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Intensive Cultivation for Root Production and Technology for Harvesting Roots of Five Medicinal Trees of *Dasamoola*

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ABSTRACT OF THE PROJECT PROPOSAL

1. Project No	:	KFRI/406/03
2. Title	:	Intensive Cultivation for Root Production and Technology for Harvesting Roots of Five Medicinal Trees of *Dasamoola*
3. Objectives	:	• To develop a technology for intensive cultivation of the medicinal trees and harvesting of roots. • To assess the phytochemicals of the roots for use in Ayurvedic Medicine Industry
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5. Date of completion	:	March 2004
6. Funding Agency	:	KFRI Plan Fund
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8. Project Assistants	:	P Subin Jose, Research Fellow CB Santhosh Kumar, Technical Assistant

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ABSTRACT

The demand for herbal medicines, particularly Ayurveda, the most prevalent herbal system in Kerala is increasing every year. The raw drugs for manufacturing Ayurvedic medicines are collected mostly from the wild. The continued exploitation of raw drugs from wild sources has resulted in their depletion. In order to meet the increasing demand, cultivation is inevitable. Traditional cultivation practices of tree species are not practical for extracting roots. The present study suggests a rapid root technology for Dasamoola which is very convenient for farmers. Among the various containers used in the study, PVC pipes of size 100 x 15 cm were found to be ideal. They have several advantages for root production. The container can be reused further. There is absolutely no loss of roots while extracting.

There is a notion that mature roots of older trees are superior and therefore preferred in the preparation of medicines. In order to assess the suitability of roots produced by rapid root production methods, comparative phytochemical analyses were carried out. The TLC profile obtained for phenolics, alkaloids and flavanoids show more or less similar pattern for the roots of young and mature *Brehetpanchamoola* trees. These findings indicate that roots of young trees are qualitatively as good as those from mature trees and can be used in the preparation of Ayurvedic medicines.

1. INTRODUCTION

The Ayurvedic medicine industry in Kerala uses about 400 raw drugs for the production of 450 medicinal preparations. The raw drugs consist of plants/parts, minerals, metals, animal products etc. Among the plants/parts used, root and rhizome constitute 42 per cent of the total consumption, followed by fruits and seeds (20 per cent); whole plants (13 per cent); leaves (7 per cent); bark (6 per cent); wood and stem 5 per cent each (Sasidharan and Muraleedharan, 2008). Among the roots, the consumption of *Brehetpanchamoola* (Pathiri, Palakapayyani, Kumizhu, Koovalam and Munja) is the highest. The annual consumption of the root of Pathiri in 2006 in Kerala is 215 tonnes; Koovalam, 355 tonnes; Kumizhu, 115 tonnes; Palakapayyani, 104 tonnes and Munja, 212 tonnes (Sasidharan and Muraleedharan, 2008). Collection of roots of trees is always destructive and leads to its death. Further, collection of root of trees is laborious and results in the loss of a substantial quantity. Uprooting trees for collecting roots is not practical. Therefore, there is scarcity for the roots of medicinal tree species. Due to the scarcity, substitution is prevalent with related or unrelated species. It is noticed that, though root is the preferred part, stem is also used. Farmers are reluctant to take up the cultivation of trees for root production because of the long duration and less remuneration as compared to other cash crops.

There is a notion that roots from older trees are superior to that of young trees. *Oushadhagrahyabhagam* (the section dealing with part of the plant to be used in medicinal preparations) of *Shargadhara samhitha* (Achutha Warrier, 1959) mentions contradicting explanations for the plant part to be used in the case of *A. marmelos* and *G. arborea*. The term *'thachombujii'* denotes root bark where as the term *'thwachonothu'* is root without bark. There is no mention about the age of the plant from which roots are to be taken. Although studies with species such as asokam (*Saraca asoca*) indicate that age of the plant does not influence the quality of the preparation. However, this cannot be applied to dasmoola trees without evidence.

Plants possess medicinal properties due to the presence of biologically active compounds like polyphenolics, terpenes, alkaloids, flavonoids etc. (Fransworth, 1984; Cragg *et al.*, 1997). Polyphenolics and flavonoids have the ability to activate free radical scavenging enzymes. Hence, analysis of the polyphenolics and flavonoid profile has great significance. It is often assumed that mature roots possess more beneficial compounds than younger roots. The present study was undertaken to look in to the possibilities of intensive cultivation of *Brehetpanchamoola* plants to facilitate their root harvesting in a convenient manner.

The project had the following two objectives.

(i) To develop a technology for intensive cultivation of the medicinal trees and harvesting of roots.

(ii) To assess the phytochemicals of the roots for use in Ayurvedic Medicine Industry.

2. MATERIALS AND METHODS

2.1. Cultivation

Cultivation of *Aegle marmelos* was first attempted in nursery beds at close spacing. Based on the results, nursery trials were carried out with and without various types of containers. Further nursery trials were conducted using rigid and flexible tubes.

Planting in nursery beds: Seedlings of *A. marmelos* were planted in nursery beds at different espacements such as 10 x 10 cm, 20 x 20 cm, 40 x 40 cm, 15 x 15 cm. Container seedlings with containers intact, after removing containers and without containers were planted in the nursery. Similarly container seedlings of *Gmelina arborea* were also planted in nursery beds at different espacement like 40 x 40 cm, 20 x 20 cm, 10 x 10 cm, 15 x 15 cm, with and without containers, 25 x 25 cm. In the case of *Stereospermum colais* the seedlings were planted in nursery beds at 25 x 25 cm spacing. The other two species (*Premna serratifolia* and *Oroxylum indicum*) were not planted in nursery beds.

Cultivation in tubular containers: Ten seedlings of each species were planted in PVC tubes 15 cm diameter and 100 cm length and filled with a potting mixture of soil, sand and dry cow dung in 1:1:1 ratio.

Figure 1. Rapid root production of Dasamoola trees

Cultivation in field: All the five species were planted in the field at 1 x 1 m spacing in 30 cm x 30cm x 30 cm size pits. There were 22 plants for each of the species.

2.2. Root Harvesting

The plants in nursery beds were excavated at 6, 12, 18, and 24 months after planting. The roots were separated and categorized into diameter classes as below 2 mm, 2-5 mm, 5-10 mm, 10-20 mm and 20-50 mm, and depth class as 0-15 cm, 15-30 cm, 30-45 cm, 45-60 cm, 60-75 cm, 75-90 cm, 90-105 cm. The plants grown in the PVC pipes were harvested at intervals of 4 and 6 months after planting by pushing the tubes aside and putting the seedling out of the tube after cutting the root at ground level. Roots were categorized into diameter classes as below 2 mm and 2-5 mm and juvenile tender roots.

Aegle marmelos

Gmelina arborea

Oroxylum indicum

Stereospermum colais

2.3. Phytochemical Studies

Roots from twelve months and twenty four months old *Aegle marmelos* plants grown on raised nursery beds at 20 x 20 cm spacing and six months old Koovalam (*Aegle marmelos*) plants grown in PVC pipes were used for the study along with root samples from 25 year old tree, growing in the medicinal garden of the institute at Peechi and from a 30 year old tree grown in a homestead.

Root samples of nine month and twenty-month-old *Gmelina arborea*, grown in nursery beds and in different container sizes at different spacing and six-month-old *Gmelina arborea* plants grown in PVC pipes were used for analysis along with the root sample collected from a 12 year old tree.

Root samples of six month-old *Oroxylum indicum* and *Stereospermum colais* plants, grown on PVC pipes were used for analysis.

The roots were separated into different diameter classes, (below 5 mm diameter and over 10 mm diameter) dried at 40-45^0 C and powdered.

Extraction of root samples

One gram of the powdered root sample was extracted with 5 ml methanol (for eighteen-month-old *Aegle marmelos* plants) and 5 ml methanol containing 1% sodium carbonate (for eighteen-month-old *Aegle marmelos* plants and three-month-old *Aegle marmelos* plants) by heating at 60^{0}C with continuous stirring for 4 days. The extracts were fractionated through thin layer chromatography (TLC) by various solvent systems and analyzed for polyphenolics, flavonoids and alkaloids profile.

Reagents and chemicals

Cyclohexane, Chloroform GR, Toluene, Ethyl acetate, Acetic acid glacial 100%, Dragendorff reagent, Folin-ciocalteu's phenol reagent, Methanol, Diethylamine, Acetone and Silica gel G were obtained from Merck Limited, Mumbai. All other reagents and chemicals used in the present study were of analytical grade.

Thin layer chromatography

Thin layer chromatography is a form of solid-liquid adsorption chromatography and one of the valuable and versatile method for analysis of wide range of bio molecules. In TLC, fixed phase is a solid (for eg: alumina, magnesium oxides, silica gel etc.) and mobile phase is a liquid. The separation takes place on a layer of finely divided solid that is fixed on a flat (glass or alumina) surface. The principle of TLC is based on the differences in the adsorption co-efficients.

A thin layer plate was prepared as slurry of silica gel (Merck) with water and applied to a glass plate as a thin uniform layer by means of a spreader. The

plates with thin layer of silica gel were air dried for 10-15 minutes and over dried at 100^0C-110^0C for 30 minutes to activate the adsorbent. The root extracts (30µl) were spotted using capillary tubes or micropipette at 1.5 cm distance between them.

The developing chamber was filled with the solvent to a depth of 1.5 cm and equilibrated for about 1 hour. The thin layer plate was then placed gently in the chamber with enough solvent to come to a level just below the original sample spot. The solvent migrates up the plate due to the capillary action carrying with it the components of the mixture at different rates. As the solvent front reaches about 1-2 cm from the top of the plate, the plate was removed. Finally, the separated compounds on TLC plate were detected by appropriate spray reagents.

3. RESULTS

3.1.Root Yield

3.1.1. Root yield from nursery beds

In the case of *Aegle marmelos*, the highest root yield after 24 months from one m^2 was 2.07 kg (dry weight) for the treatment 25 plants/m^2 at an espacement of 20 x 20 cm (Table 1). The best root yield of 2.57 kg (dry weight) for *Gmelina arborea* was for the treatment 44 plants/m^2 at an espacement of 15 x 15 cm after 18 months (Table 2). For *Stereospermum colais*, 1.087 kg dry root was obtained from one m^2 at an espacement of 25 x 25 cm after 18 months (Table 3).

Table 1. *Aegle marmelos*

Planting stock and Planting method	Plants per m^2	Period between planting and harvesting (months)	Survival at harvest (%)	Root fresh weight (kgm^{-2})	Root dry weight (kgm^{-2})	Dry weight – Fresh weight ratio	Mean Annual Dry root productivity kgm^{-2}
Polybag seedlings, on raised nursery beds, at 20x20cm spacing	25	18	100	1.775	0.810	0.46	0.54
Bare seedlings, on raised nursery beds at 40x40cm	6	18	100	0.579	0.243	0.42	0.162
Polybag seedlings on raised nursery beds at 20x20cm	25	24	100	1.782	0.815	0.46	0.407
Bare seedlings on raised nursery beds at 40x40cm	6	24	100	1.409	0.6	0.43	0.30
Polybag seedlings on raised nursery beds at 10x10cm	100	24	30	1.832	0.795	0.43	0.397
Bare seedlings on raised nursery beds at 20x20cm	25	24	68	4.387	2.074	0.47	1.037
Plot	1	24	100	0.292	0.125	0.43	0.010

Table 2. ***Gmelina arborea***

Planting stock and Planting method	Plants per m^2	Period between planting and harvesting (months)	Survival at harvest (%)	Root fresh weight (kgm^{-2})	Root dry weight (kgm^{-2})	Dry weight – Fresh weight ratio	Mean Annual Dry root productivity kgm^{-2}
Bare seedlings on raised nursery beds at 20x20cm	25	6	72	2.957	1.585	0.54	2.378
Bare seedlings on raised nursery beds at 40x40cm	6	6	100	0.594	0.348	0.59	0.522
Bare seedlings on raised nursery beds at 10x10cm	100	6	69	5.846	4.036	0.69	6.023
Bare seedlings on raised nursery beds at 40x40cm	6	12	100	1.916	1.493	0.78	1.493
Polybag Seedlings on raised nursery beds at 40x40cm	6	12	100	2.615	1.543	0.59	1.543
Polybag seedlings on raised nursery beds at 20x20cm	25	12	68	5.891	2.578	0.44	2.578
Polybag seedlings on raised nursery beds at 10x10cm	100	12	71	4.638	2.241	0.48	2.241
Bare Seedlings on raised nursery beds at 20x20cm	25	12	96	2.973	1.769	0.59	1.769
Bare seedlings on raised nursery beds at 20x20cm	25	12	100	0.058	0.016	0.28	0.016
Polybag seedlings on raised nursery beds at 20x20cm	25	18	40	3.089	1.109	0.36	0.739
Polybag seedlings on raised nursery beds at 15x15cm	44	18	86	3.022	1.194	0.40	0.796
Polybag seedlings on raised nursery beds at 15x15cm	44	18	53	1.712	0.513	0.30	0.342
Polybag seedlings on raised nursery beds at 25x25cm	16	18	50	1.51	0.465	0.31	0.310
Root trainer seedlings on raised nursery beds at 20x20cm	25	18	38	0.276	0.087	0.32	0.058
Plot	1	24	100	1.21	0.532	0.44	0.266

Table 3. *Stereospsermum colais*

Planting stock and Planting method	Plants per m^2	Period between planting and harvesting (months)	Survival at harvest (%)	Root fresh weight (kgm^{-2})	Root dry weight (kgm^{-2})	Dry weight – Fresh weight ratio	Mean Annual Dry root productivity kgm^{-2}
Polybag seedlings on raised nursery beds at 25x25cm	16	18	81	3.689	1.087	0.29	0.725
Plot	1	24	100	0.664	0.23	0.35	0.115

The roots yield of PVC grown *Gmelina arborea, Oroxylum indicum, Aegle marmelos, Stereospermum colais* and *Premna serratifolia* harvested at intervals of three months and six months are given in Table 4.

