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Evaluation of Antibacterial effect of *Corymbia ptychocarpa*

Antony Adaikalam A*[1] and M. Arumugam[2]

[1-2] J. J. College of Arts and Science (Autonomous), (Affiliated to Bharathidasan University, Tiruchirappalli), J. J. Nagar, Sivapuram, Pudukkottai - 622422, Tamil Nadu, India

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Abstract

Antibiotic resistance poses a serious global public health threat, necessitating the discovery of new antimicrobial agents. This study explores the antibacterial potential of *Corymbia ptychocarpa*, an ethnomedicinal Australian plant used in traditional medicine but not scientifically investigated. Phytochemical screening of leaf and stem extracts revealed a diversity of bioactive compounds like flavonoids, tannins, glycosides, and alkaloids with known antimicrobial properties. Gas chromatography-mass spectrometry (GC-MS) analysis identified major antibacterial, anti-inflammatory, and anticancer candidates in the leaf (ethyl acridine) and stem (indolizine). The extracts were tested against ESKAPE pathogens using disk diffusion assays. Both leaf and stem extracts exhibited promising antibacterial activity, with inhibition zone diameters ranging 2-7 mm compared to antibiotic controls. Leaf extracts showed broad spectrum inhibitory activity, especially against *Klebsiella pneumoniae* and *Salmonella typhimurium.* Stem extracts were most potent against *E. coli* and *E. faecalis*. The observed antibacterial effects validate the traditional use of *C. ptychocarpa* and reveals its potential for new antibiotic drug discovery.

***Key words*:** *Corymbia ptychocarpa,* Ethnomedicinal plants, Phytochemical screening, Antibacterial activity

The introduction of antibiotics and chemotherapeutics revolutionized the treatment of infectious diseases in the 20th century. However, overuse and misuse of antibiotics over decades has led to a dramatic rise in antibiotic resistance, posing one of the most serious threats to public health globally [1]. Multi-drug resistant (MDR) variants of bacterial pathogens have emerged, causing infections that do not respond to first-line antibiotics. The CDC estimates over 3 million antibiotic-resistant infections occur in the US annually, resulting in approximately 50,000 deaths [2].

The ESKAPE pathogens, comprising *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *and Enterobacter spp*., are responsible for a majority of hospital-acquired infections and effectively 'escape' the effects of antibacterial drugs through various resistance mechanisms [3]. These are a group of bacteria known for their ability to "escape" the effects of antibacterial drugs. These include enzymatic inactivation of antibiotics via beta-lactamases, aminoglycoside-modifying enzymes and carbapenemases; reduced cell wall permeability and increased efflux pump expression; target site modifications in ribosomes, DNA gyrase and folic acid synthesis; and ability to form biofilms [4]. Worryingly, the antibiotic drug development pipeline is running dry, with most major pharmaceutical companies exiting R&D efforts due to high costs, long timelines, tedious regulatory approvals and lack of economic incentives [5]. As conventional antibiotic discovery approaches stagnate, there is an urgent need to explore unconventional sources for new antibiotic scaffolds capable of evading existing resistance mechanisms [6].

For millennia, plants have been widely used in traditional medicine systems and remain a prolific resource for bioactive molecules. Phytochemicals serve as defense compounds in plants and many exhibits antimicrobial effects against pathogens [7]. Historically, over 80% of antibiotic drug leads have been derived from natural sources, including penicillin, tetracycline and erythromycin [8]. Plant biodiversity represents an abundant reservoir of structurally diverse secondary metabolites with enormous molecular plasticity conferring potential antibacterial activity [9].

Ethnopharmacological knowledge provides vital clues for identifying plant species with purported antibacterial uses. Systematic exploration of such plants can provide phytochemical leads to develop into new antibiotics, particularly against drug-resistant strains [10]. *Corymbia ptychocarpa* is one such ethnomedicinal plant native to Australia belonging to the Myrtaceae family. Myrtaceae plants are known to contain antimicrobial compounds like terpenoids, tannins and flavonoids [11]. However, *C. ptychocarpa* remains scientifically uninvestigated as a source of antibiotic scaffolds. This study aims to fill this gap through phytochemical characterization and evaluating its antibacterial potency against MDR ESKAPE pathogens. Positive results can pave the way for developing standardized phytotherapeutics and new antibiotic drug leads from this novel plant resource. *C. ptychocarpa*'s untapped chemical diversity, ethnomedical history and taxonomic uniqueness provide a compelling rationale for exploring its antimicrobial potential, highlighting the continuing need to prospect medicinal plants as renewable resources for discovering new antibiotics.

