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PHYTOCHEMICAL ASSESSMENT AND METABOLIC FINGERPRINTING OF *BOERHAVIA DIFFUSA* AND *CEPHALANDRA INDICA* FROM INDIAN SUB-CONTINENT

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Keywords:

Boerhavia diffusa, *Cephalandra indica*, Phytochemical analysis, HPTLC, GC-MS

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ABSTRACT: The medicinal plants, *Boerhavia diffusa* and *Cephalandra indica* are both perennial climbing herbs commonly used in traditional system of medicine for the treatment of several ailments because of their healing properties. The present study aimed to identify the phytochemical constituents of methanolic extracts of these plants, followed by their metabolic fingerprinting, which was carried out by HPTLC and GC-MS analysis. Total phenol and flavonoid content were measured, followed by a radical scavenging assessment using 1,1, Diphenyl-2-picrylhydrazyl (DPPH) radical system. This preliminary data showed encouraging results for the presence of a significant amount of flavonoid, phenols, and anti-oxidant activities. The HPTLC chromatogram of both methanolic extracts showed a variety of metabolites having different R_f value suggesting the diverse nature of phytoconstituents. Further, the GC-MS analysis revealed the manifestation of at least 23 and 22 metabolites of *B. diffusa* and *C. indica*, respectively. These metabolites were identified as fatty acids, sugar alcohols, large carbohydrates, phenols, and flavonoids possessing potential antioxidant, antitumor, anti-hyperglycemic, and antimicrobial properties. The results of the present study confirm the utilization of these plants in conventional therapeutic setups, and it also shows the possibility of designing novel semi-synthetic compounds based on these identified bioactive components.

INTRODUCTION: Plants either alone or in combination have been in use for medicinal purposes since time immemorial all over the world, notably in developing countries. The World Health Organisation has estimated that at least 80% of the world population relies on traditional treatment regimens.

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Plants also hold a very significant place in the field of drug discovery as the active compounds isolated from plants provide with a lead for chemical synthesis of drugs for numerous illnesses.

Indeed, almost 60% of chemically synthesized drugs presently in clinics have been derived from products of plants and other natural origins [1]. The myriad of bio-active molecules or secondary metabolites present in plants includes phenols, polyphenols, flavonoids, isoflavonoids, stilbenes, lignans, terpenoids and tannins. These components impart anti-oxidant, anti-inflammatory, anti-microbial, anti-hyperglycemic, anticancer and anti-infectious properties, among others [2].

Apart from this, the detrimental side-effects and high toxicity associated with conventional drugs have also diverted the attention towards the use of medicinal plants in mainstream therapeutic strategies. Despite the extensive utilization of medicinal plants and their products as therapeutic agents, they remain largely unexploited reservoir of medicines [3, 4]. This is mainly due to the improper or inadequate characterization and quantification of medicinal plants and their biologically active components [5]. The presence of countless compounds of diverse nature in plants available from different environments makes their characterization a crucial step towards drug discovery and development. The metabolite profiling and characterization of plants are possible through various analytical chromatographic or spectrometric techniques [6]. In this context, the present study aimed to identify the chemical components of two ethnopharmacologically important medicinal plants, *Boerhavia diffusa,* and *Cephalandra indica* found in the Indian Sub-Continent.

Boerhavia diffusa belonging to the family Nyctaginaceae is an abundant perennial climbing herb widely used in Unani and Ayurvedic therapeutics for its efficacy against diseases such as inflammation, jaundice, asthma, diabetes, abdominal pain, elephantiasis, bacterial and viral infections [7]. The root or whole plant is used and has been reported to treat epilepsy, night blindness, and hepatic disorders [8, 9, 10]. It is found in tropical and sub-tropical regions of Africa, Australia, Asia, and America and is rich in alkaloids, flavonoids, isoflavonoids, xanthones, lignans, phenols, and saturated fatty acids [11].

Similarly, *Cephalandra indica* is a perennial climbing herb belonging to the Cucurbitaceae family and commonly known as Ivy gourd [12]. In vernacular terminology, it is known as kundru, Tundika or Bimbu and is found in several regions of Indian sub-continent [13]. This plant has been widely used in Homeopathy and Ayurveda for the treatment of diabetes mellitus and accumulating data have validated that this herbal plant contains an exorbitant amount of triterpenoids, tannins, saponins, lignans, alkaloids and flavonoids which accounts for its antioxidant, anti-inflammatory, antibacterial, analgesic, hepatoprotective, anti-pyretic and anti-malarial properties [13, 14]. It's thick, and tuberous roots are mostly used as a therapeutic agent.