Table 4. Root Yield From PVC Potted Dasamoola Plants

Sl. No.	**Species**	**Period between Planting and Harvesting (Months)**	**Mean Fresh wt. per plant (g)**	**Mean dry wt. per Plant (g)**	**Dry wt. / Fresh wt. ratio**	**Mean Fresh wt. per m^2 ($kg\ m^{-2}$)**	**Mean dry wt. per m^2 ($kg\ m^{-2}$)**	**Dry wt. / Fresh wt. ratio**
1	*Gmelina arborea*	3 months	5.25	2.75	0.52	0.131	0.069	0.52
		6 months	55	11.4	0.20	1.375	0.285	0.20
2	*Oroxylum indicum*	3 months	26.25	9	0.34	0.656	0.225	0.34
		6 months	58.2	11.1	0.19	1.455	0.278	0.19
3	*Aegle marmelos*	3 months	8.25	4	0.48	0.206	0.100	0.48
		6 months	23.7	7.4	0.31	0.593	0.185	0.31
4	*Stereospermum colais*	3 months	12.75	4	0.31	0.319	0.100	0.31
		6 months	35.3	4.3	0.12	0.883	0.108	0.12
5	*Premna serratifolia*	3 months	3.75	2.25	0.6	0.094	0.056	0.6
		6 months	14.5	3.7	0.25	0.363	0.093	0.25

The ratio of dry/fresh root yield was 36 for *Gmelina arborea* (Figure 1), 34 each for *Aegle marmelos* and *Premna serratifolia* (Figure 2), 35 for *Stereospermum colais* (Figure 3) and 37 for *Oroxylum indicum* (Figure 4).

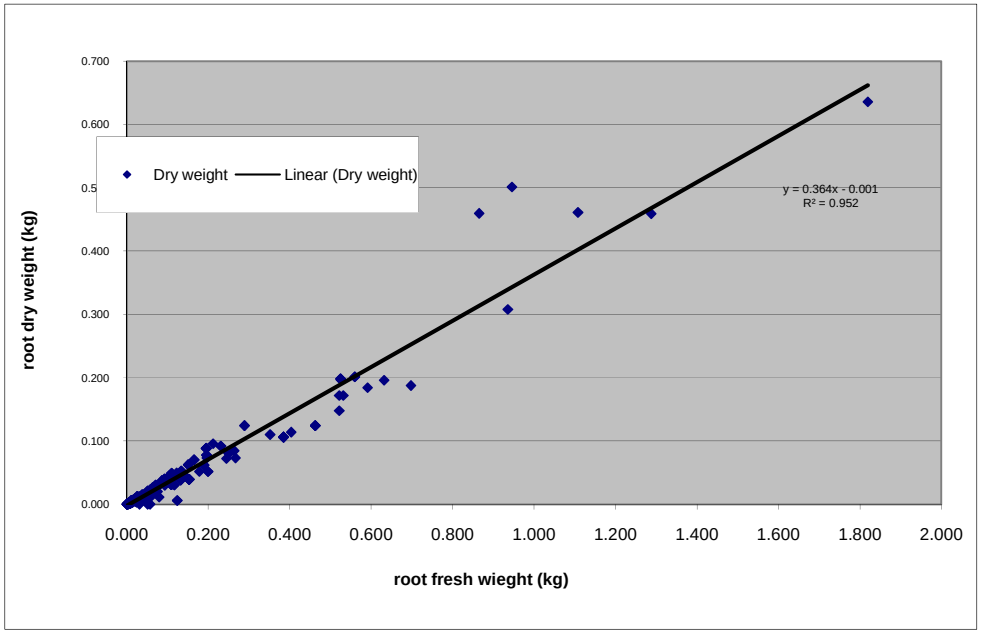

Figure 2. Fresh weight-dry weight relationship in roots of *Gmelina arborea*

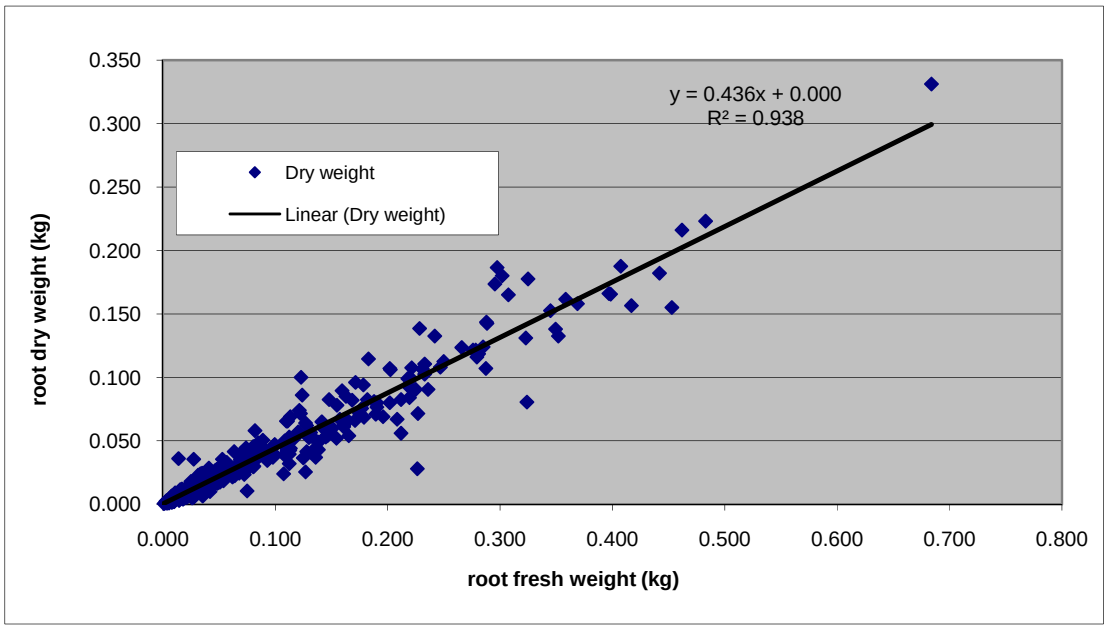

Figure 2. Fresh weight-dry weight relationship in roots of *Aegle marmelos*

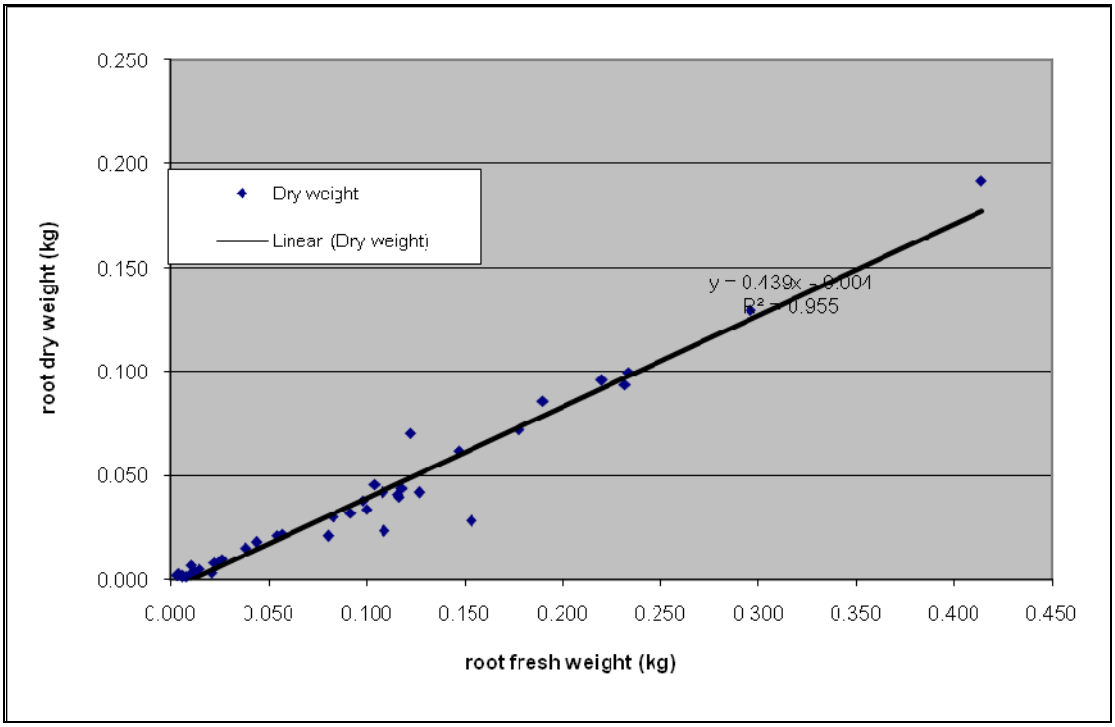

Figure 3. Fresh weight-dry weight relationship in roots of *Premna serratifolia*

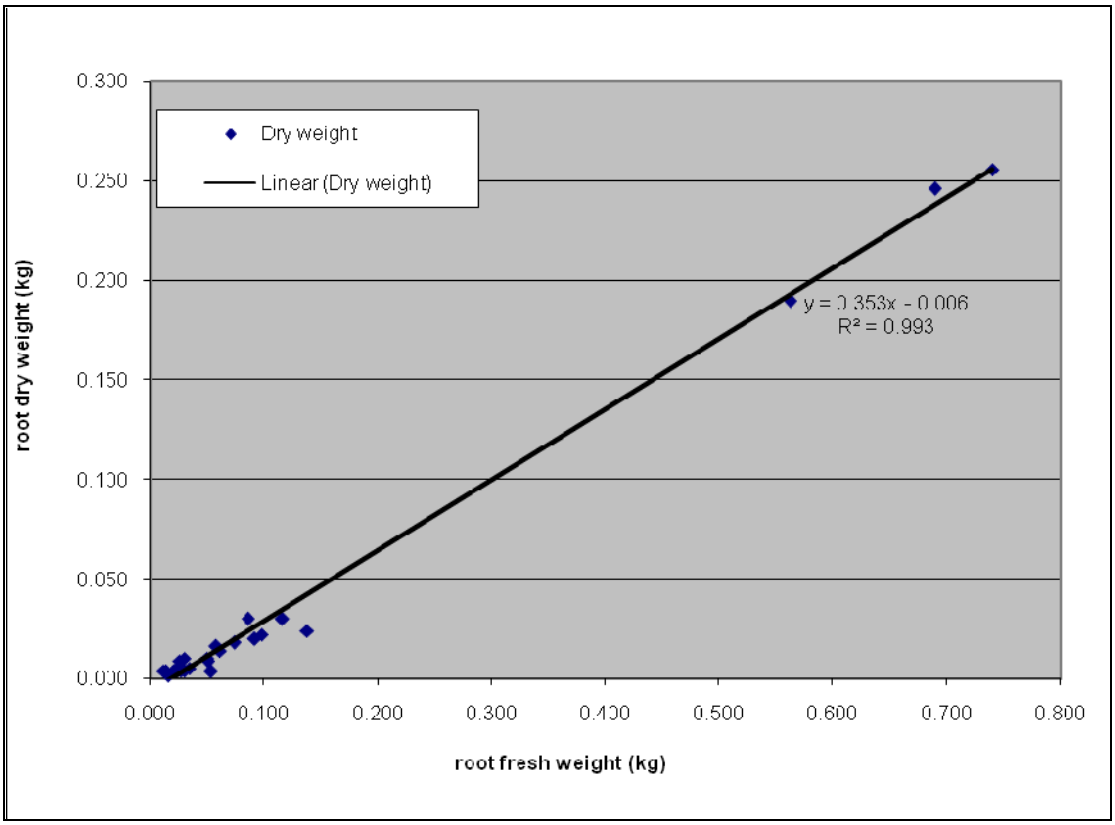

Figure 4. Fresh weight-dry weight relationship in roots of *Stereospermum colais*