***Correspondence to:** Antony Adaikalam A, E-mail: antonyadaikalama@gmail.com; Tel: +91 8610874671

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MATERIALS AND METHODS

Collection of samples: *Corymbia ptychocarpa* a member of the Myrtaceae family, was the subject of this study. Mature and healthy leaves and stem of *C. ptychocarpa* were collected from Narimedu, Pudukkotai District, Tamil Nadu, India during December 2020. Specimen identification was performed using the Flora of Presidency of Madras [12], Flora of Tamil Nadu Carnatic [13], and Bulletin, Madras Government Museum [14].

Preparation of plant extract

Air-dried plant samples were used to prepare extracts. Specifically, 30 grams of the leaf and stem portions were subjected to separate extraction processes. Each extraction involved the use of 250 milliliters of two different solvents: petroleum ether and Ethyl acetate and water. Each extract sample was diluted in its respective solvent to achieve a 1/10 solution. This dilution would make it easier to work with the extracts in subsequent experiments and ensure that the concentrations were consistent for accurate testing and analysis.

Preliminary phytochemical screening

The plant extracts were tested for the presence of bioactive compounds such as terpenoids, alkaloids, glycosides, steroids, phenols, tannins, flavonoids and saponins by standard methods (Table 1) [15].

Table 1 Preliminary phytochemical analysis of *Corymbia ptychocarpa*.

S. No	Experiment	Observation indicates the positive / presence
1.	*Test for carbohydrates*: Take about 2ml of extract, add 5 drops of Napthalin and add 2ml of alcohol, 1ml of H_2SO_4	Formation of violet ring.
2.	*Test for anthocyain*: About 2 ml of Leaf extract was added with 1 ml of 2N NaOH and heated for 5min at 100	Formation of bluish green colour.
3.	*Test for alkaloids*: (Pazhanisamy and Ebenezer, 2013) About 2 ml of plant extract was dissolved in conc. HCL and add few drops of Mayer's reagent	Formation of white precipitate or green colour.
4.	*Test for steroids*: (Phytosteriods): To 0.5ml of extract, 2 ml of chloroform and 1 ml of Sulphuric acid was added	Formation of reddish-brown ring.
5.	*Test for tannins*: (Dhanasekaran*et al.,* 2013) About 0.5 ml of extract added to 2ml of 0.1% $FeCl_2$ solution (Segelman *et al.* 1969)	Appearance of brownish green or dark blue colour.
6.	*Test for saponins*: Take about 2ml of extract, 2ml of Dis. H_2O was added and shaken well.	Foam appears for 10 min.
7.	*Test for flavonoid*: Take about 1ml of extract. And add 1 ml of 2N NaOH solution	Appearance of yellow colour.
8.	*Test for quinones*: Take about 1ml of the extract, 1ml of Conc. Sulpuric acid was added	Formation of red colour.
9.	*Test for glycosides*: Take about 2ml of extract, 3ml of chloroform and 1ml of 10% ammonium, solution was added.	Formation of pink colour.
10.	*Test for cardiac glycosides*: Take about 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added and 1 ml of conc. sulphuric acid was added.	Formation of brown ring.
11.	*Test for terpenoids*: (Segelman*et al.,* 1969) Take about 0.5ml of the extract, 2ml of chloroform was added and conc. sulphuric acid was added carefully	Formation of reddish-brown colour.
12.	*Test of phenols*: Take about 1ml of the extract 2ml of distilled water was added followed by few drops of 10% ferric chloride	Formation of blue or black colour.
13.	*Test for proteins/amino acids: Nin hydrin test*: Take about 2ml of extract. Few drops of 0.2% Nin hydrin solution was added, and heat for 5 min.	Formation of blue colour.
14.	*Test for anthraquinone*: (Stanczyk, 2007) Take about 1ml of extract, few drops of 10% ammonium solution was added.	Appearance of Pink colour.
15.	*Test for phlobatannins*: Take about 1ml of extract and add 1ml of 10% NaOH solution.	Formation of yellow colour.
16.	*Test for coumarins*: Take about 1 ml of extract and add 1ml of 1N NaOH. The test tube was kept in boiling water bath for few min and shaken well.	Formation of yellow colour.