Therefore, the present study focuses on assessing the total phytoconstituents of these plants by quantification of polyphenols and flavonoids. Furthermore, the plants were characterized and profiled for verification of bioactive metabolites responsible for their medicinal potency by HPTLC and GC-MS analysis.

MATERIALS AND METHODS:

Collection and Identification of Plant Materials: *Cephalandra indica* was obtained as a gift from Mr. Sharma, Biotech Park Lucknow, Uttar Pradesh, India, whereas *Boerhavia diffusa* were collected from herbal drug market located in Old Delhi, India. The specimens of plants were deposited in the National Institute of Science Communication and Information Resources (NISCAIR) under voucher herbarium specimen no. Ref. NISCAR/RHMD/ Consult/- 2010-11/1671/269 for identification and authentication. It was identified by Dr. B. Singh, Head Raw Material Herbarium and Museum NISCAIR.

Extraction and Stock Preparation: Whole plant materials were properly dried at 50 °C in a hot air oven, powdered and passed through sieve no. 60 to obtain a uniform coarse powder. Powdered drugs were extracted in Soxhlet apparatus, using methanol as an extraction solvent for 72 h at 70-80°C. Further, the liquid extract was filtered through Whatman filter paper twice; concentrated in rotavapour; freeze-dried and stored at -80 °C till further use. Later, respective stock solutions of each lyophilized powder were made in DMSO; the un-dissolved matter was removed by mild centrifugation at 2000 rpm / 5 min, followed by filtering the stock using 0.45 µm syringe filter.

Phytochemical Screening of the Plant Extract:

Total Phenolic Content Determination: Total phenol content was determined by the Folin-Ciocalteu method with slight modifications. Briefly, 0.5 ml of each extract and the phenolic standard was mixed with 5 ml FCR (diluted 1:10 v/v) and 4 ml of 1M aqueous Na_2CO_3. Solutions were incubated at 50 °C in a water bath for 15 min, and total phenol content was determined

spectrophotometrically at 765 nm, using a UV- Vis spectrophotometer (Shimadzu 1601). A calibration curve was constructed using gallic acid solutions as standard, and total phenolic content of the extract was expressed in terms of mg of gallic acid equivalents per gram dry weight of the extract.

Total Flavonoid Content Determination: Flavonoid content was determined by the aluminum chloride calorimetric method. Briefly, 0.5 ml of each extract and flavonoid standard was mixed with 1.5 ml of methanol, 0.1 ml each of 10% aluminum chloride and 1M potassium acetate followed by 2.8 ml of distilled water. The whole mixture was incubated at room temperature for 30 minutes, and absorbance read at 415 nm, using a UV- Vis spectrophotometer (Shimadzu 1601). A calibration curve was plotted using quercetin solutions as standard, and total flavonoid content of the extract was expressed in terms of mg of quercetin equivalents per gram dry weight of the extract.

Antioxidant Activity Determination: The antioxidant activity of the two plant extracts was evaluated by the (1,1-diphenyl-2-picryl-hydrazil DPPH) radical scavenging assay according to Ismail *et al.* [15] The absorbance was measured at 515 nm and Ascorbic acid, a potent antioxidant was used as a positive control. Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

% Free radical scavenging activity= Absorbance of control - Absorbance of sample / Absorbance of control ×100

Then, the percent inhibitions against concentrations were plotted, and IC_{50} was calculated.

Metabolite Profiling of Plant Extracts:

HPTLC Fingerprinting: HPTLC studies were carried out following the method already described, with some modifications. After trying several TLC in different solvent systems by hit and trial method, the presence of spot/s was confirmed by TLC in a specific solvent system. Briefly, 10 mg of each extract was dissolved in 1 ml methanol and filtered through 0.45 µm membrane filter, and the respective samples (8 µl each) were spotted on pre-activated and precoated silica gel 60 F_{254} TLC plates (E. Merck, Germany) (0.2 mm thickness). The sample solution was applied to 6 mm wide band using Camag Linomat-V automated TLC applicator with the nitrogen flow providing a delivery speed 150 nL/s from the syringe. A mobile phase of toluene: ethyl acetate: formic acid (9:4:1, v/v/v) was employed in a CAMAG glass twin trough chamber (20 × 10 cm), which was pre-saturated with the solvent before 15 min. The plates were developed horizontally in a Camag horizontal developing chamber (10 × 10 cm) at the room temperature. Plates were scanned at different wavelengths such as 250 nm, 366 nm. After derivatization of the plates with 5% anisaldehyde sulphuric acid in methanol, the scanning was carried out at 540 nm with a Camag TLC scanner III using the Wincats1.2.3 software.