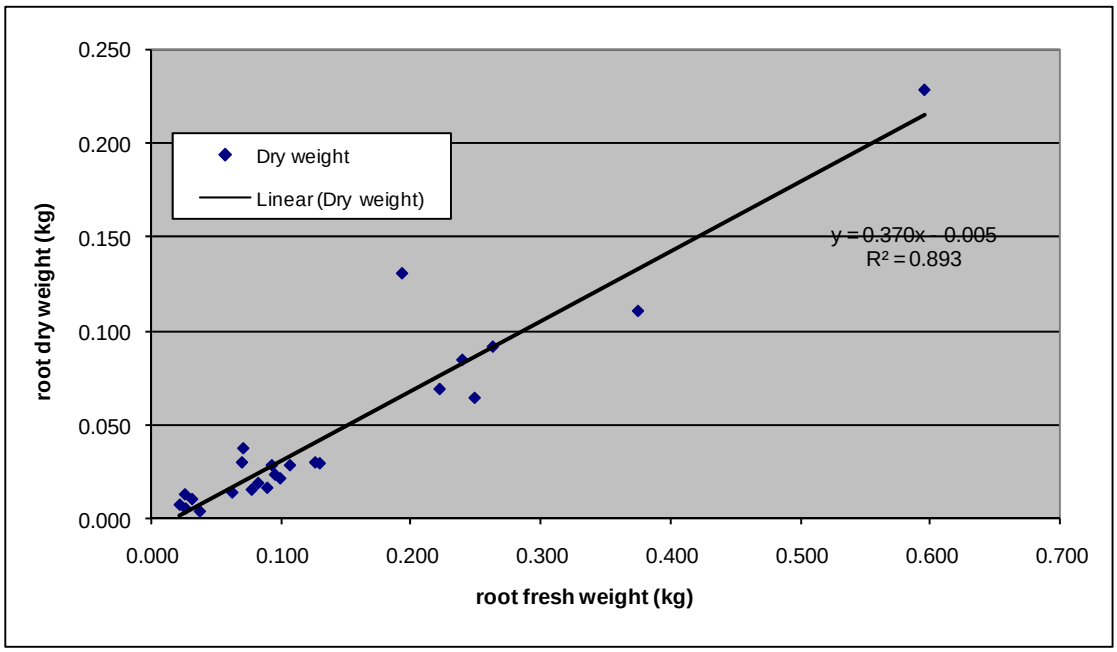

Figure 5. Fresh weight-dry weight relationship in roots of *Oroxylum indicum*

3.2 Phytochemical Studies

3.2.1. *Aegle marmelos*

Six-month-old plants

Polyphenolics: Two solvent systems, toluene: chloroform: methanol (4:4:1) and cychlohexane: chloroform: acetic acid (45:45:10) gave good separation profiles. Approximately, 9 bands were observed in all the samples after spraying with Folin Ciocalteu's phenol reagent (Figures 6, 7).

Flavonoids: Among the solvent systems used, toluene: chloroform: methanol (4:4:1) and cychlohexane: chloroform: acetic acid (45:45:10) gave good separation profile. Approximately, 12 bands were observed under UV (256nM) in all the samples separated (Figures 8, 9).

Alkaloids: Among the solvent systems used, chloroform: acetic acid (9:1) and ethyl acetate: methanol (9:1) gave good separation profile. Approximately, 2 bands were observed in all the samples (except the 3rd sample) after spraying with Dragendorff reagent (Figures 10, 11).

Table 5. Treatment modalities of six-months-old *Aegle marmelos* root samples and mature trees (25-year and 30-year old)

Sample No.	Age of plant	Root part used for analysis	Site condition and planting method
1	Six Months	Root with bark (Juvenile root) <5 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe
2	Six Months	Root bark <5 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe
3	Six Months	Root after bark removal <5 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe
4	25 years	Root with bark >10 mm diam.	Grown in the medicinal garden along with other trees
5	25 years	Root bark >10 mm diam.	Grown in the medicinal garden along with other trees
6	25 years	Root after bark removal >10 mm diam.	Grown in the medicinal garden along with other trees
7	>30 years	Root with bark >10 mm diam.	Grown in homestead along with other trees
8	>30 years	Root bark >10 mm diam.	Grown in homestead along with other trees
9	>30 years	Root after bark removal >10 mm diam.	Grown in homestead along with other trees

TLC Profile of compounds extracted from six-month-old *Aegle marmelos* plants

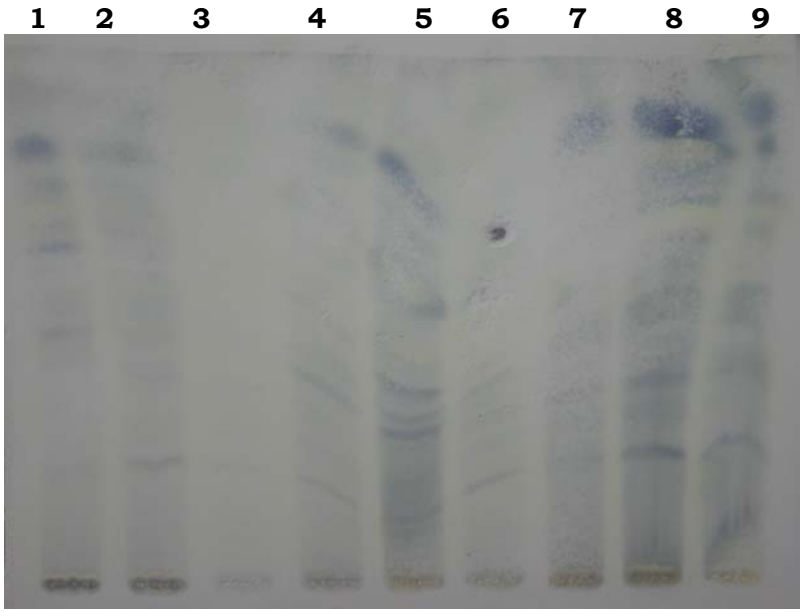

Figure 6. TLC Profile of polyphenolic compounds extracted in methanol + sodium carbonate with the solvent system toluene: chloroform: methanol (4:4:1) Spray reagent: Folin Ciocalteu's phenol reagent

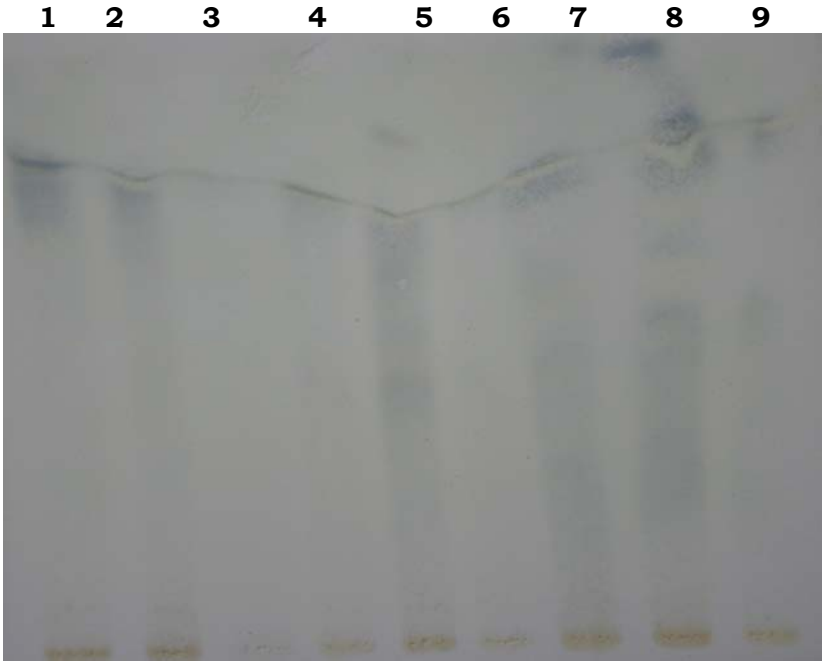

Figure 7. TLC Profile of polyphenolic compounds extracted in methanol + sodium carbonate with the solvent system cyclohexane: chloroform: acetic acid (45:45:10)
Spray reagent: Folin Ciocalteu's phenol reagent

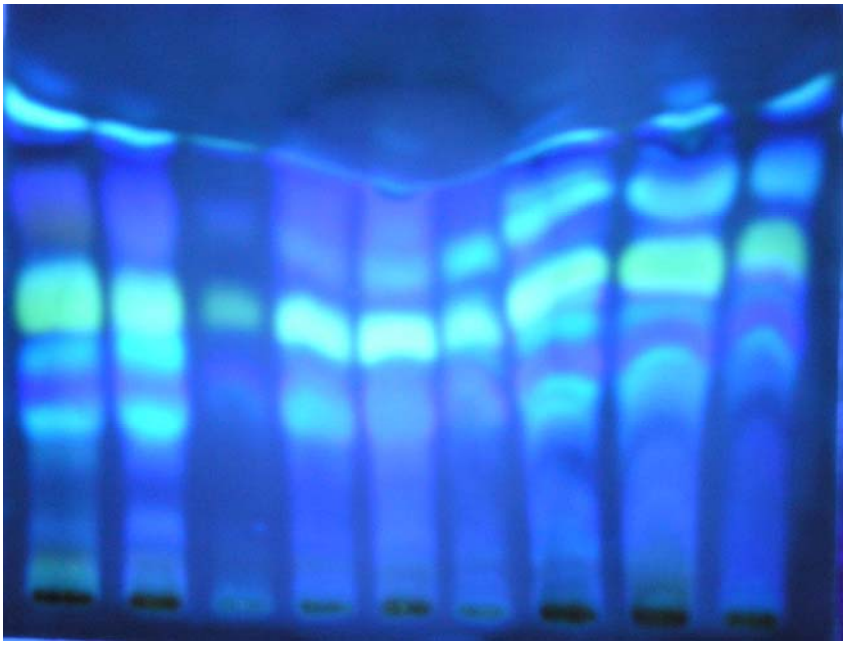

Figure 8. TLC Profile of flavonoids under UV of methanol + sodium carbonate extract of *Aegle marmelos* with the solvent system toluene: chloroform: methanol (4:4:1)

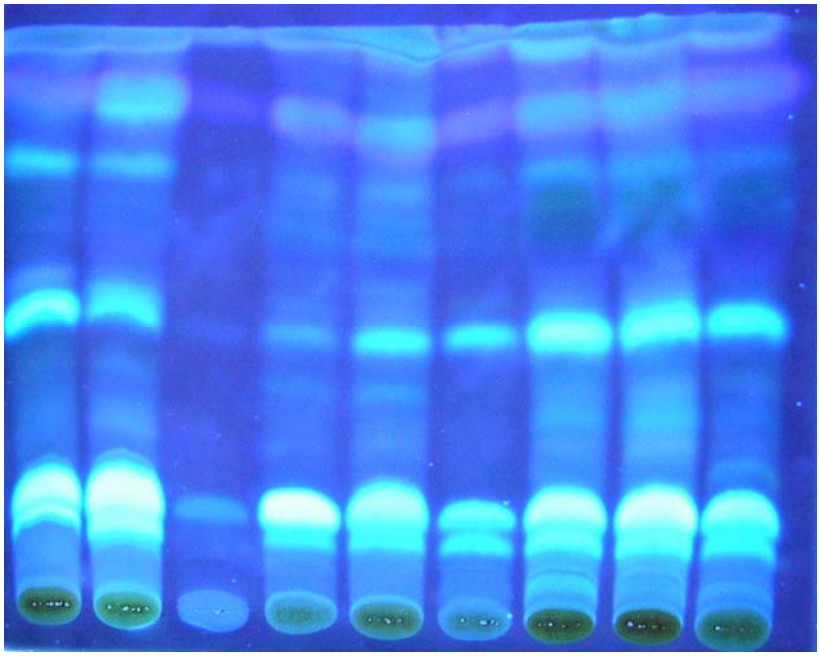

Figure 9. TLC Profile of flavonoids under UV of methanol + sodium carbonate extract of *Aegle marmelos* with the solvent system cyclohexane: chloroform: acetic acid (45:45:10)

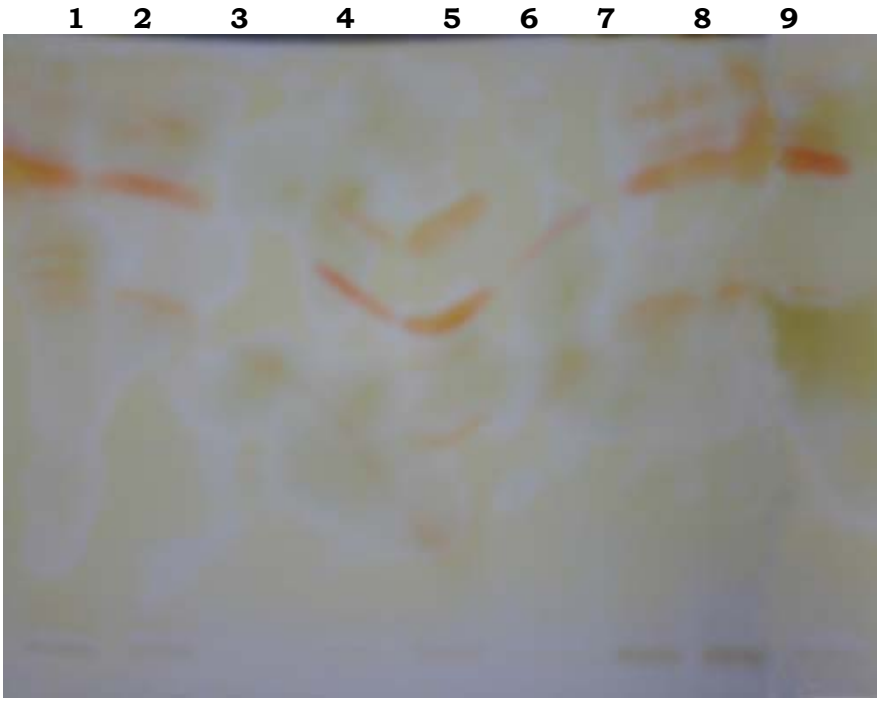

Figure 10. TLC Profile of alkaloid compounds extracted in methanol + sodium carbonate with the solvent system chloroform: acetic acid (9:1)
Spray reagent: Dragendorff reagent

