Phytochemical screening and chromatographic studies on Triphala and Trikatu

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Abstract-Triphala and Trikatu both are widely used traditional herbal formulation used in ayurveda for improving digestive disorders, respiratory disorders and in skin disease. Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. Therefore, the objective of the present study was to characterize the phytochemicals profile for various secondary metabolites using HPTLC of Triphala and Trikatu extract. HPTLC studies to explore medicinally active phytoconstituents present in different solvent extracts in these polyhedral formulations.

Key words: Triphala, Trikatu, HPTLC, Photochemical.

Introduction

Ayurveda, the traditional Indian medicinal system remains the most ancient yet living traditions with sound philosophical and experimental basis. According to charaka it is the knowledge which seeks to weigh life in the scales of wholesomeness and happiness against their opposites[(1)]. Ayurveda literally means "The knowledge" of life. In sanskrit the word ayurveda consists of two words 'ayu' meaning 'life' and 'veda' meaning knowledge or 'science'. According to WHO, about 70-80% of the world population rely on non-conventional medicines mainly of herbal sources in their healthcare[(2)].

In this study, we compared and contrasted two different triherbal traditional formulation i.e. triphala and trikatu. Talking about trikatu, trikatu is a sanskrit name indicates its meaning 'tri' stands for 'three' ans 'katu' stands for 'acrids'. Trikatu is the combination of three herbs black pepper (Piper longum), pippali (Piper longum), dry ginger (Zingiber officinalis). Trikatu acts mainly on stomach, liver and pancreas. In stomach trikatu increases the production of digestive juices therefore stimulating digestion. In liver, it is used to increase production of bile salts by stimulating gall bladder. Trikatu affects overall digestive system along with its curative affects on respiratory, urinary immunity, skin and metabolic system of our body[(3)].On the other hand triphala in sanskrit 'tri' stands for 'three' and 'phala' stands for 'fruits' Triphala is the simple equiproportional mixture of three different herbs Amla (Emblica officinalis), Harad (Terminalia chebula), Bahera (Terminalia bellerica).It is classified as a tridoshic rasayana in ayurvedic medicine as it

promotes longitively and rejuvenating in patient of all constituents and ages. Triphala represents an essential foundational formula as it promotes efficient digestion, absorption, elimination and rejuvenation. Moreover, studies have validated a number of potential uses of triphala, which include antioxidant, anti-inflammatory, antibacterial, dental caries prevention, antipyretic, analgesic, anti-mutagenic, anti-cariogenic, anti-stress, hypoglycaemic, anticancer, chemo-protective, chemopreventive, radio protective effects[4].

Material and methods

Collection and identification

The plant materials for making of triphala and trikatu were collected from the Himalaya Wellness company campus, Dehradun, UK, India and were identify by the Department of Pharmacognosy. The Himalaya Wellness Company, Dehradun, UK.

Preparation of powder

The collected plant material were dried and powdered using mixer grinder for triphala powder Amla, Harad, Bahera powder were mixed in equiproportional ratio and for trikatu powder Black pepper, Pippali, Saunth powder were mixed in same ratio. In plants alkaloids redox reactions of nicotinamide adenine dinucleotide (NAD) reduces its pyridine ring into dihydropyridine. (Lin SX and Sperry 2020; Pollak and Ziegler 2007).*The plant Senna* spp. is a source of natural alkaloids of the piperidine and pyridine classes.(Francisco et al. 2012)

Extraction of plant material

For phytochemical screening 5g of both sample triphala trikatu dissolve in each solvent water, methanol, acetone, hexane and mixed properly. Leave them overnight and then filtered using whatman's filter paper.

Phytochemical studies

The phytochemical screening of triphala and trikatu in all four solvent (Aqueous, Methanol, Acetone, Hexane) were carried out according to different phytochemicals were estimated qualitatively and quantitatively by using following procedure[5-8].

