



Research Article

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HPTLC PROFILING OF USEFUL PARTS OF FIFTEEN PLANTS IN AYURVEDA WITH COLD POTENCY

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ABSTRACT

Any drug to act as a medicine essentially should be potent. Potency is an expression of the activity of a drug in terms of the concentration or amount of the drug required to produce a defined effect, whereas clinical efficacy judges the therapeutic effectiveness of the drug. Potency is a good preclinical marker of the therapeutic potential of a drug. Ayurveda mentions a term 'Veerya' in context of modus operandi of a drug which indicates the 'potency of the drug'. Dual potency either as cold or hot is also the contributory theory of Ayurveda. Possibility to identify the nature of potency through current sophisticated tools is interrogated in the present communication. The paper focuses on HPTLC finger print profile of useful parts of 15 medicinal plants possessing Sheeta Veerya (cold potency) according to Ayurveda. For ethanol extract of randomly selected 15 plants of cold potency, a plate was developed in mobile phase of Toluene: Ethyl acetate: (8:2), scanned under UV at 254 nm, 366 nm, and derivatized after dipping in vanillin-sulphuric acid. R_f value, colour of spots and densitometric scan were recorded. 15 test samples followed a typical HPTLC fingerprint pattern on densitometric scanning at different wavelengths, which was further analyzed based on principles of Dravyaguna including panchamahabhoota principle to rationalize specificity of R_f value, and found relevant. The experiment is an evidence to authenticate the concept of 'Cold Potency' delineated in Ayurveda facilitating the global acceptance of its principles.

Keywords: Ayurveda, Plants, Sheeta veerya, Cold potency, HPTLC finger print profile.

INTRODUCTION

Veerya (Potency) of any drug makes it perform an action¹. It is the most debated topic in history till date². Various opinions have been put forth by various scientists in olden and recent years about the concept of the potency. A few Scientists also have worked through physico-chemical and experimental approach as to ascertain this concept³, but a definite remark is still awaited. Concept of Veerya along with other concepts of Rasapanchaka (the five functional units of a drug) is an ancient tool in Ayurveda to define and understand a medicine.⁴ Attempt for the accreditation the Concept of Veerya (potency) will facilitate the drug study in Ayurveda and also help re-validation of ancient concepts in Ayurveda as to globally present it as evidence based.

Chromatography is an analytical method which establishes qualitative, as well as quantitative profiles of a crude drug constituents thus confirming the quality control and quality assurance.⁵ It is a physical process where mixture of components are separated into individual components through distribution between two phases. It is based on the differences in the rate at which the components of the mixture move through porous medium (called stationary phase) under the influence of moving phase which is solvent⁶. The main adoption of High Performance Thin Layer Chromatography is standardization and isolation of active principles in field of medicine. Current study holds on to the basic principle behind the elution with regards to its affinity towards either stationary or mobile phase. The

fingerprint pattern for fifteen plants of cold potency is studied with Ayurvedic parlance. The work is an attempt to match the both HPTLC and Dravyaguna principles and try bridging it in context of concept of veerya (potency) with special reference to Sheeta veerya. (Cold potency)

MATERIALS AND METHODS

Collection of Plant material

Dry, raw samples of useful parts of 15 plants described in literature of Ayurveda processing Sheeta Veerya ^{7,8} namely

1. Amalaki phala (Fruit of *Emblia officianalis*)
2. Asana twak (Bark of *Pterocarpus marsupium*)
3. Arjuna twak (*Bark of Terminalia arjuna*)
4. Dhataki pushpa (Flowers of *Woodfordia fruticosa*)
5. Gokshura phala (Fruit of *Tribulus terrestris*)
6. Jatamamsi mula (Rhizomes of *Nardostachys jatamamsi*)
7. Kapikacchu phala (Fruit of *Mucuna pruritis*)
8. Katuki mula (Rhizomes of *Picrorhiza kurroa*)
9. Kadira twak (Bark of *Acacia catechu*)
10. Musta rhizomes (Rhizomes of *Cyperus rotundus*)
11. Shatavari mula (Roots of *Asparagus racemosus*)
12. Sariva mula (Roots of *Hemidesmus indicus*)
13. Usheera mula (Roots of *Vetiveria zizanioides*)
14. Udumbara twak (Bark of *Ficus racemosa*)
15. Yastimadhu mula. (Roots of *Glyzyrrhiza glabra*)

Samples were collected from the raw drug section of SDM Ayurveda Pharmacy, Udupi, identified and authenticated based

on macroscopic examination after comparison with botanical descriptions⁹ in the flora and monographs.