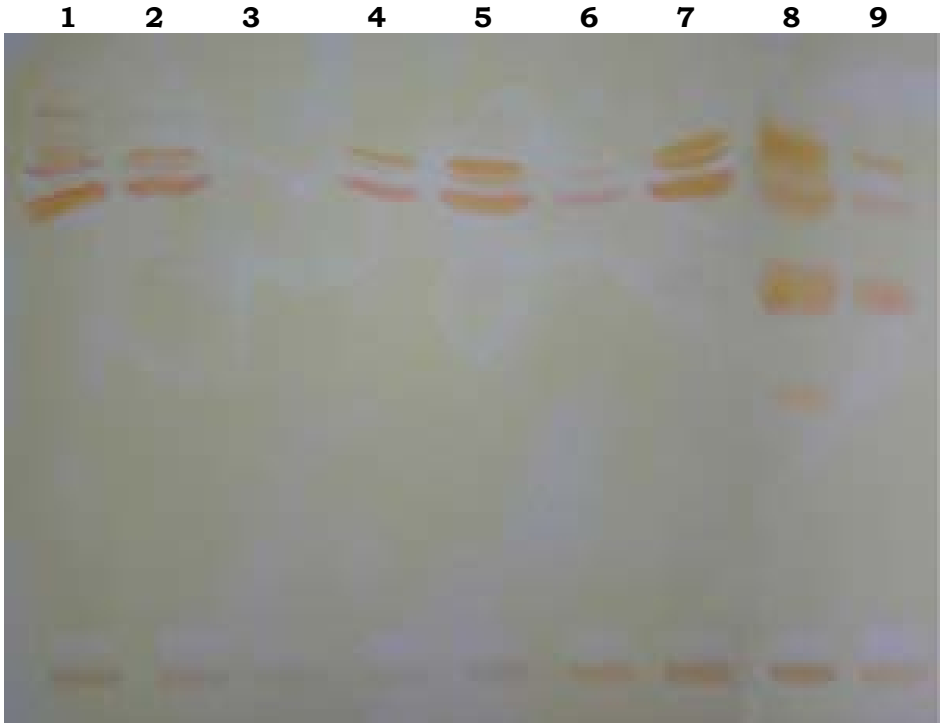

Figure 11. TLC Profile of alkaloid compounds extracted in methanol + sodium carbonate with the solvent system ethylacetate: methanol (9:1)
Spray reagent: Dragendorff reagent

Twelve-month-old plants

Polyphenolics: Among the solvent systems, toluene: chloroform: methanol (4:4:1) and cychlohexane: chloroform: acetic acid (45:45:10) gave good separation profiles. Approximately, 12 bands were observed in all the samples after spraying with Folin Ciocalteu's phenol reagent (Figures 12, 13).

Flavonoids: Among the solvent systems used, toluene: chloroform: methanol (4:4:1) and cychlohexane: chloroform: acetic acid (45:45:10) gave good separation profile. Approximately, 13 bands were observed under UV (256nM) in all the samples separated (Figures 14, 15).

Table 6. Treatment modalities of Twelve-month-old *Aegle marmelos* root samples

Sample No:	Age of plant	Root part used for analysis	Site condition and planting method
1	12 months	Root with bark <5 mm diam.	Nursery grown; Small poly bag; planted at 20 x 20 cm spacing
2	12 months	Root bark <5 mm diam.	Nursery grown; Small poly bag; planted at 20 x 20 cm spacing
3	12 months	Root after bark removal <5 mm diam.	Nursery grown; Small poly bag; planted at 20 x 20 cm spacing
4	12 months	Root with bark >10 mm diam.	Nursery grown; Small poly bag; planted at 20 x 20 cm spacing
5	12 months	Root bark >10 mm diam.	Nursery grown; Small poly bag; planted at 20 x 20 cm spacing
6	12 months	Root after bark removal >10 mm diam.	Nursery grown; Small poly bag; planted at 20 x 20 cm spacing

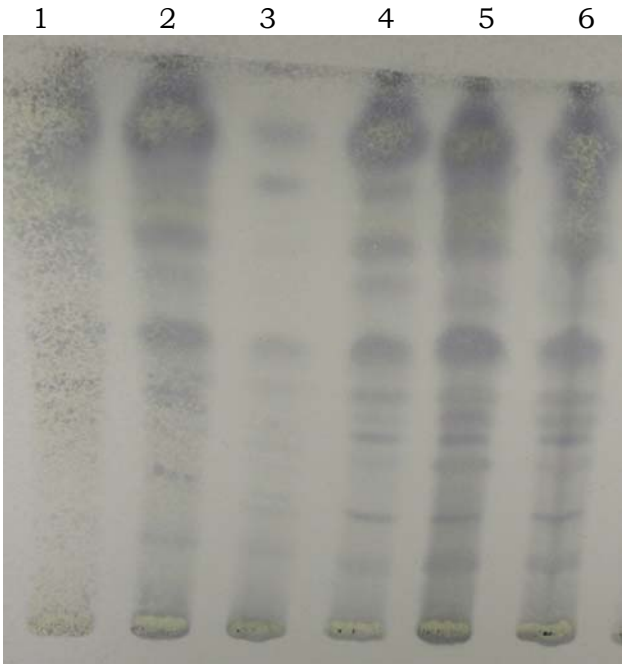

Figure 12. TLC Profile of polyphenolic compounds extracted in methanol with the solvent system toluene: chloroform:methanol (4:4:1)
Spray reagent: Folin Ciocalteu's phenol reagent

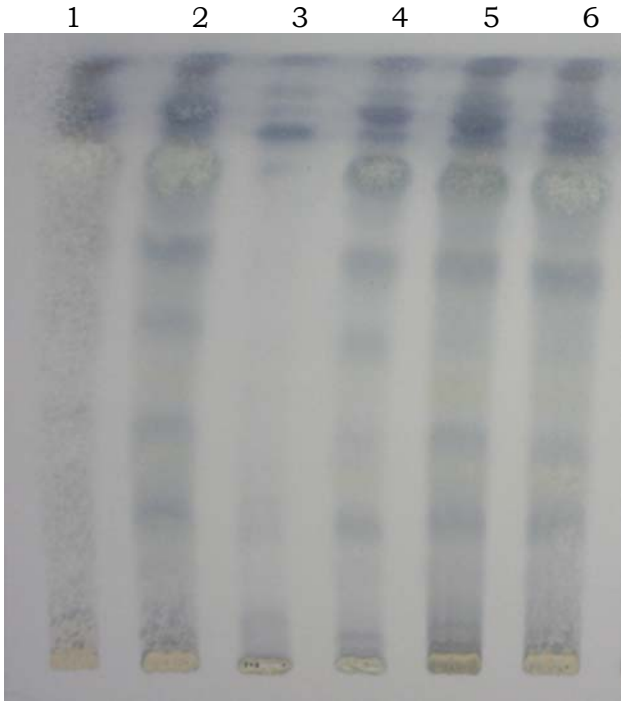

Figure 13. TLC Profile of polyphenolic compounds extracted in methanol with the solvent system cychlohexane: chloroform: acetic acid (45:45:10)
Spray reagent: Folin Ciocalteu's phenol reagent

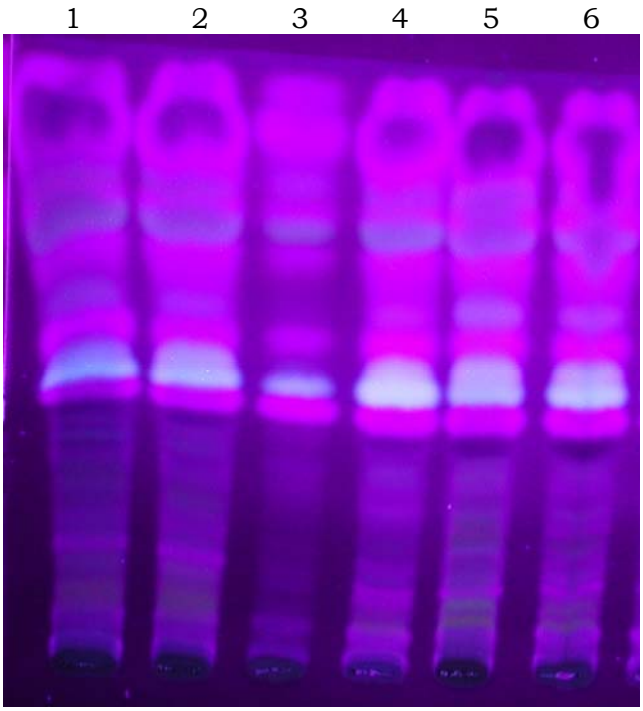

Figure 14. TLC Profile of flavonoids under UV of methanolic extract of *Aegle marmelos* with the solvent system toluene: chloroform: methanol (4:4:1)

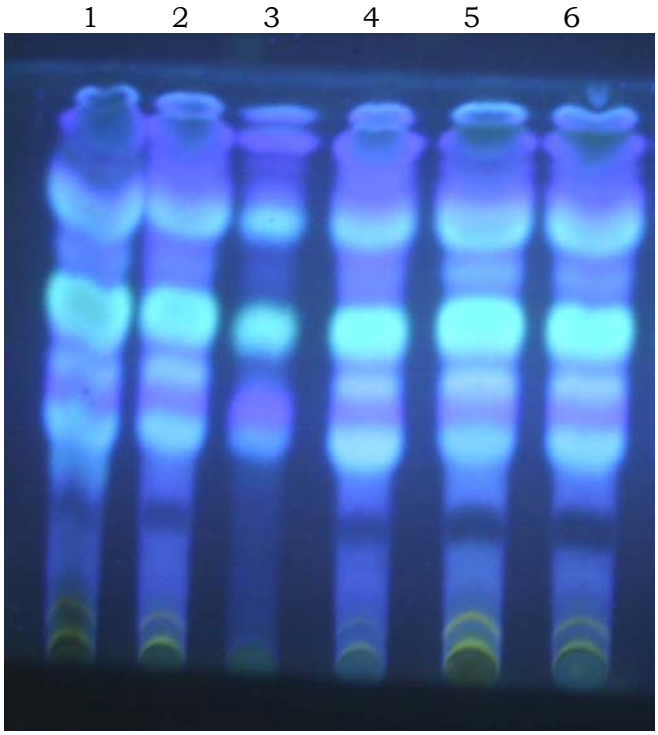

Figure 15. TLC Profile of flavonoids under UV of methanolic extract of *Aegle marmelos* with the solvent system cychlohexane: chloroform: acetic acid (45:45:10)

Twenty-four-month-old plants

Polyphenolics: Two solvent systems, toluene: chloroform: methanol (4:4:1) and chloroform: methanol (9:1) gave good separation profiles. Approximately, 10 bands were observed in all the samples after spraying with Folin Ciocalteu's phenol reagent (Figures 16, 17).

Flavonoids: Among the solvent systems used, toluene: chloroform: methanol (4:4:1) and cychlohexane: chloroform: acetic acid (45:45:10) gave good separation profile. Approximately, 13 bands were observed under UV (256nM) in all the samples separated (Figures 18, 19).

Alkaloids: Two solvent systems, toluene: chloroform: methanol (4:4:1) and chloroform: methanol (9:1) gave good separation profile. Approximately, 5 bands were observed in all the samples after spraying with Dragendorff reagent (Figures 20, 21).

