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Original Article

DETERMINATION OF ANTIBACTERIAL, ANTIFUNGAL, BIOACTIVE CONSTITUENTS OF TRIPHALA BY FT-IR AND GC-MS ANALYSIS

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ABSTRACT

Objectives: To characterize the number of phytoconstituents present in triphala using FT-IR and GC-MS.

Methods: Antibacterial activity was measured by disc diffusion method, antifungal activity were analyzed by poisoned food technique, organic analysis was done by FT-IR, phytocomponents were identified by GC-MS analysis.

Results: The major bioactive components were present in methanolic extracts, further screened by GC-MS analysis revealed the presence of 10 bioactive compounds. The results were presented that triphala contains richly 1,2,3-Benzenetriol, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Furfural, 2H-Pyran-2,6(3H)-dione, D-Allose, n-Hexadecanoic acid, DL-Proline, 5-oxo-, methyl ester, Undecanol-5, 9-Phenanthrenol

Conclusion: Present findings indicated promising antimicrobial and phytocomponents are present and having remarkable number of qualities.

Keywords: Triphala, Antimicrobial activity, FT-IR and GC-MS.

INTRODUCTION

Triphala is an equiproportional mixture of fruits of three medicinal herbs*, Phyllanthus emblica, Treminalia bellirica and Terminalia chebula*[1]. It is mild, non-habit forming and rejuvenative and strengthens different tissues of the body, prevents aging and promotes health and immunity. It corrects constipation, cleanses and tonifies the gastro intestinal tract and also detoxifies the whole body, and improves digestion and assimilation [2]. It exhibits antiviral, antibacterial, antifungal and antiallergic properties [3]. Triphala contains several compounds responsible for health benefits including gallic acid, chebulagic acid and chebulinic acid [4]. Triphala exerts a marked heart-protective and cardio-tonic effect, improves digestion, improves liver function and hepato protective [5]. Indian traditional medical system, triphala strengthens the different tissues of the body, prevents aging, promotes health and immunity [6]. Triphala shows immunomodulatory properties and helps in improving the body's defense system [7]. Triphala and its constituents act as cardiotonic, control blood pressure, improve blood circulation and reduce cholesterol levels [8]. *Terminalia bellirica* has more bioactive principles and it constitutes rich source of medicine [9]. *Terminalia chebula* has phytoconstituents such as tannin, flavanoids, alkaloids, carbohydrates, glycosides, saponin, protein, aminoacids, polyphenol, quinine, caumarin[10]. Numerous studies have shown antimicrobial activity of *Terminalia chebula* fruit extracts against a number of microorganisms [11].

MATERIALS AND METHODS

Sample Collection and authentication

Fresh fruits of *Terminalia chebula*, *Terminalia bellirica and Phyllanthus emblica* were obtained from hill areas, the same was identified and authenticated by Dr. John Britto, Rapinet Herbarium, St. Joseph's College Trichy, Tamilnadu, India and given the Voucher Specimen No.VEA/001/2013, VEA/002/2013 and VEA/003/2013 respectively.

Preparation of extracts

Triphala fruits were shade dried, deseeded and pounded into fine powder in 1:1:1 ratio using stainless steel blender. Extracts were prepared by using Soxhlet extractor and 95% ethanol filtrates were individually pooled and each solvent removed at 40°C under reduced pressure by rotary evaporator. The methanol, acetone and aqueous extracts were subjected to preliminary screening of various plant constituents [12].

FT-IR analysis

Fourier transform infrared spectroscopy (Thermo Scientific Nicolet 1S5 FTIR) was used to analyze the fruit extract of Triphala in the Department of Chemistry, Annamalai University, Chidambaram, Tamilnadu, India. The spectrum was focused in the IR ranges between 600cm-4000cm by KBr pellet technique. The spectrum of fruit extract was recorded.

Preparation of Microorganism

The bacterial strains used in this study were gram positive bacteria *Staphylococcus aureus* (MTCC 3160) gram negative bacteria *Psuedomonas aeruginosa* (MTCC 1934) and *Klebsiella pneumoniae* (MTCC 4030). Fungal strains *Aspergillus flavus* (MTCC 277), *Aspergillus niger* (MTCC 282) and *Candida albicans* (MTCC 183) were procured from MTCC, Chandigarh, India. All chemical, media components and antibiotic impregnated discs used in this study from Hi Media, Mumbai, India.