Gas Chromatography and Mass Spectrometry (GC-MS) Analysis: The metabolomic finger-printing of methanolic extracts were carried out to find out nature and content of polar metabolite/s to be used for the activity. The polar metabolomic fingerprinting of *Cephalandra indica* and *Boerhavia diffusa* was carried out as described. GC-MS was carried out using Gas Chromatograph system, equipped with dual split/ splitless inlet along with a recommended Mass spectrometer detector (Agilent technologies, 6890). GC was performed on a 30-m SPB-50 column with 0.25-mm film thickness (Superlco, Bellfonte, CA). The injection temperature was set at 230 °C, the interface at 250 °C, and the ion source adjusted to 200 °C. The analysis was performed under the following temperature program: 5 min of isothermal heating at 70 °C, followed by a 5 °C/ min oven temperature ramp to 310 °C, and a final 1 min of heating at 310 °C. The system was then temperature equilibrated for 6 min at 70 °C before the injection of the second sample. Mass spectra were recorded at 2 scan/ sec with a scanning range of 50 to 600 m/z. Identification of individual components was achieved using the Wiley and National Institute of Standards and Technology (NIST) Library.

Statistical Analysis: All experiments were performed in triplicates at least three times independently and are presented as mean ± SD.

RESULTS:

Phytochemical Screening of the Plant Extracts: Total phenol and flavonoid contents were measured

as part of the assessment for total phyto-constituents. The total phenol content was measured as gallic acid equivalent (GAE), and it was seen that *Boerhavia diffusa* showed higher phenol as well as flavonoid contents as shown in **Table 1**.

Antioxidant Activity Determination: Further, DPPH radical scavenging activity was performed and the percent inhibition of DPPH radical by extract/s calculated against concentration **Fig. 1**. IC_{50} values were calculated as shown in **Table 2**. Overall higher inhibition in DPPH oxidation was shown by standard ascorbic acid followed by *Cephalandra indica* though *Boerhavia diffusa* showed a more or less similar reduction **Fig. 1**. The difference in percent reduction in DPPH radical was more at lower concentrations; however, at higher concentrations, this difference got decreased **Fig. 1**.

Metabolite Profile of Plant Extracts:

HPTLC Fingerprinting: HPTLC profile of methanolic extracts of *Boerhavia diffusa* and *Cephalandra indica*, was recorded **Fig. 2-3** and **Table 3-4**. The conspicuous blue zones in each chromatogram were recorded under UV C/A and visible light after derivatization with anisaldehyde-sulfuric acid. The HPTLC chromatogram of *Boerhaavia diffusa* showed 20, 19, 09 numbers of metabolites at 250, 366, 540 nm respectively with a total of 4 metabolites common **Fig. 2** and **Table 3**. Likewise, the HPTLC fingerprinting of *Cephalandra indica* showed 18, 11, 11 numbers of metabolites at 250, 366, 540 nm respectively, out of which a total of 6 metabolites were common **Fig. 3** and **Table 4**.

TABLE 1: THE TOTAL PHENOLIC AND FLAVONOID CONTENT OF METHANOLIC EXTRACTS OF *BOERHAVIA DIFFUSA* AND *CEPHALANDRA INDICA*

Medicinal plants	Total Phenolic content (mg/g of GAE)	Total Flavonoids (mg/g of QE)
B. diffusa	92.78±8.3	34.38±9.5
C. indica	70.63±10.5	18.54±6.4

TABLE 2: *IN-VITRO* ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *B. DIFFUSA* AND *C. INDICA* IN TERMS OF RADICAL SCAVENGING EFFECT OF DPPH FREE RADICAL ACTIVITY IN COMPARISON TO ASCORBIC ACID

Medicinal plants	IC_{50} (µg/mL)	R^2
Ascorbic acid	19.15	0.998
Boerhavia diffusa	160.6	0.9771
Cephalandra indica	111.9	0.9729