Table 7. Treatment modalities of 24-month-old *Aegle marmelos* root samples

Sample No.	Age of plant	Root part used for analysis	Site condition and planting method
1	24 Months	Root with bark <5 mm diam.	Nursery grown; Small poly bag; planted at 10x10 cm spacing
2	24 Months	Root after bark removal <5 mm diam.	Nursery grown; planted at 10x10 cm spacing
3	24 Months	Root bark <5 mm diam.	Nursery grown; planted at 10x10 cm spacing
4	24 Months	Root after bark removal >10 mm diam.	Nursery grown; planted at 10x10 cm spacing
5	24 Months	Root bark >10 mm diam.	Nursery grown; planted at 10x10 cm spacing
6	24 Months	Root after bark removal >10 mm diam.	Nursery grown; planted at 10x10 cm spacing
7	24 Months	Root bark >10 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe
8	24 Months	Root after bark removal >10 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe
9	24 Months	Root bark >10 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe

TLC Profile of compounds extracted from 24--month-old *Aegle marmelos* plants

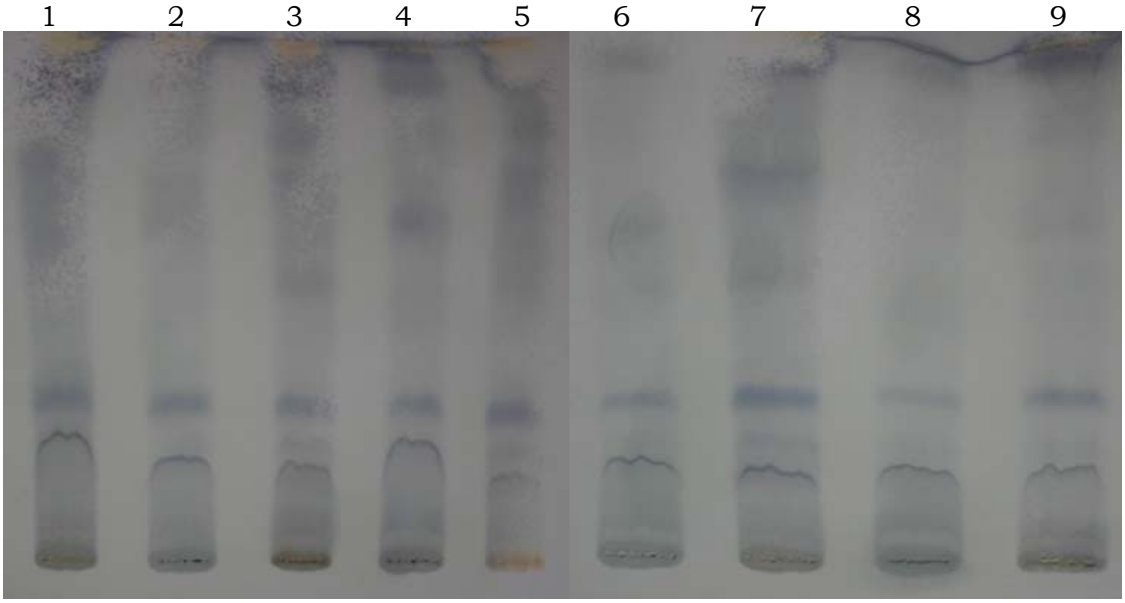

Figure 16. TLC Profile of polyphenolic compounds extracted in methanol + sodium carbonate with the solvent system toluene: chloroform: methanol (4:4:1)
Spray reagent: Folin Ciocalteu's phenol reagent

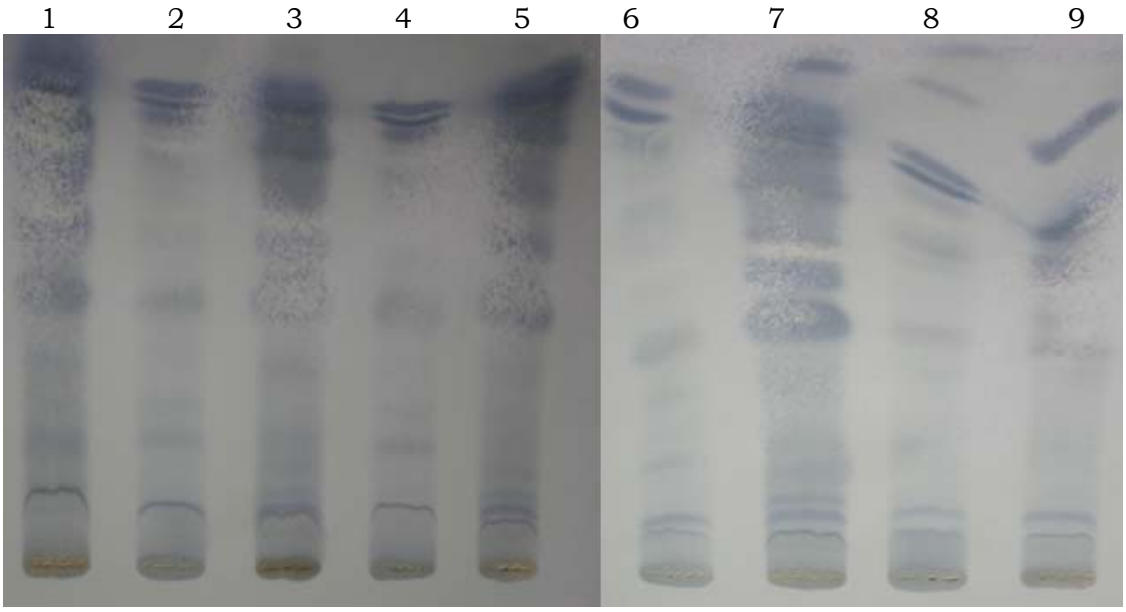

Figure 17. TLC Profile of polyphenolic compounds extracted in methanol + sodium carbonate with the solvent system chloroform: methanol (9:1)
Spray reagent: Folin Ciocalteu's phenol reagent

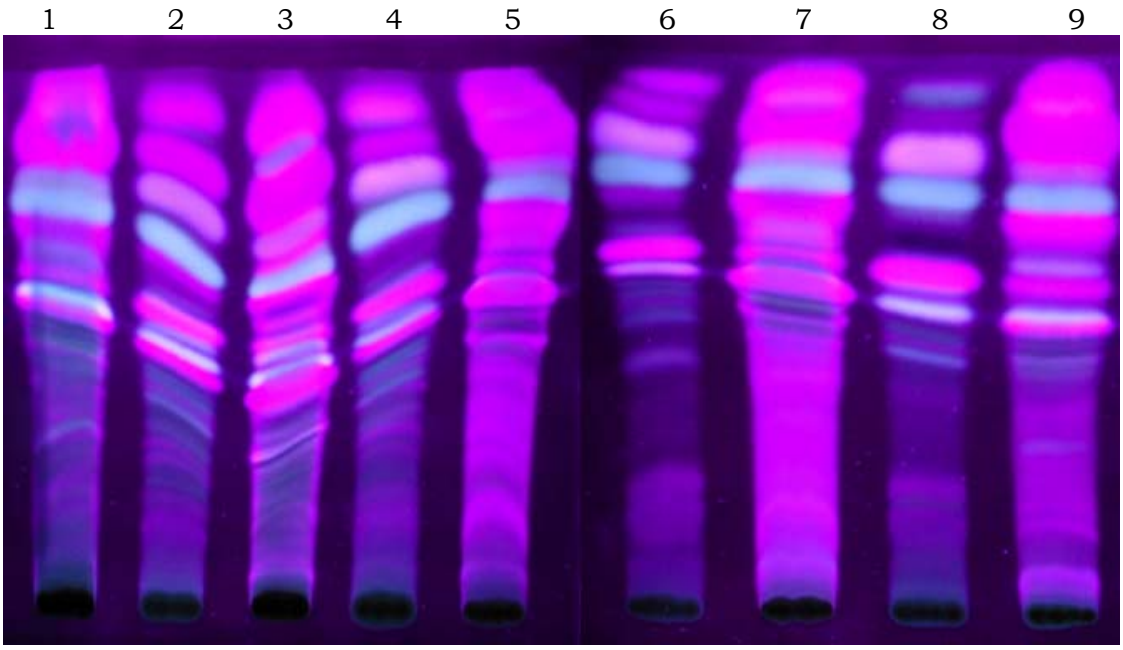

Figure 18. TLC Profile of flavonoids under UV of methanolic extract of *Aegle marmelos* with the solvent system toluene: chloroform: methanol (4:4:1)

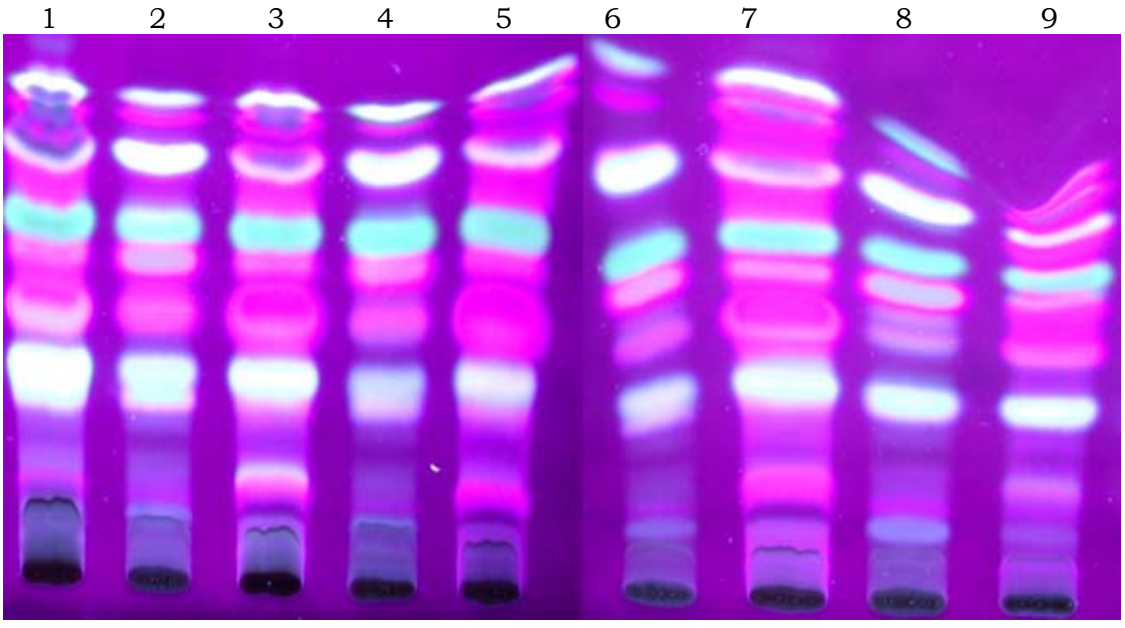

Figure 19. TLC Profile of flavonoids under UV of methanolic extract of *Aegle marmelos* with the solvent system chloroform: methanol (9:1)

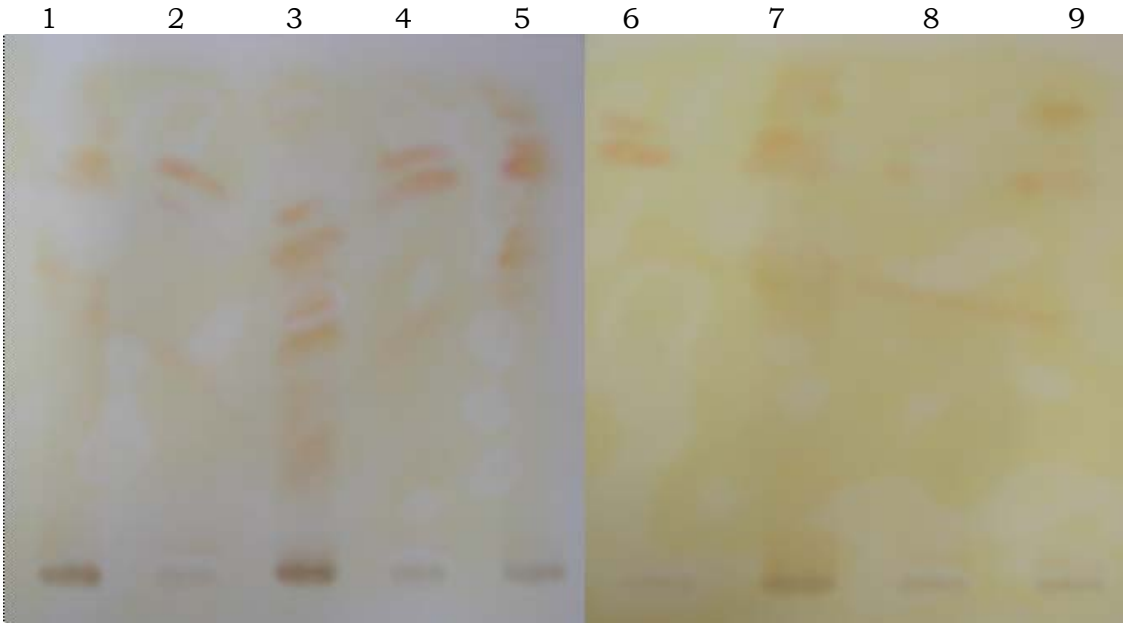

Figure 20. TLC Profile of Alkaloid compounds extracted in methanol + sodium carbonate with the solvent system toluene: chloroform: methanol (4:4:1)
Spray reagent: Dragendorff reagent