Extraction of plant material

One gram of powdered samples of dry useful parts of the 15 plants were extracted with 10 ml ethanol by cold percolation for 24h and filtered in SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi.

HPTLC Study

CAMAG HPTLC system (Switzerland) comprising of Linomat 5 TLC applicator was used in the study carried out in SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka. 5 and 10 µl of the sample extracts were applied on aluminium plates pre-coated silica gel F₂₅₄ as a band width of 6 mm. The speed of the application of extracts was maintained at 5 sec/µl. The plate was developed in CAMAG visualizing chamber under short UV and using mobile phase of Toluene: Ethyl acetate: (8:2). Toluene, ethyl acetate was purchased from SRL chemicals, Mumbai, India. TLC plates were obtained from Merck, Bombay, India. The developed plates were air dried and then visualized in UV 254, 366 and

then derivatised with Vanillin Sulphuric acid reagent and scanned under UV 254 and 366 nm. R_f value, colour of the spots and densitometric scan were recorded. (Figure 1-5)

Method of Analysis

To streamline the analysis in Ayurvedic purview, the R_f value exhibited at 254 nm, 366nm and after polarization is categorized in to two categories with regards to the Pancha mahabhoota theory. Values 0.05 to 0.50 are considered for first category and 0.51 to 1.00 are considered for second category. Both categories are evaluated for the assumed pancha-mahabhoota Zone (PMB Zone).

First category signifies that affinity of drugs are more towards stationary phase and for the purpose of analysis it is referred as possessed with Prithvi mahabhuta (pro-element of earth) which has Sheetha Guna (cold) in built with Stira Guna (stability). Second category where drugs leave stationary phase and migrate with mobile phase is either Vayu (pro-element of wind) or Jala (pro-element of water) along with Sheeta guna as in built along with Chala guna (mobility).

Chromatography Experiments

Stationary phase: pre-coated silica gel F₂₅₄ on aluminum plates

Mobile phase: Toluene: Ethyl Acetate (8:2)

Application: Volume 7µl ; band width 6 mm; speed of the application 5 sec/µl

Tracks:

1- Arjuna twak; 2- Asana twak; 3- Amlaki phala; 4- Dhataki pushpa; 5- Gokshura phala; 6- Jatamamsi mula; 7- Katuki rhizome; 8- Kapikacchu phala 9- Kadira twak; 10- Musta rhizomes; 11- Shatavari mula; 12- Sariwa mua; 13- Udumbara twak ; 14- Usheera mula; 15- Yastimadhu mula.

Photo Documentation: Figure 1, Figure 2, Figure 3

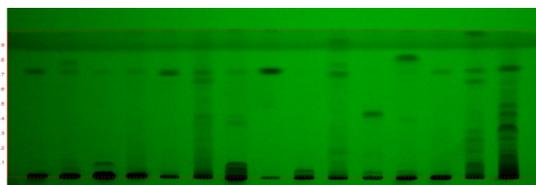


Figure 1: TLC photo of ethanol extracts of 15 sheetha veerya drugs at 254nm

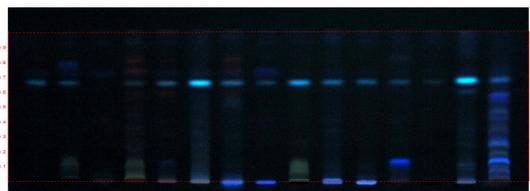


Figure 2: TLC photo of ethanol extracts of 15 sheetha veerya drugs at 366nm



Figure 3: TLC photo of ethanol extracts of 15 sheetha veerya drugs Post Derivatisation

