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Integrated Phytochemical Evaluation of Triphala Extract: TLC Detection and GC–MS Elucidation of Bioactive Compounds

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ABSTRACT

Triphala, a classical Ayurvedic polyherbal formulation composed of *Terminalia chebula*, *Terminalia bellirica*, and *Phyllanthus emblica*, is well known for its therapeutic efficacy and wide range of biological applications. Its pharmacological potential is attributed to a rich diversity of phytoconstituents, including alkaloids, flavonoids, tannins, and phenolic compounds. Scientific validation and standardization of such formulations require systematic phytochemical evaluation using both conventional and advanced analytical techniques. The present study was undertaken to investigate the phytochemicals composition of Triphala extract using TLC and GC-MS. TLC was done to detect gallic gallic acid a major component of Triphala. GC-MS analysis was carried out enabling the separation and identification of volatile components based upon the retention time and mass fragmentation patterns. Several compounds of pharmacological relevance were identified including phenolic acids fatty acids and flavonoid derivatives which are consistent with the known antioxidant and therapeutic properties of Triphala. This integrated approach combining TLC and GC-MS along with standard biochemical testing provided both qualitative and confirmatory data on the phytoconstituents of Triphala. The fidings not only support the traditional claims but also contribute to a scientific validation ensuring quality and paving the way for further pharmaceutical and nutraceutical approaches.

Keywords: Triphala Phytoconstituents, TLC, GC-MS, Gallic acid

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INTRODUCTION

Herbal medicines have played a vital role in traditional systems of healthcare, particularly in Ayurveda, Siddha, and Unani. Among the classical Ayurvedic formulations, *Triphala* is one of the most widely used and scientifically explored. The term *Triphala* means "three fruits," referring to the dried fruits of *Terminalia chebula* (Haritaki), *Terminalia bellirica* (Bibhitaki), and *Phyllanthus emblica* (Amalaki)[1]. These fruits, individually valued for their therapeutic efficacy, are known for properties such as antioxidant, antimicrobial, anti-inflammatory, and rejuvenating effects. When combined, they exert synergistic actions, making *Triphala* a unique formulation for maintaining health and treating a wide spectrum of ailments, including gastrointestinal disorders, ocular conditions, metabolic syndromes, and as a general immunomodulatory agent[2].

The pharmacological potential of Triphala is attributed to its rich content of phytochemicals. Alkaloids, flavonoids, tannins, glycosides, and phenolic compounds are among the bioactive constituents reported to contribute significantly to its activity[3]. Alkaloids are known for their antimicrobial and neuroactive properties while flavonoids possess strong antioxidant anti-inflammatory and cardioprotective roles[4-5]. Hence identifying and characterizing these phytoconstituents is crucial not only for understanding the therapeutic basis of Triphala but also for ensuring quality control and standardization of herbal medicine.

Preliminary phytochemical screening techniques remain indispensable in herbal research. TLC is a rapid cost effective and reliable method widely employed for detecting phytoconstituents. It enables identification of specific classes of compounds such as alkaloids and flavonoids based on their retention factor and characteristic reactions with detecting reagents. TLC serves as an important tool for comparative profiling and provides preliminary evidence of the phytochemical make up of herbal extracts.

In- contrast advanced instrumental methods like gas chromatography -mass spectrometry provides detailed insights into the molecular composition of plant extract .GC-MS combines efficient chromatographic separation with mass spectral identification, enabling precise detection of volatile and semi volatile compounds in complex mixtures. This technique is particularly valuable in phytochemical studies as it not only identifies individual compounds but also helps corelate their presence with pharmacological activities'.

The present study aims to explore the phytochemical profile of Triphala extract using both TLC and GC-MS approaches. This integrated methodology provides both preliminary and confirmatory data thereby supporting the scientific validation , standardization and potential therapeutic applications of Triphala.