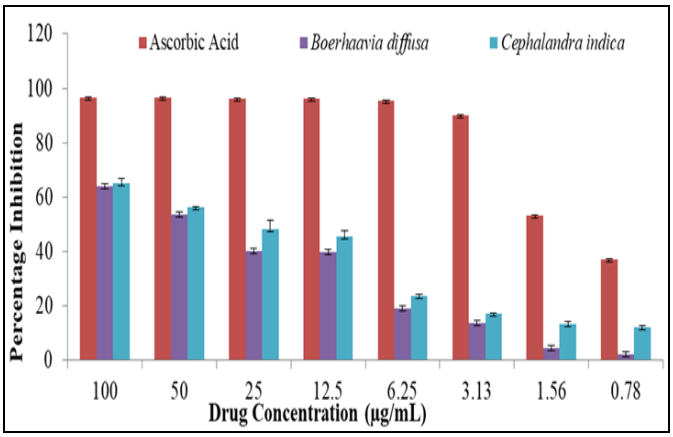

FIG. 1: DPPH FREE RADICAL INHIBITORY EFFECT OF *B. DIFFUSA* AND *C. INDICA* IN COMPARISON TO ASCORBIC ACID

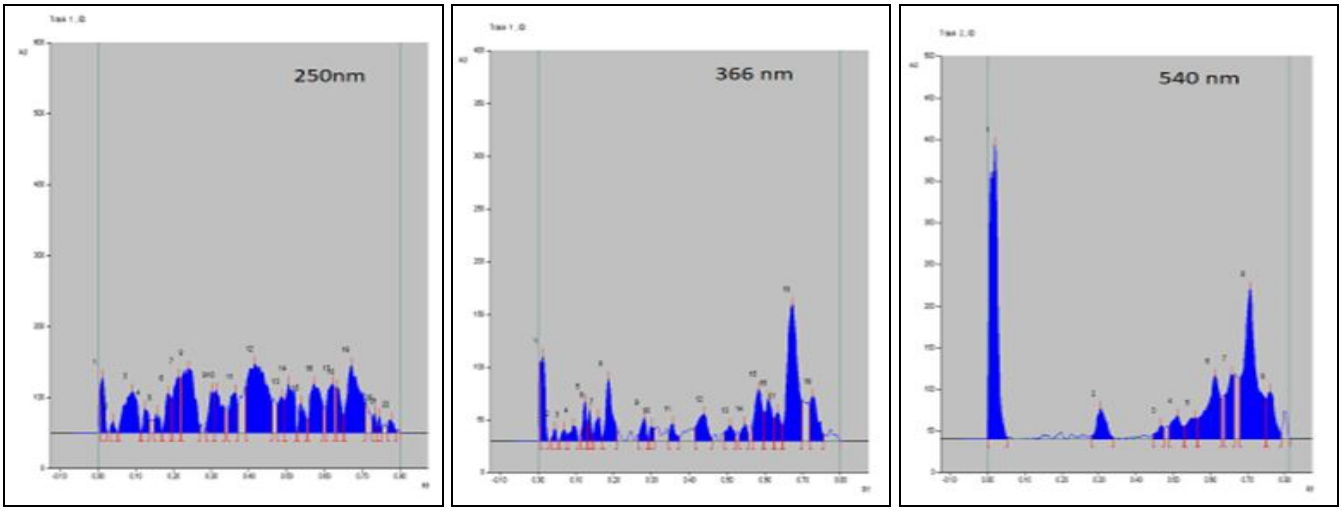

FIG. 2: HPTLC CHROMATOGRAM OF *B. DIFFUSA* AS OBSERVED AT 250; 366 and 540 nm WAVELENGTHS, SHOWING 20, 19, 9 METABOLITES OUT OF WHICH 4 METABOLITES WITH R_f VALUES OF 0.51, 0.57, 0.61 AND 0.67 WERE COMMON

