Journal of Pharmaceutical Research International

33(35A): 197-203, 2021; Article no.JPRI.67959
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Comparative Evaluation of Phytochemical Constituents by GC-MS and Antitubercular & Antimicrobial Potential of *Ceiba pentandra* and *Parmotrema perlatum* against Resistant Strains

K. Varalakshmi Devi[1], E. Bhargav[2], G. Swaruparani[3] and M. Vijaya Jyothi[2*]

[1]SKU College of Pharmaceutical Sciences, SK University, Anantapuramu, India.
[2]Department of Pharmaceutical Chemistry, Raghavendra Institute of Pharmaceutical Education and Research, K. R. Palli Cross, Chiyyedu Post, Anantapuramu - 515721, Andhra Pradesh, India.
[3]JSS College of Pharmacy, Ooty, Tamilnadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author MVJ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KVD and EB managed the analyses of the study. Author GS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i35A31889
Editor(s):
(1) Dr. Aurora Martínez Romero, Juarez University, Mexico.
Reviewers:
(1) Nazir Aslam, University of Gujrat, Pakistan.
(2) Meryem Slighoua, University Sidi Mohammed Ben Abdellah, Morocco.
Complete Peer review History: http://www.sdiarticle4.com/review-history/67959

Original Research Article

Received 15 May 2021
Accepted 15 June 2021
Published 05 July 2021

ABSTRACT

Ceiba pentandra and *Parmotrema perlatum* lichen are two illustrious spices. Apart from their benefit as aromas, These are known for various therapeutic activities. Both are well known as appetizers. Decoctions of theses powders are used to relieve cough, anorexia and helminthiasis etc. Though several studies on antimicrobial activities of these two plants are available, studies on resistant microorganisms and anti tubercular activity are very limited. Hence, the author made an attempt to identify the phytochemical constituents present in methanolic and n-hexane extracts of *Ceiba pentandra* and *Parmotrema perlatum* using phytochemical tests and GC-MS method then to evaluate antibacterial activity against 5 resistant microorganisms by microtitre broth dilution method using ciprofloxacillin & streptomycin as reference standards. Methanolic extracts of *Parmotrema perlatum* and *Ceiba pentandra* had shown a considerable antibacterial activity than n-

**Corresponding author: E-mail: drmvjyothiriper@gmail.com;*

hexane extracts and exhibited 90% growth inhibition against H37Rv resistant strain of mycobacterium at 80 & 320 micrograms/ml respectively. N-hexane extracts of both species were found to be poorly effective against H37 Rv.

Keywords: Ceiba pentandra; Parmotrema perlatum; antimicrobial activity; anti tubercular activity; microtitre broth dilution method; micro plate Alamar blue assay.

1. INTRODUCTION

Medicinal plants serve as a rich source for various therapeutically active molecules. The ayurvedic system being a traditional practice generally utilizes Phyto molecules to cure various diseases and ailments. Indian spices are well-known remedies to treat gastrointestinal problems including helminthiasis, GIT infections etc. These spices are not only known to contribute flavor to food but also dispense therapeutically active molecules to the body. Secondary metabolites like saponins, tannins and alkaloids being bitter and acrid principles generally able to kill or sustain the growth of localized microorganisms hence many medicinal herbs are popularized as anti-infectives. Though several synthetic anti-bacterial drugs are available, antimicrobial resistance is the serious and most concern problem for today's global health. Hence it has become an emerging field of research and a challenge to the medicinal chemist to discover new molecules with a safe therapeutic index.

In the present study, author had selected the following 5 resistant microorganisms and reported the most common infections caused by them and the extent of resistance as follows.