MATERIALS AND METHODS

MATERIALS

Triphala powder was sourced from Baidyanath Ayurved Pvt. Ltd, Gallic acid was purchased from online store (labwale.in). Mayers reagent, Dragendroff Reagent and Wagners reaget was sourced from online store ibuychemicals . Toluene, ethyl acetate, acetic acid was purchased from Molychem Hyderabad.

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METHODS

Preparation of Aqueous Extract of Triphala[6-7]

Triphala powder sourced locally was accurately weighed for six grams and was transferred in a round-bottom flask. 90ml of distilled water and 10 ml of ethanol was added to the round bottom flask and the mouth of the round bottom flask was sealed using aluminum foil and was left undisturbed in a dry environment for two days. The rationale behind leaving the powder soaked in hydro-alcoholic mixture is to completely extract all the water soluble and alcohol soluble active components from the triphala powder. After soaking it completely for 2 days the round bottom flask along with the contents were attached to a reflux system and was heated on a low flame for about 30 minutes, after cooling it down the contents were heated on a medium flame on a water bath for 5 minutes to evaporate the ethanol, now the contents of the RBF were filtered using Whatman filter paper and the obtained liquid is stored in a sterile glass container. The whole process beginning with weighing of ingredients to filtration is carried out in sterile environment to avoid any contamination.

Chemical evaluation of triphala extract[8-12]

Chemical evaluation of the triphala extract is done to detect the presence of of alkaloids, flavonoids, tannins etc in the extract. These are the pharmacologically active components of the extract.

i. Test for alkaloids

a) Dragendroffs Test

2ml of the triphala extract was mixed with 2ml of dilute hydrochloric acid and was heated for a few minutes. The resultant was cooled and filtered and Dragendorffs reagent was added. A orange or reddish-brown precipitate indicates presence of alkaloids

b) Mayers test

2 ml of the triphala extract is mixed with 2 ml of Mayers reagent, a creamish or white colored precipitate indicates presence of alkaloids

c) Wagners Test

2 ml of the Triphala extract is mixed with Wagners reagent, a reddish-brown color indicates presence of alkaloids.

ii. Test for Tannins (Ferric chloride test)

2ml of the Triphala solution is mixed with 10% ferric chloride solution, a dark bluish color change indicates presence of tannins/phenols

iii. Test for flavonoids

a. Shinoda Test

2ml of the Triphala extract is taken and a small quantity of magnesium turnigs or powder is mixed. To this add few drops of concentrated hydrochloric acid and shake. A pink or red color indicates presence of flavonoids.

b. Lead acetate test

2ml of Triphala extract is mixed with 10% lead acetate solution, a yellow-colored precipitate confirms the presence of flavonoids

The presence or absence of the above components hints about the efficiency of triphala extraction further confirming the presence of active components in the solution.

Thin Layer Chromatography Of Triphala Extract[13]

a. Preparation of standard solutions of gallic acid

10 mg of gallic acid was accurately weighed on a analytical balance and was dissolved in 10ml of methanol in a volumetric flask to prepare standard solutions of concentration 1mg/ml

b. Sample Preparation

100 mg of Triphala powder was mixed with 100ml of acetone and was left undisturbed for one hour and was subsequently heated over a water bath for 15 minutes. The extract was filtered and dried completely. The residue was redissolved in 10ml of acetone and was used as sample.

c. Mobile phase

Toluene :Ethyl acetate :Methanol: Acetic acid taken in ratio of 4:4:5:5 was used as the mobile phase

d. Stationary Phase

Dried silica plates were used as stationary phase

e. Procedure for TLC

The silica plates were cut for the desired size (8x4cm). In a beaker the mobile phase was added to reach a length of 1cm from the bottom of the beaker and was kept aside closed for 2 hours to saturate the chamber. The silica plates were marked for TLC analysis, 1 cm from the bottom was marked as the baseline and 0.5 cm from the right and left side were left. A capillary was used to mark the sample and standard spots on the baseline, each sample was placed 1 cm away from each spot. The plate was then carefully placed in the beaker containing mobile phase such that the mobile phase just touches below the baseline mark and the mobile phase was allowed to elute through the silica plate so that the separation process can take place. Once the mobile phase has travelled 75% of the plate length the plate was removed, dried and was observed under UV light for the detection of spots followed by calculation of the Rf values and comparing with Rf values with that of standards.

d) Calculation Of Rf Value

Rf value was calculated using the following formula and were compared with Rf values of he standard.