TABLE 3: PEAK TABLE FROM CHROMATOGRAM OF *B. DIFFUSA* ALONG WITH AREA AND R_f OF THE CORRESPONDING PEAKS OBTAINED

S. no.	R_f	Area		
		250 nm	366 nm	540 nm
1	0.01	757.5	847.4	
2	0.02			6789.9
3	0.05	149	89.8	
4	0.07		116.8	
5	0.09	1803.5	222.7	
6	0.13	415	327.8	
7	0.14		209.8	
8	0.16		300.3	
9	0.19	733.9	893.7	
10	0.21	1291.3		
11	0.24	2750.8		
12	0.28		267.1	
13	0.30	705.1	104.9	
14	0.31	1101.5		838.1
15	0.36		223	
16	0.42	4544.3		
17	0.44		617.1	
18	0.47			275.3
19	0.49	784.9		
20	0.51	1336.9	271.1	723.9
21	0.54	577.3	233.4	
22	0.57	1817.8	891.2	625.4
23	0.61	1218.9	656.3	2423
24	0.63	820.1	391.9	
25	0.67	2968	3172.9	1526.8
26	0.71			5522.5
27	0.73	258.7	838	
28	0.75	156.5		1126.4
29	0.78	249		

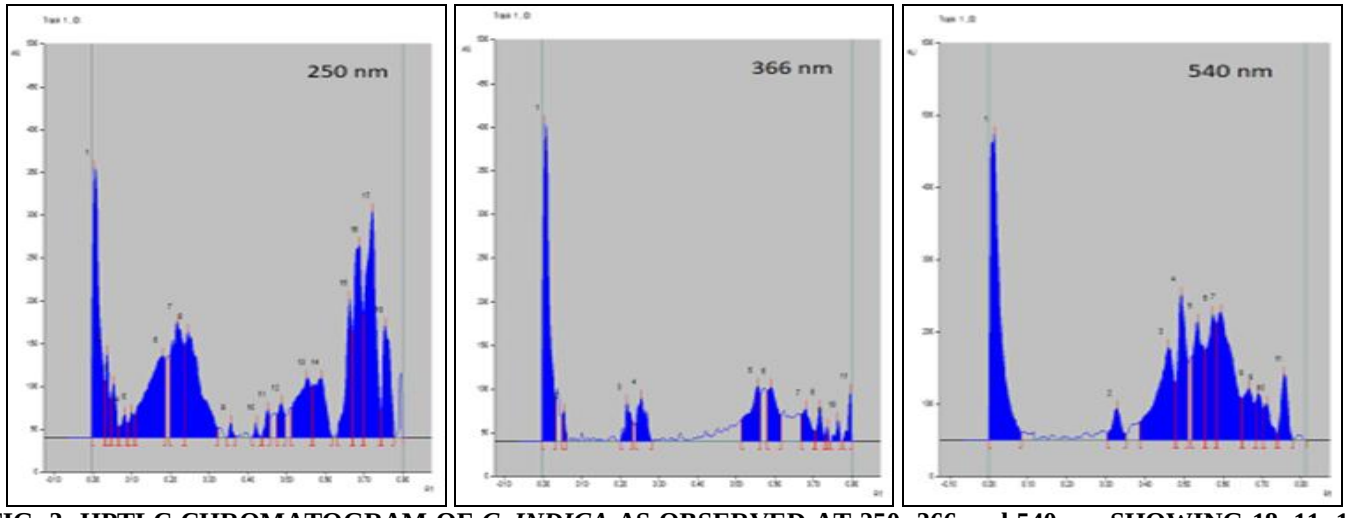

FIG. 3: HPTLC CHROMATOGRAM OF *C. INDICA* AS OBSERVED AT 250; 366 and 540 nm SHOWING 18, 11, 11 METABOLITES OUT OF WHICH 6 METABOLITES WITH R_f VALUES OF 0.01, 0.55, 0.59, 0.69, 0.72 AND 0.76 WERE COMMON

TABLE 4: PEAK TABLE FROM CHROMATOGRAM OF *C. INDICA* ALONG WITH AREA AND R_f OF THE CORRESPONDING PEAKS OBTAINED

S. no.	R_f	Area		
		250 nm	366 nm	540 nm
1	0.01	4860.3	5265.5	9798.5
2	0.04	786.3		
3	0.05	704.7	276.8	
4	0.08	338.6		

5	0.10	363.4		
6	0.18	4202.4		
7	0.22	3579.5	590.6	
8	0.25	4548	963.4	
9	0.33			888.3
10	0.36	123		
11	0.42	117		
12	0.45	341		5230
13	0.49	616		3791.2
14	0.55	2307.2	1659.7	3950.2
15	0.57			3326.8
16	0.59	2004.7	1457.4	6519.9
17	0.66	2323.4		1666.3
18	0.69	4137.6	757.9	955.4
19	0.72	6155.7	419	827.1
20	0.74		93.9	
21	0.76	2321.2	195	1437.3
22	0.80		372.2	

GC-MS Based Metabolomics: Metabolic profiling was done by comparing relative retention time and the mass spectra of the present compounds with those of authentic ones from the MS library.

