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RESEARCH ARTICLE

Preliminary phytochemical characterization and *in vitro* antioxidant and antimicrobial potential screening of an ayurvedic drug combination *Panchatikta*

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Abstract

Panchatikta is an Ayurvedic drug combination of 5 bitter-tasting drugs, namely *Azadirachta indica*, *Tinospora cordifolia*, *Justicia adhatoda*, *Solanum virginianum* and *Trichosanthes dioica*. In this study, estimations of the phenolic, flavonoids and tannin contents, HPTLC profile and *in vitro* antioxidant and anti-microbial potential of 3 extracts of the Panchatikta were carried out. The total phenolic content of the Panchatikta combination was almost the same in ethanol, water and hydroalcoholic extracts. Flavonoid content and tannin content were highest in ethanol extract. HPTLC profiling of the 3 extracts of Panchatikta showed the maximum number of peaks for the ethanolic extract. Water extract showed a comparatively smaller number of peaks. The maximum number of peaks was seen after derivatisation. In the DPPH assay, gallic acid has IC_{50} value of 5.50 ± 0.38 µg/mL; ethanol and hydroalcoholic extracts have IC_{50} close to 60 µg/mL and water extract has around 90 µg/mL. The ABTS assay showed standard ascorbic acid has a IC_{50} value of 67.33 ± 0.58 µg/mL while Panchatikta extracts have a much higher IC_{50}. In nitric oxide assay also, tested samples have a higher IC_{50} when compared with that of ascorbic acid (177.33 ± 5.09). In well diffusion assay for antibacterial screening, all 3 extracts have a significant zone of inhibition at concentrations of 500 µg and 1000 µg. *Pseudomonas aeruginosa* was found to be resistant to all 3 test samples and standard antibiotic discs. Minimum activity was obtained against *Klebsiella pneumonia*. Panchatikta extracts at concentrations of 500 µg and 1000 µg showed significant activity against the fungal strains *Aspergillus niger* and *Fusarium oxysporum*. In conclusion, the results of the study can provide scientific support for the traditional usage of the Ayurvedic drug combination Panchatikta for various ailments.

Keywords

Panchatikta; HPTLC; DPPH; ABTS; antioxidant; antimicrobial; flavonoid; phenol; Ayurveda

Introduction

Ayurveda, an ancient system of medicine that is said to have originated more than 5000 years ago in the Indian sub-continent has a significant role in ensuring holistic well-being. Ayurveda is regarded as 'the science of life literally', as the word itself is a combination of two Sanskrit words, namely, 'ayu' (life) and 'veda' (knowledge). The revered tradition of Ayurveda covers the immense interrelationship between mind, body and spirit. Ayurveda stands for the maintenance of equilibrium between mind and body follow-

ing the belief that true well-being happens only when these elements are in parallel. In an era when modern medicine has its own remarkable pace, Ayurvedic medicine has significance through its holistic approach, preventive concern, personalised care and recognition of the individual's unique constitution. As per the ayurvedic concept, 'there is no substance in the world that has no medicinal value'. The majority of Ayurvedic medicines are plant-based. India is a rich country in its vast biodiversity and more than 10000 plants are reported to be used by various systems of medicine for medicinal preparations. But Ayurvedic pharmacopoeia covers a list of less than 2000 medicinal plants (1). Most of the Ayurvedic medicines are polyherbal, with 3 -30 medicinal plants being used for each formulation. Polyherbal formulations are proven to be more effective than single formulations, because, in a combination, 1 or 2 drugs play a key role and others have a supportive role. Drugs of different actions act in equilibrium to catalyse the proper absorption and transportation of major chemical constituents of a formulation and also reduce toxicity. Panchatikta is an Ayurvedic drug combination of 5 bitter-tasting drugs, namely stem and bark of *Azadirachta indica*, stem of *Tinospora cordifolia*, roots of *Justicia adhatoda*, whole plant of *Solanum virginianum* and the aerial parts of *Trichosanthes dioica* (2). As we are aware that each medicinal plant has its own chemical composition and when we combine 2 or more plants together, the therapeutic potential of the end product may be due to the synergic action of various constituents present in ingredient drugs. Reports are there mentioning the importance of phytonutrients such as phenolics and flavonoids in maintaining health and well-being in human beings. Phenols are compounds having an aromatic structure with a single or multi hydroxyl group attached to it. More than 5000 naturally occurring phenolic compounds are reported, of which half are flavonoids. The presence of phenolic compounds in herbal extracts proved to be the major reason for their antioxidant, antimutagenic and anticarcinogenic activities as well as their capacity to alter gene expression. Flavonoids can occur in plants either in their free state or as glycosides and they are the largest naturally occurring phenolics in plants. Its 2 benzene rings alternate with propane. Phenolic compounds are reported to be the reason for many of the pharmacological properties of medicinal plants. Tannins, a major group of polyphenolic compounds, are readily available in the barks and leaves of many medicinal plants. The interaction between cellular macromolecules and free radicals produced by mutagens or carcinogens can be reduced by means of the antimutagenic activity of tannin molecules (3). High Performance Thin Layer Chromatography (HPTLC) has wide acceptance in herbal drug standardisation and quality control. It can be applied for analysing crude drug samples containing multiple ingredients. This makes it an inevitable Ayurvedic drug analysis. The antioxidant properties of drugs are responsible for preventing cellular oxidative damage. The present study is aimed at estimating the phenolic, flavonoid and tannin content of various extracts of the ingredient drugs and combinations, performing HPTLC profiling of three extracts of panchatikta and checking their *in vitro* antioxidant and anti-microbial activities through various assays. It is proposed that the results of the study can be used to validate the traditional Ayurvedic usage of Panchatikta combinations against various disease conditions.

Materials and Methods

Plant materials

Stem bark of *A. indica* (CMPR Herbarium - 12015), stem of *T. cordifolia* (CMPR Herbarium - 12024), root of *J. adhatoda* (CMPR Herbarium - 12012) and root of *S. virginianum* (CMPR Herbarium - 12017) were collected from different natural habitats in Kerala and aerial parts of *T. dioica* (CMPR Herbarium - 12021) were collected from Gujarat. All the materials were authenticated, herbariums were deposited in the 'CMPR Herbaria' and dried materials were deposited in CMPR Raw Drug Repository.

Microbial Cultures

Source

Microbial Type Culure Collection, Chandigarh, India.