Rf= Distance travelled by the solute

Distance travelled by the solvent

GC-MS analysis of the Triphala Extract[14,15]

GC- MS is an analytical tool that is used to detect the presence of components in a given material. 10 µl of sample (50 mg/ml) was taken in a separating funnel and shaken by adding 10 ml of water and ethyl acetate in the ratio of 1:4 (add 2.5 µl water to 7.5 µl Ethyl Acetate). Upper layer was collected and concentrated to 1 ml in the rotary evaporator.50 µl N, O-Bis (trimethylsilyl)trifluoroacetamide and trimethylchlorosilane (BSTFA+TMCS) was added and then finally 10µl of Pyridine was also added. For BSTFA+TMCS, make 100µl solution of 99µl of BSTFA and 1µl of TMCS. Samples were transferred to GC vial and dried using nitrogen gas. Finally, sample was dissolved in methanol before GC-MS analysis. Acquired samples were programmed as described below:

- Analytical conditions (instrument GC-2010) (Table 1)
- Oven temperature Program (Table 2)
- GC Program (GCMS-QP2010) (Table 3)
- MS Table (Table 4)

RESULTS AND DISCUSSION

Preparation of Aqueous Extract of Triphala

The extract of Triphala was prepared as per the specified procedure. The obtained solution was a dark brown colored liquid. The final

Table 1. Analytical conditions of GC-MS instrument

Column Oven Temp.	120.0 °C
Injection Temp.	260.00 °C
Injection Mode	Split
Injection Volume	2 µl
Flow Control Mode	Linear Velocity
Pressure	99.3 kPa
Total Flow	16.3 mL/min
Column Flow	1.21 mL/min
Linear Velocity	41.3 cm/sec
Purge Flow	3.0 mL/min
Split Ratio	10
High Pressure Injection	OFF
Carrier Gas Saver	OFF
Carrier Gas	Helium
Splitter Hold	OFF
Size of GC-Vial	2 ml
Size of Capillary Column	Length- 30 meter and Inner diameter – 0.25 mm

Table 2. Oven temperature program

Rate	Temperature (°C)	Hold Time (min) -
	120	2
10	300	20

Table 3. GC-Program

Ion Source Temp	220.00 °C
Interface Temp.	270.00 °C
Solvent Cut Time	3.50 min
Detector Gain Mode	Relative
Detector Gain	+0.00 kV
Threshold	1000

Table 4. MS Conditions

Start Time	4.30 min
End Time	39.98 min
ACQ Mode	Scan
Event Time	0.20sec
Scan Speed	3333
Start m/z	40
End m/z	650

Table 5. Chemical evaluation to check presence of different components

SR.NO	TEST	RESULT	INFERENCE
1	TEST FOR ALKALOIDS		
a	Dragendroffs Test	Reddish brown precipitate	alkaloids present
b	Mayers Test	Creamy precipitate	alkaloids present
c	Wagners Test	Red-brown precipitate	alkaloids present
2	TEST FOR TANNINS	Bluish black color	Tannins present
3	TEST FOR FLAVONOIDS		
a	Shinoda Test	Pink Color	Flavonoids present
b	Lead Acetate Test	Pale Yellow precipitate	Flavonoids present