Preparation of Medium

Cultures were procured from MTCC, nutrient agar media had been used for *Staphylococcus aureus* and *Psuedomonas aeruginosa,* LB medium for *Klebsiella pneumoniae* and Czapek Yeast Extract Agar for *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans.* These microbes were sub-cultured for the antimicrobial and antifungal activity.

Antibacterial Activity

Required numbers of petriplate were prepared and autoclaved at 121°C for 15 minutes and allowed to cool under laminar airflow chamber. Aseptically transferred about 20ml of media into each sterile petridish and about 1ml inoculum suspension were spread equivalently over the agar medium using sterile glass rod to get uniform distribution of bacteria. The readily prepared sterile discs were loaded with plant extract as triplicates and incubated at 5°C for 1 hour to permit good diffusion and then transferred to the incubator at 37°C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (mm)[13].

Antifungal Activity

Extracts were screened for antifungal activity by Food Poisoning method against *A. flavus, A. niger, C. albicans*. Extract concentration (1000µm) was mixed with sterilized medium in separate flasks and transferred into petriplates and allowed to solidify. In aseptic condition 6mm diameter of fungal culture disc were taken and inoculated to the center of petriplates containing extract asceptically and radial growth of colony was measured after incubation period [14**].**

GC-MS Analysis

GC-MS technique was used in this study to identify the phytochemicals present in the extract; it was carried out in SASTRA University, Thanjavur, Tamilnadu. GC-MS analysis of this extract was performed using Perkin Elmer Clarus 500 and gas chromatograph interfaced with a mass spectrometer equipped with capillary column Elite-5MS(30MX250µM) composed of 5% Phenyl 95% dimethylpolysiloxane. For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas was used as carrier gas at constant flow rate of 1ml/min and injection volume 1.4µl was employed split ratio of 10:1. Injector temperature was 280°, ion source temperature 200°C, 150°C for 20 minutes. Total GC running time was 20minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatogram was a Turbo Mass Ver.5.2.0.

Compound identification

Interpretation of mass spectrum GC-MS was conducted using the data base of National Institute Standard and Technique (NIST) having more than 62000 patterns. The spectrum of the unknown components were compared with the spectrum of the known components stored in the NIST library, Name, Molecular Weight, Structure of the component of the test material was ascertained.

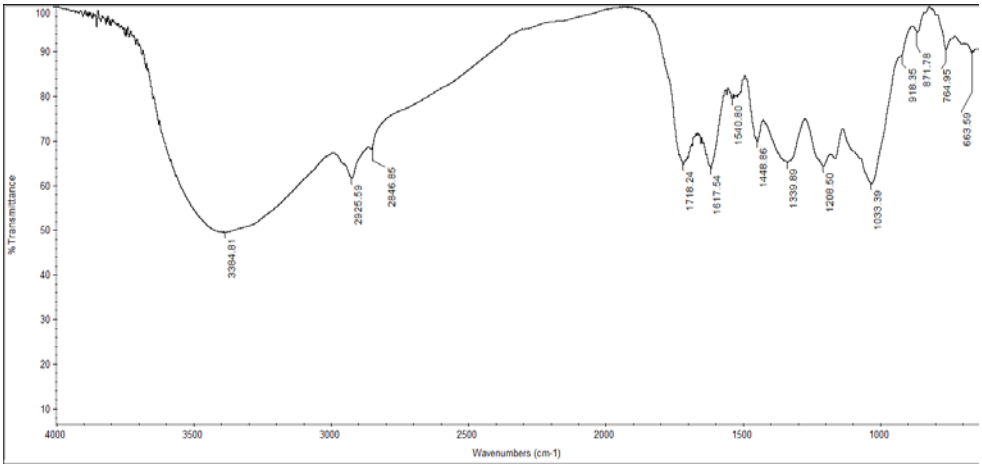

Fig. 1: FTIR peaks of Triphala fruits extract

Table 1: FTIR peaks of Triphala fruits extract

Functional Group	OH Alcohol	C-H Alkane	=C-H Aldehyde	Acyclic Ketone	N-H Amide	C=C Aromatic	C-N Amine	C-F Alkyl Halide	=C-H Alkene	C-Cl Alkyl Halide
Wavelength	3384.81	2925.59	2485.85	1708.24	1607.54 1542.82	1445.88	1339.59	1288.50 1033.33	918.35 871.78 764.95	668.59