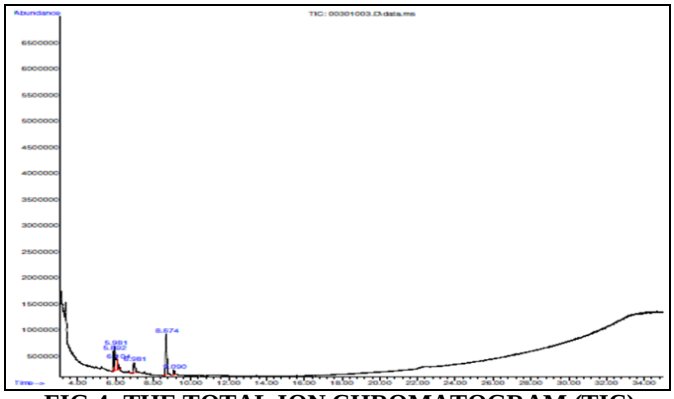

FIG 4: THE TOTAL ION CHROMATOGRAM (TIC) OBTAINED FROM THE METHANOLIC EXTRACT OF *BOERHAAVIA DIFFUSA*

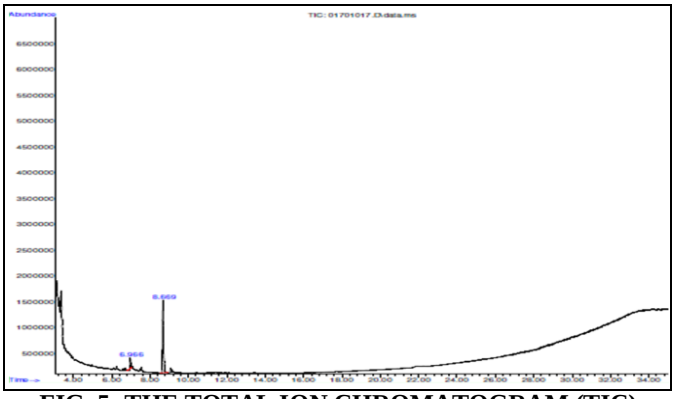

FIG. 5: THE TOTAL ION CHROMATOGRAM (TIC) OBTAINED FROM THE METHANOLIC EXTRACT OF *C. INDICA*

TABLE 5: TOTAL POLAR METABOLITES IDENTIFIED FROM METHANOLIC EXTRACT OF B.DIFFUSA DETECTED BY GC-MS AFTER DERIVATISATION OF THE METHANOLIC EXTRACT

S. no.	RT	Area %	Library ID
1	5.757	0.54	Xylonic acid, 2,3,4-tris-O-(trimethylsilyl)-, .delta.-lactone, D-
2	5.842	1.77	1,2-O-Isopropylidene-3,5,6-tri-O-trimethylsilyl-D-glucofuranose
3	5.939	1.41	Sorbopyranose, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-, LSORBOSE5TMS
4	6.017	1.29	SORBOSE 5TMS
5	6.162	2.37	Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)
6	6.289	20.36	3,7-Dioxa-2,8-disilanonan-5-one, 2,2,8,8-tetramethyl
7	6.537	1.52	3-dibromomethylene-2,3-dihydrothiophen 1,1-dioxide
8	6.621	1.5	Farnesyl bromide
9	6.694	1.31	1-Ethyl-4-phosphorinanone thiosemicarbazone
10	6.748	15.63	1,2-O-Isopropylidene-3,5,6-tri-O-trimethylsilyl-D-glucofuranose
11	6.887	5.08	1,2-O-Isopropylidene-3,5,6-tri-O-trimethylsilyl-D-glucofuranose
12	7.099	1.71	D-Xylopyranose, 1,2,3,4-tetrakis-O-(trimethylsilyl)
13	7.244	0.77	Hexadecanoic acid
14	7.328	1.88	Ethylmalonate Mono Et Ester 1TMS
15	7.492	7.6	Isosteviol
16	7.969	1.03	Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, muco-
17	8.948	14.3	Inositol,O,O,O,O,O,O-TMS
18	17.75	4.1	3-(10-Methyl-10H-phenothizine)methylenepropanedinitrile
19	17.95	0.87	Silane, (1,2,4,5-cyclohexanetetrayltetraoxy)tetrakis[trimethyl
20	19.18	0.97	d-(-)-Fructose, pentakis(trimethylsilyl) ether
21	19.88	12.37	Methyl Tetra-O-TMS-.Alpha.-D-Galactofuranocide
22	20.84	0.62	1-Ethyl-4-phosphorinanone thiosemicarbazone
23	22.23	0.99	Di-triethylsilyl ether