Tests for qualitative analysis

Test for carbohydrates (Molish's test): To two ml of molish's reagent, 2ml of extracts were added and shaken well. To this another 2ml of concentrated sulphuric acid was added carefully through the sides of the test tube. Appearance of a reddish violet ring at the junction of the two layers indicate the presence of carbohydrates.

Test for tannins: To the extracts, a few drops of 10% ferric chloride solution were added. appearance of a green or blue colour indicates the presence of tannins.

Test for steroids: Leaf extracts were mixed with 1 ml of chloroform and 2-3 drops of conc. H_2SO_4were added to it. Appearance of a pink or red colour indicate the presence of steroids.

Test for terpenoids (Salkowiski test): Five ml of the extinct were mixed with 2ml of chloroform and 3ml of conc. H_2SO_4, solution. A reddish-brown colour at the interphase indicate the presence of terpenoids.

Test for alkaloids (Mayer's test): Extracts were treated with mayer's reagent (potassium mercuric chloride). Formation

of a yellow coloured precipitate indicates the presence of alkaloids.

Test for flavonoids: The extracts were treated with conc. H_2SO_4 and formation of a yellowish orange colour indicate the presence of flavonoids.

Test for proteins (Xanthoprotein test): To the leaf extracts 20% NaOH solution were added and the formation of an orange colour confirms the presence of proteins which is characteristic for ammonia formation.

Test for cardiac glycosides (Keller-killani test): Five ml of test extracts were treated with 2ml of glacial acetic acid containing 2-3 drops of ferric chloride solution and 1 ml of conc. H_2SO_4 solution. Appearance of a green ring initially which first turns violet and then to brown at the interphase indicates the presence of cardiac glycosides.

Test for Saponins (Foam test): Two mi of the extracts were diluted with 20ml of distilled water, shaken vigorously and was observed for a stable persistent froth.

Test for phenolic compounds (Ferric chloride test): Two ml of diluted extracts were treated with dil. FeCl, solution. Appearance of a violet colour indicate the presence of phenol like compounds.

Test for triterpenes: The extract were treated with chloroform then add cons H_2SO_4 and shake lower layer changes into yellow colour indicates the presence of triterpenes.

Test for starch: Take 0.015g of iodine and 0.75g of potassium iodide and mix them then add 5ml distilled water and 2ml of extract appearance of blue colour indicates the presence of starch[3].

Test for anthraquinone glycoside: Take extract and add 1ml of H_2SO, and boiled it for 5 min. then filtered it (while hot) and add 1 ml of chloroform, 0.5 ml dilute ammonia. Rose pink to red colour indicates the presence of anthraquinone glycosides.

Chromatographic studies

HPTLC (High Performance Thin Layer Chromatography)

Sample preparation

Aqueous- Measure accurately 3gm of sample in 250ml round bottom flask add 20ml of distill water and reflux it by immersing in a water bath at 80-100^{O}C for 30 minutes. Filter the extracts through whatman no. 1 filter paper into a conical flask.

Methanol-Measure 3gm of sample in 250ml round bottom flask add 20ml of methanol and

reflux it by immersing in a water bath at 70-80^{O}C for 30 minutes. Filter the extract through whatman no.1 filter paper into a conical flask.

Hexane- Measure 3gm of sample in 250 ml round bottom flak add 20ml of hexane and reflux it by immersing in a water bath at 40-50^{O}C for 30 minutes, Filter the extract through whatman no.1 filter paper into a conical flask.

Chromatogram layer- TLC plates, silica gel 60 F254, 10 X 10cm.

Chemicals required-Methanol, Hexane, Chloroform.

Mobile phase- Chloroform: Methanol (90: 10)

Application

Apply the sample and standard solution as 12mm band, in a distance of 12mm from the bottom of a precoated thin layer silica plate of uniform thickness, made a mark up to distance of 8.5 cm from the application point as a development mark using pencil.

