1023.9	2.96	unknown *

Sample 8- Microwave blanched and dried in vacuum drier

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	0.9	0.01	292.5	14.45	0.07	57 5	10130.5	16.27	unknown *
2	0.08	38.8	0.09	58.4	2.88	0 10	8.2	936.2	1.50	unknown *
3	0.11	8.9	0.15	127.1	6.28	0.16	113.8	3286 1	5.28	unknown *
4	0.16	115.1	0.21	339.1	16.75	0.26	7.0	16759.1	26.91	unknown *
5	0.26	7.2	0.27	23.3	1.15	0.28	1.6	340.4	0.55	unknown *
6	0.29	1.8	0.31	61.5	3.04	0.32	36.3	1111.3	1.78	unknown *
7	0.32	37.9	0.35	142.1	7.02	0.37	92.2	4199.1	6.74	unknown *
8	0.38	85.3	0.39	132.6	6.55	0.42	74.2	4086.2	6.56	unknown *
9	0.42	75.6	0.43	111.5	5.51	0.45	53.9	2594.9	4.17	unknown *
10	0.45	54.0	0.46	89.1	4.40	0.47	63.6	1520.6	2.44	unknown *
11	0.47	64.6	0.49	131.0	6.47	0.52	63.6	4293.5	6.89	unknown *
12	0.52	64.7	0.52	73.9	3.65	0.54	30.3	1308.0	2.10	unknown *
13	0.56	48.3	0.59	103.2	5.10	0 60	92 9	3438.9	5.52	unknown *
14	0.60	94.1	0.61	99.9	4.94	0.63	60.3	2203.2	3.54	unknown *
15	0.64	57.6	0.65	66.5	3.28	0.68	42.8	1955.5	3.14	unknown *
16	0.71	39.0	0.73	83.0	4.10	0.76	49 9	3385.5	5.44	unknown *
17	0.76	50.0	0.77	90.2	4.45	0.78	3.5	729.5	1.17	unknown *

Sample 9- Unblanched and dried in cabinet drier

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	0.4	0.01	285.5	19.21	0.03	85.1	7418.5	17.42	unknown *
2	0.05	52.9	0.06	70.3	4.73	0.08	20.5	1116.9	2.62	unknown *
3	0.08	21.8	0.08	36.3	2.44	0.09	29.7	537.3	1.26	unknown *
4	0.11	29.3	0.12	47.4	3.19	0.13	12.3	572.1	1.34	unknown *
5	0.15	24.0	0.18	65.3	4.39	0.21	5.9	1943.0	4.56	unknown *
6	0.22	1.2	0.24	30.2	2.03	0.26	2.9	646.8	1.52	unknown *
7	0.26	3.1	0.27	21.7	1.46	0.28	3.9	248.5	0.58	unknown *
8	0.28	4.3	0.30	47.4	3.19	0.32	20.5	768.3	1.80	unknown *
9	0.32	21.4	0.34	109.6	7.37	0.36	42.5	2856.1	6.71	unknown *
10	0.37	28.0	0.40	198.1	13.33	0.43	55.7	5474.4	12.86	unknown *
11	0.43	56.0	0.45	65.1	4.38	0.46	50.4	1681.4	3.95	unknown *
12	0.47	49.6	0.50	148.2	9.97	0.54	28.2	6363.6	14.94	unknown *
13	0.54	28.6	0.60	163.7	11.01	0.66	13.3	9610.3	22.57	unknown *
14	0.72	2.5	0.75	84.7	5.70	0.76	76.8	2531.2	5.94	unknown *
15	0.76	77.4	0.77	112.9	7.60	0.78	0.0	813.4	1.91	unknown *

Sample 10- Microwave blanched and shade dried

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	1.9	0.00	284.7	20.01	0.03	48.7	7004.6	16.38	unknown *
2	0.03	49.1	0.03	70.7	4.97	0.05	18.7	1044.5	2.44	unknown *
3	0.05	18.9	0.07	35.5	2.50	0.08	2.8	542.6	1.27	unknown *
4	0.10	0.4	0.12	27.5	1.93	0.12	26.1	351.0	0.82	unknown *
5	0.14	23.5	0.14	30.6	2.15	0.16	15.7	427.1	1.00	unknown *
6	0.16	15.9	0.19	47.9	3.37	0.22	9.1	1606.0	3.75	unknown *
7	0.23	18.2	0.24	24.8	1.75	0.26	19.1	621.3	1.45	unknown *
8	0.28	18.6	0.29	68.8	4.84	0.31	22.2	1282.8	3.00	unknown *
9	0.31	22.6	0.34	76.1	5.35	0.35	63.4	1941.1	4.54	unknown *
10	0.35	64.0	0.37	68.4	4.81	0.38	56.7	1566.0	3.66	unknown *
11	0.38	58.2	0.40	143.8	10.11	0.44	60.4	5570.4	13.02	unknown *
12	0.45	59.8	0.45	64.4	4.52	0.47	52.8	1205.0	2.82	unknown *
13	0.47	52.9	0.50	146.8	10.32	0.54	39.2	6059.9	14.17	unknown *
14	0.54	49.9	0.59	149.9	10.54	0.65	8.2	8932.2	20.88	unknown *
15	0.69	2.0	0.71	56.5	3.97	0.72	55.4	1032.4	2.41	unknown *
16	0.72	55.4	0.77	126.1	8.86	0.78	0.3	3588.2	8.39	unknown *

