Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

JPP Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(6): 114-127
Received: 10-09-2021
Accepted: 12-10-2021

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Phytochemical evaluation of young roots of Brihatpanchmoola species comparative to bark

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Abstract
Brihatpanchmool is an integral part of Dashmoola herb which is a combination of ten ayurvedic herbs i.e. Bilva, Patala, Agnimantha, Shyonaka, Gambhari, Bruhati, Kantakari, Prishniparni, Shalparni, Gokshura, where part used are moola of all the drugs in a group. The first five herbs of Dashmoola are collectively called Brihatpanchmoola which is used traditionally in various ayurvedic formulations like Dashmolarishta, Dashmool ghritam, chywanprash, Mahanarayan Tail etc.
Aim of study: Due to their wide range of therapeutic actions and to meet the enormously increasing demand, procuring of these herbs has been boosted significantly resulting in an increase in pressure on their wild resources. The present study has been taken up with a view to identify the most appropriate plant part (especially young roots) which can be used in place of current practice of using root bark/stem bark.
Material and Methods: Phytochemical screening of stem bark, root bark, leaves and young roots of Brihatpanchmoola species were performed using standard methods. Quantification of biomarker compounds of aqueous extracts of each plant part was done using high performance thin layer chromatography (HPTLC) and High Performance Liquid Chromatography (HPLC) techniques.
Results: The aqueous extract of young roots of Brihatpanchmoola species showed comparable phytochemical profiles to stem bark and root bark samples.

Keywords: *Aegle marmelos* (L) Correa, Brihatpanchmool, *Clerodendrum phlomidis* L.f, Dashmool, *Gmelina arborea* Roxb, high performance liquid chromatography, *Oroxylum indicum* (L) Benth. Ex. Kurz., *Stereospermum chelonoides* (L.f.) DC

1. Introduction
Brihatpanchmool constitutes an important part of Dashmoola which comprises of a combination of 10 herbs used in combination. Dashmool consists of two categories as Brihatpanchmool which includes *Aegle marmelos, Clerodendrum phlomidis, Oroxylum indicum, Stereospermum suveolens, Gmelia arborea* while Laghu panchmul includes; *Solanum indicum, Solanum xanthocarpum, Uraria picta, Desmodium gangeticum and Tribulus terrestris* [1]. It is used in various ayurvedic remedies like for strengthening and detoxification of body, tonic for liver and kidney, in infertility, uterine tonic, used for anorexia, edema, anaemia, potent antioxidant and in various disorders of nerves, bones, joints and muscles [2]. Now days, there has been an increased global interest in traditional medicine and traditional therapies are being used worldwide to cure various ailments. So, we need to promote our therapies with more extensive scientific research and evidence base
Authors observed that Brihatpanchmoola, a very effective and important plant species needs to be examined thoroughly using scientific approach so that traditional literature and modern science can be explored jointly to have a global acceptance. Also, due to enormously increasing demand of Brihatpanchmool, it has been categorized as red listed species which is on the verge of extinction [3]. There are also various complexities associated with this species. Initially, root bark of the tree species was being used as a part of Brihatpanchmoola. Usage of stem bark in lieu of root bark or roots came into vogue long ago though the origin of this practice is not trace-able. There is no scientific evidence behind this switchover.
The present research work is based on the hypothesis that young roots could be used as alternative plant parts in lieu of root bark/stem bark. Leaves were also examined in some cases depending on the availability. The findings of our research work support this hypothesis. The age of young roots is standardized basis phytochemical data. Further, the production of young roots can be done through tissue culture and High-Density short term plantation scheme.

2. Material and Methods

2.1 Collection and Identification of raw materials

Stem bark, root bark, leaves and young roots of Brihatpanchmoola species (*Aegle marmelos* (L) Correa, *Clerodendrum phlomidis* L.f., *Oroxylum indicum* (L) Benth. Ex. Kurz., *Stereospermum chelonoides* (L.f.) DC and *Gmelina arborea* Roxb.) were received from the Bioresource Development Group, Dabur Research & Development Centre against Voucher Nos. DRDC-1258-BRD/AM, DRDC-1259-BRD/CP, DRDC-1260-BRD/SS, DRDC-1261-BRD/GA and DRDC-1262-BRD/OI). The plant materials were identified by Dr. G.P. Kimothi & Dr. C.S. Rana, Taxonomist, Dabur Research & Development Centre, Sahibabad, Ghaziabad. A voucher specimen has been retained in the department for future reference.

2.2 Chemicals and reagents

All solvents used were HPLC grade, and reagents were analytical grade. Water was purified using a Milli-Q Academic A10 water purification system (Millipore, France).

2.3 Physicochemical Studies

The fine powdered samples were analyzed for physico-chemical parameters such as the foreign organic matter, loss on drying, ash values and extractive values [4]. The samples were also screened for presence of secondary metabolites *viz.* alkaloids, glycosides, total phenols and flavonoids [5].

2.4 Preparation of Extracts

Aqueous extract of the plant materials were prepared using the decoction process. Around 200 g of the dried crushed plant materials were taken in different vessels respectively and sufficient quantity of double distilled water was added and proceeded further for extraction. The solution obtained was then concentrated and filtered. It was finally dried and collected in bottles and stored in refrigerator for further testing and evaluation.

2.5 Chromatographic Studies

2.5.1 Development of fingerprint profiles Sample preparation

1 g of extract was taken, added 5 ml of methanol, sonicated for 10 minutes and then volume made up to 10 ml in a volumetric flask with methanol.

Stationary phase

Precoated silica gel 60F254 TLC aluminum sheets obtained from E. Merck was employed as stationary phase.

Solvent system

Different solvent system used for each plant species

Procedure

Specific quantity of the each sample was applied with the help of Linomat V as bands on the TLC plate and developed with mobile phase up to 90mm. The developed chromo- plate was dried by dryer. The spots were found to be visible under UV 254 nm. Then the plate was sprayed with vanillin spraying reagent and dried in hot oven at 105 ºC for 5-10 min. Then photo documented with the help of digital camera. Rf value of each sample was then calculated [6].

2.5.2 Estimation of marker compounds using high performance liquid chromatography (HPLC) [7]

2.5.2.1 Quantification of Marmelosin Content in Bilva by HPLC

Sample/Extract preparation: 100 mg of extract was sonicated with methanol in a 50 ml volumetric flask for 20 min. and volume made up to 50 ml with methanol.

Standard preparation: Around 2.5 mg of marmelosin standard (Sigma Aldrich) was dissolved in methanol in a 10 ml volumetric flask and made up to volume with methanol. 1ml aliquot from this solution was again diluted up to 10 ml with methanol.

Column: C18, Hypersil, 5μ, 250 × 4.6 mm

Mobile phase: Gradient
A: 10 mM ortho phosphoric acid;
B: Methanol

Flow: 1 ml/min

λmax: 247 nm

2.5.2.2 Quantification of Oroxylin-A Content in Syonak-B by HPLC

Sample/Extract preparation: Approx. 1.0 gm -2.0 gm of extract was weighed into a 25 mL volumetric flask, 15 ml of methanol was added and sonicated for 15 min. Final volume was made up with methanol.

Standard preparation: 2-3 mg of standard was weighed in a 50 ml volumetric flask. The standard was dissolved completely using around 10 ml methanol and final volume was made up using methanol.

Column: Phenomenex C18, 5μ, 250 × 4.6 mm

Mobile phase: 0.1 %TFA: ACN (60:40)

Flow: 1 ml/min

λmax: 274 nm

2.5.2.3 Quantification of Lupeol Content in Padal by HPLC

Sample/Extract preparation: Approx 1-2 gm of extract was weighed in a 20 mL volumetric flask, 15 ml of methanol was added and sonicated to dissolve. Final volume was made up with methanol.

Standard preparation: 3-5 mg of standard was weighed in a 50 ml volumetric flask. The standard was dissolved completely using 10 ml methanol and final volume was made up using methanol.

Column: Phenomenex C18, 5μ, 250 × 4.6 mm

Mobile phase: Methanol: ACN (30:70)

Flow: 1 ml/min

λmax: 210 nm

2.5.2.4 Quantification of Apigenin Content in Gambhar by HPLC

Sample/Extract preparation: Approx 1-2 gm of extract was weighed in a 25 mL volumetric flask. The extract was dissolved in 1 ml DMSO and final volume was made up with ethanol.

Standard preparation: 2 mg of standard was weighed in a 50 ml volumetric flask. The standard was dissolved completely using 1 ml DMSO and final volume was made up using ethanol.

Column: Phenomenex C18, 5μ, 250 × 4.6 mm

Mobile phase: 0.1 %TFA:ACN (60:40)

Flow: 1 ml/min

λmax: 377 nm

3. Results and Discussion

3.1 Physicochemical Observations

Results of physicochemical observation are shown in Figure 1-5.

The physicochemical parameters selected for comparative analysis are ash content, acid insoluble ash, water soluble and alcohol soluble extractive values, total alkaloids, flavonoids, total phenols and glycosides.The determination of total ash is a very important parameter as it tells about the presence of inorganic matter present in the plant. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

The acid-insoluble ash indicates the presence of siliceous matter in the drug. The total ash and acid insoluble ash content observed in almost all of the five species is found be comparatively lesser in younger roots compared to bark samples. However, it has also been found that it varies between different batches of the same part of the plant. This may be due to the process of collection and many other factors like storage condition etc.

Extractive value determines the amount of active constituents extracted with solvents from a given amount of samples. This is also an important parameter which reveals the amount of various active constituents [8]. The extractive values in younger roots are found to be comparable to bark samples in all of the five species studied. From the data obtained, we can say that young roots have a comparable water and alcohol soluble extractive values to bark samples. From the data of secondary metabolites (alkaloids, flavonoids, glycosides and phenols) we can infer that it varies in different batches of the same part of the plant which gives an indication that herbs are prone to variation in their chemical nature depending on the source (wild or cultivated) and also on the basis of geographical region. However, we can infer that young roots also contain a substantial quantity of active constituents.