Preparation of development tank

Camag made twin through development tank (10X10) was used. Covered one side of the inside chamber with required size of whatman no.41 filter paper. Measured 20ml of mobile phase and transferred into chamber from the side of filter paper.

Visualization and documentation

Visualized the dried plate under UV 254 nm and 366nm using cabinet and capture the image.

Table-1 Detection of secondary metabolites in Polyherbal Trikatu

Class of compounds	Aqueous	Methanol	Acetone	Hexane
Taninn	+	+	+	-
Flavinoid	+	+	-	+
Saponin	+	-	+	-
Alkaloids	-	+	+	-
Starch	-	-	-	-
Protein	+	+	+	+
Carbohydrate	+	+	+	+
Triterpenes	+	-	-	-
Cardiac glycosides	+	+	+	+
Phenolic compounds	+	-	-	-
Steroid	+	-	-	-
Anthra quinone glycosides	-	-	-	-
Terpenoids	+	-	+	-

(+)- Positive: (-)- not Detected

Table-2 Detection of secondary metabolites in polyherbal Triphala

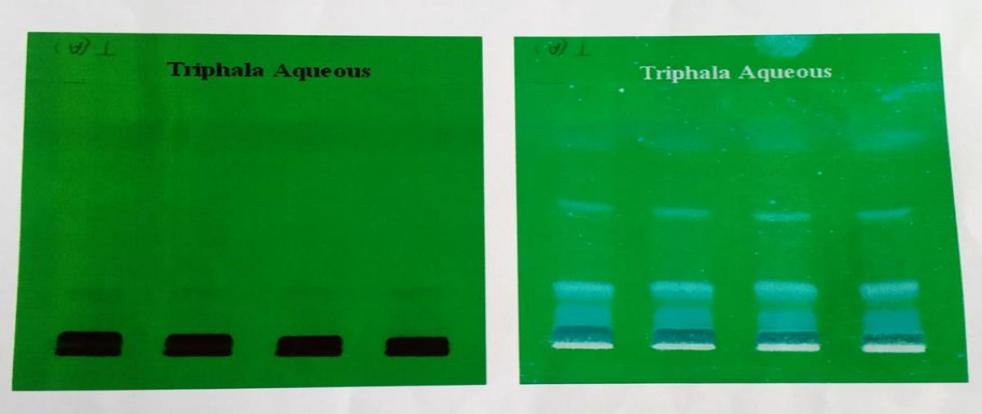
Class of compounds	Aqueous	Methanol	Acetone	Hexane
Taninn	+	+	+	-
Flavinoid	+	+	+	-
Saponin	+	+	+	-
Alkaloids	-	+	+	-
Starch	-	-	-	-
Protein	+	+	+	+
Carbohydrate	+	+	+	+
Triterpenes	+	-	-	+
Cardiac glycosides	+	+	+	+
Phenolic compounds	+	+	+	+
Steroid	-	-	-	+
Anthra quinone glycosides	-	-	-	+
Terpenoids	+	+	+	-

(+)-Positive; (-)-Not Detected

Knowledge of the chemical constituent of plants is desirable because such information may be of great value in revealing new sources of compounds and precursors for the synthesis of new chemical constituent, which can be used in drugs (Mentha et al. 2017). Preliminary analysis is an indication of the presence or absence of phytochemicals in a plant extract based on visual inspection of colour or precipitation reaction; whereas, HPTLC chemo profile accurately and efficiently confirms the presence of these constituents. While traditional TLC is based on visual inspection of the chromatographic plate and its documentation by either tracing or photography, HPTLC features highly sensitive scanning densitometry for rapid chromatogram evaluation and documentation (Mukherjee 2008).