Sample 11- Steam blanched and shade dried

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	1.2	0.01	276.8	20.73	0.04	22.8	7043.8	16.86	unknown *
2	0.04	23.9	0.05	29.7	2.23	0.06	17.3	423.3	1.01	unknown *
3	0.08	19.1	0.09	55.6	4.17	0.09	36.7	606.9	1.45	unknown *
4	0.10	38.3	0.10	54.5	4.08	0.11	6.9	550.2	1.32	unknown *
5	0.14	19.0	0.18	66.0	4.94	0.20	10.1	2223.1	5.32	unknown *
6	0.23	12.0	0.24	19.8	1.48	0.25	0.2	239.7	0.57	unknown *
7	0.26	0.1	0.29	30.6	2.29	0.31	14.5	881.0	2.11	unknown *
8	0.31	14.6	0.33	61.4	4.60	0.35	45.2	1885.1	4.51	unknown *
9	0.37	51.4	0.40	120.4	9.02	0.42	55.0	4247.1	10.17	unknown *
10	0.43	53.7	0.44	61.8	4.63	0.46	47.7	1631.9	3.91	unknown *
11	0.46	49.5	0.49	148.9	11.15	0.52	51.0	5852.8	14.01	unknown *
12	0.52	51.1	0.53	56.2	4.21	0.54	36.1	774.3	1.85	unknown *
13	0.54	36.1	0.60	132.2	9.90	0.67	4.8	10027.2	24.00	unknown *
14	0.69	0.3	0.74	100.0	7.49	0.75	90.5	3742.1	8.96	unknown *
15	0.75	90.8	0.77	121.4	9.09	0.77	0.0	1649.5	3.95	unknown *

Sample 12- Hot water blanched and dried in cabinet drier

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	0.8	0.00	258.6	21.02	0.07	21.8	7160.2	21.31	unknown *
2	0.07	22.0	0.08	41.5	3.37	0.09	1.0	540.8	1.61	unknown *
3	0.13	6.5	0.14	26.8	2.18	0.15	16.6	512.2	1.52	unknown *
4	0.16	16.5	0.18	36.7	2.98	0.19	0.1	778.3	2.32	unknown *
5	0.21	0.5	0.23	11.5	0.94	0.23	10.1	119.7	0.36	unknown *
6	0.26	1.8	0.29	18.4	1.50	0.30	15.1	389.9	1.16	unknown *
7	0.31	16.8	0.34	29.4	2.39	0.34	18.0	659.5	1.96	unknown *
8	0.37	23.5	0.40	149.5	12.15	0.42	72.1	3990.5	11.88	unknown *
9	0.42	72.9	0.43	75.4	6.13	0.46	37.8	2452.7	7.30	unknown *
10	0.46	38.2	0.49	136.7	11.11	0.53	33.4	5331.4	15.87	unknown *
11	0.54	33.5	0.59	96.3	7.82	0.59	91.5	3326.7	9.90	unknown *
12	0.59	91.8	0.60	96.9	7.87	0.62	60.3	2380.2	7.08	unknown *
13	0.66	64.6	0.67	66.3	5.39	0.70	47.2	2138.1	6.36	unknown *
14	0.70	47.3	0.72	69.2	5.63	0.74	62.7	2346.8	6.98	unknown *
15	0.75	60.7	0.77	117.4	9.54	0.78	1.0	1471.8	4.38	unknown *