Pneumonia, septicaemia, Urinary tract infections and intra abdominal infections are the most serious infections caused by Gram negative bacteria called *Pseudomonas aeruginos* [1]. However recent drugs of carbapenam category were also found to be ineffective due to drug resistance developed by pseudomonas pathogens. Including Pneumonia, blood stream infections, meningitis and pyogenic lever abscess are the life threatening conditions caused by a gram negative *Klebsiella pneumoni* [2] bacteria and known for its resistant [3] against carbapenams and other antibiotics. *Streptococcus pyogenes* [4] is a gram-positive Bacterium by which causes scarlet fever, severe pharyngitis and tonsillitis. Generally, macrolide antibiotics are used to treat the infections successfully. But recent reports have revealed that this pathogen had developed resistance to newer macrolides. *Clostridium difficile* is a spore forming gram positive bacteria. It causes severe inflammation in colon. Though cephalosporins are the effective antibiotics to treat various forms of colon infections, recent studies reported the resistance [5] of *Clostridium* pathogens against newer cephalosporins. H37Rv is the most virulent strain of tuberculosis and also a usual biomarker to study antitubercular potential of new antitubercular leads with respect to dose and other invitro pharmacodynamic effects.

Based on the above illustrations, the researcher had viewed to identify phytochemical constituents present in methanolic and n-hexane extracts of two Indian spices namely seeds of *Ceiba pentandra* [6] and lichen of *Parmotrema perlatum* [7] and to demonstrate their antibacterial and antimycobacterial activities. *Ceiba pentandra* is commonly known as Kapok belongs to the family Malvaceae. It is a very tall deciduous tree grows in rainfall forest areas. *Parmotrema perlatum* is lichen which is commonly known as Black stone flower belongs to the family Parmeliaeceae. It occurs throughout northern and southern hemispheres. Both of these spices are commonly used in India and China. *Ceiba pentandra* is known for antioxidant [6], anticancer, anti-inflammatory and other activities [8]. *Parmotrema perlatum* was reported for antibacterial activity where as evidences for phytoconstituents [9] and other biological activities [10] are very limited. So the author had focussed the present study on phytoconstituents and biological activities of *Parmotrema perlatum*.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fruits of Kapok and black stone flower samples were purchased in local market of Trivendrum (City), Kerala (state), India then herbariums were authenticated by Dr. K. Madhava Setty, Associate professor, Taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, Andhra pradesh, India as their Voucher specimen Numbers: 0429 & 0776 for *Ceiba pentandra* and *Parmotrema perlatum*

respectively at S.V. university Herbarium. All the chemicals used for extraction and estimations were purchased from Sigma Aldrich, Bengaluru.

2.2 Methods

Extracts of n-hexane and methanol were prepared separately by cold maceration [11] as specified below. Fruits of kapok were broken to collect seeds. Seeds were sieved to remove debris and fibrous. Then cleaned seeds were crushed in to a coarse powder. Two samples each containing 150 grams of powder were weighed and transferred into two separate round bottom flasks and numbered the flasks as (1) & (2). 450 ml of Methanol was transferred to flask (1) and 450 ml of n-hexane was transferred to flask (2). Methanol extract was named as CP(M)E and n-hexane extract was named as CP(N)E.

Black stone flower material was cleaned to remove fibres, sand etc. Then it was cleaned under running tap water to remove adhered dust after that immediately it was dried under sunshade. Then it was crushed to produce coarse material for the preparation of extracts of methanol and n-hexane extracts as mentioned in the extraction procedure for kapok. The methanol extract was named as PP(M)E and n-hexane extract was named as PP(N)E. Then all the flasks were kept aside for 7days. At 8^{th} day solvents were recovered under reduced pressure using a rotary vacuum evaporator. The extracts were then used for further studies.

Phytochemical tests [12] were performed on methanolic and n-hexane extracts of *Ceiba pentandra* and *Parmotrema perlatum* extracts to identify the presence of secondary metabolites. The results obtained are represented in Table 1.