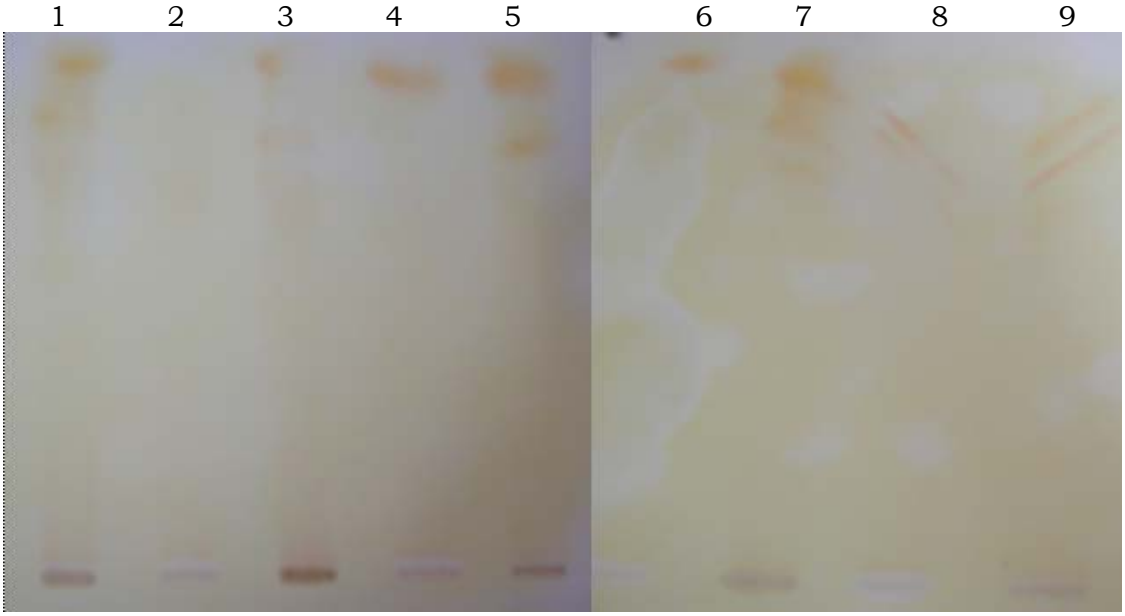

Figure 21: TLC Profile of alkaloid compounds extracted in methanol + sodium carbonate with the solvent system chloroform: methanol (9:1)
Spray reagent: Dragendorff reagent

4.2 *Gmelina arborea*

Six-month-old plants

Polyphenolics: Toluene: chloroform: methanol (4:4:1) gave good separation profiles. Approximately, 5 bands were observed in all the samples after spraying with Folin Ciocalteu's phenol reagent (Figure 22).

Flavonoids: Among the solvent systems used, toluene: ethylacetate: diethylamine (7:2:1) and toluene: chloroform: methanol (4:4:1) gave good separation profile. Approximately, 6 bands were observed under UV (256nM) in all the samples separated (Figures 23, 24).

Alkaloids: Two solvent systems, chloroform: methanol (9:1) and chloroform: acetic acid (9:1) gave good separation profile. Only one band was observed in the 5th sample after spraying with Dragendorff reagent (Figures 25, 26).

Table 8. Treatment modalities of six-month-old *Gmelina arborea* root samples

Sample No:	Age of plant	Root part used for analysis	Site condition and planting method
1	Six months	Root with bark (Juvenile root) <5 mm diam.	Nursery grown; planted in 15 cm x 100cm PVC Pipe
2	Six months	Root bark <5 mm diam.	Nursery grown; planted in 15 cm x 100cm PVC Pipe
3	Six months	Root after bark removal <5 mm diam.	Nursery grown; planted in 15 cm x 100cm PVC Pipe
4	12 years	Root with bark >10 mm diam.	Field grown along with other trees
5	12 years	Root bark >10 mm diam.	Field grown along with other trees
6	12 years	Root after bark removal >10 mm diam.	Field grown along with other trees

TLC Profile of compounds extracted from roots of six-month-old and 12-year-old *Gmelina arborea*

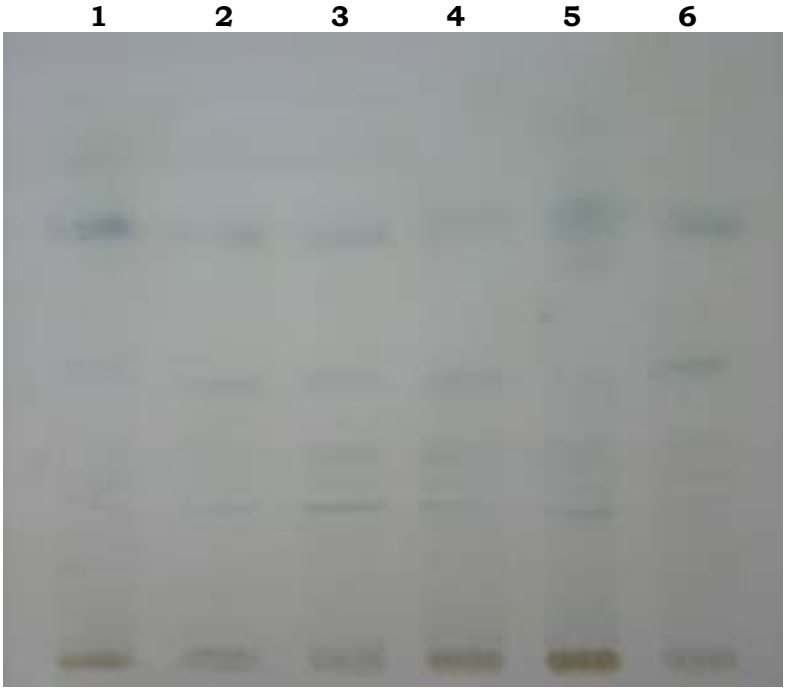

Figure 22. TLC Profile of polyphenolic compounds extracted in acetone with the solvent system toluene: chloroform: methanol (4:4:1)
Spray reagent: Folin Ciocalteu's phenol reagent

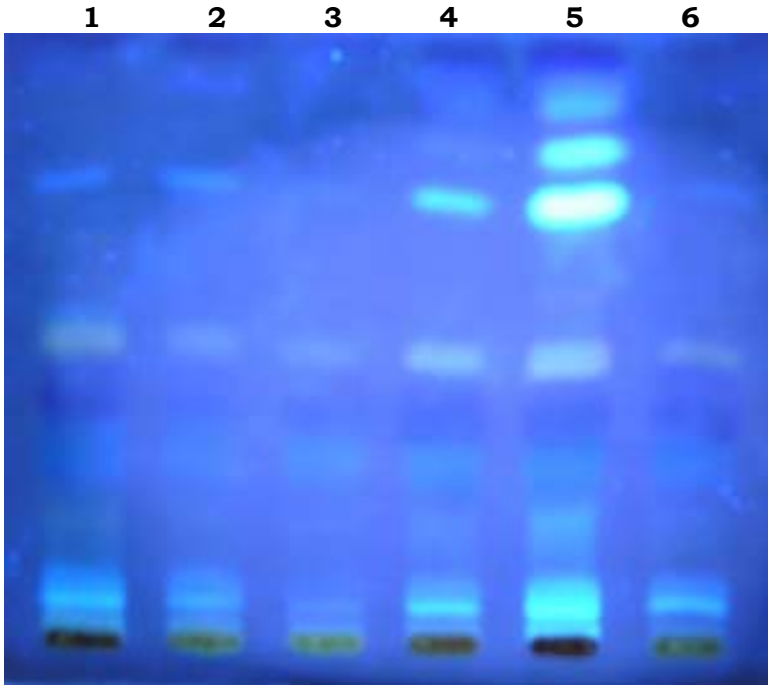

Figure 23. TLC Profile of flavonoids under UV of acetone extract of *Gmelina arborea* with the solvent system toluene: ethyl acetate: diethylamine (7:2:1)

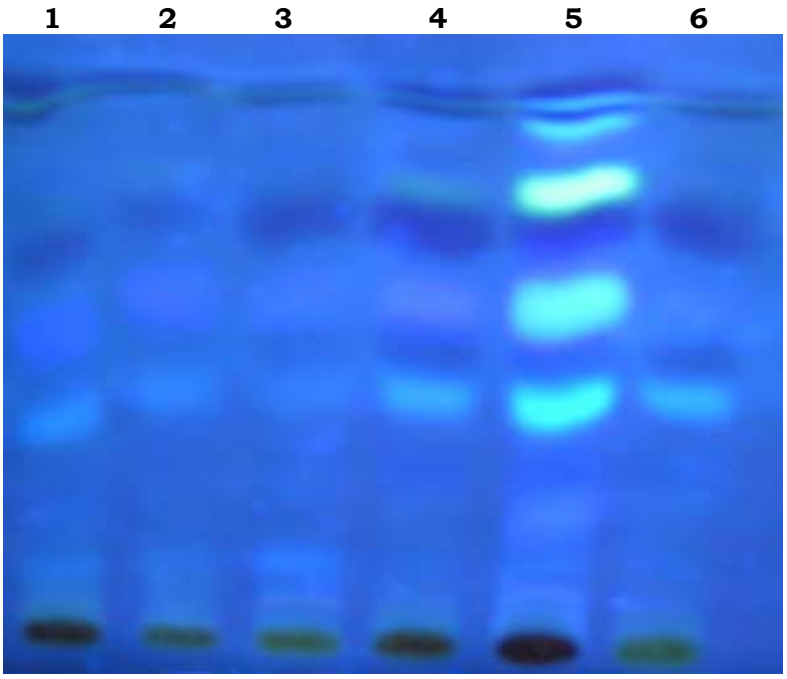

Figure 24. TLC Profile of flavonoids under UV of acetone extract of *Gmelina arborea* with the solvent system toluene: chloroform:methanol (4:4:1)

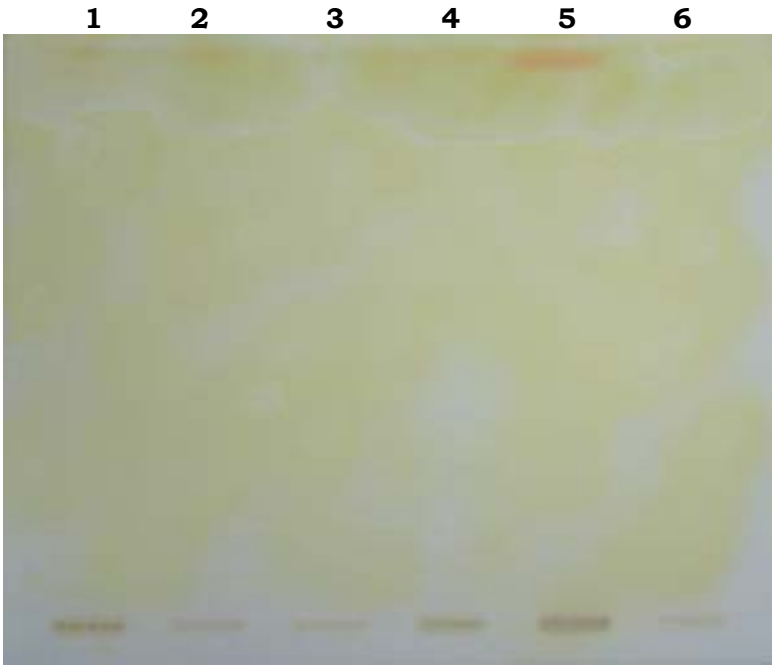

Figure 25. TLC Profile of alkaloid compounds extracted in methanol + sodium carbonate with the solvent system chloroform: methanol (9:1)
Spray reagent: Dragendorff reagent

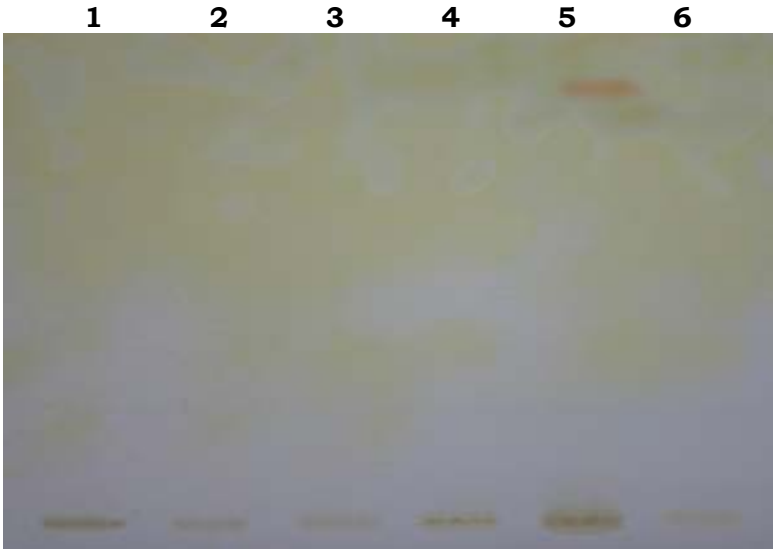

Figure 26. TLC Profile of alkaloid compounds extracted in methanol + sodium carbonate with the solvent system chloroform: acetic acid (9:1)
Spray reagent: Dragendorff reagent

Twelve-month-old plants

Polyphenolics: Three solvent systems, chloroform: acetic acid (9:1), toluene: chloroform: methanol (4:4:1) gave good separation profiles. Approximately, 10 bands were observed in all the samples after spraying with Folin Ciocalteu's phenol reagent (Figures 27, 28).