Staphylococcus aureus (MTCC 3160), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 40), *Klebsiella pneumoniae* (MTCC 3384), *Pseudomonas aeruginosa* (MTCC 741). All the bacterial cultures were maintained in nutrient agar and stored at 4 ˚C.

Fungal isolates were subcultured on potato dextrose agar and prepared for the antifungal assessment. The fungi proposed for the study include *Aspergillus niger (*MTCC 281), *Penicillium citrinum* (MTCC 1256), *Fusarium oxysporum* (MTCC 284).

Extraction of phytochemicals

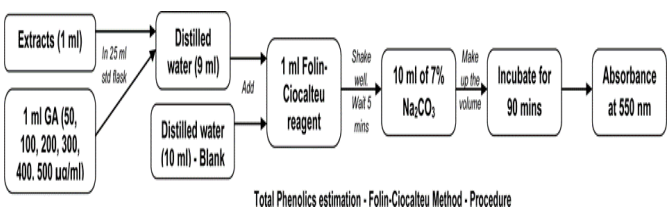
The dried materials were powdered and subjected to extraction using ethanol, water and hydro alcohol (50:50) as per standard protocol (6). For the extraction, 5 g each of the 5 ingredient drugs as well as the combination were taken in a round bottomed flask and refluxed with 100 mL of the solvent, continuously for 3 h and the extracts were filtered. The residue was refluxed again at 50 °C continuously for 3 h. The extract was filtered, concentrated and dried.

Total Phenolic Estimation

Folin Ciocalteu assay method as per earlier reports was followed for phenolic content estimation (4). Results were obtained from the calibration curve of the graph in mg gallic acid equivalents (mg GAE).

Total Flavonoid Estimation

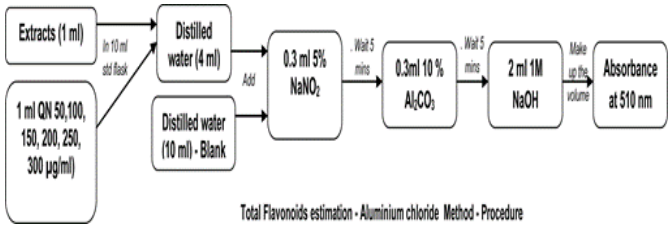
Aluminium chloride colorimetric assay was done as per protocol (5). Results were obtained in mg quercetin equivalents (mg QE).



Total Phenolics estimation - Folin-Ciocalteu Method - Procedure

Total Tannin Estimation

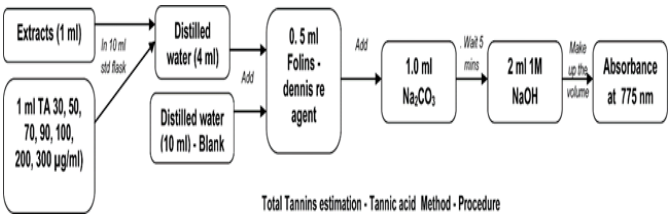
Tannic acid calorimetric assay as per Ayurvedic Pharmacopoeia was followed (6). By plotting a graph the corresponding concentration of tannin calculated. Value obtained after plotting a graph is termed as mg Tannic acid Equivalents (mg TAE).



Total Flavonoids estimation - Aluminium chloride Method - Procedure

HPTLC Profiling

Chromatographic profiling was done using the Camag HPTLC system (6). Each of the extracts (10 µL) was applied to Merck TLC plates (20 x 10 cm) using the CAMAG TLC Sampler ATS IV, developed under the solvent system Toluene: Ethylacetate: Formic acid: Methanol (7:3:3:1) in a twin -trough glass chamber at room temperature, images were captured at 254 nm, 366 nm and 550 nm using the CAMAG TLC Visualizer.



Total Tannins estimation - Tannic acid Method - Procedure

Antioxidant Assays

DPPH radical scavenging assay:

1 mL of Panchatikta extracts at concentrations of 500, 250, 125, 61.5, 31 and 15 µg/mL and standard gallic acid (100, 50, 25, 12.5, 6.25 and 3.125 µg/mL) were added to 1 mL of 0.004 % DPPH and mixed well. The absorbance at 517 nm was measured using UV spectrophotometer. DPPH scavenging activity was calculated using the formula scavenging activity = [(Ac−As) ÷ Ac] × 100; where Ac = Absorbance of Control, As= Absorbance of Sample. Calibration curves of standard and test samples were drawn, the % of inhibition was calculated and the IC_{50} value estimated (7).

Free radical-scavenging activity on ABTS

7 mM ABTS solution was mixed with 2.45 mM potassium persulfate solution and kept in the dark at room temperature for 12-16 h to produce ABTS radical cation (ABTS+). The absorbance was measured at 732 nm after the addition of extracts (1000, 500, 100 µg/mL) and ascorbic acid (100, 50, 10 µg/mL) (8). ABTS scavenging activity was calculated using the formula scavenging activity = [(Ac−As) ÷ Ac] × 100; where Ac = Absorbance of Control, As= Absorbance of Sample. Calibration curves of standard and test samples were drawn, the % of inhibition was calculated and the IC_{50} value estimated.

Nitric oxide quenching activity

1 mL of 10 mM sodium nitroprusside was added with 1.5 mL of 0.2 M phosphate buffer. Extracts (1000, 500, 100 µg/mL) and standard ascorbic acid (100, 50, 10 µg/mL) solution were mixed separately with it. Absorbance at 546 nm was measured after mixing 1 mL reaction mixture and 1 mL Griess reagent. NO quenching activity was calculated using the formula quenching activity = [(Ac−As) ÷ Ac] × 100; where Ac = Absorbance of Control, As= Absorbance of Sample. Calibration curves of standard and test samples were drawn, the % of inhibition was calculated and the IC_{50} value was estimated (9).

Anti-microbial Assays

The agar well diffusion method (10) was followed for the present study. MHA was used as the medium for both assays. Extracts were poured into the respective wells prepared and the zonal diameters for each organism were measured and recorded. Values obtained were compared with those of standard antibiotics chloramphenicol (30 mg) and gentamicin (10 mg) for bacteria and Erythromycin (15 µg) and Cotrimaxazol (25 µg) for fungus.

Results

Total Phenolics, Flavonoids and Tannins

Estimation showed that *Azadirachta indica* stem bark contains the highest phenolic, flavonoids and tannin content among the 5 ingredients of *Panchatikta*. The total phenolic content of the Panchatikta combination was almost the same in all 3 extracts, i.e., ethanol, water and hydroalcohol. Flavonoid content and tannin content were highest in ethanol extract. The detailed reports are given in Table 1 and Fig. 1.