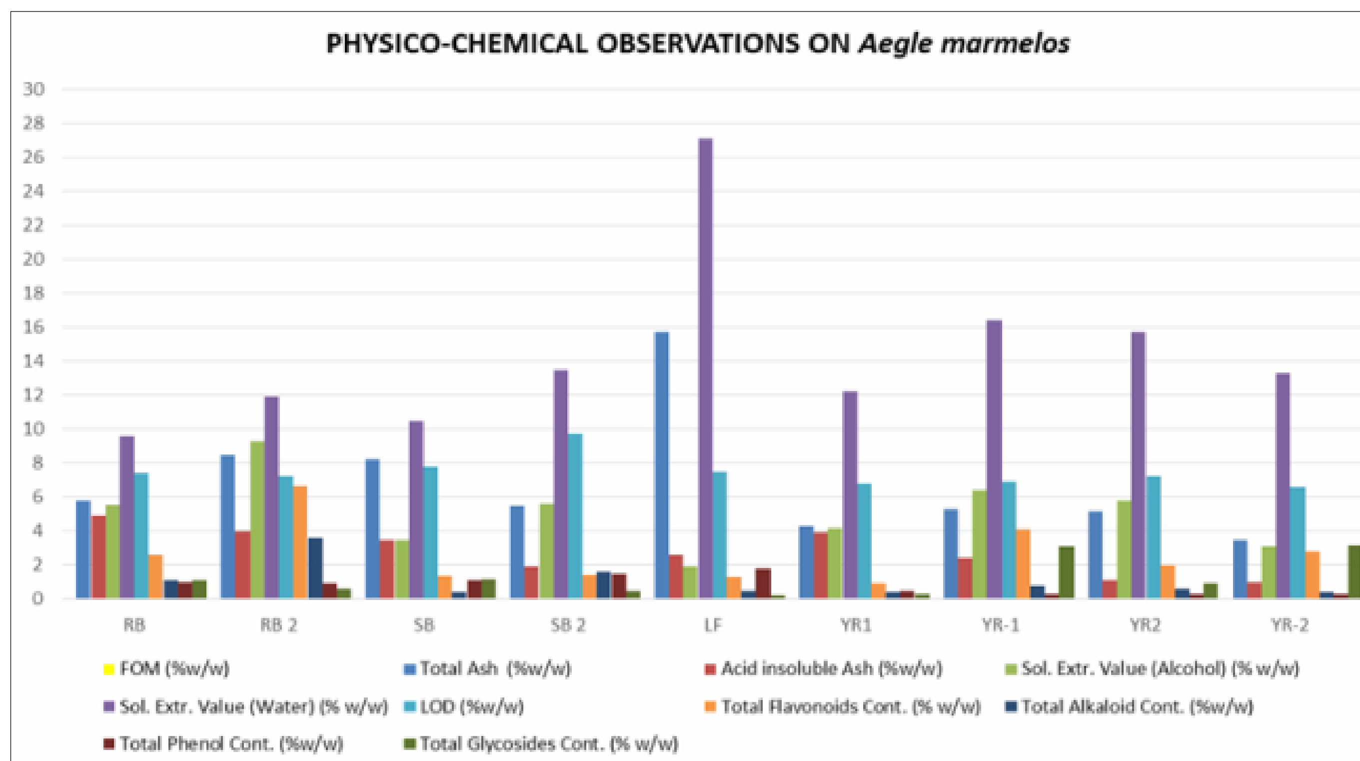

Fig 1: Physicochemical observations on *Aegle marmelos*

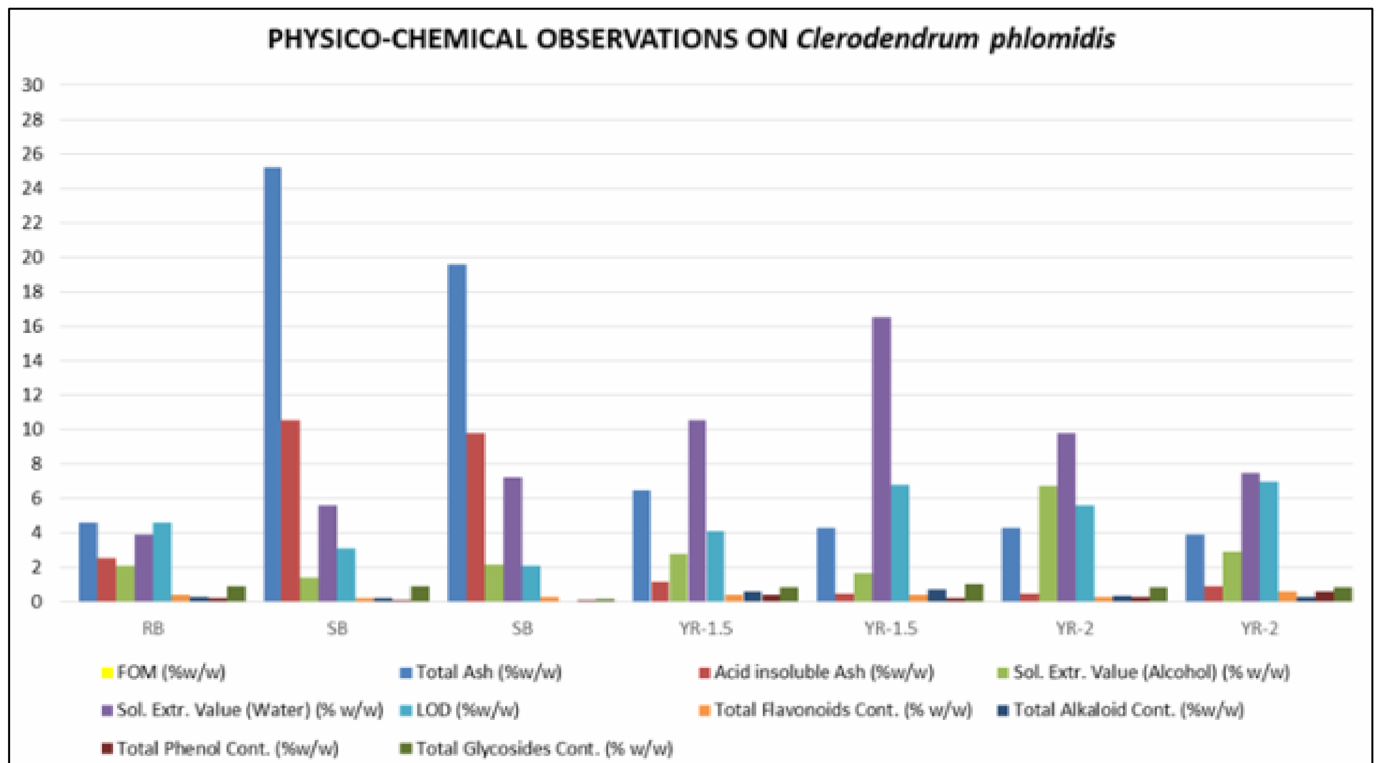

Fig 2: Physicochemical observations on *Clerodendrum phlomidis*

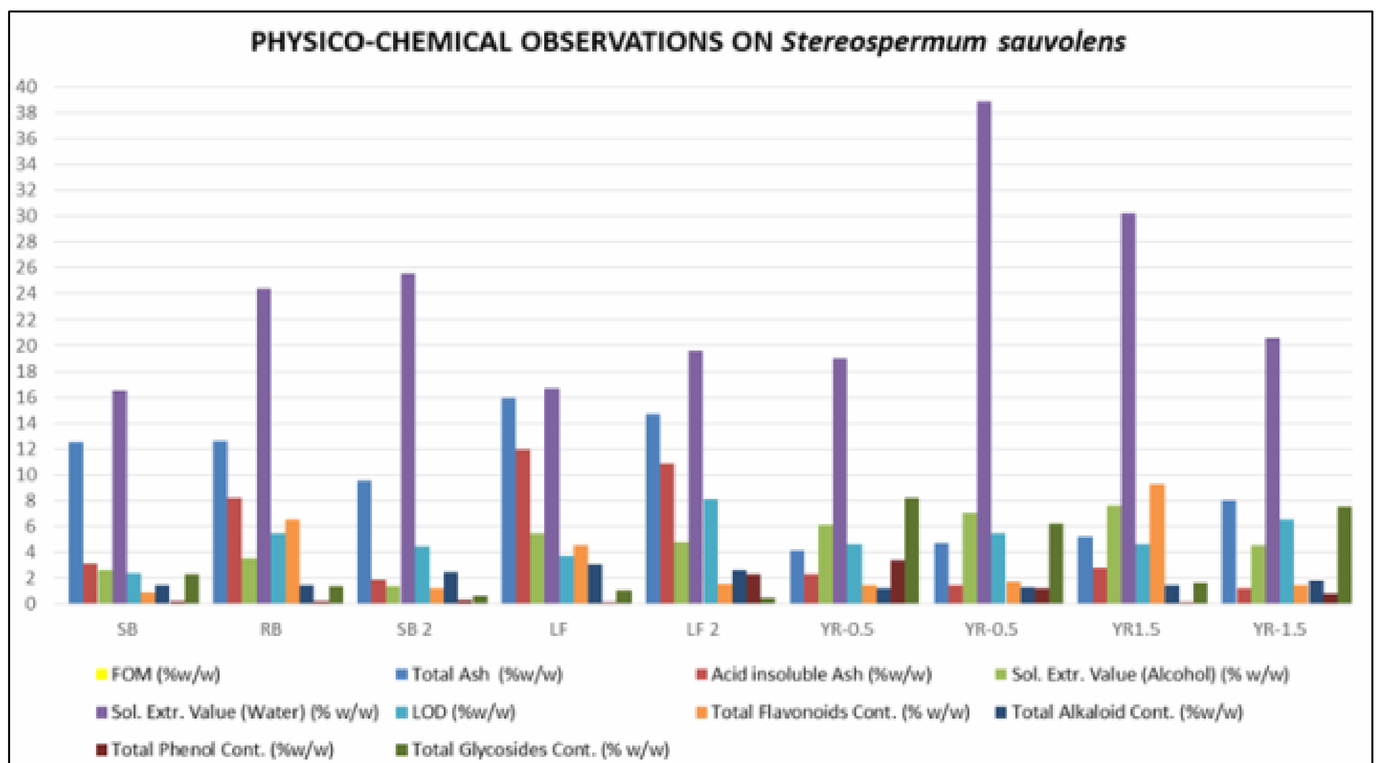

Fig 3: Physicochemical observations on *Stereospermum sauveolens*

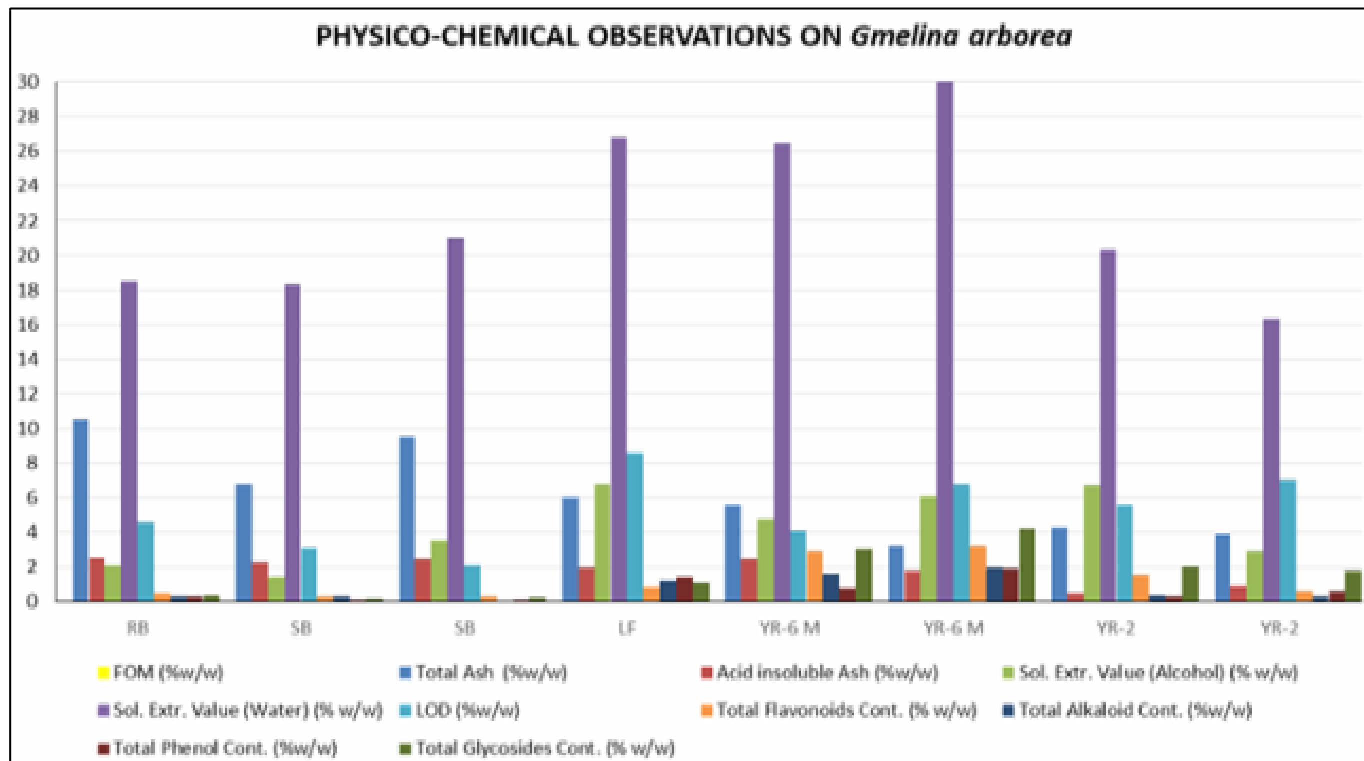

Fig 3: Physicochemical observations on *Stereospermum sauveolens*