Table 1. Qualitative analysis on phytochemical constituents

Chemical test	*Ceiba pentandra*		*Parmotrema perlatum*	
	CP(M)E	CP(N)E	PP(M)E	PP(N)E
Mayer's test	+++	++	+++	++
Dragendorff's test	+++	++	+++	++
Wagner's test	+++	++	+++	++
Hager's test	+++	++	+++	++
Tannic acid test	+++	++	+++	++
Froth test	++	++	+++	++
Hemolysis test	++	+	++	+
Ferric chloride test	++	+	++	+
Shinoda test	+++	++	+++	++
Zinc-Hydrochloride reduction test	+++	++	+++	++
Alkaline reagent test	++	++	+++	++
Millons test	++	+	-	-
Ninhydrin test	+	+	-	-
Molisch's test	+++	++	-	-
Benedicts test	+++	++	-	-
Fehlings test	+++	++	-	-
Steroids & Terpenoids test	+++	++	+++	++
Phosphates test	++	+	++	+
Sulphates test	++	+	++	+

+ + +: High presence of pyhtochemical constituents
+ +: Moderate presence of pyhtochemical constituents
+: Low presence of phytochemical constituents
-: Absence of pytochemical constituents

2.2.1 GC-MS analysis

The Gas Chromatography-Mass spectroscopy (GC–MS) analysis [13] of the components were carried by equipping Mass Selective Detector (MSD) (HP6890 GC and HP7673) autosampler, operated at 65eV using acquisition scan mode with HP-5MS at 100° C oven temperature held initially for 1 min and then increased to 280°C by 20°C/min and held for 10 min. 250°C was the injector temperature, and Helium was used as the carrier gas at a constant column flow rate of 1.5 ml/min. By split, less mode technique 2*µl* aliquot of the sample extract was injected. From 10 chromatograms, target components were selected, and Comp Extractor software was used for the registration of chromatograms.

2.2.2 Determination of MIC 90 by micro titre broth dilution method

Stock solutions of each test compound (PP(N)E, CP(N)E, PP(M)E and CP(M)E) were prepared in DMSO to obtain a concentration of 1 mg/ml. Each primary stock solution of PP(N)E, CP(N)E, PP(M)E and CP(M)E were diluted to obtain the final concentrations of 5,10,20,40,80,160 and 320 µg/ml. Resistant bacterial strains namely Carbapenam resistant *Pseudomonas aeruginosa (Cr-P.a)* Carbapenam resistant *Klebsiella pneumoniae (Cr-K.p)* Cephalosporin resistant *Clostridium difficile (C.r-C.d)* and Macrolide resistant *Streptococcus pyogenes (Mr-S.p)* were cultured as per standard Protocol. An aliquot of 100 µl of each test sample was added to a 96-welled (12 x 8) microtitre plate, to obtain test concentrations along with an aliquot of 75 µl of MH broth, an aliquot of 20-µl of bacterial inoculum (10^9 CFU/ml) and a 5-µl aliquot of 0.5% of 2,3,5-triphenyl tetrazolium chloride (TTC). After pouring all of the above into a well, the microplate was incubated at 37°C for 18 h. The development of pink colouration due to TTC in a well indicated bacterial growth, and the absence of the colour was taken as mean inhibition of bacterial growth. The first well of the microplate was the control without any additional molecules and second well contains Ciprofloxacin as (+)ve control. The MIC value was noted at the well, where no colour was manifested. 90% of growth inhibition was considered as MIC which was calculated based on Non-linear regression method of the test sample as per standard protocol [14,15].

2.2.3 Determination of anti tubercular activity using Microplate Alamar Blue Assay (MABA)

The anti-tubercular activity of test samples PP(N)E, CP(N)E, PP(M)E and CP(M)E were assessed against *M. tuberculosis* using Microplate Alamar Blue Assay (MABA). This methodology is nontoxic, uses a thermally stable reagent and shows good precise and accurate measurements inhibitory activity of strain. Sterile deionised water (200µl) was added to all outer perimeter wells of sterile 96 well plates to minimum evaporation of medium in the test wells during incubation. The 96 well plates received 100µl of Middle brook 7H9 broth and serial dilutions of test samples (PP(N)E, CP(N)E, PP(M)E and CP(M)E) were added to obtain the final concentrations of 5,10,20,40,80,160 and 320 µg/ml (The preparation of dilutions were described above). Plates were covered and sealed with parafilm and incubated at 37°C for five days. After 5 days of incubation, 25µl of freshly prepared 1:1 mixture of alamar blue reagent and 10% tween 80 was added to the plate and incubated for 24 hours. A blue colour in the well was interpreted as no bacterial growth and pink colour was scored as growth. The Minimum Inhibitory Concentration (MIC) at 90% growth inhibition was defined as the lowest extract concentration, which prevented the colour change from blue to pink. Streptomycin was used as standard anti-TB drug. MIC 90 was calculated based on Non-linear regression method using Win-analyst software [16,17].