Table 1. Total phenolics, flavonoids and tannins of different extracts of Panchatikta plants.

	Total Phenolics (mg/g GA Eq.)			Total Flavonoids (mg/g QT Eq.)			Total Tannins (mg/g TA Eq.)		
	Water	Ethanol	W+E	Water	Ethanol	W+E	Water	Ethanol	W+E
AI	20.99±0.82	22.29±0.83	23.86±1.00	4.26±0.57	6.72±1.73	6.94±1.17	9.61±0.88	8.71±0.13	8.64±0.30
TC	8.06±0.82	7.95±0.48	7.91±0.24	1.00±0.09	1.70±0.34	1.54±0.30	2.63±0.17	3.01±0.17	4.50±0.13
JA	9.32±0.32	8.48±0.74	8.24±0.30	0.80±0.03	1.29±0.37	1.16±0.29	3.79±0.16	4.89±0.10	5.45±0.10
SV	6.97±1.33	6.35±0.67	6.39±0.34	0.78±0.28	0.72±0.20	0.98±0.15	1.25±0.23	1.30±0.24	1.51±0.26
TD	8.01±0.83	6.62±0.59	7.45±1.07	0.81±0.26	1.31±0.36	0.97±0.33	2.49±0.11	2.37±0.10	2.02±0.11
PTC	8.62±1.10	8.56±0.37	8.39±0.46	1.29±0.19	2.37±0.42	1.74±0.12	3.34±0.29	5.16±0.46	4.66±0.25

Tests done in triplicates; ± Standard deviation; AI, *Azadirachta indica*; TC, *Tinospora cordifolia*; JA, *Justicia adhathoda*; SV, *Solanum virginianum*; TD, *Trichosanthes dioica*; PTC, Panchatikta Combination; GA Eq., Gallic acid Equivalents; QT Eq. Quercetin Equivalents; TA Eq. Tannic acid Equivalents.

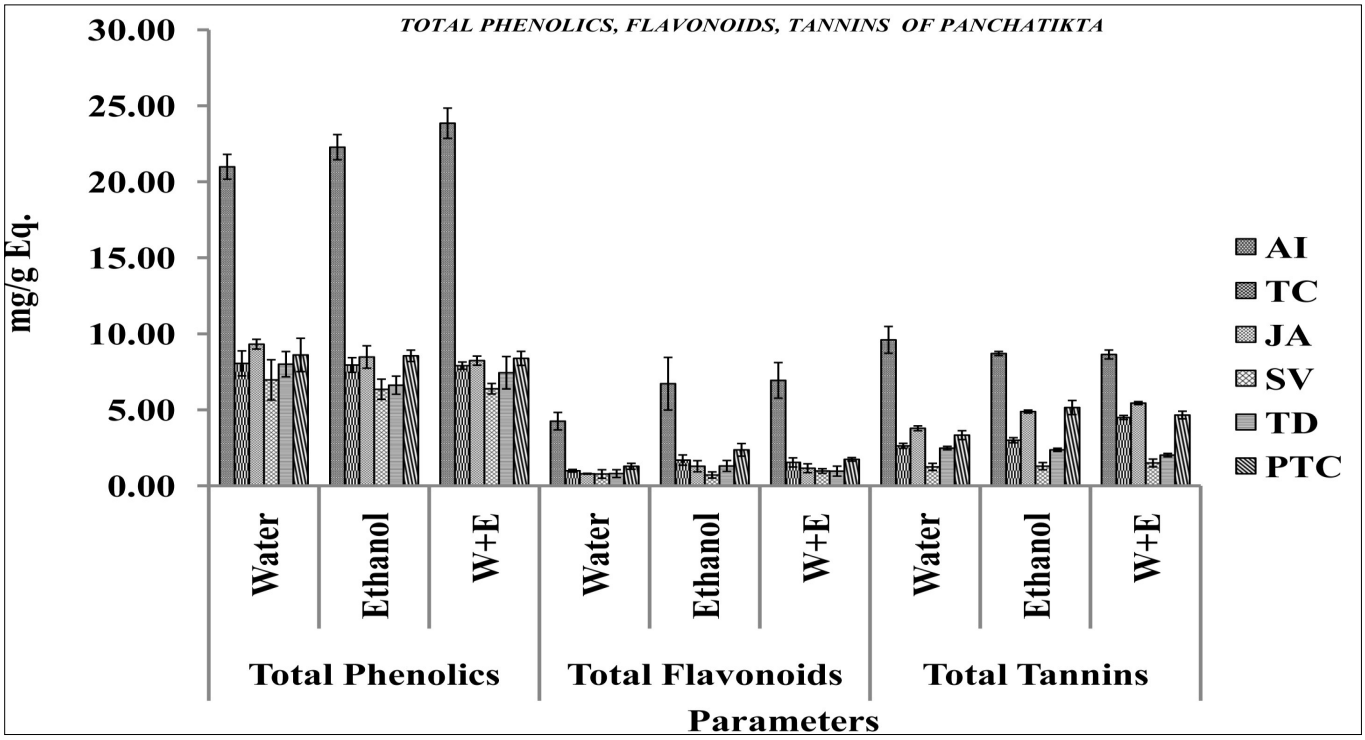


Fig. 1. Comparison of total phenolics, flavonoids and tannins of different extracts of Panchatikta plants. Tests done in triplicates; ± Standard deviation; **AI**- *Azadirachta indica*; **TC**- *Tinospora cordifolia*; **JA**- *Justicia adhathoda*; **SV**- *Solanum virginianum*; **TD**-*Trichosanthes dioica*; **PTC**- Panchatikta combination.