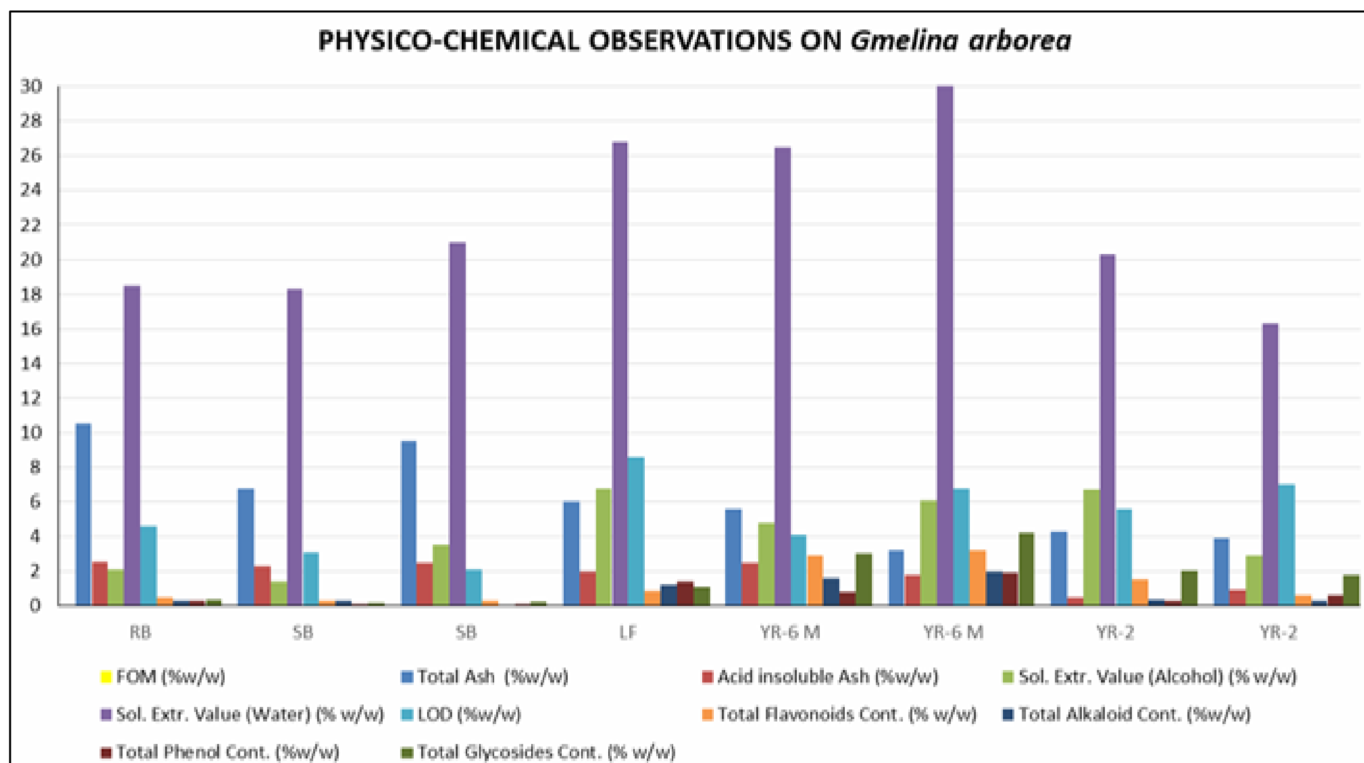

Fig 4: Physicochemical observations on *Gmelina arborea*

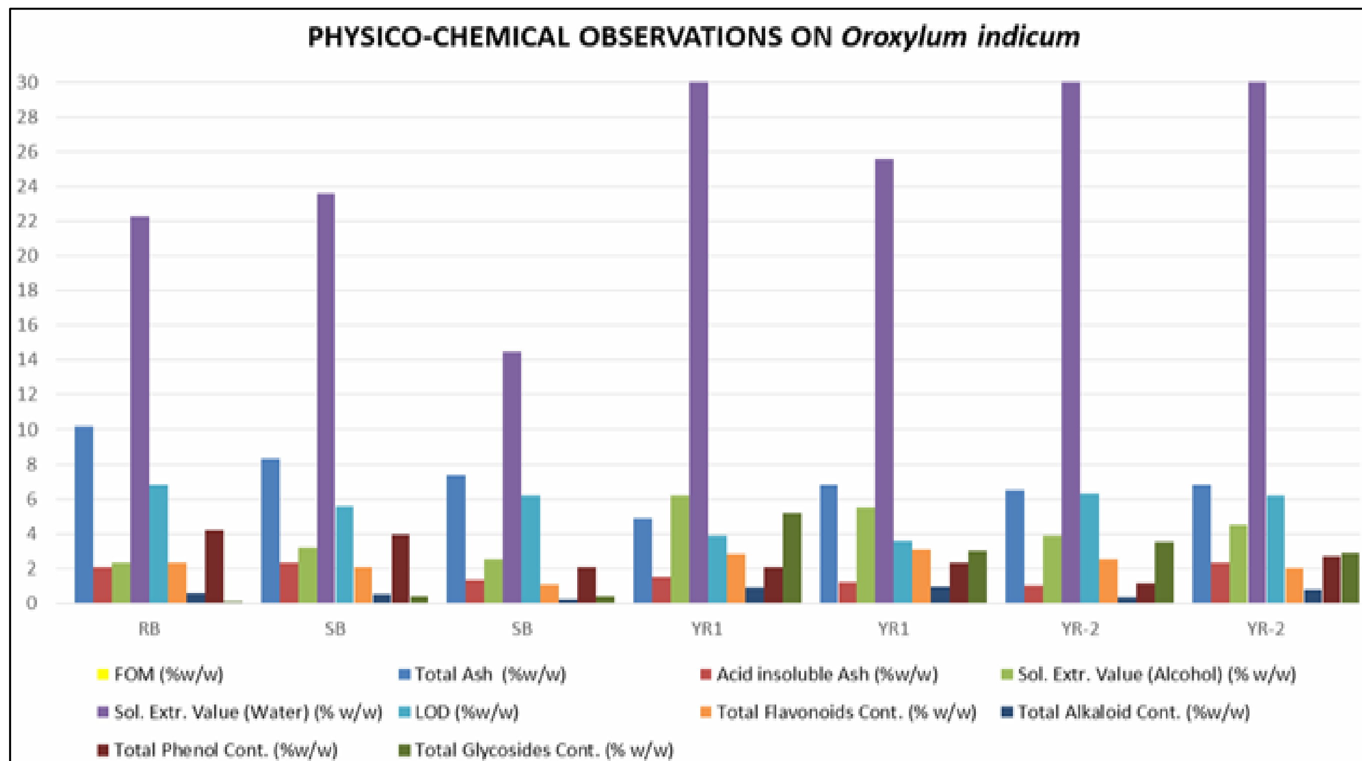

Fig 5: Physicochemical observations on *Oroxylum indicum*

3.2 HPTLC Scanning Profile

One to One comparison of HPTLC profiling is shown in Figure 6-17. HPTLC profiling shows many common peaks between young root extract and bark extract of all the species although the intensity of peaks varies.