3. RESULTS AND DISCUSSION

Alkaloids, terpenoids, flavonoids, saponins, tannins, steroids, phosphates and sulphates were identified as major constituents in methanolic extracts of *Parmotrema perlatum* whereas carbohydrates, and proteins were found to be absent in both extracts of *Parmotrema perlatum.* Methanolic extract of *Ceiba pentandra* contains alkaloids, tannins, saponins and flavonoids, carbohydrates, phosphates and sulphates as the constituents whereas n-hexane extract has given the positive result for alkaloids, saponins, tannins and carbohydrates. Steroids were absent in both *Ceiba pentandra* extracts (Table 1).

3.1 GC-MS Study

GC-MS study had revealed the presence of some of the following important constituents on

comparison of the compound libraries with Retention time and peak areas. *Parmotrema perlatum* contains a notable amount of terpenes and sesquiterpenes like Carvone, Copaene, Cubebene, Viridifloral and Rishitin. Sambucol is an immune stimulant anthocyanin. Longidione is a tricyclic hydrocarbon, Platambin is a naphthalene derivative. Stigmastan 3,5-diene is a phytosterol. *Ceiba pentandra* extracts contain saturated dicarboxylic acids like azelaic and Suberic acid, limonene a liquid phenol and Limonene-6-ol, a monocyclic monoterpene (Table 2).

3.2 Antibacterial Activity

Methanolic extract of *Parmotrema perlatum* was found to exhibit good antibacterial activity (less than 200 *µg/mL* against *Klebsiella pneumonia, Pseudomonas aeruginosa and Clostridium difficile.* t had shown intermediate activity (200-350 *µg/mL*) against *Streptococcus pyogenes. Parmotrema* perlatum had shown intermediate activity against *Clostridium difficile* whereas methanolic extract of *Ceiba pentandra* exhibited intermediate activity against *Klebsiella pneumonia and Pseudomonas aeruginosa.* N-hexane extract of ceiba pentandra had not shown any notable antibacterial and antitubercular activities between 500 to 800 µg/mL (Table 3).

3.3 Anti Tubercular Activity

Methanolic extract of *Parmotrena perlatum had shown* 90% growth inhibition against H37 Rv at 80 micrograms/ml and intermediate activity was observed with methanolic extract of

Table 2. GC-MS data of phytoconstituents

S. No	Compound name	Molecular formula	Retention time	Peak area%
Phyto constituents of n-hexane extract of Parmotrema perlatum				
1	D-carvone	$C_{10}H_{14}O$	9.65	0.41
2	Sambucol	$C_{15}H_{22}O_4$	9.83	0.12
3	Copaene	$C_{15}H_{24}O_4$	11.58	0.12
4	Cubebene	$C_{15}H_{24}$	11.58	1.45
5	Viridifloral	$C_{15}H_{26}O$	11.97	0.10
6	Rishitin	$C_{14}H_{22}O_2$	12.86	0.11
7	Esculetin	$C_9H_6O_4$	15.86	0.28
8	Daphnetin	$C_9 H_6O_4$	15.86	0.28
Phyto constituents of Methanolic extract of Parmotrema perlatum				
9	5,7-dihydroxy 4-methyl coumarin	$C_{10}H_8 O_4$	15.62	5.92
10	Platambin	$C_{15}H_{22}O_2$	19.03	0.08
11	Longidione	$C_{15}H_{22}O_2$	19.03	0.08
12	Gamolenic acid	$C_{18}H_{30}O_2$	23.79	0.12
13	Stigmastan 3,5-diene	$C_{29}H48$	25.56	0.45
Phyto constituents of n-hexane extract of Ceiba pentandra C(P)-H				
14	Undecanol	$C_{11}H_{24}O$	8.14	0.06
15	Azelaic acid	$C_9H_{16}O_4$	14.13	2.29
16	9-Eicosyne	$C_{18}H_{30}O_2$	16.97	0.31
17	1(+) Asorbic acid 2,6-dihexa deconate	$C_{38}H_{68}O_8$	18.27	6.29
18	8,11,14-Eicosa trienoic acid	$C_{21}H_{36}O_2$	18.49	0.49
19	8-Propoxy cedrane	$C_{18}H_{32}O$	24.31	0.15
Phyto constituents of Methanolic extract of Ceiba pentandraC(P)-M				
20	Isoserine	$C_3H_7NO_3$	4.66	0.37
21	Erythritol	$C_4H_{10}O_4$	7.81	4.95
22	dl-threitol	$C_8H_{10}O$	7.92	7.03
23	Eugenol	$C_{10}H_{12}O_2$	11.18	0.11
24	Subericacid	$C_9H_{16}O_4$	12.91	2.93
25	Limonene-6-ol	$C_{15}H_{24}O_2$	17.17	0.13