HPTLC Fingerprinting

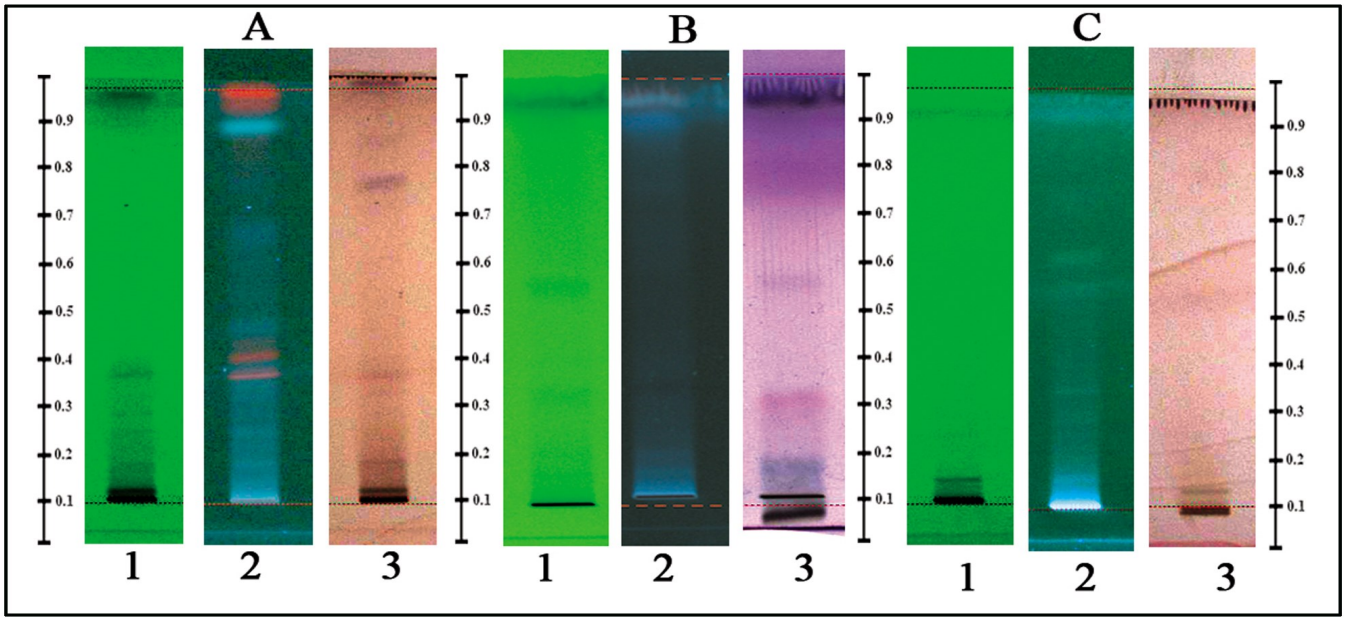
HPTLC profiling of the ethanol, hydroalcohol and water extracts of Panchatikta showed the maximum number of peaks for ethanolic extract. 6 peaks were present under UV 254 nm, 5 peaks under UV 366 nm and 7 peaks after spraying with ANS. Water extract showed a comparatively smaller number of peaks. The maximum number of peaks was seen after derivatisation. The details are given in Table 2 and Fig. 2.

Antioxidant activity

The antioxidant activity of 3 different extracts of Panchatikta was screened using 3 different assay methods. As per the results of the DPPH, ABTS and NO assay methods, ethanol and hydroalcoholic extracts possess higher antioxidant activity when compared to water extracts, but when compared with standard drugs, gallic acid and ascorbic acid, the activity is less and significant activity is obtained at higher concentrations. In the DPPH assay, gallic acid has

Table 2. HPTLC Rf values of compounds separated from different extracts of Panchatikta.

Extract	UV 254	UV 366	Derivatised
Ethanol	0.02, 0.16, 0.20, 0.29, 0.70, 0.96 (6 Nos.)	0.29, 0.33, 0.70, 0.94, 0.96 (5 Nos)	0.01, 0.08, 0.28, 0.69, 0.74, 0.85, 0.98 (7 Nos)
Hydro alcohol	0.06, 0.25, 0.32, 0.51 (4 Nos)	0.09, 0.11, 0.25 (3 Nos)	0.04, 0.07, 0.23, 0.51, 0.78, 0.90 (6 Nos)
Water	0.05, 0.51, 0.56, 0.93, 0.96 (5 Nos)	0.90 (1 No)	0.04, 0.14, 0.50, 0.60, 0.79, 0.96 (6 Nos)



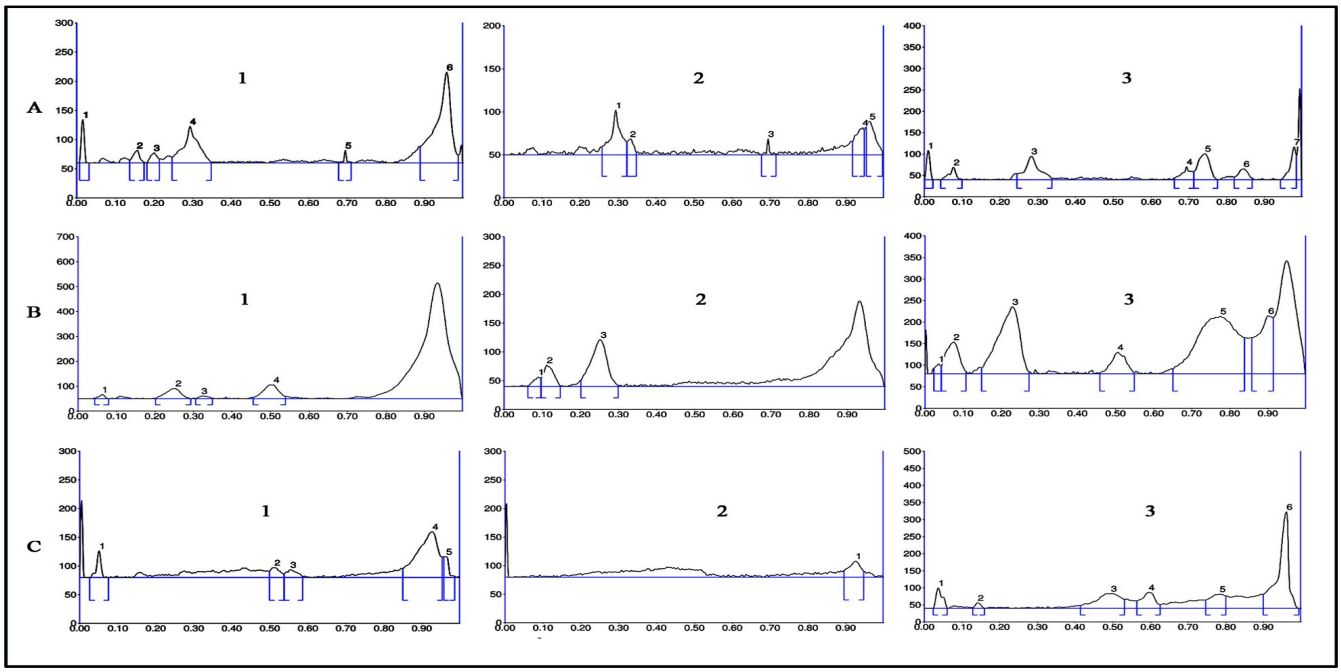


Fig. 2. HPTLC fingerprint profile of three different extracts of Panchatikta. **A**. Ethanol extract; **B**. Hydro alcohol extract; **C**. Water extract; **1**- at UV 254; **2**- at UV 366; **3**- profile after derivatisation.