Since, Brihatpanchmoola is generally used as fixed dose combination and rarely used individually, all the five extracts were also combined in 1:1 ratio and evaluated for HPTLC.HPTLC profiling of extracts mixture shows many common peaks, however more number of peaks are observed in young roots mix extracts compared to bark extract mixture

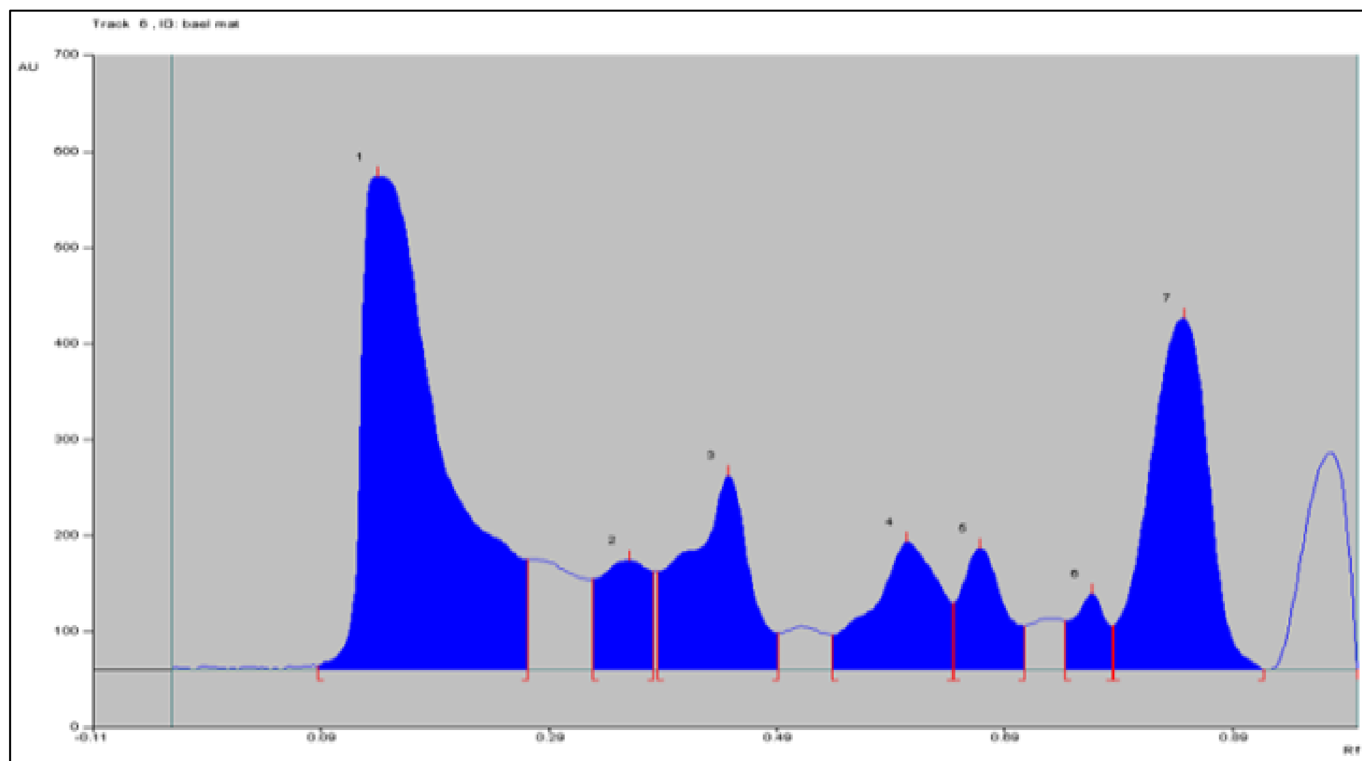

Fig 6: HPTLC chromatogram obtained from aqueous extract from of Bael Chhal

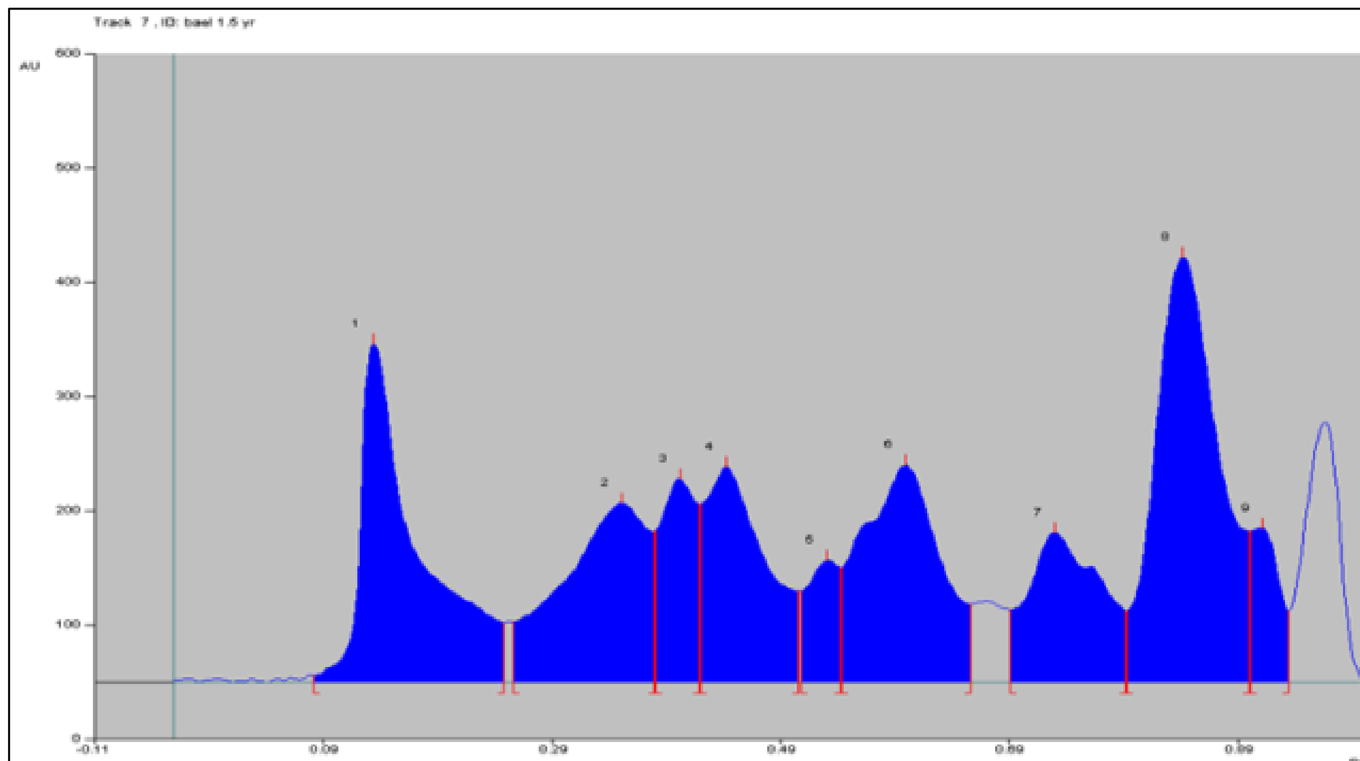