Table 9: Treatment modalities of 12-month-old *Gmelina arborea* root samples

Sample No:	Age of plant	Root part used for analysis	Site condition and planting method
1	12 months	Root with bark <5 mm diam.	Field grown along with other trees
2	12 months	Root bark <5 mm diam.	Nursery grown; Small poly bag; planted at 10x10 cm spacing
3	12 months	Root after bark removal <5 mm diam.	Nursery grown; bare root plants; planted at 40x40 cm spacing
4	12 months	Root with bark >10 mm diam.	Nursery grown; large poly bag; planted at 25x25 cm spacing
5	12 months	Root bark >10 mm diam.	Nursery grown; large poly bag; planted at 20x20 cm spacing
6	12 months	Root after bark removal >10 mm diam.	Field grown along with other trees

TLC Profile of compounds extracted from nine-month-old *Gmelina arborea* plants

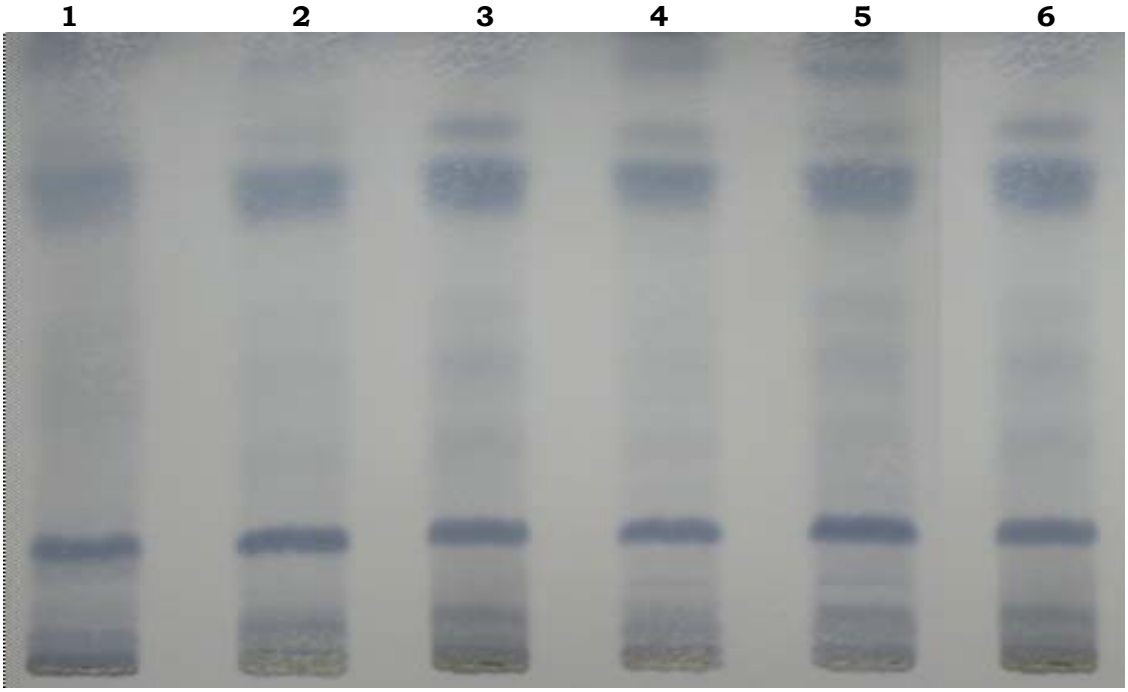

Figure 27. TLC Profile of polyphenolic compounds extracted in acetone with the solvent system chloroform: acetic acid (9:1)
Spray reagent: Folin Ciocalteu's phenol reagent

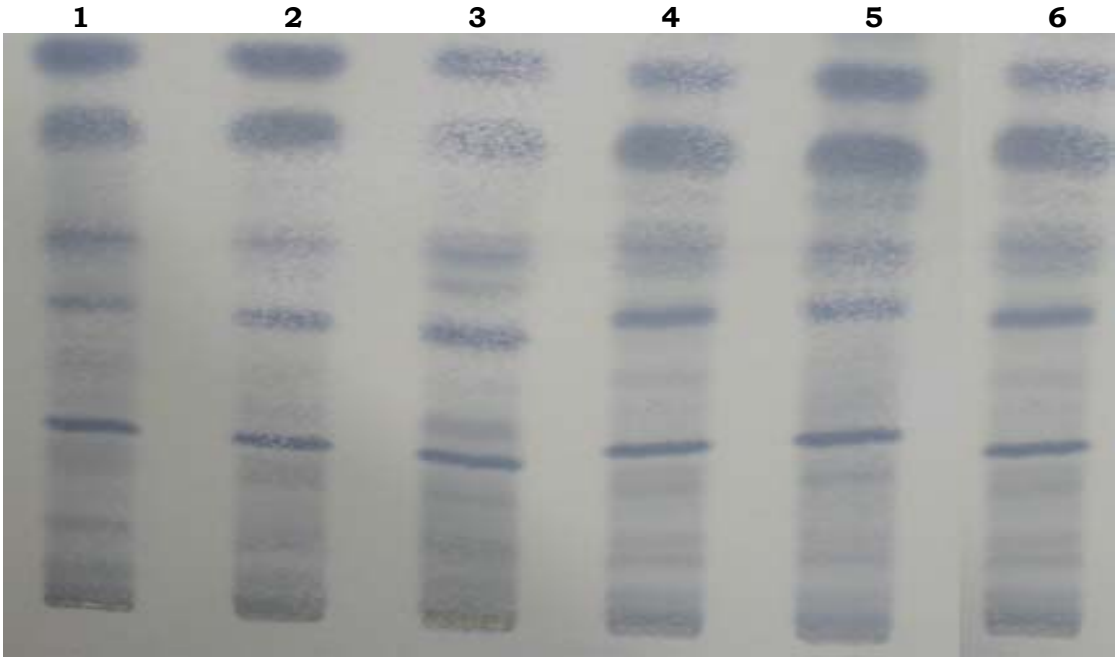

Figure 28. TLC Profile of polyphenolic compounds extracted in acetone with the solvent system toluene: chloroform: methanol (4:4:1)
Spray reagent: Folin Ciocalteu's phenol reagent

Twenty-four-month-old plants

Flavonoids: Among the solvent systems used, toluene: ethylacetate: diethylamine (7:2:1) and cychlohexane: chloroform: acetic acid (45:45:10) gave good separation profile. Approximately, 7 bands were observed under UV (256nM) in all the samples separated (Figures 29 & 30).

Table 10. Treatment modalities of 24-month-old *Gmelina arborea* root samples

Sample No:	Age of plant	Root parts used for analysis	Site condition and planting method
1	24 Months	Root with bark <5 mm diam.	Nursery grown; without poly bag; planted at 10x10 cm spacing
2	24 Months	Root bark <5 mm diam.	Nursery grown; without poly bag; planted at 10x10 cm spacing
3	24 Months	Root after bark removal <5 mm diam.	Nursery grown; without poly bag; planted at 10x10 cm spacing
4	24 Months	Root with bark >10 mm diam.	Nursery grown; without poly bag; planted at 25x25 cm spacing
5	24 Months	Root bark >10 mm diam.	Nursery grown; without poly bag; planted at 25x25 cm spacing
6	24 Months	Root after bark removal >10 mm diam.	Nursery grown; without poly bag; planted at 25x25 cm spacing

TLC Profile of compounds extracted from 20-month-old *Gmelina arborea* plants

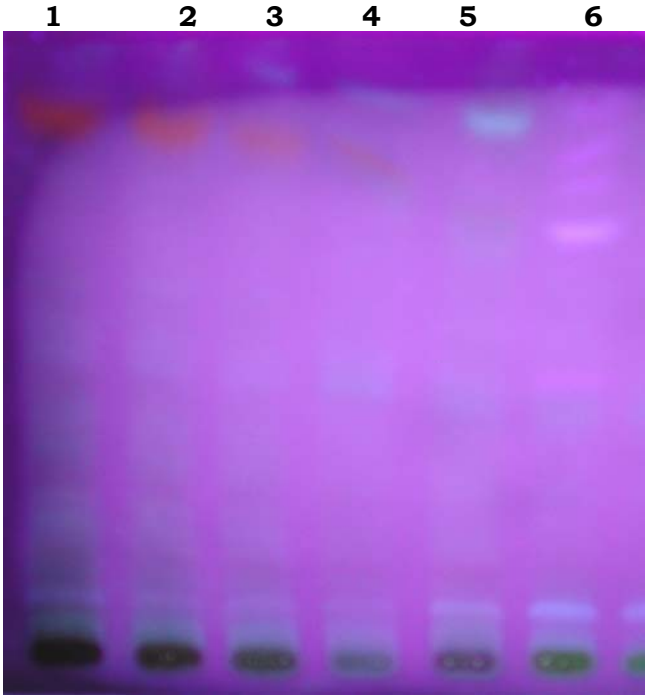

Figure 29. TLC Profile of flavonoids under UV of acetone extract of *Gmelina arborea* with the solvent system toluene: ethyl acetate: diethylamine (7:2:1)

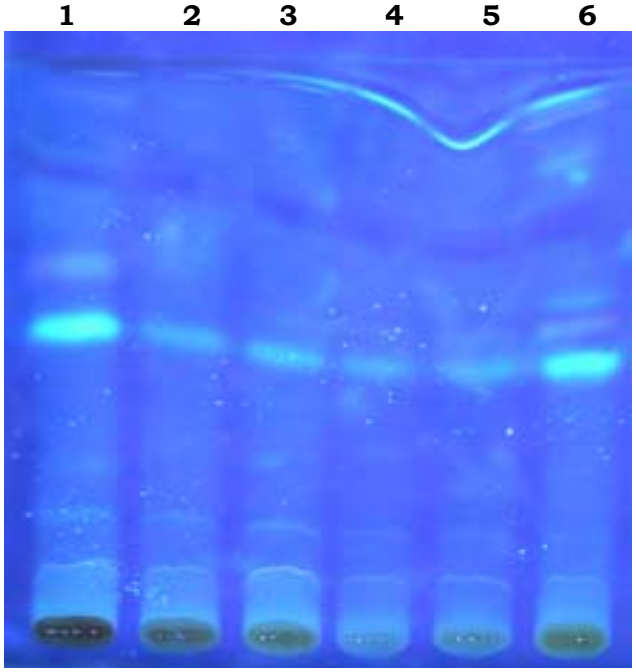

Figure 30. TLC Profile of flavonoids under UV of acetone extract of *Gmelina arborea* with the solvent system cyclohexane: chloroform: acetic acid (45:45:10)

4.3 *Oroxylum indicum*

Six-month-old plants

Polyphenolics: Two solvent systems, chloroform: acetic acid (9:1) and cyclohexane: chloroform: acetic acid (45:45:10) gave good separation profiles. Approximately, 4 bands were observed in all the samples after spraying with Folin Ciocalteu's phenol reagent (Figures 31, 32).

Flavonoids: Among the solvent systems used, chloroform: acetic acid (9:1) and cychlohexane: chloroform: acetic acid (45:45:10) gave good separation profile. Approximately, 6 bands were observed under UV (256 nM) in all the samples separated (Figures 33, 34).

Table 11: Treatment modalities of six-month-old *Oroxylum indicum* root samples

Sample No:	Age of plant	Root part used for analysis	Site condition and planting method
1	Six months	Root with bark <5 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe
2	Six months	Root after bark removal <5 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe
3	Six months	Root bark <5 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe
4	Six months	Root after bark removal >10 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe
5	Six months	Root bark >10 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe
6	Six months	Root after bark removal >10 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe

TLC Profile of compounds extracted from three-month-old *Oroxylum indicum* plants

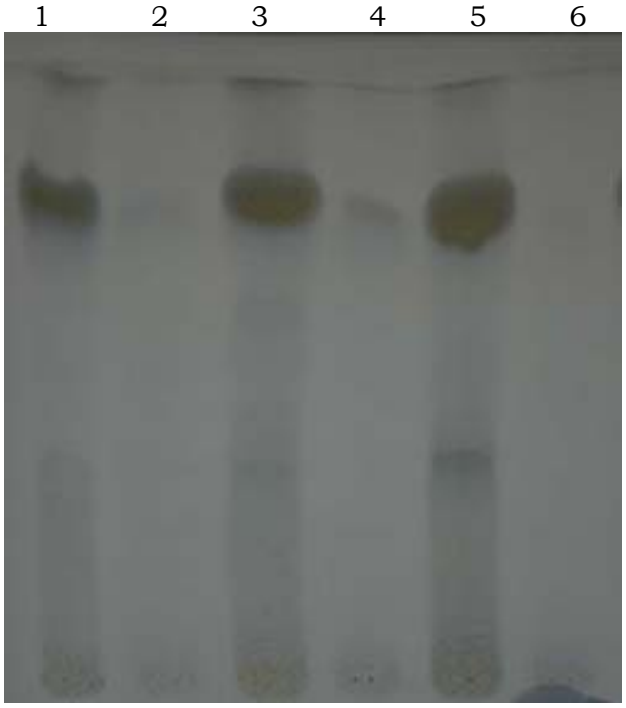

Figure 31. TLC Profile of polyphenolic compounds extracted in methanol with the solvent system chloroform: acetic acid (9:1)
Spray reagent: Folin Ciocalteu's phenol reagent