an IC_{50} value of 5.50 ± 0.38 µg/mL; ethanol and hydroalcoholic extracts have IC_{50} close to 60 µg/mL and water extract has around 90 µg/mL (Table 3, Fig. 3). The ABTS assay showed standard ascorbic acid has an IC_{50} value of 67.33 ± 0.58 µg/mL. Comparing to the standards, Panchatikta extracts have a much higher IC_{50} (Table 4, Fig. 3). In NO assay, test samples also have a higher IC_{50} when compared with that of ascorbic acid (177.33 ± 5.09 µg/mL) (Table 5, Fig. 3).

Table 3. Percentage of inhibition of different extracts of *Panchatikta* in DPPH assay.

Conc. (µg/mL)	% Inhibition Panchatikta			% Inhibition Gallic acid	
	Ethanol	Hydro-Alcohol	Water	Conc. (µg/mL)	% Inhibition
500	79.43±1.29	78.93±1.32	69.39±0.30	100	80.18±2.84
250	75.95±0.82	75.27±0.61	67.50±0.86	50	77.18±1.17
125	74.23±0.53	73.34±1.05	66.08±0.27	25	67.71±1.20
61.25	50.57±1.36	51.40±0.12	37.81±1.83	12.5	67.78±1.07
31	23.81±0.45	21.48±2.08	28.54±0.75	6.25	53.54±1.86
15	20.23±0.72	19.02±0.75	21.02±2.01	3.125	22.81±0.94
IC_{50} (µg/mL)	60.30±1.53	60.60±0.10	90.10±0.12	IC_{50} (µg/mL)	5.50±0.38

Tests done in triplicates ; ± Standard deviation.

Table 4. Percentage of inhibition of different extracts of *Panchatikta* in ABTS assay.

Conc. (µg/mL)	% Inhibition Panchatikta			% Inhibition Ascorbic acid	
	Ethanol	Hydro-Alcohol	Water	Conc. (µg/mL)	% Inhibition
1000	54.07±0.36	62.71±0.83	52.51±1.09	100	55.95±0.28
500	53.36±0.64	55.90±0.82	43.49±0.58	50	46.54±0.37
100	37.16±1.63	41.56±0.76	36.75±0.42	10	40.35±0.52
IC_{50} (µg/mL)	405.00±13.23	333.33±11.55	880.00±17.32	IC_{50} (µg/mL)	67.33±0.58

Tests done in triplicates ; ± Standard deviation.

Table 5. Percentage of inhibition of different extracts of *Panchatikta* in NO assay.

Conc. (µg/mL)	% Inhibition Panchatikta			% Inhibition Ascorbic acid	
	Ethanol	Hydro-Alcohol	Water	Conc. (µg/mL)	% Inhibition
1000	65.06±0.83	60.86±1.72	56.12±1.24	200	52.25±0.82
500	47.92±0.50	48.77±1.64	34.80±0.62	100	42.45±0.36
100	23.37±1.21	37.56±1.09	15.91±0.92	50	30.15±0.49
IC_{50} (µg/mL)	556.67±15.28	536.67±25.17	866.67±15.28	10	22.06±0.68
				IC_{50} (µg/ml)	177.33±5.09

Tests done in triplicates ; ± Standard deviation.

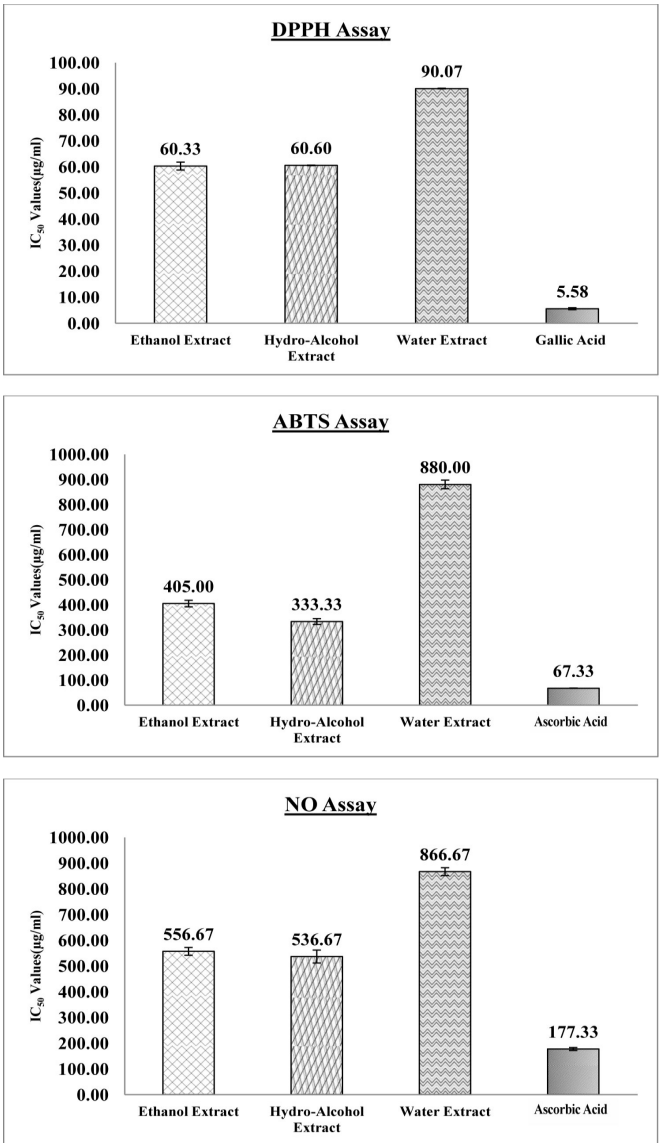


Fig. 3. Comparison of IC_{50} values of different extracts of *Panchatikta* in various antioxidant assay methods. Tests done in triplicates; Error bar showing standard deviation.