GCMS analysis: Dried *Corymbia ptychocarpa* leaves and stems (60g) were refluxed with 250ml of Ethyl acetate for 5 hours and concentrated. This extract was analyzed using a Shimadzu GC-MS-QP 2010 (DBI column, He carrier gas, 0.7 ml/min) programmed for temperature ramping. Injector and detector temperatures were 280°C and 290°C, respectively. 1µL TMS ether derivatives in n-hexane (2%) were injected with a 3:0 split ratio. The mass spectrometer operated with 70eV ionization potential, 200°C ion source temperature, 100°C quadrupole temperature, and 3000V eV voltage.

Identification relied on retention times and mass spectra. These facilitated compound identification through computerized library searches (NIST '98 MS) comparing retention times and mass spectra. This GC-MS analysis revealed the chemical composition of the Ethyl acetate extract of stem and leaf.

Antibacterial activity

Preparation of microbial strains: The antibacterial activity of plant extract pellets was tested by the Agar disc diffusion method. The indicator strains such as *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus* and *Enterococcus faecalis*. The overnight broth culture of each bacterial indicator strains was adjusted to 0.5 McFarland.

Preparation of sterile assay discs: Micropipettes dispensed 200-500 µl of plant extract onto 5mm, sterile Whatman No. 1 filter paper discs. Precautions ensured the extract remained confined to the disc before incorporation into the agar diffusion assay.

Antibacterial activity assessment: Plant extracts were evaluated for antibacterial potential using the CLSI-guided disc-diffusion method [16]. Sterile 9mm paper discs imbued with 5mg extract each were placed onto Mueller-Hinton II agar pre-inoculated with test bacteria. Solvent and 10mcg streptomycin discs served as negative and positive controls, respectively. After 18h incubation at 35°C, the diameters of clear zones surrounding each disc, reflecting extract-induced bacterial growth inhibition, were measured to quantify antibacterial activity.

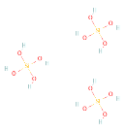
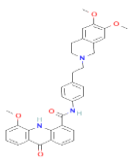
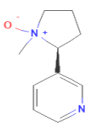
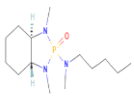
RESULTS AND DISCUSSION

Preliminary phytochemical screening: The preliminary Carbohydrates and Phytosteriods are the most abundant compounds in *Corymbia ptychocarpa*, found in all extracts. Anthocyanins and Phenols are exclusively present in the ethyl acetate extracts, indicating their selective solubility. Interestingly, Anthraquinone is absent in all extracts, suggesting it may not be a constituent of this plant. Compounds like Flavonoids and Coumarins show variability, being present in some extracts and absent in others, highlighting the influence of extraction methods on compound detection (Table 2).

Table 2 Preliminary phytochemical analysis *Corymbia ptychocarpa*

S. No.	Experiment	Leaves			Stem		
		Petroleum ether	Ethyl acetate	Water	Petroleum ether	Ethyl acetate	Water
1.	Carbohydrates	+	+	-	+	+	+
2.	Anthocyain	-	+	-	-	+	-
3.	Alkaloids	-	+	-	+	+	-
4.	Phytosteriods	+	-	+	+	+	+
5.	Tannins	+	+	+	-	-	-
6.	Saponins	+	+	-	+	+	-
7.	Flavonoid	+	-	+	-	+	+
8.	Quinones	+	-	+	+	+	+
9.	Glycosides	+	-	-	-	-	-
10.	Cardiac glycosides	-	-	+	+	-	-
11.	Terpenoids	-	-	+	+	+	+
12.	Phenols	+	+	-	-	+	_
13.	Proteins / amino acids	+	+	+	-	-	+
14.	Anthraquinone	-	-	-	-	-	-
15.	Phlobatannins	-	-	+	+	+	-
16.	Coumarins	+	-	+	-	+	+

Table 3 GC-MS analysis of ethyl acetate of leaf extract of *Corymbia ptychocarpa*