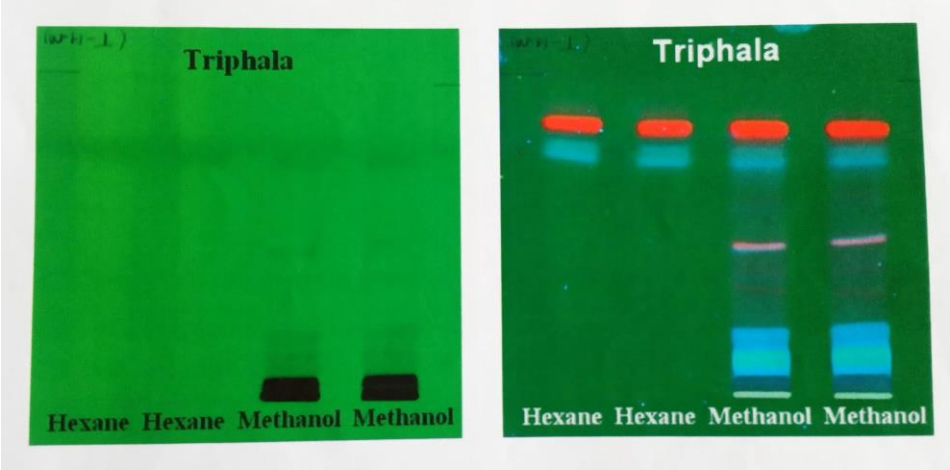
In the current study, Triphala and Trikatu extracts were evaluated for the detection of thirteen main classes of secondary phytocompounds namely Tannin, alkaloids, starch, protein, carbohydrate, flavonoids, saponins, triterpenes, cardiac glycosides, phenolic compound, Anthraquinones glycosides, steroid and terpenoids (**Table-1** and **Table-2)**.