Antibacterial activity

Antibacterial activity was screened against *S. aureus* and *B. subtilis* (Gram $^{+ve}$) and *E. coli*, *K. pneumonia* and *P. aeruginosa* (Gram $^{-ve}$). In all 3 extracts, a significant zone of inhibition was observed at concentrations of 500 µg/mL and 1000 µg/mL. *P. aeruginosa* was found to be resistant to all 3 test samples and standard antibiotics, Chloramphenicol and Gentamycin. Minimum activity was obtained against *K. pneumonia*. The maximum zone of inhibition was obtained for ethanol extract against *E. coli*, which ranged from 2 mm to 20 mm followed by water extract against *S. aureus* (1 mm to 18 mm) and hydroalcoholic extract against *B. subtilis* (3 mm to 17 mm) at concentrations ranging from 100 µg/mL to 1000 µg/mL (Table 6-9, Fig. 4).

Antifungal activity

The antifungal activity of 3 *Panchatikta* extracts was screened against 3 strains: *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium citrinum*. Both the antibiotic discs selected, *Erythromycin* and *Cotrimaxazol*, didn't show any resistance against any of these 3 strains. Panchatikta extracts at concentrations of 500 µg/mL and 1000 µg/mL showed significant activity against *Aspergillus niger* and *Fusarium oxysporum*. Against *A. niger* ethanol extract showed a zone of inhibition ranging from 1 mm to 16 mm, hydroalcoholic extract showed a zone of 5 mm to 39 mm and water extract had a zone of 2 mm to 39 mm at concentrations ranging from 100 µg/mL to 1000 µg/mL. Against *F. oxysporum* ethanol extracts' zone was between 7 mm to 17 mm, hydro alcohol extract has zone ranging from 4 mm to 22 mm and the organism was resistant against water extract. Against *P. citrinum*, only the water extract showed some activity and had a zone ranging from 5 mm to 12 mm.

Table 6. Antibacterial activity of ethanol extract of *Panchatikta.*

Sl. No.	Organism	Zone of Inhibition (mm)				
		50	100	200	500	1000
1	*SA*	-	-	-	0.67±0.58	3.00±1.00
2	*BS*	-	4.33±0.58	9.33±1.15	12.33±2.52	13.33±2.89
3	*EC*	2.67±1.15	8.00±0.00	9.67±0.58	12.33±2.52	20.00±0.00
4	*KP*	-	-	-	-	-
5	*PA*	-	-	-	-	-

Tests done in triplicates; ± Standard deviation; SA, *Staphylococcus aureus* (MTCC 3160); BS, *Bacillus subtilis* (MTCC441); EC, *Escherichia coli* (MTCC40); KP, *Klebsiella pneumoniae* (MTCC3384); PA, *Pseudomonas aeruginosa* (MTCC741).

Table 7. Antibacterial activity of hydro-alcohol extract of *Panchatikta.*

Sl. No.	Organism	Zone of Inhibition (mm)				
		50	100	200	500	1000
1	*SA*	-	-	-	5.67±0.58	5.60±0.00
2	*BS*	-	3.00±1.73	8.00±1.00	12.33±2.52	17.33±2.08
3	*EC*	-	-	5.67±1.52	8.00±1.00	14.33±1.83
4	*KP*	-	-	0.67±1.35	-	1.67±0.58
5	*PA*	-	-	-	-	-

Tests done in triplicates; ± Standard deviation; SA, *Staphylococcus aureus* (MTCC 3160); BS, *Bacillus subtilis* (MTCC441); EC, *Escherichia coli* (MTCC40); KP, *Klebsiella pneumoniae* (MTCC3384); PA, *Pseudomonas aeruginosa* (MTCC741).

Table 8. Antibacterial activity of water extract of *Panchatikta.*

Sl. No.	Organism	Zone of Inhibition (mm)				
		50	100	200	500	1000
1	*SA*	-	1.00±0.00	9.67±1.15	15.00±1.00	18.00±2.65
2	*BS*	-	4.67±1.53	7.00±1.73	12.33±2.52	12.67±2.08
3	*EC*	2.67±1.15	0.67±1.15	5.33±1.52	9.00±1.73	16.33±1.52
4	*KP*	-	-	-	2.00±0.00	-
5	*PA*	-	-	-	-	-

Tests done in triplicates; ± Standard deviation; SA, *Staphylococcus aureus* (MTCC 3160); BS, *Bacillus subtilis* (MTCC441); EC, *Escherichia coli* (MTCC40); KP, *Klebsiella pneumoniae* (MTCC3384); PA, *Pseudomonas aeruginosa* (MTCC741).

Table 9. Antibacterial activity of standards.

Sl. No.	Organism	Zone of Inhibition (mm)	
		Chloramphenicol (30 µg)	Gentamycin (30 µg)
1	*SA*	19.33±1.15	16.33±0.58
2	*BS*	23.67±1.15	20.00±0.00
3	*EC*	37.00±1.00	15.67±0.58
4	*KP*	27.33±2.08	-
5	*PA*	-	-

Tests done in triplicates; ± Standard deviation; SA, *Staphylococcus aureus* (MTCC 3160); BS, *Bacillus subtilis* (MTCC441); EC, *Escherichia coli* (MTCC40); KP, *Klebsiella pneumoniae* (MTCC3384); PA, *Pseudomonas aeruginosa* (MTCC741).