S. No.	Retention time (RT)	Name of the compound	Molecular formula (MF)	Molecular weight (MW)	Structure	Peak area (%)	Biological activity
1.	16.909	Silicic acid, diethyl bis(trimethyl lsilyl) ester	$H_{12}O_{12}Si_3$	288.34Da		40.59	Histamine H_2 receptor against, hyponitrite reductase inhibitor and mucositis treatment
2.	17.027	2- Ethyl acridine	$C_{34}H_{33}N_3O_5$	563.6Da		35.60	Acid phosphatase inhibitor, anti-diarrheal, antifungal, anti-hypoxic, anti-mycobacterial, anti-protozoal and anti-ulcerative
3.	17.213	3-Amino-&-nitro-1,2,4-brnotriazine 1-oxide	$C_{10}H_{14}N_2O_5$	178.23Da		15.13	Antifungal, anti-inflammatory, anti-protozoal, arylesterase inhibitor and antiviral
4.	17.295	Trimethyl[(4-2-methyl-4-oxo-2-pentyl)phenoxy] silane	$C_{14}H_{30}N_{30}O$ P	287.38 Da		21.39	Arylesterase inhibitor, apyrase inhibitor, anti-ulcerative, anti-naupathic and antibacterial

Medicinal plants, such as *Corymbia ptychocarpa*, are crucial in various medical fields due to their bioactive compounds, which are essential for drug development. These compounds, including saponins, glycosides, flavonoids, and alkaloids, exhibit antibiotic properties [17]. Saponins, for instance, have been associated with activities like hemolysis,

antifungal properties, anti-inflammatory effects, fungistatic activity, and molluscicidal properties [18-22]. Phytochemicals are known to accumulate in different plant parts and serve various protective and functional roles, influencing aspects such as plant color, odor, and flavor. These plant-derived chemicals play a vital role in safeguarding plant cells against environmental stressors [23].

GCMS analysis

GC-MS analysis revealed distinct chemical profiles in *Corymbia ptychocarpa*'s leaf and stem extracts. Leaf extracts contained four major compounds (Table 3, Fig 1), notably ethyl acridine and 1-ethylacridine, known for anti-inflammatory, anticancer, and antimicrobial properties [24]. Stem extracts contained two major compounds (Table 4, Fig 2), including Indolizine, which has potential for anticancer drug discovery [25]. Other identified phytochemicals, such as cyclotrisiloxane hexamethyl and palmitic acid, have established antimicrobial, anti-inflammatory, and antioxidant properties [26]. These findings highlight the diverse bioactive compounds in *Corymbia ptychocarpa* and their potential pharmacological applications.

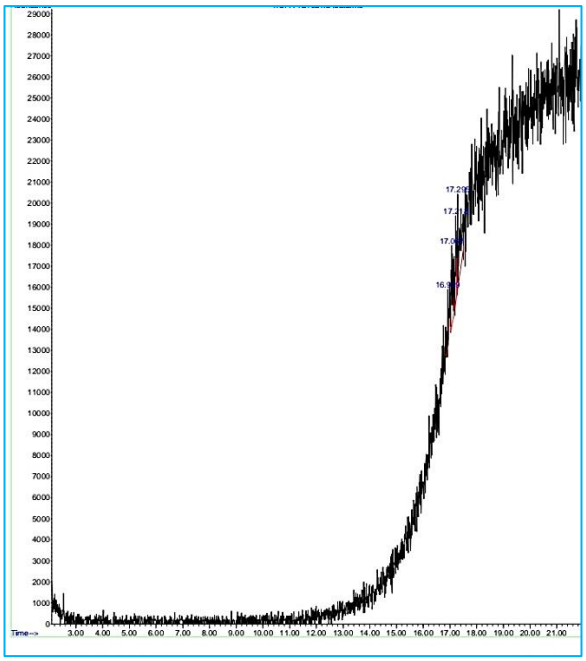


Fig 1 GCMS analysis of ethyl acetate leaf extract of *Corymbia ptychocarpa*

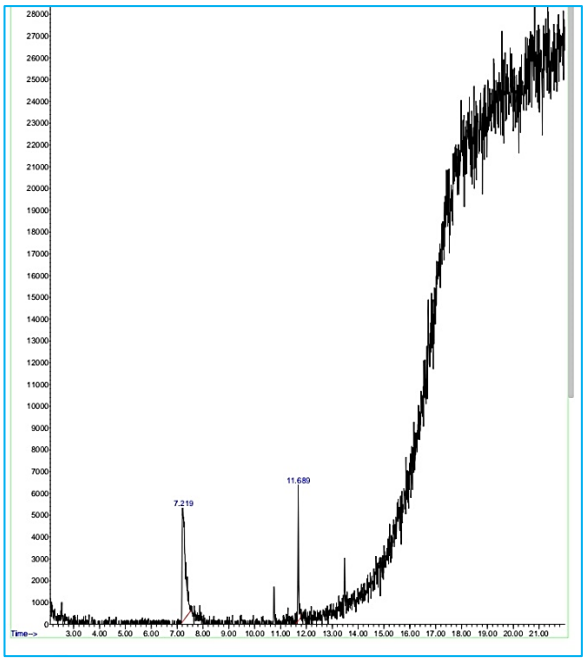
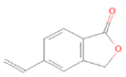
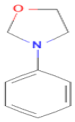