Table 6. Components identified in the GC-MS analysis of Triphala extract

PEAK	R. TIME	AREA	AREA%	NAME
1	9.527	231006	1.58	DODECANOIC ACID, METHYL ESTER
2	11.883	303013	2.07	Methyl tetradecanoate
3	14.013	1249215	8.54	Hexadecanoic acid, methyl ester
4	15.002	79128	0.54	DIMETHYL ESTER OF EICOSAN-1,20-DIOIC ACID
5	15.651	757247	5.18	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
6	15.713	3536531	24.18	9-Octadecenoic acid, methyl ester, (E)-
7	15.949	696987	4.77	Methyl stearate
8	16.124	183751	1.26	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
9	16.363	103036	0.7	9,12,15-OCTADECATRIENOIC ACID, 2,3-DIHYDROXYPROPYL ESTER, (Z,Z,Z)-
10	16.431	220405	1.51	Hexadecanamide
11	16.512	262487	1.8	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
12	17.409	97435	0.67	n-Nonadecanol-1
13	17.724	155612	1.06	EICOSANOIC ACID, METHYL ESTER
14	18.018	887845	6.07	9-Octadecenamide, (Z)-
15	18.229	170318	1.16	Dodecanamide
16	18.927	332907	2.28	Formamide, N-(4-[2-(1,1-dimethylethyl)-5-oxo-1,3-dioxolan[1]4-yl]butyl)
17	19.061	504703	3.45	1H-Indene, 1-hexadecyl-2,3-dihydro[1]
18	23.768	234468	1.6	STIGMAST-5-EN-3-OL, OLEAT
19	20.597	105534	0.72	1H-Indene, 1-hexadecyl-2,3-dihydro
20	19.362	262543	1.8	Docosanoic acid, methyl ester
21	24.395	263376	1.8	Desmosterol
22	25.088	322600	2.21	Kolavenol
23	26.127	1322865	9.05	.gamma.-Sitosterol
24	26.784	593402	4.06	A'-NEOGAMMACER-22(29)-ENE
25	26.993	209108	1.43	4-Cholesten-3-one semi carbazone
26	27.413	368488	2.52	METHYL COMMATE B
27	31.6643	64986	2.5	1,2 ,6A,6B,9,9,12A-HEPTAMETHYL-1,3,4,5,6, 6A, 6B,7,8,8A,9,12,12A,12B,13,14B HEXADECAHYDRO-2H[1]PICENE-4A-CARBOXYLIC ACID METHYL ESTER
28	33.249	279857	1.91	Urs-12-en-28-al
29	33.249	5241563.	3. 58	A'-Neogammacer-22(29)-en-3-one

volume of the obtained extract was 75ml and was stored in a sterile glass container.

Chemical evaluation of triphala solution

The chemical evaluation of the extract was performed to check the presence of various active components (Table 5).

Thin Layer Chromatography of Triphala Ghrita

The TLC process was conducted as per the specified procedure. Post TLC analysis visualization of spots was done under UV light and both sample and standard had travelled more than 75% of the plate length and the spots were distinctive and visible under UV. The spots of sample and the standard were at the same distance from the bottom line which proved the presence of the standard gallic acid in the Triphala sample (Figure 1).