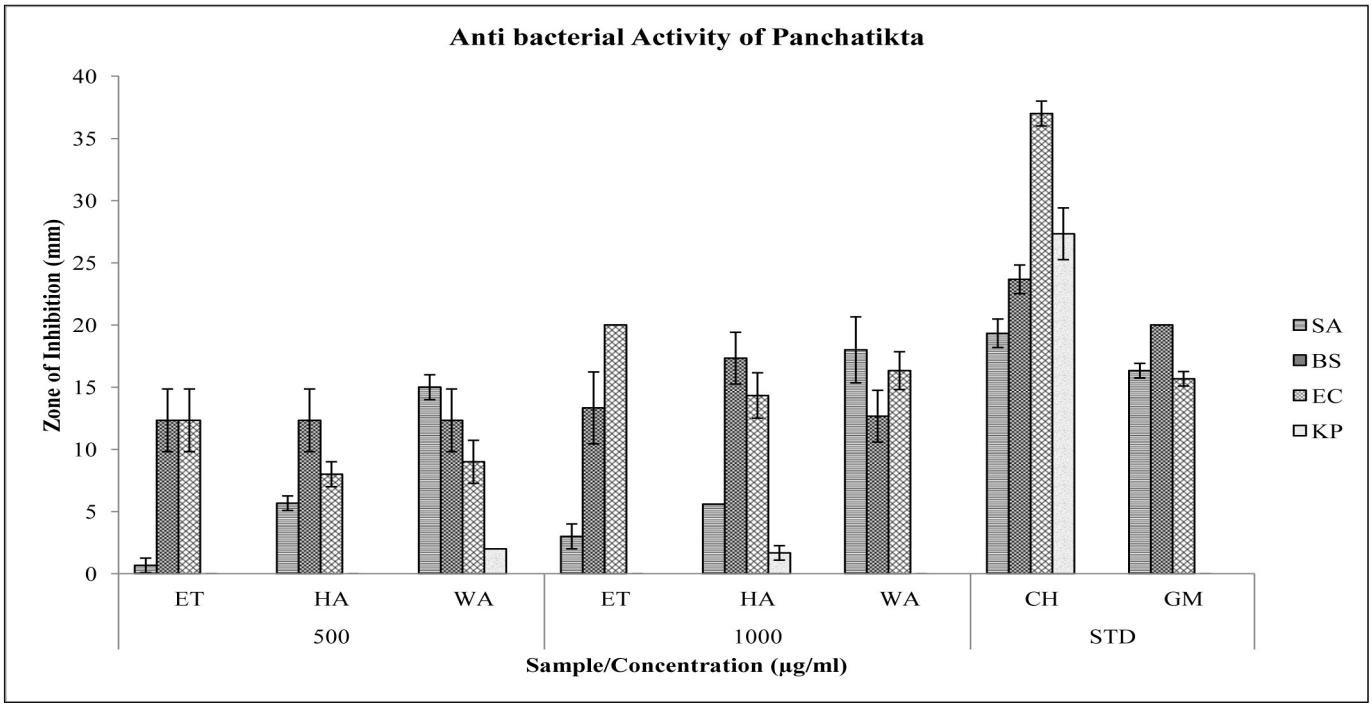


Fig. 4. Comparative antibacterial activity of different extracts of *Panchatikta* at two different concentrations with standard antibiotic discs. Tests done in triplicates; ± Standard deviation; **SA**. *Staphylococcus aureus* (MTCC 3160); **BS**. *Bacillus subtilis* (MTCC441); **EC**. *Escherichia coli* (MTCC40); **KP**. *Klebsiella pneumoniae* (MTCC3384); **PA**. *Pseudomonas aeruginosa* (MTCC741); **ET**. Ethanol extract; **HA**. Hydro alcohol extract; **WA**. Water extract; **CH**. Chloramphenicol (30 µg); **GM**. Gentamycin (30 µg). Tests done in triplicates; Error bar showing standard deviation.

Table 10. Antifungal activity of ethanol extract of *Panchatikta.*

Sl. No.	Organism	Zone of Inhibition (mm)				
		50	100	200	500	1000
1	*AN*	-	1.33±0.58	8.33±0.58	15.34±0.58	16.33±1.53
2	*FO*	-	-	7.00±1.73	15.00±0.00	17.67±2.52
3	*PC*	-	-	0	-	-

Tests done in triplicates; ± Standard deviation; AN, *Aspergillus niger* (MTCC 281); FO, *Fusarium oxysporum* (MTCC 284); *Penicillium citrinum* (MTCC 1256).

Table 11. Antifungal activity of hydro-alcohol extract of *Panchatikta.*

Sl. No.	Organism	Zone of Inhibition (mm)				
		50	100	200	500	1000
1	*AN*	-	5.00±0.00	10.00±2.00	18.67±1.15	39.00±3.60
2	*FO*	-	4.00±1.73	12.32±2.52	22.67±2.52	22.67±2.52
3	*PC*	-	-	0	-	5.00±0.00

Tests done in triplicates; ± Standard deviation; AN, *Aspergillus niger* (MTCC 281); FO, *Fusarium oxysporum* (MTCC 284); *Penicillium citrinum* (MTCC 1256).

Table 12. Antifungal activity of water extract of *Panchatikta.*

Sl. No.	Organism	Zone of Inhibition (mm)				
		50	100	200	500	1000
1	*AN*	-	2.00±0.00	9.67±0.58	21.67±2.89	39.00±1.73
2	*FO*	-	-	-	-	-
3	*PC*	-	-	5.00±0.00	10.00±2.00	12.00±2.65

Tests done in triplicates; ± Standard deviation; AN, *Aspergillus niger* (MTCC 281); FO, *Fusarium oxysporum* (MTCC 284); *Penicillium citrinum* (MTCC 1256).