Table 3. Antibacterial activity

Compound code	MIC90			
	Cr-P.a	*Cr-K.p*	*Cr-C.d*	*Mr-S.p*
	MIC in µg/mL against Cr-P.a	MIC in µg/mL against Cr-K.p	MIC in µg/mL against Cr-C.d	MIC in µg/mL against Mr S.p
PP(N)E	1310.84	1106.66	238.64	1027.64
CP(N)E	1286.15	1039.59	1328.51	996.33
PP(M)E	185.10	177.84	186.28	202.60
CP(M)E	241.54	218.54	636.99	335.35
CIPRO	37.54	28.28	33.23	41.31

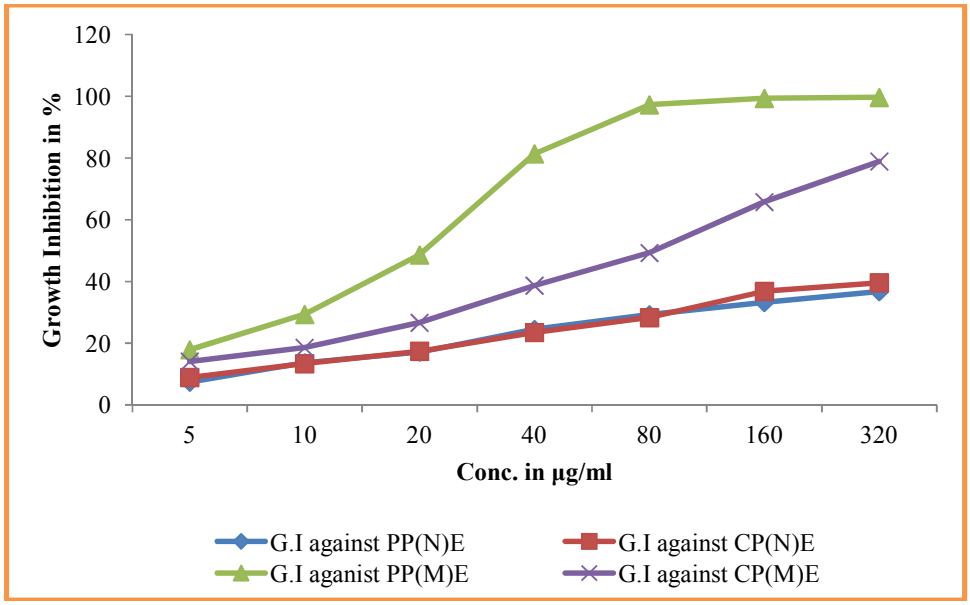

Fig. 1. Antitubercular activity

Ceiba pentandra at 320 micrograms/ml. n-hexane extracts of both samples had shown only about 30% growth inhibition even at 320 micrograms/ml. Parmotrema perlatum has terpenes and steroids as chief chemical constituents as per the GC-MS study hence contributed the remarkable antibacterial and antitubercular activities. Isolation of active constituents and exploration of structures of principle constituents in the future study may lead to discover natural antitubercular and antimicrobial leads (Fig. 1).