Table 2: Antibacterial activity of Triphala from various solvents

Test Samples and Extracts	Zone of Inhibition (mm)							
	Gram Positive		Gram Negative				Mean	S.D
	S. aureus	%	*P. aeruginosa*	%	*K. pneumoniae*	%	$\bar{x}$	σ
Acetone	12.25	35.50	12.25	34.50	10	28.98	11.50	1.06
Methanol	10	33.89	9.5	32.20	10	33.89	9.83	0.23
Aqueous	14	40.29	10.75	30.94	10	28.71	11.58	1.74
Antibiotic	10	27.02	10	27.02	17	45.94	12.3	3.35

Table 3: Antifungal activity of Triphala from various solvents

Test Samples and Extracts	Zone of Inhibition (mm)							
	Fungal Strain						Mean	S.D
	C.albicans	%	*A.flavus*	%	*A.niger*	%	$\bar{x}$	σ
Acetone	7	28	7	28	15	31.9	8.33	1.88
Methanol	7	28	7	28	14	29.78	8.33	1.88
Aqueous	11	44	11	44	18	38.29	15.7	1.7
Antibiotic	10	29.4	9	26.47	15	44.1	11.3	2.62

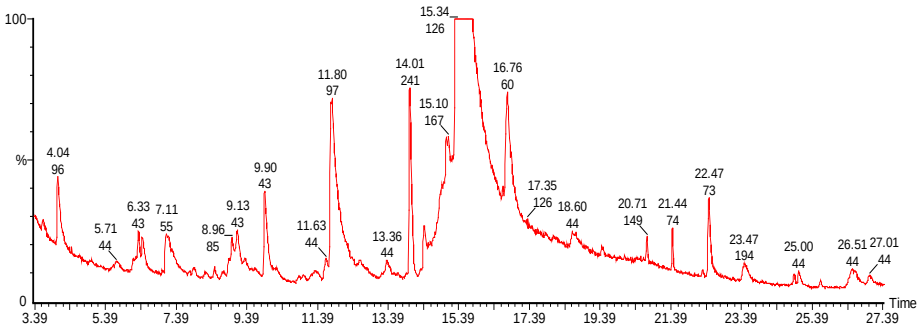

Fig. 2: GC-MS Chromatogram of fruits extract of Triphala

Table 4: Phytoconstituents identified in the fruits extract of Triphala by GC-MS:

Compound Name	RT	%	Molecular Formula	Compound Nature	Activity
1,2,3-Benzenetriol	15.34	69.62	$C_6H_6O_3$	Aromatic Alcohol	Antiseptic,Antioxidant, Antidermatitic,Fungicide, Insecticide, Candidicide
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	11.80	9.174	$C_6H_6O_3$	Aldehyde Compound	Antimicrobial, Preservative
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.90	3.233	$C_6H_8O_4$	Flavanoid Fraction	Antimicrobial, Anti-inflammatory
Furfural	4.04	3.108	$C_5H_4O_2$	Aldehyde Compound	Antiseptic,Flavour,Fungicide, Pesticide, Insecticide, Irritant
2H-Pyran-2,6(3H)-dione	7.11	2.974	$C_5H_4O_3$	Ketone	Not known.
D-Allose	16.76	2.789	$C_6H_{12}O_6$	Carbohydrate	Antisecretory, Preservative
n-Hexadecanoic acid	21.44	2.092	$C_6H_{32}O_2$	Fatty Acid ester compound	Antioxidant, Hypercholesterolemic,Cancer Preventive,Hepatoprotective, Nematicide,Insecticide, Anticaronary, Antiandrogenic
DL-Proline, 5-oxo-, methyl ester	14.40	0.9499	$C_6H_9NO_3$	Flavanoid Fraction	Antioxidant, Anti-inflammatory
Undecanol-5	13.36	0.8314	$C_{11}H_{24}O$	Fatty Alcohol	Flavour, Perfumery
9-Phenanthrenol	23.47	0.8254	$C_{14}H_{10}O$	Fatty Alcohol	Antimicrobial,Dermatitic, Antifungal