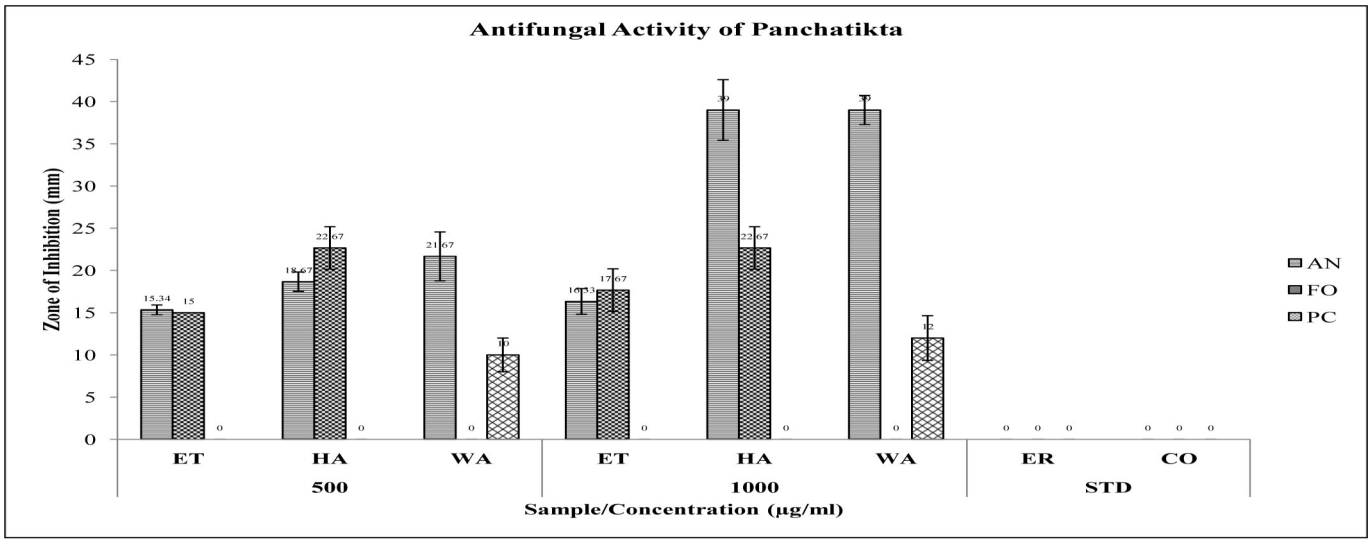


Fig. 5. Comparative antifungal activity of different extracts of *Panchatikta* at two different concentrations with standard antibiotic discs. Tests done in triplicates; ± Standard deviation; **AN**. *Aspergillus niger* (MTCC 281); **FO**. *Fusarium oxysporum* (MTCC 284); *Penicillium citrinum* (MTCC 1256); **ER**. Erythromycin (15µg); **CO**. Cotrimaxazol (25 µg); Tests done in triplicates; Error bar showing standard deviation.

Discussion

Panchatikta is a combination that is included in a variety of medicines that are used for conditions like skin problems, tumors, osteoporosis etc. Clinical reports depicting the effectiveness of medicines involving panchatikta combinations are available. But no specific reports pointing towards the chemical composition and *in vitro* activities of the panchatikta combination are available. Evidence based validation of traditional herbal medicine is an essential one in this world of globalisation. India has a rich tradition of herbal medicine system, which play a crucial role in rural livelihood healthcare. Systems like Ayurveda are being accepted globally and have the potential to withstand widespread needs (11). Since panchatikta lacks such information, this study is an attempt to fill that space. Study reports are available substantiating the importance of polyphenolic compounds in the therapeutic efficacy of medicinal plants. Phenolic groups have rich anti-inflammatory and antioxidant capacity, which in turn regulates metabolic pathways, promotes health and decreases the risk of chronic diseases (12). This suggests that the phenolic, flavonoids and tannin contents of a drug directly point towards its medicinal properties. Phenolic, flavonoid and tannin content have a linear correlation with potentials like antioxidant activity (13). All 5 ingredient drugs are reported to have rich phenolic, flavonoid and tannin contents. As per the available reports, *A. indica* stem bark extract has high phenolic and flavonoid content and has significant antioxidant and antimicrobial activities (14). In our study, also, *A. indica* stem bark extracts showed high levels of phenolics, flavonoids and tannins. A recent study on *T. cordifolia* stem extract portrays its remarkable antioxidant and antimicrobial potential (15). Phytochemical screening of *S. virginianum* was done recently and its *in vitro* antioxidant and antimicrobial properties were evaluated (16). The phenolic content and total flavonoids of *T. dioica* were remarkable as per the present study. The results obtained comply with a study reporting the chemical composition and antimicrobial potential of *T. dioica* (17). Though there are no significant studies on the phytochemical composition of the panchatikta combination, all the ingredient drugs are reported to have rich phenolic and flavonoid content. In the present study, different extracts of Panchatikta, like ethanol, hydroalcohol and water were ana-

lysed for their polyphenolic composition. As per the results, all 3 extracts have almost the same amount of phenolic flavonoid and tannin contents, though the ethanolic extract has a slight higher flavonoid and tannin content than the others. In Ayurveda, the majority of medicines are water extracts and as per this study, steps can be taken to study the possibility of ethanol and hydro alcoholic extracts to check their efficacy and toxicity. HPTLC profiling has importance in ensuring the quality and efficacy of polyherbal formulations. In the case of Panchatikta, there are no reports on the TLC profile of the combination. For herbal extraction, acetone proved to be the best solvent, followed by ethanol and hydroalcoholic extract (18). Since acetone extraction cannot be applied to medicine preparation, the maximum separation of chemical constituents was found to be in ethanol when compared to water. In our study, HPTLC fingerprinting of ethanol extracts also showed maximum separation of chemical constituents. In water extracts, separation was less. Due to the maximum extraction of polyphenolic compounds in ethanol and hydroalcoholic extracts, antioxidant activity is also expected to be the maximum in these. In the present study, the maximum % of inhibition was observed for ethanol and hydroalcoholic extracts (IC_{50} values close to 60 µg/mL in DPPH, close to 350 µg/mL in ABTS and close to 500 µg/mL in NO). In a clinical study report published in 2017, it was reported that panchatikta is effective in healing wounds due to its antimicrobial potential, but no *in vitro* study reports are available on its effectiveness against particular organisms (19). In anti-bacterial screening, all three extracts showed a significant zone of inhibition at concentrations of 500 µg and 1000 µg. *Pseudomonas aeruginosa* was found to be resistant to all 3 test samples and standard antibiotic discs. Minimum activity was obtained against *Klebsiella pneumonia*. Panchatikta extracts at concentrations of 500 µg and 1000 µg showed significant activity against the fungal strains *A. niger* and *F. oxysporum* while organisms were found to be resistant to standard antibiotic discs. In anti-bacterial screening, water extracts also showed significant activity against some of the selected organisms at higher concentrations when compared to standard discs. Thus, the present study results follow earlier reports of this kind and have helped in filling the gaps present in available reports.

Conclusion

The present study is a first approach to scientifically validating the therapeutic potential and screening the phytochemical composition of an Ayurvedic drug combination, panchatikta. The total phenolic content of the Panchatikta combination was almost the same in ethanol, water and hydroalcoholic extracts. Flavonoid content and tannin content were highest in ethanol extract. HPTLC profiling of the 3 extracts of Panchatikta showed the maximum number of peaks for the ethanolic extract. In antioxidant assays, the maximum % of inhibition was observed for ethanol and hydroalcoholic extracts. Panchatikta extracts showed a significant zone of inhibition at concentrations of 500 µg and 1000 µg against bacterial strains *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* and fungal strains *Aspergillus niger* and *Fusarium oxysporum*. Preliminary phytochemical analysis, including HPTLC profiling, can be used as tools for the quality control of the drug combination. A major issue concerning Ayurvedic medicines is the lack of scientific data and the results presented here can be used to represent Panchatikta scientifically and also to support advanced studies in this regard.

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Authors' contributions

HCM designed the article, analysed the samples, drafted the article; DM supervised and guided the phytochemical part; GSP corrected the draft article, suggestions were made on pharmacological part; AK corrected the entire article, advised and guided throughout the work, modified the study designs.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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