Densitometric Scan

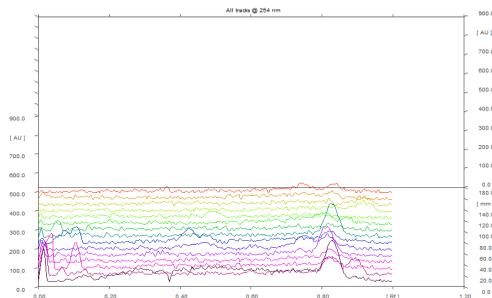


Figure 4: Three-D Display of all the tracks at 254nm

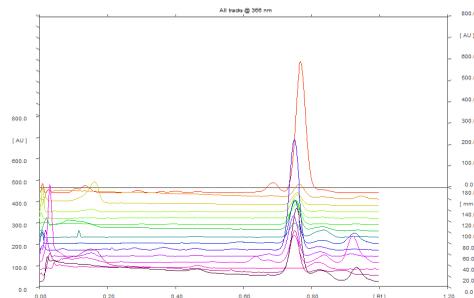


Figure 5: Three-D Display of all the tracks at 254nm

RESULTS AND DISCUSSION

Fine powders of fifteen drugs with respect to their useful parts are taken for the study and analyzed according for the spots exhibited in HPTLC keeping their rasapanchaka (five basic pharmacological faculties) as the criteria.¹⁰ Gokshura (*Tribulus terrestris*), and Yastimadhu (*Glyzyrrhiza glabra*) with qualities like madhura (sweet) guru (heavy) snigdha (unctuous), madhura vipaka (final transformation of the drug is sweet) have its spots maximum in Prithvi-Jala zone thus substantial. Shatavari (*Asparagus racemosus*), with madhura (sweet)- tikta (bitter) with guru (heavy) snigdha (unctuous) qualities, madhura vipaka (final transformation of the drug is sweet) has shown maximum spots in Prithvi-Jala zone. Here tikta (bitter) components are submissive. Sariva (*Hemidesmus indicus*) too is, guru (heavy) snigdha (unctuous) with madhura vipaka (final transformation of the drug is sweet), but here the tikta (bitter) rasa has taken the predominance spots are exhibited in Vayu zone. Kapikacchu (*Mucuna pruritis*) is madhura (sweet) tikta (bitter) with guru (heavy) snigdha (unctuous) and madhura Vipaka (final transformation of the drug is sweet). But spots are maximum in Vayu zone and it may be the reason that the tikta (bitter) taste is also potent and active. Amalaki (*Emblia officianalis*) is sour predominant, salt devoid material with 5 tastes, also a Vichitra prathyayarabdha dravya (complexly structured) with laghu (light), ruksha (dry) qualities, madhura Vipaka (final transformation of the drug is sweet) and sheeta veerya (cold potency). As all its mahabhuta (pro-elements) are equally predominant, equal number of spots is exhibited in both Prithvi-Jala and Vayu zone. Katuki (*Picrorhiza kurroa*) is bitter tasting drug with laghu (light) ruksha (un-unctuous) qualities katu vipaka (final transformation of the drug is pungent) and has shown maximum spots in Vayu zone which is substantial. Usheera (*Vetiveria zizanioides*) tastes bitter and sweet, with laghu (light) ruksha (un-unctuous) qualities, katu Vipaka (final transformation of the drug is pungent) showing maximum spots in Prithvi-Jala zone. This may be because of the dominant sweet taste. Musta (*Cyperus rotundus*) has bitter pungent taste, laghu (light) ruksha (un-unctuous) qualities, katu Vipaka (final transformation of the drug is pungent) and shows maximum spots in Prithvi-Jala zone. Though it has basic qualities indicating possessing Vayu mahabhuta, the spots in Prithvi-Jala zone may be due to underlying moisture because of its moist habitat. Jatamamsi (*Nardostachys jatamamsi*) is with bitter and astringent taste, laghu (light), teekshna (quick, sharp) qualities, katu vipaka (final transformation of the drug is pungent) and shows maximum spots in Vayu zone. Khadira (*Acacia catechu*) is bitter astringent, with laghu (light) ruksha (un-unctuous) qualities, katu vipaka (final transformation of the

drug is pungent) and shows maximum spots in Prithvi zone because of dominant astringent taste. Arjuna (*Terminalia arjuna*) Asana (*Pterocarpus marsupium*) are astringent taste predominant, laghu (light), ruksha (un-unctuous) qualities, katu vipaka (final transformation of the drug is pungent) and substantiates the maximum exhibition in Vata zone. Udumbara (*Ficus racemosa*) has predominant astringent taste, possesses guru (heavy) ruksha (un-unctuous) qualities, katu vipaka (final transformation of the drug is pungent), showing the maximum spots in Vayu zone. Guruta (heaviness) is not active here, thus no spot in Prithvi-Jala zone. Dhataki (*Woodfordia fruticosa*) is kashaya katu, laghu ruksha guna, katu vipaka and shows maximum spots in Vayu zone which is substantial.