HPTLC of Triphala and Trikatu extract



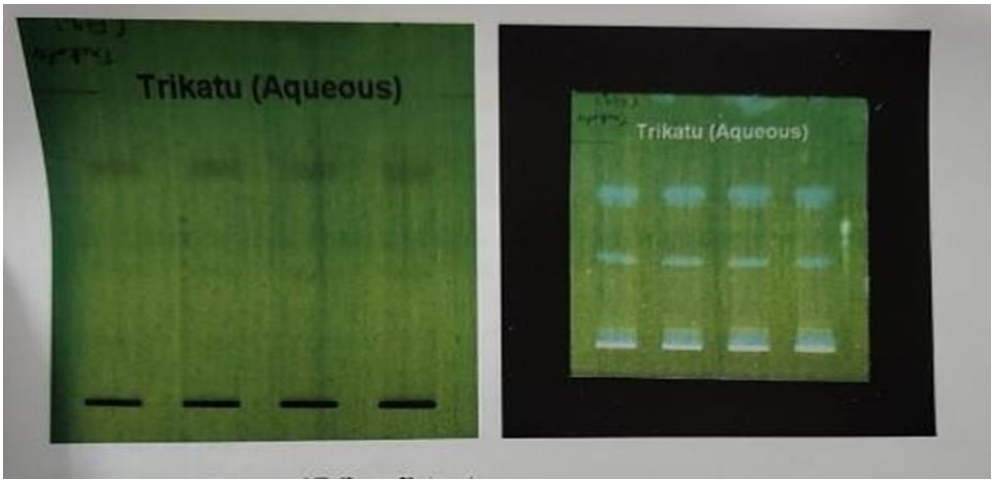
@254nm **@366nm**

Figure: 1 Aqueous extract of Triphala



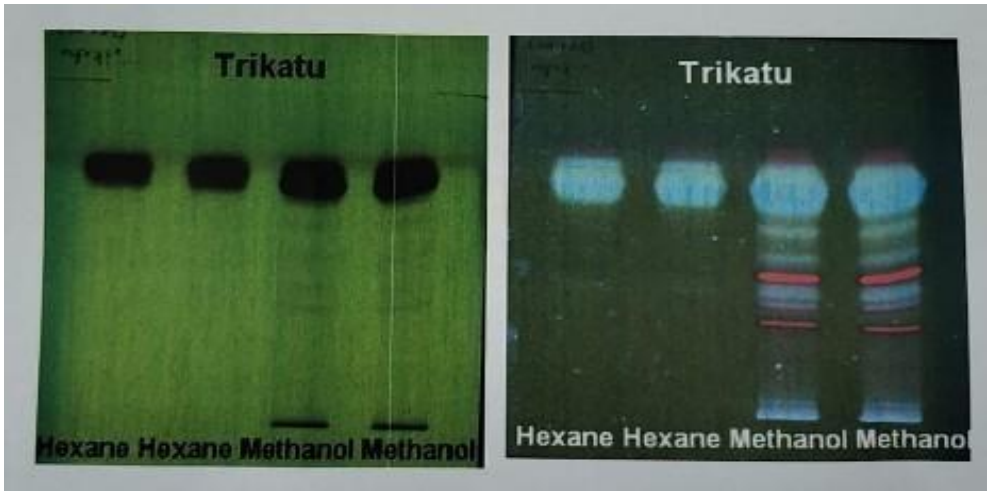
@254nm **@366nm**

Figure: 2 Hexane and methanol extract of Triphala



@254nm **@366nm**

Figure : 3 Aqueous extract of Trikatu



@254nm **@366nm**

Figure: 4 Hexane and methanol extract of Trikatu

Conclusion

The results obtained in the present study indicate Triphala and Trikatu different extracts namely aqueous, methanol, hexane and acetone are rich source of secondary metabolites as most of the class of compounds were found to be present in all these three plant parts? These findings indicate the presence of various phytochemicals in Triphala and Trikatu may be responsible for its pharmacological activities. However, there is a need to further carry out advanced studies to isolate and identify the pure active chemical compounds, and elucidate the structure of these compounds. Furthermore, these data may be handy in probing of biochemistry of this plant in the future.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

References

1. Chauhan, A.; Semwal, D.K.; Misra, S.P. and Semwal, R.B. Ayurvedic research and methedology: present status and future strategies. *Ayu.*, 2015, Oct-Dec. p$_{p.}$364-369.

2. Jaqui, W. Herbal products are often contaminated, study finds. *BMJ*., 2013.

3. Rajendra, S. S.; Santhi, N. and Jennifer, A. HPTLC Method devlopment and Validation for Standarization of Ayurvedic Formulation, Trikatu Churna. *International Journal of Pharmaceutical and Application (UPRA),* November, 2021, Volume 6, Issue 6, p$_{p.}$379-385.

4. Baliga, M.S. et.al. Scientific validation of the ethnomedicinal properties of the Ayurvedic drug Triphala: A review. *Chin. J. Integr. Med*., 2012, p$_{p.}$946-954.

5. Kumar, Neethu S.; Nair, Arun S.; Murali, Megha and Devi, Sreeja P.S. Qualitative phytochemical analysis of triphala extracts. *Journal of pharmacognosy and phytochemistry*, April, 2017, p$_{p.}$248-251

6. Sofowora, A. J. Complement. Med,. *Altern.* 1996, 2:365-372

7. Harbone, J. B. Phytochemical method; A guide to modern technique of plant analysis. *Chapman and Hall*, New York, 1998, 3 Edition,.

8. Kokate, C. K. A texy book of practical pharmacognosy. 5th Edition, vallabh prakashan, New Delhi, 2005, p$_{p.}$ 107-111.

9. Mehta, S.; Singh, P.S. and Saklani, P. Phytochemical screening and TLC profiling of various extracts of Reinwardtia indica. *Int. J. Pharmacogn. Phytochem. Res*., 2017, 9:523-527. [Google Scholar]

10. Mukherjee, P.K. Quality control of herbal drugs: an approach to evaluation of botanicals. India: Business Horizons Pharmaceutical Publishers, 2008 [Google Scholar].