Table 7. Compounds showing their anti-inflammatory and anti-oxidant properties

Compound Name	Type	Anti-inflammatory	Antioxidant
Dodecanoic acid, methyl ester	Fatty acid methyl ester	✔ (mild)	✖
Methyl tetradecanoate	Fatty acid methyl ester	✔ (mild)	✖
Hexadecanoic acid, methyl ester (Methyl palmitate)	Fatty acid methyl ester	✔	✔
Dimethyl ester of eicosan-1,20-dioic acid	Dicarboxylic acid ester	✖	✖
9,12-Octadecadienoic acid (Z,Z)-, methyl ester (Methyl linoleate)	PUFA methyl ester	✔	✔
9-Octadecenoic acid, methyl ester, (E)- (Methyl oleate)	MUFA methyl ester	✔	✔
Methyl stearate	Fatty acid methyl ester	✔ (mild)	✖
9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z,Z,Z) (Glyceryl linolenate)	PUFA ester	✔	✔
Hexadecanamide	Fatty acid amide	✔	✔
n-Nonadecanol-1	Long chain fatty alcohol	✖	✖
Eicosanoic acid, methyl ester	Fatty acid methyl ester	✔ (mild)	✖
9-Octadecenamide, (Z)- (Oleamide)	Fatty acid amide	✔	✔
Dodecanamide	Fatty acid amide	✔	✖
Formamide derivative (with dioxolane ring)	Synthetic amide	✖	✖
1H-Indene, 1-hexadecyl-2,3-dihydro	Alkyl indene derivative	✖	✖
STIGMAST-5-EN-3-OL, oleate (Stigmasterol ester)	Phytosterol ester	✔	✔
Docosanoic acid, methyl ester	Fatty acid methyl ester	✔ (mild)	✖
Desmosterol	Sterol precursor	✔	✔
Kolavenol	Diterpenoid	✔	✔
γ-Sitosterol	Phytosterol	✔	✔
A'-Neogammacer-22(29)-ene	Triterpenoid	✔	✔
4-Cholesten-3-one semicarbazone	Steroidal derivative	✔ (potential)	✖
Methyl commate B	Triterpenoid ester	✔	✔
Picene derivative (heptamethyl ester)	Pentacyclic triterpenoid	✔	✔
Urs-12-en-28-al	Triterpenoid aldehyde	✔	✔
A'-Neogammacer-22(29)-en-3-one	Triterpenoid ketone	✔	✔