Fig 7: HPTLC chromatogram obtained from aqueous extract from Bael Root-1.5 yr

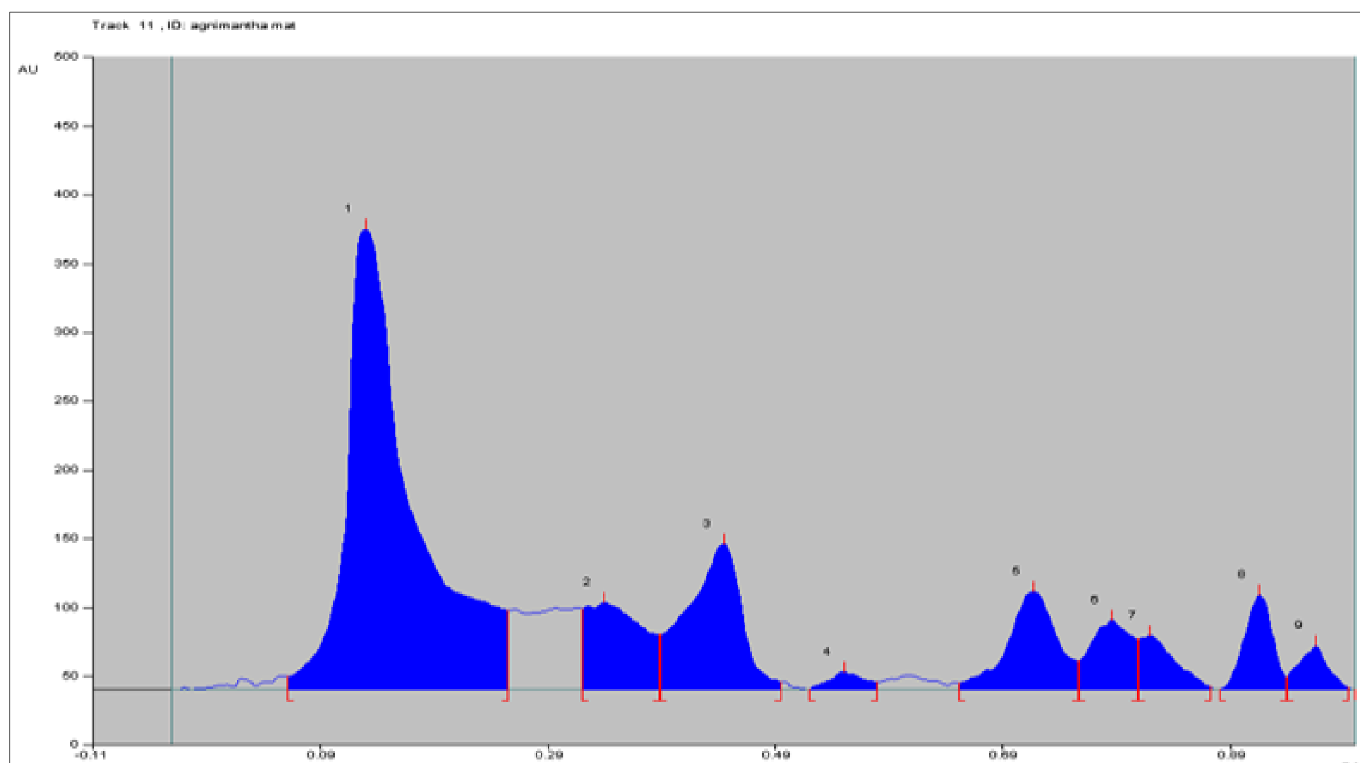

Fig 8: HPTLC chromatogram obtained from aqueous extract from Agnimantha Mature

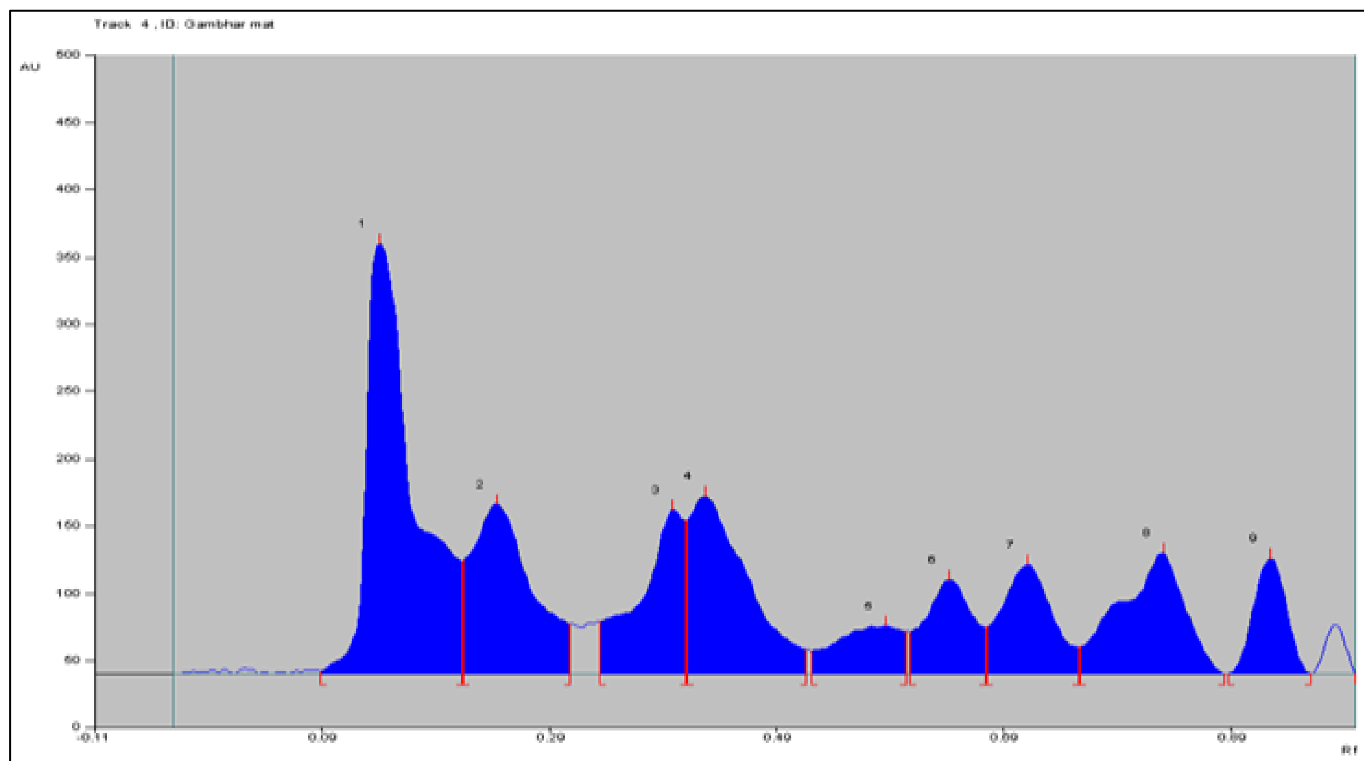

Fig 9: HPTLC chromatogram obtained from aqueous extract from Agnimantha Root-1.5 yr

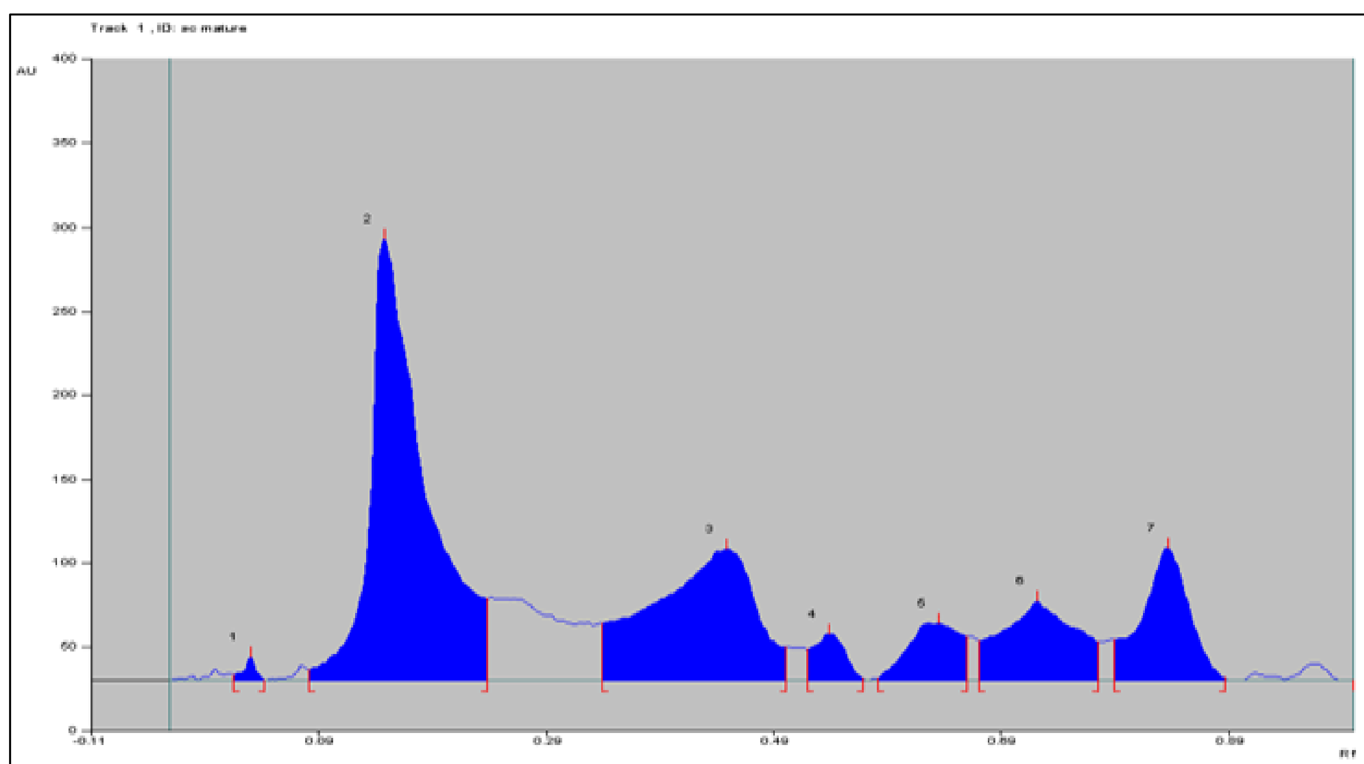

Fig 10: HPTLC chromatogram obtained from aqueous extract from Gambhar Chhal

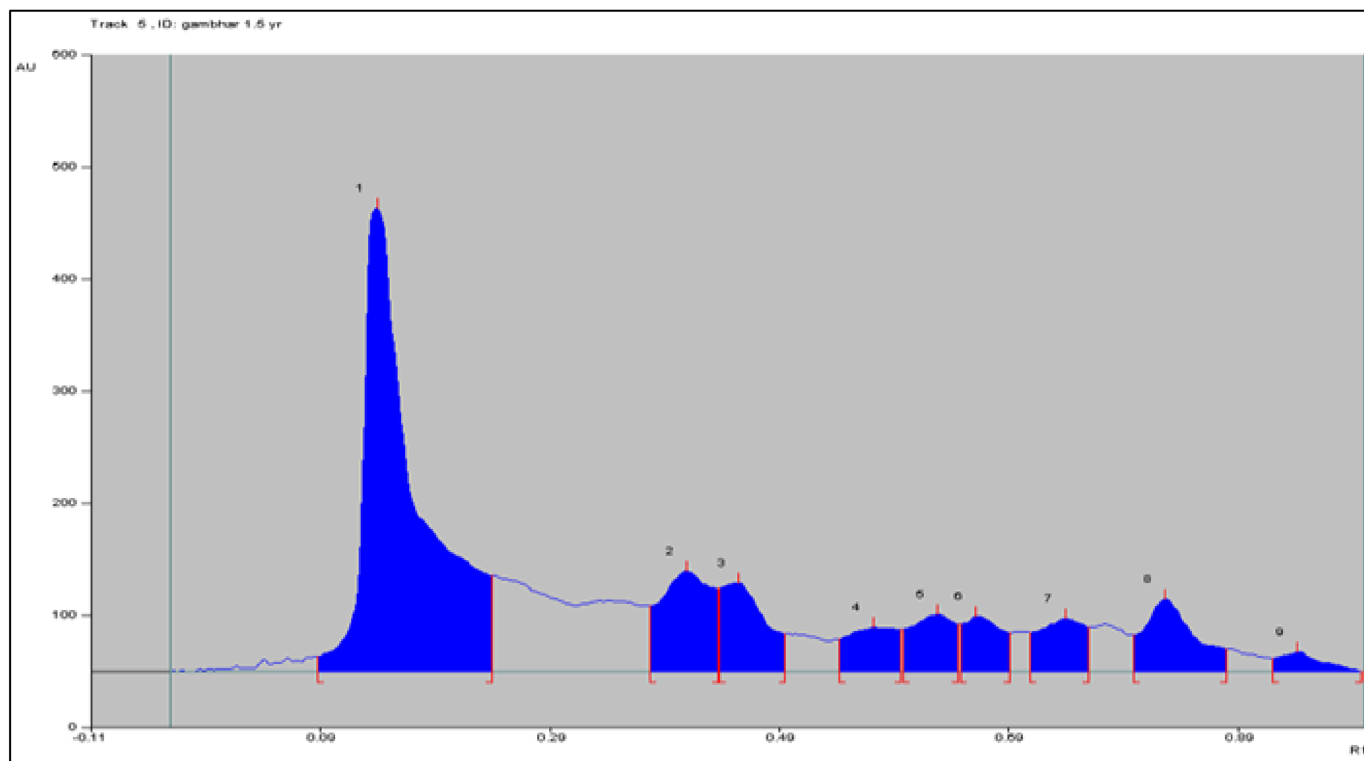

Fig 11: HPTLC chromatogram obtained from aqueous extract from Gambhar Root-1.5 yr