Drug action is known by resultant performance. Any drug will act in the body through the configuration called Rasapanchaka namely rasa (taste), guna (quality), veerya (potency) vipaka (final transformative status) and prabhava (specific effect) which is its exclusive possession.¹¹ They contribute either individually or in combination for the drug action. Responsible factor represents veerya (potency of the drug). Thus, Concept of Veerya is not indicating any one entity in the drug, instead is representing an initiating force for resultant action of the active drug¹². Thus, Utkrishta shakti sampanna guna (predominantly active attributes of the drug) which are responsible for a drug activity make the drug potent to perform an action which is understood as potency.¹³

Agni (fire element) and Soma tatva (water element) are considered to be more powerful in the universe¹⁴. Purusha (Man) is created by the combination of Sukra (sperm) which is a saumya tatva (predominant water element) and Artava (ova), a agneya tatva (predominant fire element), thus considering him too as Agni-someeya. Kala (Time) is divided as aadana (Sun predominant) and visarga (Moon predominant) as per hotness and coldness exhibited in Universe¹⁵. Similarly, the drugs too are dominant with Agni (fire) and Soma (water). And underlying potency in the drug too is considered as two, Agneya (firy) represented by Ushna veerya (hot potency) and Soumya (watery) represented by Sheeta Veerya (cold potency)¹⁶.

Cold potency of the drug is the result of prithvi (earth elements) and Jala tava (water elements)¹⁷ and it pacifies Pitta and Rakta (blood vitiation), aggravates Kapha and Vata.¹⁸ Cold potency is responsible for drug actions like Vishyandana- flowing; Sthirakarana- stabilizing; Prasadana- purifying or calming; Kledana- moistening; Hladana -causing enthusiasm, Jeevana- life promoting; Stambhana-causing stasis; Guru- causing heaviness; Balya-strengthening¹⁹. Any drug of cold potency is ruled by a

general law in Ayurvedic literatures and thus it can be assumed that there may be a common platform which can be identified in modern parameters too.

Chromatography Procedure is a physical process where the components (solutes) of a sample mixture are separated as a result of their differential distribution between stationary and mobile phases which moves in a definite direction.²⁰ During the process, the mobile phase which is a flowing stream carries the sample through a bed, layer or column containing the stationary phase. As the mobile phase flows past the stationary phase, the solutes have following possibilities:

a.) Reside only on the stationary phase (no migration); b.) Reside only in the mobile phase (migration with mobile phase); c.) Distribute between two phases (differential migration). Solutes follow affinity as the principle for the above possibilities. Those solutes with higher affinity for the stationary phase reside in the stationary phase and so migrate slower than those with lesser affinity. Those with the lesser affinity to stationary phase like to reside mostly in the mobile phase and migrate faster. Thus, the lower affinity solutes separate from solutes having greater affinity for the stationary phase.

Among the five mahabhutas (primordial elements), cold as a quality is more predominant in Ap (water element), Prithvi (earth element) and Vayu (wind element). The drugs showing more affinity towards the stationary phase indicate Partheeva (earth predominant) or Jala mahabhuta (water predominant) because of adbhagaharatwa (downwards affinity). Affinity towards mobile phase indicate Vayu mahabhuta (wind predominant) because of urdwa bhagaharatwa.(upwards affinity)

CONCLUSION

The underlying Mahabhuta (primordial element) in a drug is responsible for the activity in a body. This is appreciated in Ayurveda through Rasa, Guna, Veerya, Vipaka or Prabhava concepts. HPTLC Fingerprint of fine powders of useful parts of fifteen randomly selected drugs with cold potency display a typical presentation when subjected for the study uniformly with the same stationary and mobile phase and same plate. The pattern presented in this study possibly may be regarding the predominant mahabhuta either Jala, Prithvi or Vayu which are classically ascertained as Sheeta Veerya (cold potency). This suggests that ancient practitioners of Ayurveda had scientific evidence based perception about the concept of Sheeta Veerya which is indeed contributory to understand the similarity in drug actions by the plants with cold potency.

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