TABLE 6: TOTAL POLAR METABOLITES IDENTIFIED FROM METHANOLIC EXTRACT OF *C.INDICA* DETECTED BY GC-MS AFTER DERIVITIZATION OF THE METHANOLIC EXTRACT

S. no.	RT	Area %	Library ID
1	4.162	0.13	1H-Indole, octahydro-1-(trimethylsilyl)
2	4.283	1.2	Octanoic acid, 7-oxo-, trimethylsilyl ester
3	4.404	1.24	Hexaethyldisloxane
4	4.863	0.84	ARABITOL 5TMS
5	4.936	0.55	1-Butyl(dimethyl)silyloxypropane
6	6.217	0.53	Tetradecanoic acid, trimethylsilyl
7	6.295	8.16	Silane, [1,4-dioxane-2,3-diylbis(oxy)]bis[trimethyl
8	6.549	1.57	Erythronic Acid-.Gamma.-Lactone, Bis-O-(TRIMETHYLSILYL)
9	6.706	2.28	3-(2-Thienyl)pyridine
10	6.76	3.98	Pyridine, 3-(2-thienyl)
11	6.809	6.91	Rhamnose Meox2 4TMS
12	7.256	0.46	Hexadecanoic acid
13	7.34	24.77	Spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene
14	8.96	21.55	Inositol,O,O,O,O,O,O-TMS
15	9.407	0.08	Farnesyl bromide
16	12.4	0.38	5.beta.,7.beta.H,10.alpha.-Eudesm-11-en-1.alpha.-ol
17	15.99	0.18	D-Arabinonic acid, 2,3,5-tris-O-(trimethylsilyl)-, .gamma.-lactone
18	17.76	7.26	3-(10-Methyl-10H-phenothizine)methylenepropanedinitrile
19	17.97	0.34	.alpha.-DL-Lyxofuranoside, methyl 2,3,5-tris-O-(trimethylsilyl)
20	19.73	0.41	Arabinose, 2,3,4,5-tetrakis-O-(trimethylsilyl)
21	19.87	8.29	4-Thio-L-Lyxono-1,4-lactone silyated derivative
22	22.26	8.91	2-azathianthrene

GC-MS based metabolic profiling of the polar fractions of corresponding methanolic extracts showed conspicuous peaks at different retention times **Fig. 4-5**, which were further identified by their mass using Wiley and NIST library **Table 5-6**. In *B. diffusa*, out of 23 peaks, most prominent were 3, 7-Dioxa-2, 8-disilanonan-5-one, 2, 2, 8,8-tetramethyl, 1, 2-O-Isopropylidene-3, 5, 6-tri-O trimethylsilyl-D-glucofuranose, Inositol, O,O,O,O, O, O-TMS, Methyl Tetra-O-TMS-.Alpha.-D-Galactofuranocide, and Isosteviol **Table 5**. The Total Ion Chromatogram (TIC) of methanolic extracts of *B. diffusa* and *C .indica* are shown in **Fig. 4** & **5** respectively. Likewise, among the most prominent compounds in *C. indica* are Inositol, O,O,O,O,O,O-TMS, Silane, [1, 4-dioxane-2, 3-diylbis (oxy)] bis [trimethyl; 4-Thio-L-Lyxono-1,4-lactone silyated derivative; 2-azathianthrene; octahydro-1-(trimethylsilyl; Rhamnose Meox2 4TMS **Table 6** of all the 22 peaks.

DISCUSSION: Herbal plants hold a rich history associated with them in the context of their medicinal and pharmacological usage. Such plants have demonstrated promising anti-cancer, anti-viral, anti-inflammatory and anti-oxidant agents due to the presence of compounds and secondary metabolites which are biologically active. In this context, we intended to assess the phytochemical make-up of two ethnopharmacologically important herbal plants; *Boerhavia diffusa* and *Cephalanra indica* prevalently found in the Indian Sub-continent. In the present study, methanolic extracts of *B. diffusa* and *C. indica* revealed the presence of phenols and flavonoids in a significant amount which is in agreement with other studies that reported the presence of alkaloids, tannins, carbohydrates, saponins, glycosides, phenolic compounds, flavonoids and terpenoids in *B. diffusa* [11] and polyphenols, flavonoids and sesquiterpenes in *C. indica* [12].