Fig 2 GCMS analysis of ethyl acetate stem extract of *Corymbia ptychocarpa*

Table 4 GC-MS analysis of ethyl acetate of stem extract of *Corymbia ptychocarpa*

S. No.	Retention time (RT)	Name of the compound	Molecular formula (MF)	Molecular weight (MW)	Structure	Peak area (%)	Biological activity
1.	7.219	Benzofuran, 2-ethenyl, 5-ethenyl-2-benzofuran-1(3H)-one	$C_{10}H_sO_2$	160.17Da		21.11	Anti-diarrheal, Antibacterial, Alpha-glucuronidase inhibitor and Andropause treatment
2.	11.689	Oxazolidine, 3-phenyl- 3-Phenyloxazolidine Oxazolidine,	$C_9H_{11}NO$	149.19Da		78.89	Anti-helmintic, Anti-infective, Anti-nociceptive and Arylformamidase inhibitor

Antibacterial activity: Antibacterial activities of various extracts of *Corymbia ptychocarpa* have been presented in (Table 5). The activity of the selected extracts on the test organisms is not uniform (Table 4, Fig 3).

Leaf: The petroleum extract extracts showed a broad spectrum of activity against all the tested organisms. The zones of inhibition were ranged from 3 to 4 mm at different extracts. Maximum activity showed (3 mm) against *Proteus mirabilis*. The ethyl acetate leaf extract showed maximum activity against the bacterial strains Salmonella typhimurium and *Klebsiella pneumoniae*. Maximum zone was observed in *Salmonella typhimurium* and *Klebsiella pneumoniae* (5 and 4 mm) and the minimum zone of inhibition (2 mm) against *Enterococcus faecalis, Staphylococcus aureus* and *Proteus mirabilis* respectively.

Stem: The petroleum ether extract showed maximum zone of inhibition (2 mm) against *Escherichia coli*. The ethyl acetate stem extract showed maximum zone of inhibition in *Escherichia coli* and *Enterococcus faecalis* (6 and 7 mm) respectively.

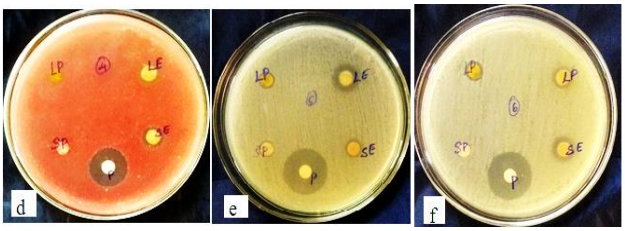
The lipophilic character of their hydrocarbon skeleton and the hydrophilic character of their functional groups are of main importance in the antimicrobial action components. Aldehydes are known to possess powerful antimicrobial activity. It has been proposed that an aldehyde group conjugated to a carbon-to-carbon double bond is a highly electronegative arrangement, which may explain their activity [27], suggesting a proportional increase of the antibacterial activity with electronegativity [28]. Secondly, there is some evidence that minor components have a critical part to play in antibacterial

activity, possibly by producing a synergistic effect between other components. Aliphatic alcohols were reported to possess strong to-moderate activities against several bacteria. The activity increased with the length of the carbon chain [29].

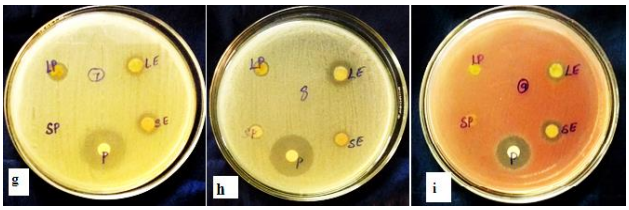
Table 4 Antibacterial activity of different solvent extracts of leaf and stem of *Corymbia ptychocarpa*.