✔ = Shows activity

✖ = Doesn't show activity

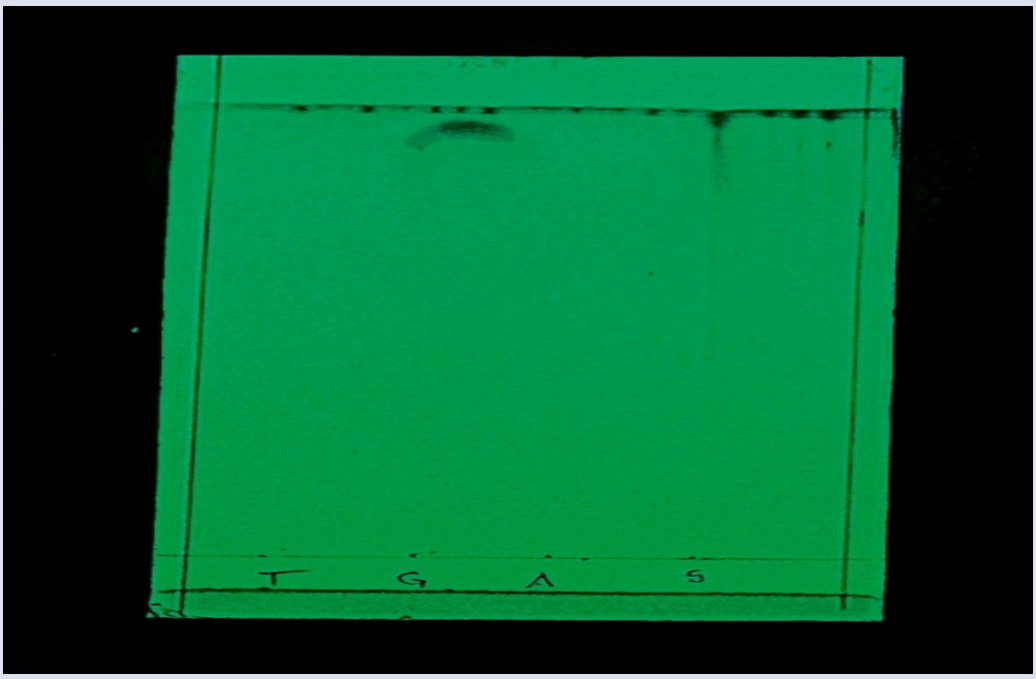

Figure 1. TLC plate visualization under UV

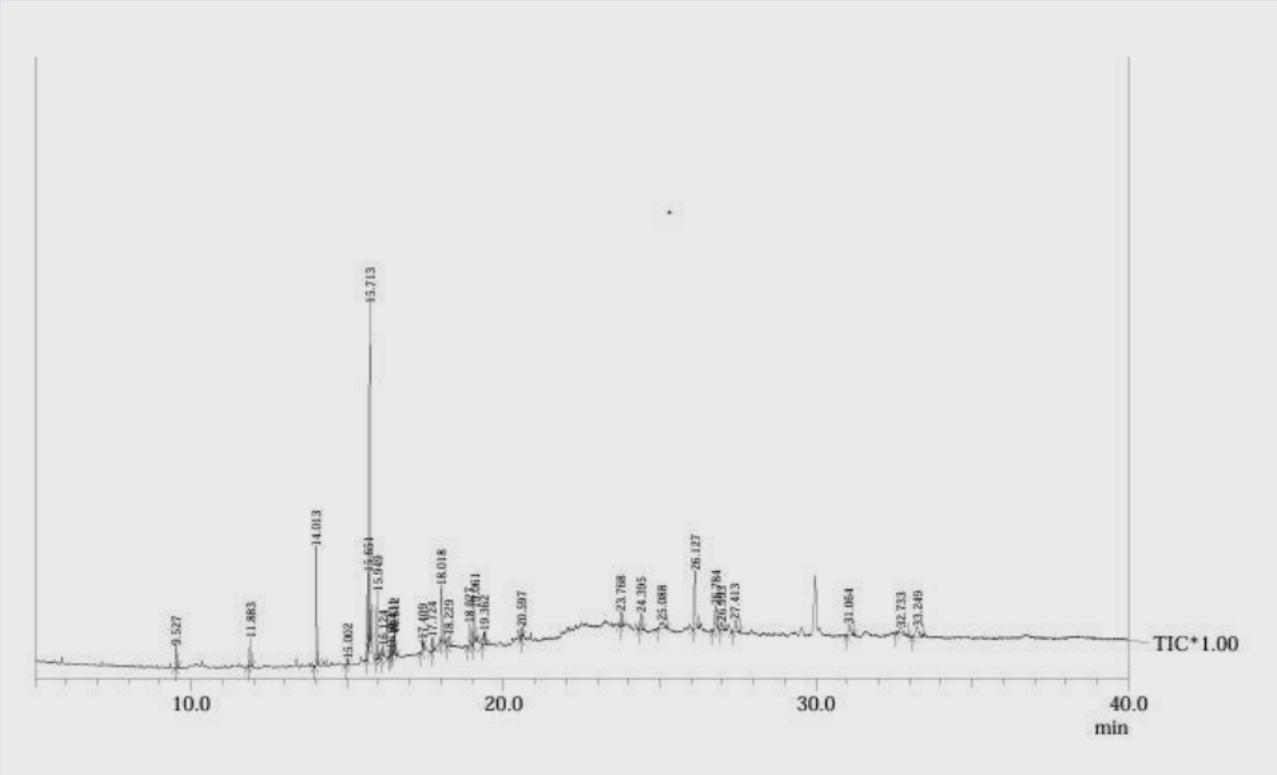

Figure 2. GC-MS scan for Triphala Solution

The calculation of Rf value was calculated as per the specified formula. The distance travelled by the solvent front was 7.3cm and the distance travelled by the standard was 6.4cm and the Rf value was found to be 0.876.

GC-MS analysis of Triphala Extract

In GCMS analysis of Sample- A revealed the presence of many phytochemical compounds. In extract of Sample A total of 29 components were identified which were a combination Fatty acid esters (FAMEs) & related acyl esters, Fatty acid amides, Long-chain alcohol, Sterols & sterol derivatives, Terpenoids (di- and triterpenes) and Aromatic hydrocarbon derivatives (Table 6) and (Figure 2).

The compounds identified in the GC-MS analysis were further identified to check which compounds possess anti-inflammatory and anti-oxidant activity (Table 7)

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