We found that *B. diffusa* showed higher phenol as well as flavonoid contents followed by *C. indica*, which can be correlated with the higher number of metabolites identified through metabolite profiling. The DPPH free radical scavenging assay was performed to determine the antioxidative capabilities of both these plants, and it was found that *C. indica* and *B. diffusa* had a somewhat similar potential of donating hydrogen to a free radical to remove the odd electron, which imparts negative connotations to the free radicals. This suggests that these plant extracts contain active compounds that are responsible for scavenging free radicals. Also, a clear picture of the metabolite composition of methanolic extracts of these herbal plants was presented by HPTLC fingerprinting and

GC-MS analysis. This helped in the identification and characterization of a myriad of phyto-chemicals, and it was determined that several similar and disparate compounds are contributing to these plant's medicinal properties. The HPTLC fingerprinting revealed methanolic extract of *B. diffusa* and *C. indica* extracts produced conspicuous peaks with retention factor (R_f) ranging from 0.01 to 0.80 at an absorbance of 250, 366, and 540 nm. This showed the presence of various bioactive molecules in the methanolic extracts of these plants.

Additionally, the polar extracts were subjected to GC-MS profiling to report the diversity of the metabolites and each extract showed many conspicuous peaks, corresponding to the presence of different compounds. Among the identified metabolites, large carbohydrates were most prominent in both the plant extracts and it has been previously reported that large carbohydrates may contribute to the anti-viral activities [16]. In the methanolic extract of *C. indica*, Spiro [5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene, a naphthalene derivative was the most abundant compound and previously it has been reported that naphthalene derivatives demonstrate strong anti-cancer potential [17]. The compound, 2-azathianthrene is known to be a phenol and is present in the methanolic extract of *C. indica* in a quite higher amount covering an area of 8.91% in the GC-MS chromatogram [18]. This compound majorly seems to provide the antioxidant activity to this plant.

Moreover, the GC-MS analysis also revealed the occurrence of various fatty acids such as octanoic acid, 7-oxo-, trimethylsilyl ester, tetradecanoic acid, hexadecanoic acid, and D-Arabinonic acid. These fatty acids are commonly used in pharmaceutical, cosmetic and food industries and are also reported to possess anti-inflammatory, anti-microbial along with anti-oxidant activities [19]. Hexadecanoic acid is also evidenced to be effective in preventing cardiovascular diseases, in addition to its anti-inflammatory properties [19, 20]. The compound Rhamnose Meox2 4TMS found in the methanolic extract of *C. indica* is a promising anti-bacterial agent [21]. On the other hand, *B. diffusa* showed more than 20 compounds and had a compound isosteviol, which is a diterpenoid derivative possessing anti-bacterial, anti-hyperglycemic, and anti-inflammatory activities [22, 23]. Metabolites of thiosemicarbazone nature also show its abundance in GC- MS chromatogram of *B. diffusa*, and it is associated with potent antimicrobial activities [24]. Inositol compounds are present in abundance in methanolic extracts of both the plants and have anti-viral activities.

Therefore, it can be stated that the presence of this variety of bioactive compounds might be responsible for the pharmacological potential of these plants. Further, the assessment of phyto-constituents in these two important medicinal plants will not only lead to the revelation of the bioactive metabolites but also aid in drug discovery and development. Although, further studies are warranted to validate the effectiveness, dose, and toxicity levels of these plants to utilize them in the clinics.

CONCLUSION: *Boerhavia diffusa* and *Cephalandra indica* are popular plants among the traditional system of medicine such as Unani and are widely used all over the Indian Sub-Continent. The comprehensive phytochemical analysis and the metabolite profiling of methanolic extract of these medicinal plants are reported for the first time in an in-depth manner in this study.

It has been revealed that these plants have a copious amount of secondary metabolites which are responsible for their medicinal properties. The presence of a variety of phytoconstituents in these plants is correlated with their high medicinal value. Besides, the metabolic fingerprinting analysis led to the exposure of bioactive compounds, and further studies is going on for isolation and purification of such compounds to decipher their pharmacological properties.

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