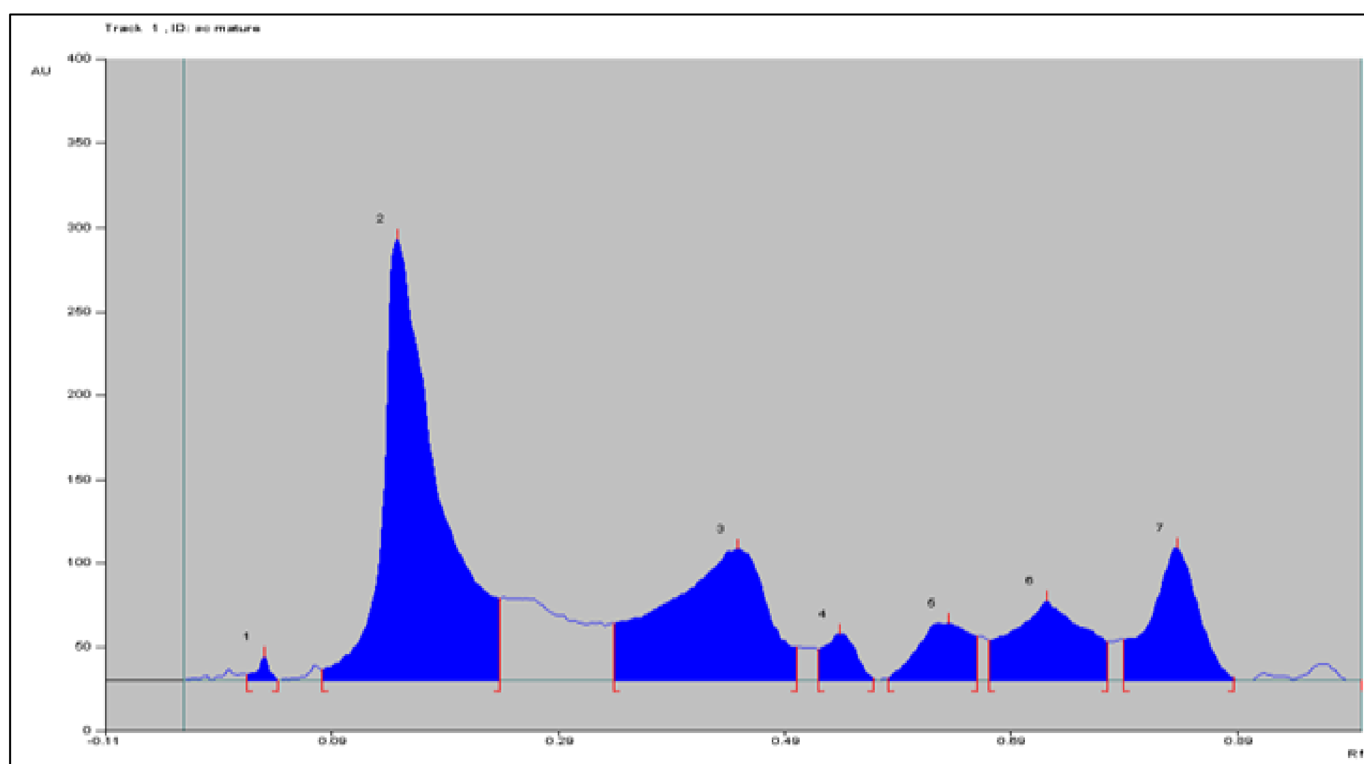

Fig 12: HPTLC chromatogram obtained from aqueous extract from Syonak Chhal

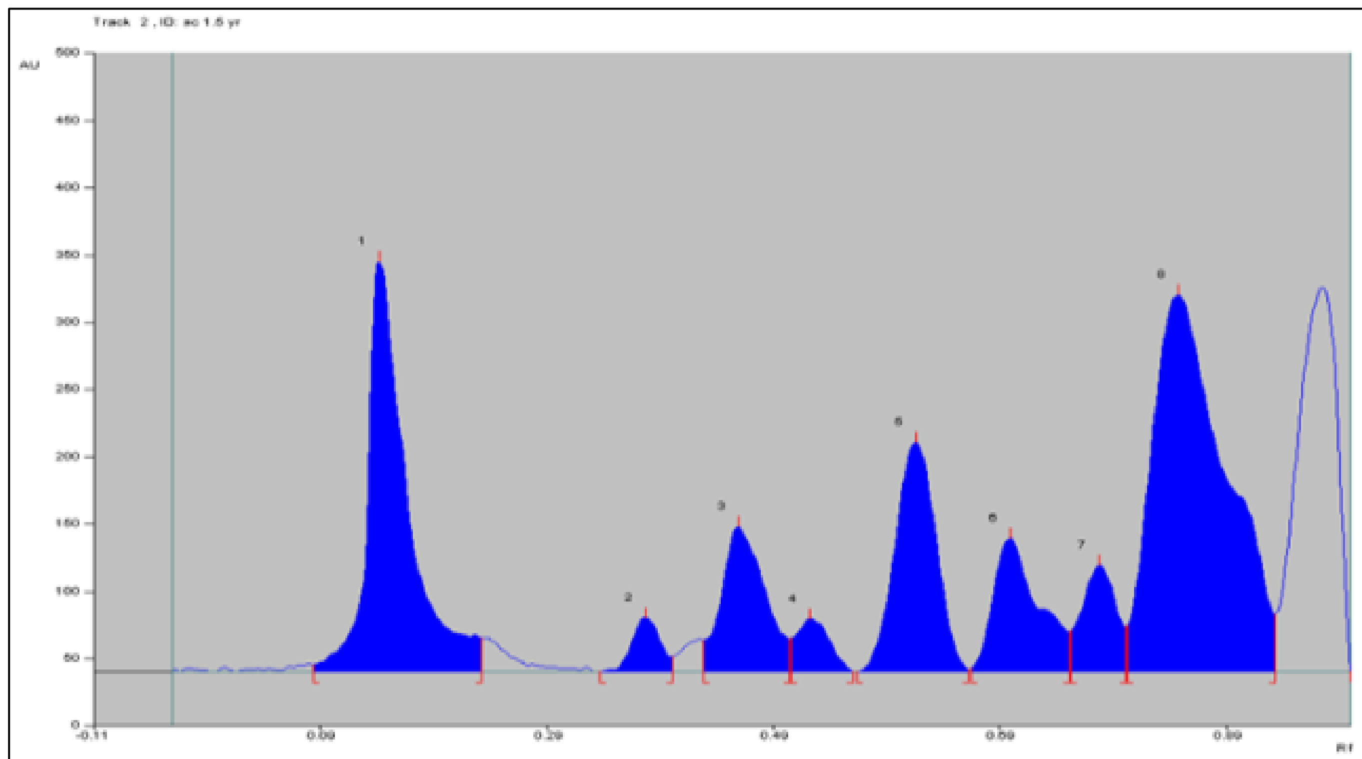

Fig 13: HPTLC chromatogram obtained from aqueous extract from Syonak Root-1.5 yr

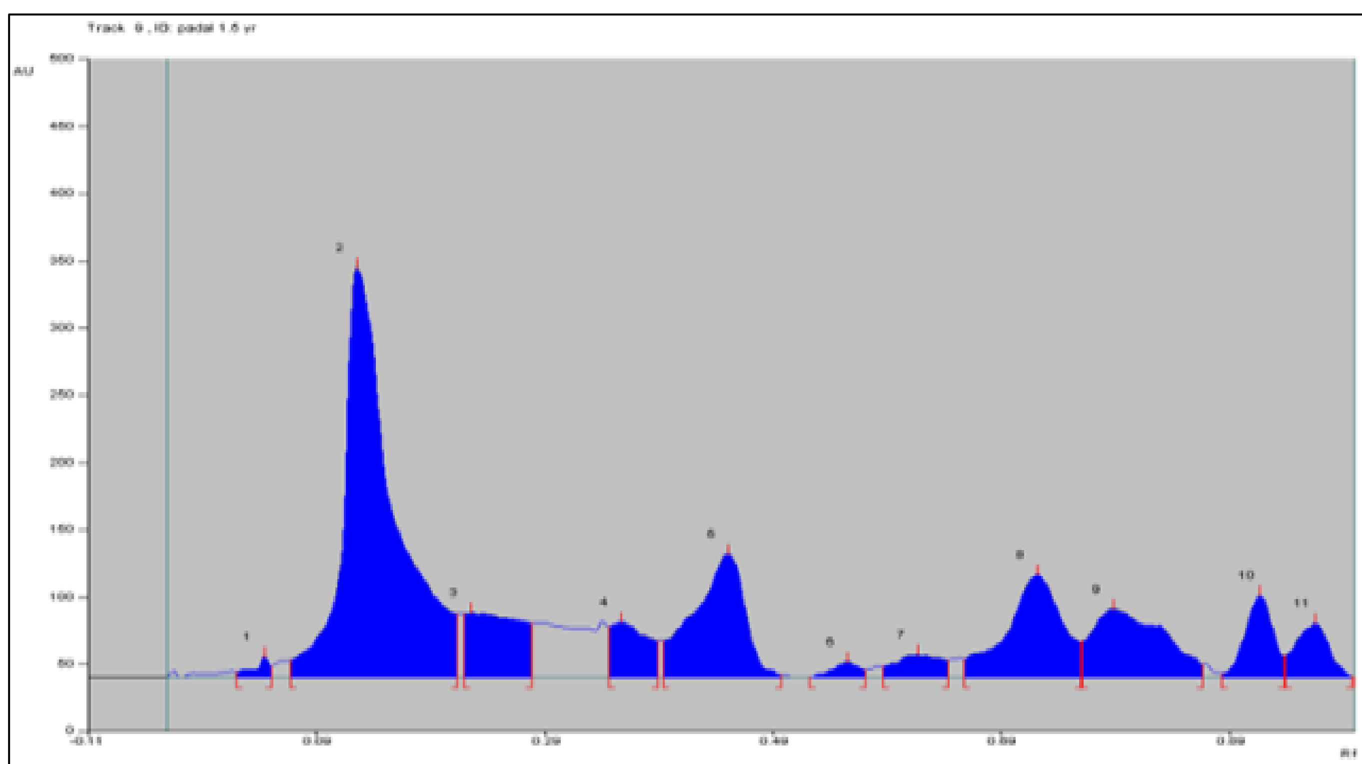

Fig 14: HPTLC chromatogram obtained from aqueous extract from Padal Chhal

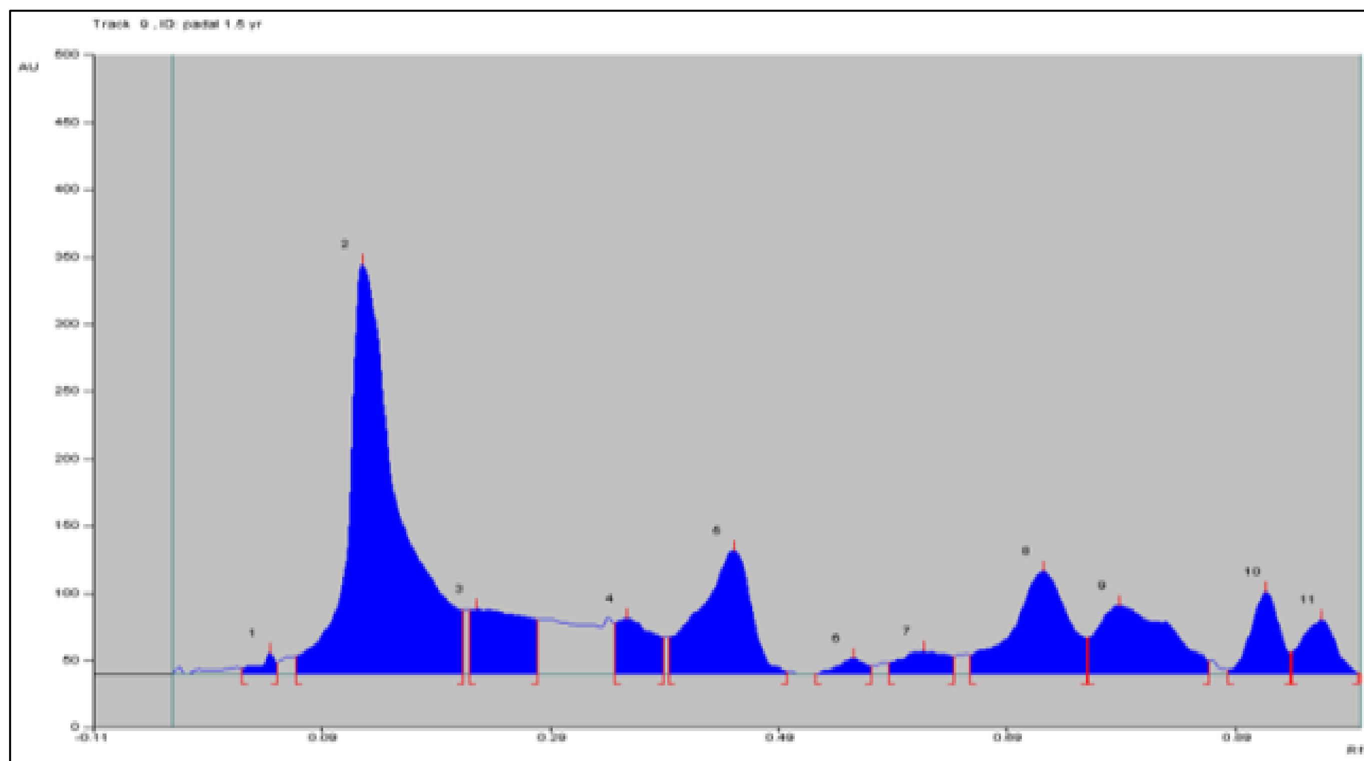

Fig 15: HPTLC chromatogram obtained from aqueous extract from Padal Root-1.5 yr

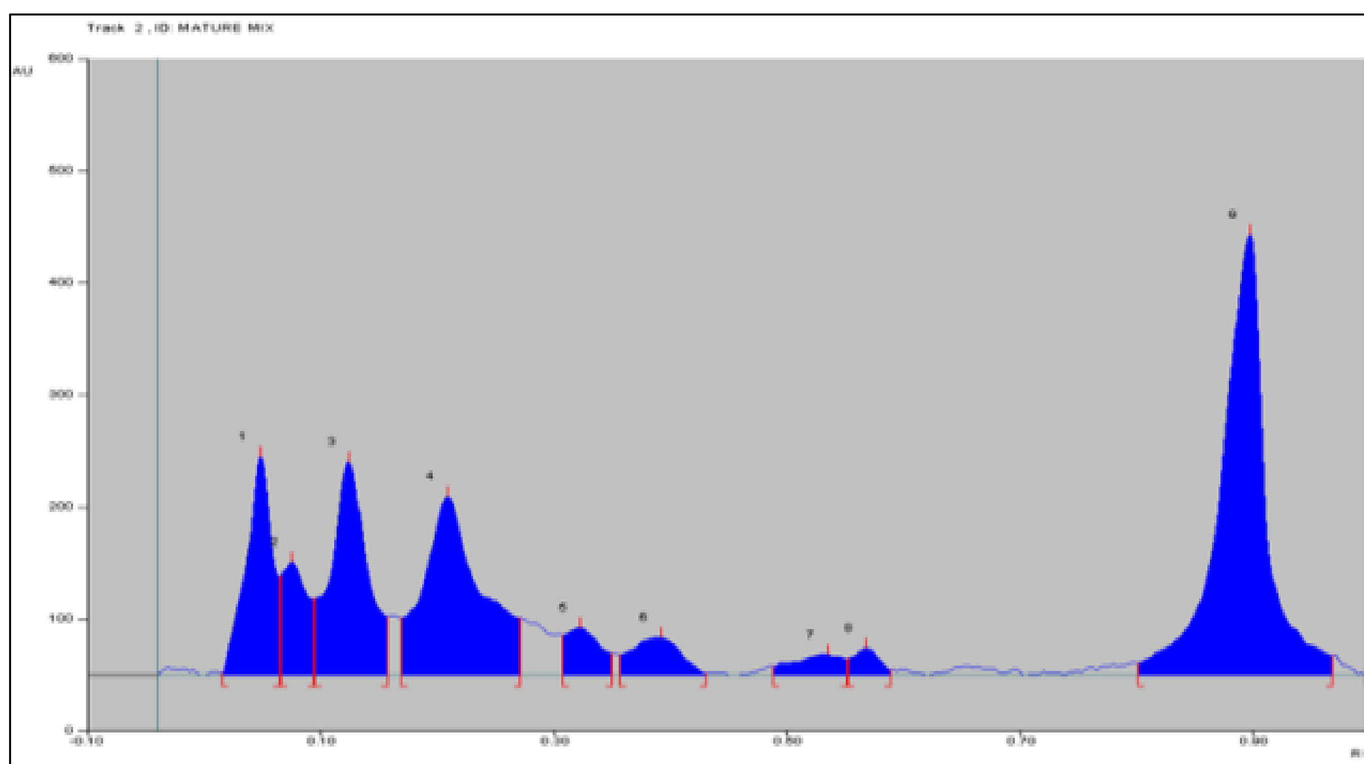

Fig 16: HPTLC chromatogram obtained from mixture of aqueous extract of bark from all Brihatpanchmoola species

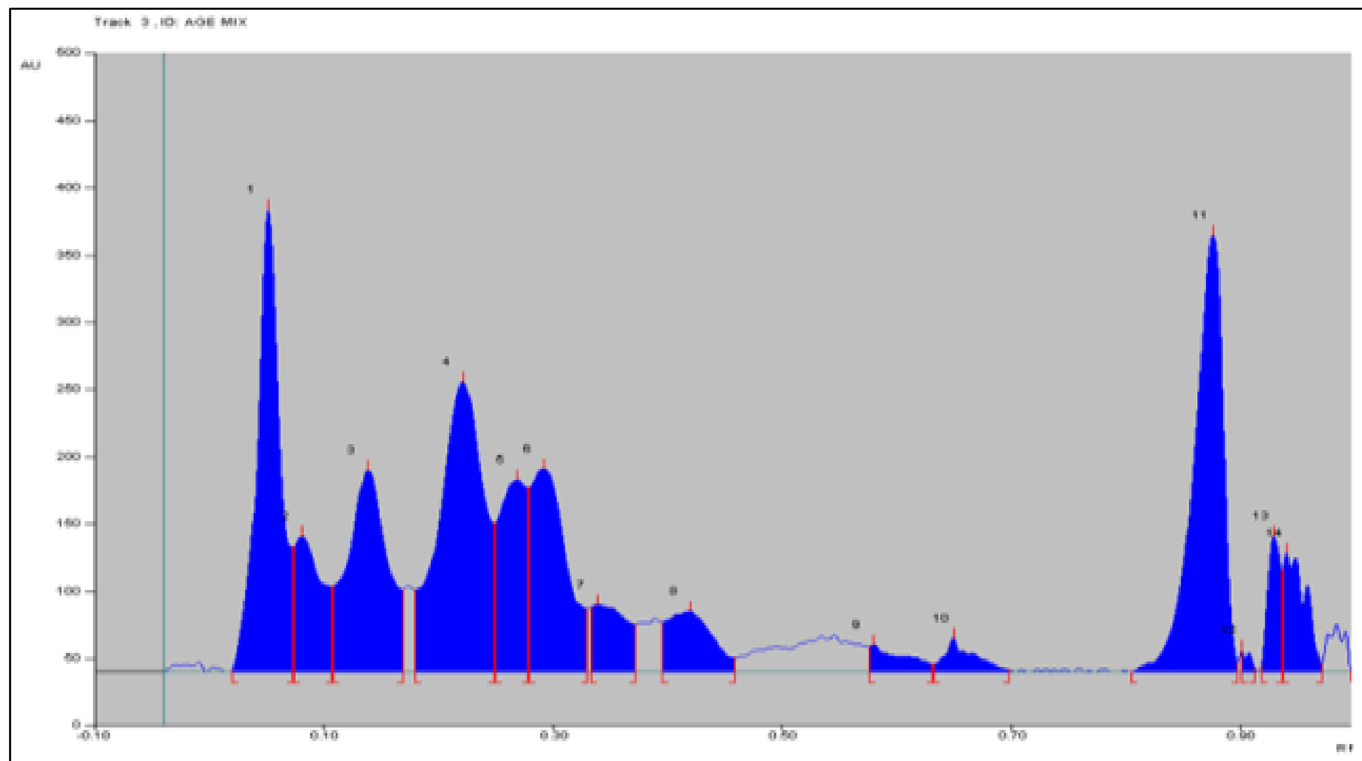

Fig 17: HPTLC chromatogram obtained from mixture of aqueous extract of young Root- 1.5 yr from all Brihatpanchmoola species

From, HPTLC analysis, it has been found that the young roots extracts mixture showed number of peaks and hence, offer logical possibility of their use. The biosynthesis of secondary metabolites is more active in the roots during initial years of tree species. This process shifts to aerial parts at a later stage only. Thus, the chemical complexity is likely to be greater in the young roots offering opportunities for a significant biological activity.

3.3 Quantification of marker compounds using HPLC

The comparative data for the presence of various marker compounds in samples are shown in Figure 18-21. The HPLC analysis shows the presence of marker compounds both in young roots and bark samples, although amount may vary in different samples.