4. CONCLUSION

It is concluded that, the study has made an attempt to identify the phytochemical constituents present in methanolic and n-hexane extracts of Ceiba pentandra and Parmotrema perlatum using phytochemical tests. These spices are not only known to contribute flavor to food but also dispense therapeutically active molecules to the body. Methanolic extracts of both spices were proven as effective antimicrobial activity against resistant microorganisms than n-hexane extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors thank DIST- FIST facility and Principal, RIPER for providing ancillary facilities to carry out the research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pachori P, Gothalwal R, Gandhi P. Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. Genes & Diseases. 2019;6(2):109-1.
2. Effah CY, Sun T, Liu S, Wu Y. Klebsiella pneumoniae: an increasing threat to public health. Ann Clin Microbiol Antimicrob. 2020;19(1):1.
3. Sakkas H, Bozidis P, Ilia A, Mpekoulis G, Papadopoulou C. Antimicrobial resistance in bacterial pathogens and detection of carbapenemases in *Klebsiella pneumoniae* isolates from hospital wastewater. Antibiotics. 2019;8(3):85.
4. Joseph JF, Dennis LS, Vincent AF. Streptococcus Pyogenes Basic Biology to Clinical Manifestations; 2016.
5. Spigaglia P, Mastrantonio P, Barbanti F. Antibiotic resistances of clostridium difficile. In: Mastrantonio P, Rupnik M, eds. Updates on Clostridium Difficile in Europe. Springer International Publishing. 2018; 1050:137-59.
6. Kiran CR, Rao DB, Sirisha N, Rao TR. Assessment of phytochemicals and antioxidant activities of raw and germinating *Ceiba pentandra* (kapok) seeds. Journal of Biomedical Research. 2015;29(5):414.
7. Shanu Hoda, Pooja Vijayaraghavan. Evaluation of antimicrobial prospective of Parmotrema perlatum hexane extract. International Journal of Pharmaceutical Research & Allied Sciences. 2015;4(2):47-53.
8. Mathangi N, Didala A, Kasarla R, Yetcharla Venkatesh. A Review on Ceiba pentandra and its medicinal features. Asian Journal of Pharmacy and Technology. 2012;3(2): 2231-5705.
9. Carlos Fernandez-Moriano, Divakar PK, Ana C, M PG-S. Antioxidant and cytoprotective potentials of parmeliaceae lichens and identification of active compounds. An Real Acad Farm. 2015; 81(2):164-78.
10. Thadhani VM, Karunaratne V. Potential of lichen compounds as antidiabetic agents with antioxidative properties: A review. Oxidative Medicine and Cellular Longevity. 2017;2017:1-10.
11. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants. 2015;04(03).
12. Altemimi A, Lakhssassi N, Baharlouei A, Watson D, Lightfoot D. Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 2017;6(4):42.14.
13. Swamy MK, Sinniah UR, Akhtar Mohd S. In vitro pharmacological activities and gc-ms analysis of different solvent extracts of lantana camara leaves collected from tropical region of Malaysia. Evidence-Based Complementary and Alternative Medicine. 2015;2015:1-9.
14. Schwalbe R, Steele-Moore L, Goodwin AC, editors. Antimicrobial susceptibility testing protocols. Crc Press; May 22, 2007.
15. Lynne S. Garcia, Henry D. Isenberg. Clinical Microbiology Procedures Handbook. 2nd ed. ASM Press. 2007;1.
16. Makane VB, Krishna VS, Krishna EV, et al. Synthesis and evaluation of α-aminoacyl amides as antitubercular agents effective on drug resistant tuberculosis. European Journal of Medicinal Chemistry. 2019;164: 665-77.
17. Sanchez JGB, Kouznetsov VV. Antimycobacterial susceptibility testing methods for natural products research. Braz J Microbiol. 2010;41(2):270-77.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/67959