S. No	Sample	Zone of Inhibition in different solvent (mm)				Control
		Leaf		Stem		
		Petroleum ether	Ethyl acetate	Petroleum ether	Ethyl acetate	
1.	*Escherichia coli*	2	3	2	6	10
2.	*Enterococcus faecalis*	1	2	1	7	11
3.	*Serratia marcescens*	2	3	1	2	8
4.	*Beta strepa*	1	3	1	3	8
5.	*Salmonella typhimurium*	2	5	1	1	10
6.	*Staphylococcus aureus*	2	2	1	3	9
7.	*Proteus mirabilis*	3	2	1	3	9
8.	*Klebsiella pneumoniae*	1	4	1	2	9
9.	*Pseudomonas aeruginosa*	0	3	0	4	7
10.	*Bacillus cereus*	1	3	0	2	10

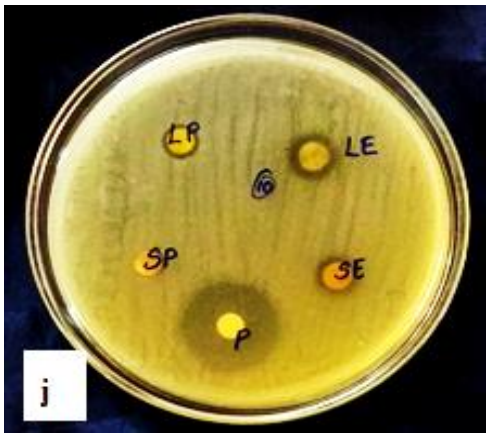
a. Antibacterial activity of various extracts of leaf and stem of *Corymbia ptychocarpa* against *Escherichia coli*
b. Antibacterial activity of various extracts of leaf and stem of *Corymbia ptychocarpa* against *Enterococcus faecalis*
c. Antibacterial activity of various extracts of leaf and stem of *Corymbia ptychocarpa* against *Serratia marcescens*



d. Antibacterial activity of various extracts of leaf and stem of *Corymbia ptychocarpa* against *Beta strepa*
e. Antibacterial activity of various extracts of leaf and stem of *Corymbia ptychocarpa* against *Salmonella typhimurium*
f. Antibacterial activity of various extracts of leaf and stem of *Corymbia ptychocarpa* against *Staphylococcus aureus*



g. Antibacterial activity of various extracts of leaf and stem of *Corymbia ptychocarpa* against *Proteus mirabilis*
h. Antibacterial activity of various extracts of leaf and stem of *Corymbia ptychocarpa* against *Klebsiella pneumonia*
i. Antibacterial activity of various extracts of leaf and stem of *Corymbia ptychocarpa* against *Pseudomonas aeruginosa*



j. Antibacterial activity of various extracts of leaf and stem of *Corymbia ptychocarpa* against *Bacillus cereus*

Fig 3 Antibacterial activity of various extracts of leaf and stem of *Corymbia ptychocarpa*

CONCLUSION

Corymbia ptychocarpa bursts onto the scene with a unique chemical profile and promising antibacterial activity. GC-MS analysis revealed distinct leaf and stem compounds, including anti-inflammatory, anticancer, and antimicrobial candidates like ethyl acridine and Indolizine. Both extracts exhibited varying degrees of inhibition against multi-drug resistant ESKAPE pathogens and other bacterial strains. *C. ptychocarpa* emerges as a valuable resource for novel antibiotic leads, and further research on its bioactive compounds could unlock the key to overcoming the growing threat of antibiotic resistance. Corymbia ptychocarpa emerges as a valuable resource for the discovery of novel antibiotics. The discovery of Corymbia ptychocarpa and its bioactive compounds represents a potential strategy for addressing the growing threat of antibiotic resistance. By identifying new sources of antibiotics and understanding their mechanisms of action, researchers may find ways to overcome bacterial resistance to existing antibiotics. *Corymbia ptychocarpa* shows promise as a source of novel antibiotics with potential activity against antibiotic-resistant bacteria.

LITERATURE CITED

1. CDC A. 2019. Antibiotic resistance threats in the United States. US Department of Health and Human Services: Washington, DC, USA.