Activity Source: Dr. Duke's Phytochemical and Enthobotanical Database

RESULTS AND DISCUSSION

The present study concretely depicts about the antibacterial, antifungal and functional group based phytoconstituents present in triphala. Invitro antimicrobial activity with microbial strains *Staphylococcus aureus, Psuedomonas aeruginosa* and *Klebsiella pneumoniae* has highest inhibitory zone in aqueous extract 14mm, 10.75mm, 10mm and the mean value of acetone, methanol and aqueous is 11.50, 9.83, 11.58 and standard deviation 1.06, 0.23, 1.74 respectively depicted in table 2. Antifungal activity also done using poisoned food technique and shown the effective growth zone to aqueous extract 11mm(2), 18mm and the mean value 8.33 (2), 15.7 and standard deviation 1.88 (2), 1.7 correspondingly presented in table 3. Aqueous extract of triphala has greater antimicrobial activity against gram negative bacteria and fungi. The essential oils from *Phyllanthus amarus* exhibited strong activity against the *B. subtilis, S. aureus* and *C. albicans*[15]. Bioactive compounds in triphala 1,2,3,4 and 10 has 69.62%, 9.17%, 3.233%, 3.1085% and 0.28% remarkable activity against gram negative microorganism.

FTIR analysis gave results that exhibit the Processing, Croom Helm, London. Presence of different functional groups ranging from O-H alcohol (3200-3600 cm^{-1} Strong, Broad), C-H alkane (2850-3000 cm^{-1} Strong), =C-H aldehyde (2820-2850 cm^{-1} medium-2 peaks), Acyclic ketone (1705-1725 cm^{-1} strong), N-H amide (1550-1640 bending), c=c aromatic (1400-1600 cm^{-1} multiple bands), C-N amine (1080-1360 cm^{-1}), C-F Alkyl Halide (1000-1400 cm^{-1} strong), =C-H alkene (675-1000 cm^{-1} strong) as functional groups illustrated in table 1 and figure 1. Through GC-MS analysis 7 bioactive compounds were identified in the methanol extracts of *Acanthus ilicifolius* leaves[16]. Dr. Dukes Phytochemical and enthobotanical database indicates 10 major compounds.

Compound name, retention time, percentage, molecular formula, nature of the compound and its activities are depicted in table 4. Compounds are 1,2,3 Benzenetriol, an aromatic alcohol compound has the nature of antioxidant, antiseptic, fungicide, insecticide. 2-Furancarboxaldehyde, 5-(hydroxymethyl)- an aldehyde compound is suggested to have antimicrobial activity and it act as a preservative. 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- a flavanoid compound used as antimicrobial, anti-inflammatory agent, Furfural an aldehyde compound is recommended to be antiseptic, flavour, fungicide, pesticide, insecticide and irritant. D-Allose the sugar moiety prescribed to be an antisecretory and preservative. n-Hexadecanoic acid, fatty acid ester compound suggested to be an antioxidant, hyper cholesterolemic, cancer preventive, hepato protective, nematicide, insecticide, anticaronary, antiandrogenic. DL-Proline, 5-oxo-, methyl ester a flavanoid compound can be used as antioxidant, anti-inflammatory. Undecanol-5 and 9-Phenanthrenol both the fatty alcohol compound is suggested to be flavour, perfumery and antimicrobial, dermatitis and antifungal properties shown in table 4 and figure 2.

CONCLUSION

This study clearly signifies that triphala fruit extract was rich in antimicrobial, antioxidant and antidiabetic properties. The FT-IR and GC-MS study also showed many phytocomponents and have more pharmacological activities and it opens a way to the phytochemical, pharmacognostical and herbal field to carry out drug formulations and research activities.

CONFLICT OF INTERESTS

Declared None

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