Sample 13- Unblanched and dried in vacuum drier

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.03	0.3	0.00	257.2	17.59	0.04	89.9	7970.5	18.79	unknown *
2	0.06	48.3	0.08	70.4	4.81	0.09	30.2	1391.6	3.28	unknown *
3	0.10	28.0	0.11	45.8	3.13	0.13	31.1	863.8	2.04	unknown *
4	0.14	35.9	0.14	42.9	2.94	0.16	12.5	686.2	1.62	unknown *
5	0.16	12.6	0.18	44.7	3.06	0.21	5.0	1165.8	2.75	unknown *
6	0.22	1.6	0.24	22.8	1.56	0.24	16.6	404.7	0.95	unknown *
7	0.27	14.9	0.28	23.2	1.58	0.29	0.3	352.6	0.83	unknown *
8	0.30	1.3	0.33	100.8	6.89	0.36	30.3	2977.0	7.02	unknown *
9	0.37	34.3	0.40	101.4	6.93	0.43	62.7	4368.1	10.30	unknown *
10	0.43	63.9	0.44	69.6	4.76	0.45	59.5	1037.2	2.44	unknown *
11	0.46	57.3	0.50	138.1	9.44	0.53	32.9	5652.4	13.32	unknown *
12	0.55	56.9	0.59	118.0	8.07	0.61	72.8	4629.5	10.91	unknown *
13	0.62	65.6	0.64	87.8	6.00	0.67	56.0	3552.1	8.37	unknown *
14	0.67	56.3	0.68	64.9	4.43	0.69	51.9	999.5	2.36	unknown *
15	0.69	51.7	0.74	125.5	8.58	0.75	119.6	4808.1	11.33	unknown *
16	0.76	118.1	0.77	149.4	10.22	0.77	1.1	1564.6	3.69	unknown *

POSTHARVEST STUDIES IN *NEELAMARI*

(*Indigofera tinctoria* L.)

By

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ABSTRACT OF THE THESIS

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Abstract

Neelamari (*Indigofera tinctoria* L.) belonging to the family Fabaceae, is a widely used medicinal crop due to its properties. The main use of the *Neelamari* leaves is in the preparation of *Neelibhrngadi Taila* which is an *Ayurvedic* formulation known for its hair tonic property. The other important ingredients of the *Neelibhrngadi Taila* are *Amla, Eclipta alba, Cardiospermum halicacabum, etc.* The availability of *Neelamari* leaves restricted to a particular season does not always coincide with the season of *Amla*, which may result in a setback in the production of *Neelibhrngadi Taila.* In this context, the present investigation "Postharvest studies in *Neelamari* (*Indigofera tinctoria* L.)" was taken up to study the effect of harvesting stage and post-harvest storage on yield and quality of leaves. The effect of drying after subjecting to various pre-treatments on leaf and oil quality was also studied.

Neelamari leaves were harvested at 70 days after transplanting (DAT), 115 DAT and 160 DAT. At each stage of harvest, the yield and quality of fresh leaves in terms of Relative Water Content (RWC), indican (Indoxyl β- D-glucoside) the glucoside responsible for the colour, total sugar and tannin were observed. The highest yield (48.05 g^{-1}plant), was obtained when harvesting was done at 160 DAT and at this stage leaves had highest tannin (1.78mg $100g^{-1}$), total sugars (4.62%) and indican (1.23%) and it was significantly different from other treatments. At each harvesting stage, the leaves were stored for 4, 8, 24 and 48 hours and observed for its quality in comparison with fresh leaves. The freshly harvested leaves recorded higher value for indican (0.913%) but there was no significant difference in tannin (1.307mg $100g^{-1}$) and total sugars (4.70%). The interaction of cutting stage and storage showed significant effect on leaf quality as indicated by the highest indican content (1.24%) in fresh leaves harvested at 160 DAT and considering it as the best, was used for further studies.

Three blanching methods including hot water, steam and microwave blanching were compared with unblanched control, followed by shade, cabinet and vacuum drying. The treated leaves were, analysed for indican content and found that, microwave blanching for 45 seconds followed by vacuum drying at 35±5°C had highest retention of indican (3.045%).

Further *Neelibhrngadi Taila* was prepared using the decoctions of dried leaf samples, along with fresh *Neelamari* leaf juice considered as control treatment as per *Ayurvedic* Pharmacopeia of India (API). The samples of *Neelibhrngadi Taila* prepared were subjected to quality assessment by estimating acid value, refractive index and performing Thin Layer Chromatography (TLC) with absolute control as the standard, as per the recommendation of API, which defines well the quality check of the medicated oils. Least acid value (1.460mg of KOH g^{-1} of sample) was a desirable feature recorded in oil sample prepared using microwave blanched and vacuum dried leaf samples and the highest acid value recorded was in the sample prepared using hot water blanched cabinet dried leaves. There was no difference in the refractive index as coconut oil was the common base oil in all the treatments and it revealed the uniform extraction of phytochemicals in to the oil in all the 13 formulations. The results from TLC showed that all the oil samples except those obtained from treatment T_{10} (steam blanched and dried in vacuum drier) and T_{11} (Hot water blanched and dried in vacuum drier) gave standard Rf value, thus conforming to the quality prescribed by API. In HPTLC performed using absolute control as standard, all the samples recorded 13-17 peaks representing different phytochemical compounds whereas the standard oil recorded 16 peaks. Further studies are required to identify the phytochemical compounds.