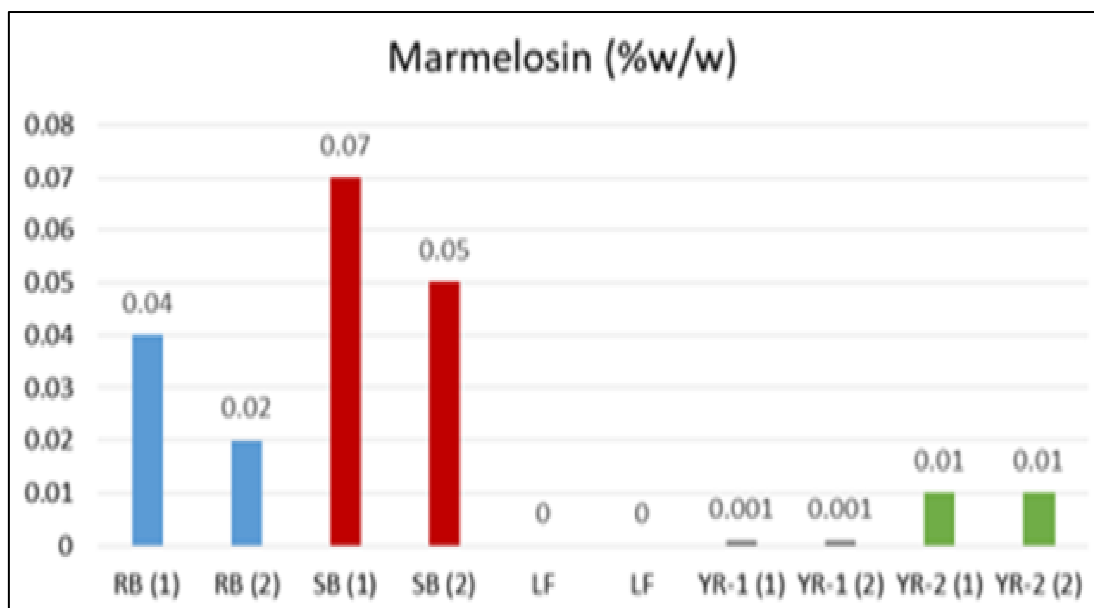

Fig 18: Marmelosin content in different part of *A. marmelos*

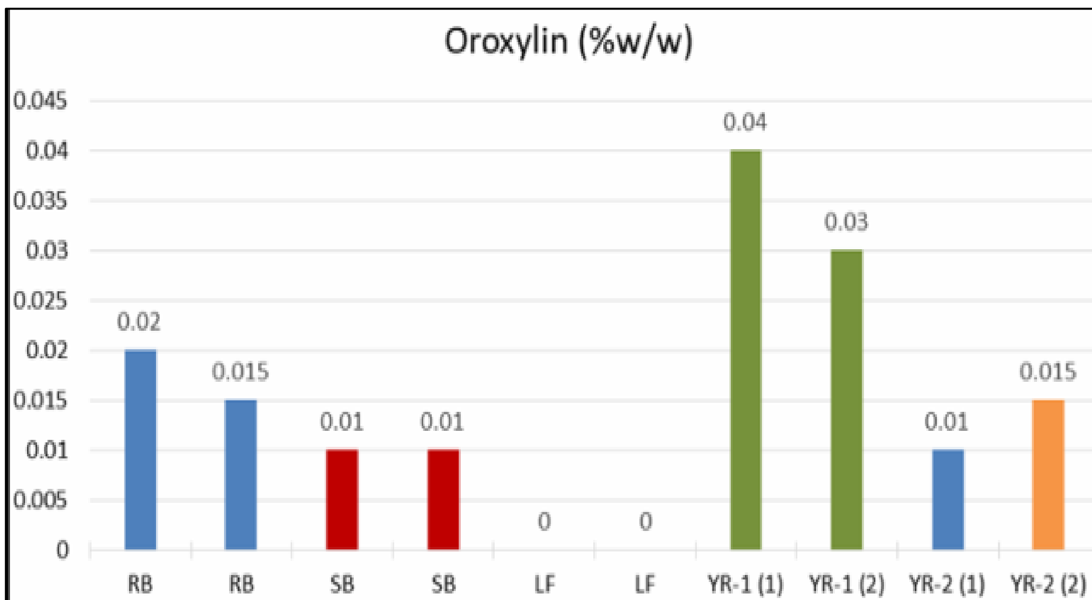

Fig 19: Oroxylin content in different parts of *O. indicum*

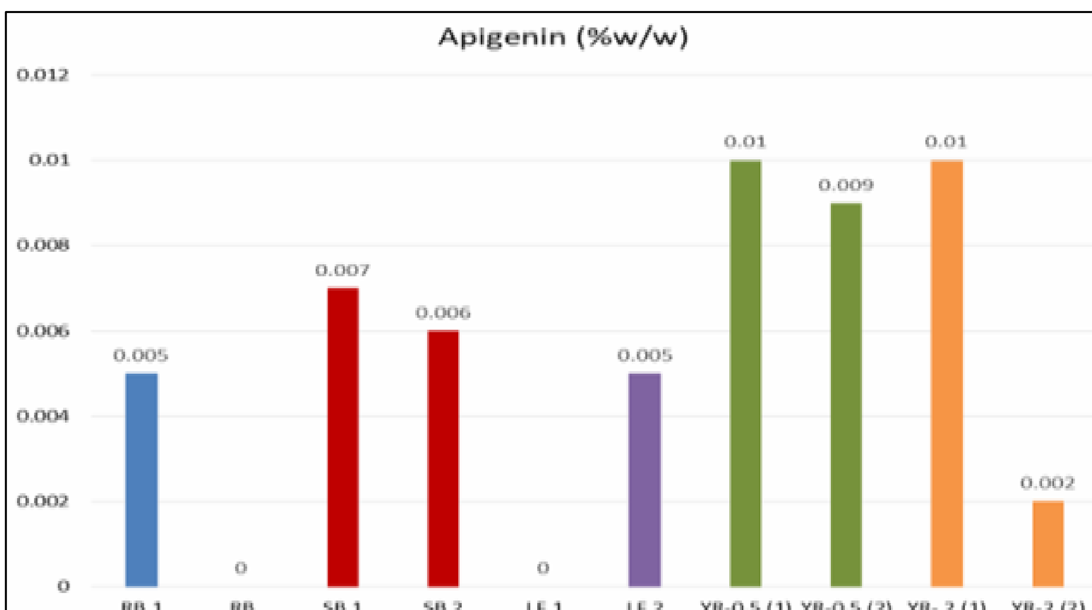

Fig 20: Apigenin content in different parts of *G. arborea*

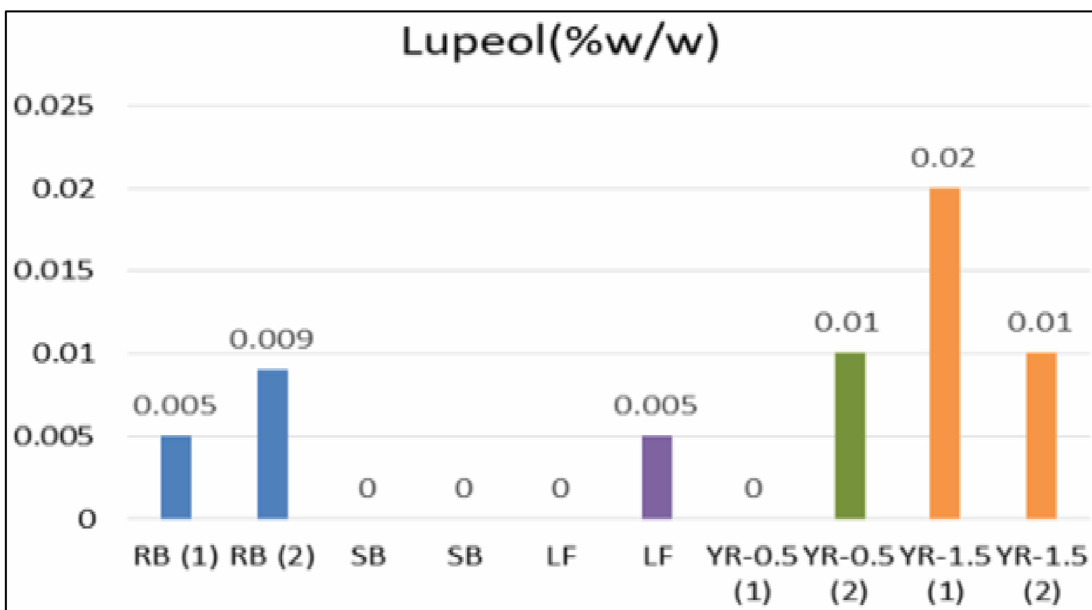

Fig 21: Lupeol content in different parts of *S. sauveolens*

Marmelosin content in *A. marmelos* is found to be highest in stem bark sample followed by root bark although the content is found to be variable between different batches of the same part. It is found to be absent in the leaf sample Young root-2 yr sample contains almost 10 times the content of marmelosin than Young root-1 yr sample.
Apigenin content in *G. arborea* is found out to be the maximum in young root-6 M sample. One batch of Young root-2 yr sample also contains maximum apigenin content compared to other samples whereas other batch of young root- 2 yr contains lowest apigenin content. Overall, we observed lot of variation in apigenin content between both of the batches studied for the same part of the plant.
Lupeol content is found to be highest in one batch of young root-1.5 yr whereas it is not detected in stem bark and one batch of leaves. Second batch of leaves extract contains 0.005 % of lupeol which is equal to one batch of root bark. Again, variation is observed between different batches of same plant part. Oroxylin content is found to be highest in Young root-1 yr samples. Root bark and young root-2 yr sample contains approximately similar oroxylin content. Lowest content is found in stem bark and it is found to be absent in the leaves samples.

4. Discussion and Conclusion

4.1 Current status of research and development in the subject

From the data obtained, most of the earlier work on Brihatpanchamoola species has been on their chemical composition and several components have been reported. Whereas, the work on young roots of Brihatpanchamoola has been sparse. The present research work is the first such attempt to re-evaluate Brihatpanchmoola species using appropriate scientific investigations. Because of a large demand of Brihatpanchmoola species, regular availability of root/stem bark is ruled out. Also, it is associated with a lot of environmental and social complexities. Therefore, there is a strong need to explore other renewable parts of Brihatpanchmoola to meet the growing demand and to preserve the wild resources and the present research work is an attempt to solve the issue.
As per the data obtained, we can infer that young roots have a comparative phytochemical profiles to bark samples of Brihatpanchmoola. Further, young roots can be made available through tissue culture technique and it will be very useful for timely availability of raw materials. The findings of the work can be further explored on a commercial scale. The study can also be extended to comparative biological activities of young roots and bark samples which offers a wide scope for future research.

5. Acknowledgement: Authors acknowledge Dr. JLN Sastry, Head Research- Dabur India Ltd, and Prof. Ramesh Kumar Goyal, Vice Chancellor, Delhi Pharmaceutical Sciences and Research University for providing necessary facilities for carrying out this research study.

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