2. Santajit S, Indrawattana N. 2016. Mechanisms of antimicrobial resistance in ESKAPE pathogens. *Biomed Research International* 2016: 2475067. doi: 10.1155/2016/2475067.
3. Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ. 2015. Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology* 13(1): 42-51.
4. Silver LL. 2011. Challenges of antibacterial discovery. *Clinical Microbiology Reviews* 24(1): 71-109.
5. Katz L, Baltz RH. 2016. Natural product discovery: past, present, and future. *Journal of Industrial Microbiology and Biotechnology* 43(2/3): 155-176.
6. Gibbons S. 2005. Plants as a source of bacterial resistance modulators and anti-infective agents. *Phytochemistry Reviews* 4: 63-78.
7. Newman DJ, Cragg GM. 2020. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products* 83(3): 770-803.
8. Koehn FE, Carter GT. 2005. The evolving role of natural products in drug discovery. *Nature Reviews Drug Discovery* 4(3): 206-220.
9. Hemaiswarya S, Kruthiventi AK, Doble M. 2008. Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine* 15(8): 639-652.
10. Batish DR, Singh HP, Kohli RK, Kaur S. 2008. *Eucalyptu*s essential oil as a natural pesticide. *Forest Ecology and Management* 256(12): 2166-2174.
11. Gamble JS, Fischer CEC. 1915-1936. *Flora of the Madras Presidency*. London: Adlard.
12. Mathew KM. 1983. *The Flora of Tamil Nadu Carnatic*. Publishers: The Rapinat Herbarium, St Joseph's College, Tiruchirapalli 620 002 & The Anglade Institute of Natural History, Shembaganur, Kodaikanal 624 104, India, Vol. 1-3.
13. Gravely FH, Mayuranathan PVP. 1931. The Indian Species of the Genus Caralluma (fam. Asclepiadaceae). Superintendent, Government Press.
14. Yadav RNS, Agarwala M. 2011. Phytochemical analysis of some medicinal plants. *Journal of Phytology* 3(12): 10-14.
15. CLSI. 2018. *Performance Standards for Antimicrobial Susceptibility Testing*. Clinical and Laboratory Standards Institute.
16. Feroz M, Ahmad R, Sindhu STAK, Shahbaz AM. 1993. Antifungal activities of saponins from indigenous *Medicago sativa* roots. *Pakistan Veterinary Journal* 13: 14.
17. Hafiza MA, Parveen B, Ahmad R, Hamid K. 2002. Phyto-chemical and antifungal screening of *Medicago sativa* and *Zinnia elegans. Online Jr. of Biol. Science* 2(2): 130-132.
18. Somolenski SJ, Silinis H, Farnsworth NR. 1974. Alkaloid screening. *V. Lloydia* 37: 506-536.
19. Takagi K, Hee PE, Histoshi K 1980. Anti-flammatory activities of hedergenin and crude saponin from *Sacchindusmukarassi* Gaertn. *Chem. Bulletin* 28(4): 1183-1188.
20. Zehavi UML, Segel RJ. 1986. Potential use of *Colocasia esculentum* and *Panicum repens* as bioindicators for environmental management of Linggi River, Malaysia. *Phytophatology* 116: 338-343.
21. Sati OP, Chandra CD, Usha R. 1987. Preliminary phytochemical analysis of some plant seeds. *Planta Medica* 5: 981-983.
22. Mathai K. 2000. Nutrition in the adult years In Krause's food, Nutrition and diet therapy. 10th Edition. Mahan LK & Escott-Stump S. 271: 274-275.
23. Kowaleska MG, Cholewin'ski G, Dzierzbika K. 2017. Recent developments in the synthesis and biological activity of acridine/ acridone analogues. *Royal Soc. Chem*. 7: 15776.
24. Sharma V, Kumar V. 2014. Indolizine: a biologically active moiety. *Medicinal Chemistry Research* 23: 3593-3606.
25. Gideon VA. 2015. GC-MS analysis of phytochemical components of *Pseudoglochidion anamalayanum* gamble: an endangered medicinal tree. *Asian Journal of Plant Science and Research* 5: 36-41.
26. Moleyar V, Narasimham P. 1986. Antifungal activity of some essential oil components. *Food Microbiology* 3(4): 331-336.
27. Kurita N, Miyaji M, Kurane R, Takahara Y. 1981. Antifungal activity of components of essential oils. *Agricultural and Biological Chemistry* 45(4): 945-952.
28. Kabelitz N, Santos PM, Heipieper HJ. 2003. Effect of aliphatic alcohols on growth and degree of saturation of membrane lipids in *Acinetobacter calcoaceticus. FEMS Microbiology Letters* 220(2): 223-227.