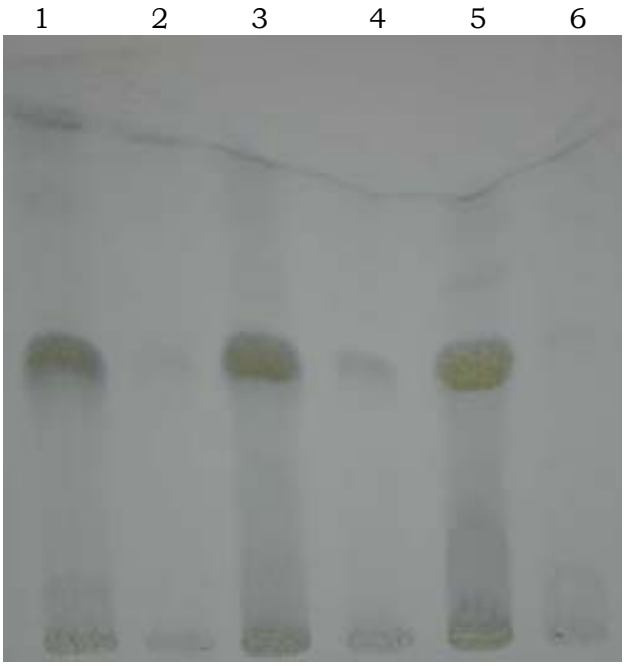

Figure 32. TLC Profile of polyphenolic compounds extracted in methanol with the solvent system cyclohexane: chloroform: acetic acid (45: 45:10)
Spray reagent: Folin Ciocalteu's phenol reagent

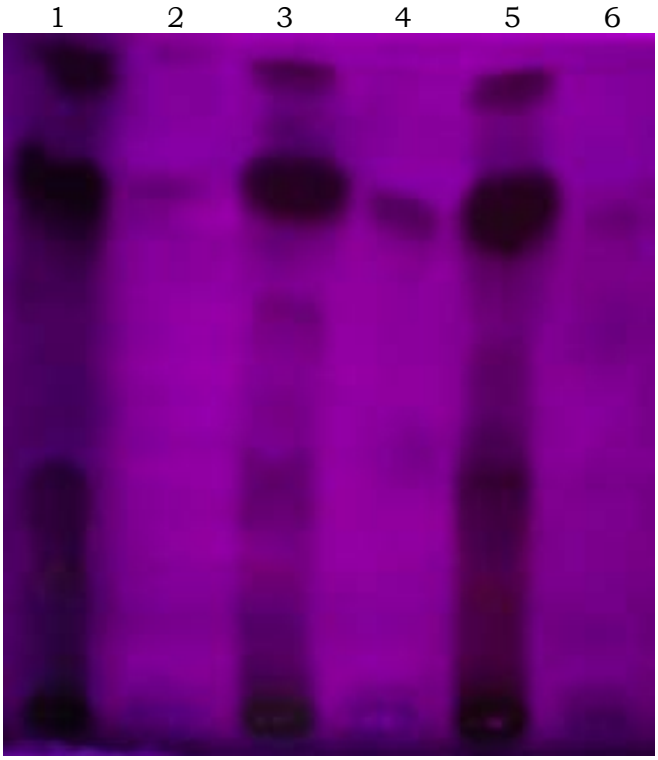

Figure 33. TLC Profile of flavonoids under UV of methanol extract of *Oroxylum indicum* with the solvent system chloroform: acetic acid (9:1)

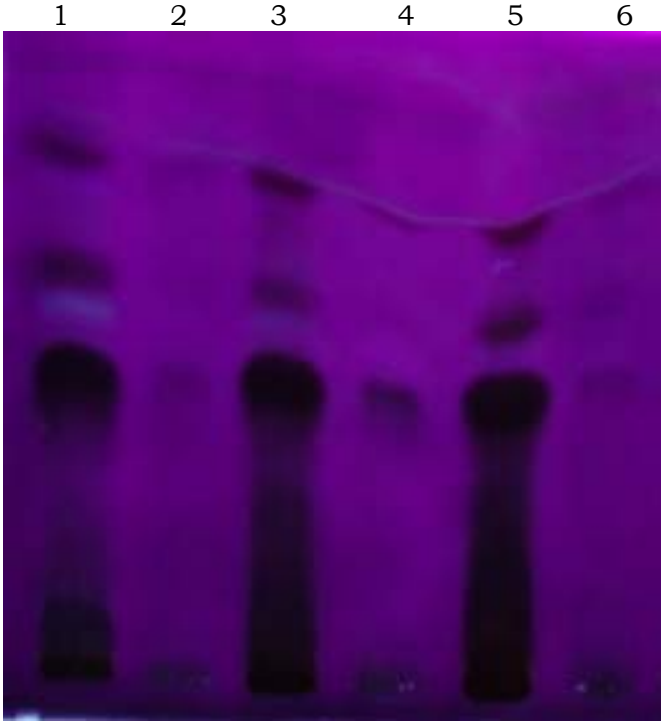

Figure 34. TLC Profile of flavonoids under UV of methanol extract of *Oroxylum indicum* with the solvent system cyclohexane: chloroform: acetic acid (45:45:10)

4.4 *Stereospermum colais*

Six-month-old plants

Flavonoids: Among the solvent systems used, chloroform: acetic acid (9:1) and cychlohexane: chloroform: acetic acid (45:45:10) gave good separation profile. Approximately, 6 bands were observed under UV (256nM) in all the samples separated (Figures 35, 36).

Table 12: Treatment modalities of six-month-old *Stereospermum colais* root samples

Sample No:	Age of plant	Root part used for analysis	Site condition and planting method
1	Six months	Root with bark <5 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe
2	Six months	Root after bark removal <5 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe
3	Six months	Root bark <5 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe
4	Six months	Root after bark removal >10 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe
5	Six months	Root bark >10 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe
6	Six months	Root after bark removal >10 mm diam.	Nursery grown; planted in 15 x 100 cm PVC Pipe

TLC Profile of compounds extracted from three-month-old *Stereospermum colais* plants

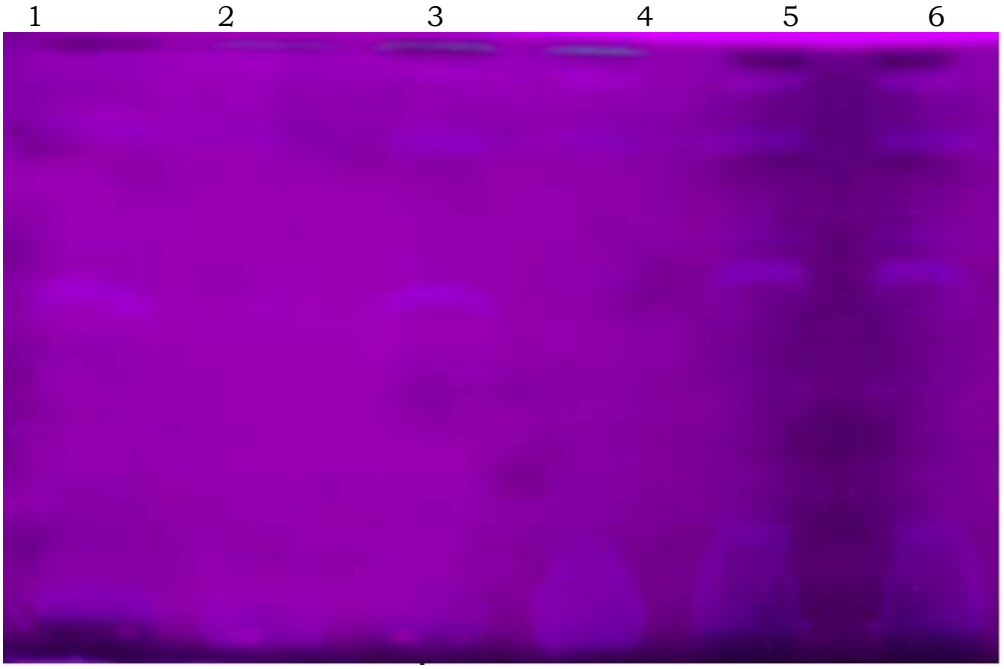

Figure 35. TLC Profile of flavonoids under UV of acetone extract of *stereospermum colais* with the solvent system chloroform: acetic acid (9:1)

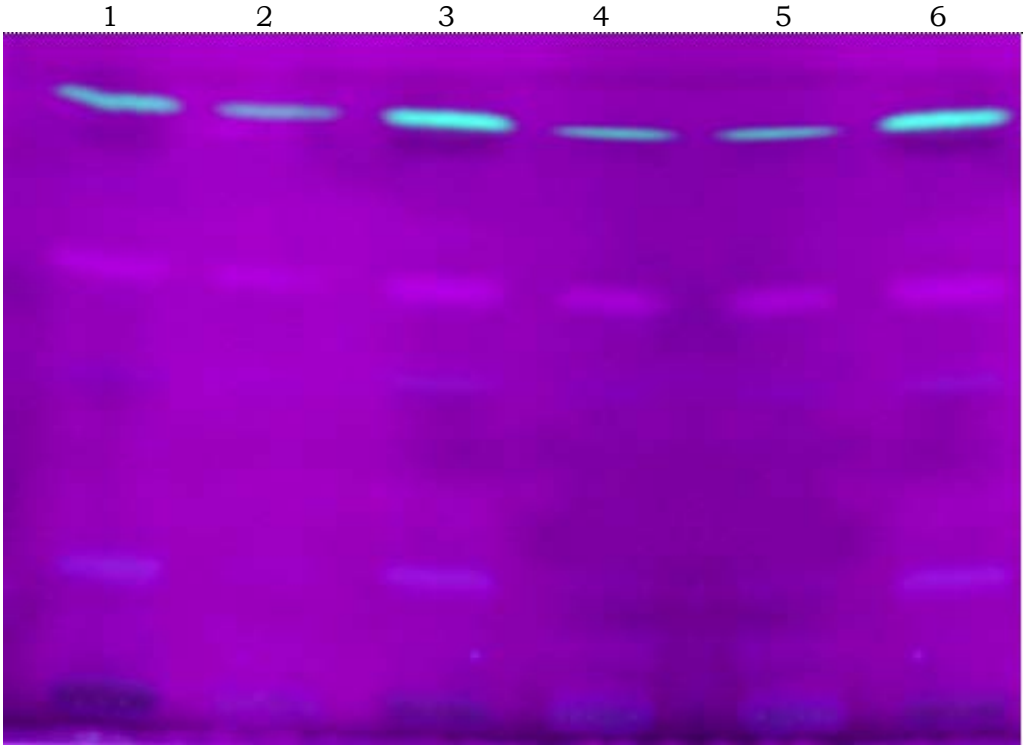

Figure 36. TLC Profile of flavonoids under UV of acetone extract of *stereospermum colais* with the solvent system cyclohexane: chloroform: acetic acid (45:45:10)

4. DISCUSSION AND CONCLUSION

The demand for herbal medicines, particularly Ayurvedic Medicines has been increasing over the years. The raw drugs for the preparation of medicines were mostly collected from the wild. The raw drugs consist of roots, stems, leaves, bark, seeds/fruits, etc. The collection of roots of trees is always destructive and will lead to the death of trees. Traditional tree cultivation for production of roots has limitations, particularly in extracting the roots. Uprooting trees for root extraction is laborious and not economical. Therefore, in the present study, rapid root production techniques for the *'Brehetpanchamoola'* trees was taken up. Various containers such as large polythene bags, earthen pots, nursery beds and PVC pipes were selected for growing the seedlings. The roots were harvested at intervals of six months, 12 months and after 24 months. As it is easy to remove the plants from the containers, there is no loss of roots. The root size varied from 2 mm to 2.5 cm in diameter. Among different containers selected for the trials, 100 cm long and 15 cm diameter size split PVC pipes were found most suitable. After harvesting the roots, the pipes can be used again.

Traditionally, the mature roots from full grown trees are preferred for the preparation of Ayurvedic Medicines. In order to assess the suitability of the roots produced in the PVC containers, phytochemical analysis was carried out. The TLC profiles obtained for polyphenolics, alkaloids and flavonoids show similar pattern for the roots of young and older age groups of *Aegle marmelos*. The TLC profiles obtained for polyphenolics show similar pattern for the young and older age groups of *Gmelina arborea*. In the case of flavonoids and alkaloids, more intense bands were obtained for the older plants of *Gmelina arborea*. Alkaloid content of *Gmelina arborea* was only present in the root bark of older plants. TLC analysis of *Oroxylum indicum* indicates that the bark region possesses more amounts of polyphenolic and flavonoid compounds than in the core part of the root. TLC analysis of *Stereospermum colais* indicates that the bark region possesses more amount of flavonoid compounds than in the core part of the root. The above findings indicate that the roots from young plants *Brehetpanchamoola* are qualitatively as good as those of mature plants in terms of polyphenolics, alkaloids and flavonoids.

5. REFERENCES

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