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INVESTIGATIONS ON THE BIO-ACTIVE CONTENTS AND ANTIBACTERIAL POTENTIALS IN *PUNCTELIA JECKERI* (ROUM). KALB. – A LICHEN SPECIES

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ABSTRACT

Lichens are composite organisms commonly found in our environment growing on various surfaces including plants, bare soils, and rocks. However, they are largely overlooked by many researchers and pharmaceutical industries due to many factors, among which could be attributed to the limited understanding of their inherent and potential usefulness. In this study, methanol extract of Punctelia jeckeri – a lichen, was prepared with a view to investigating its bioactive constituents as well as its antibacterial potential. The prepared extract was screened for the presence of bioactive substances, and was subsequently investigated for its antibacterial activities on selected bacterial species following standard methods. The results of the screening revealed the presence of important lichen substances such as tannins, alkaloids, flavonoids, phenols, saponins, and carbohydrates in the extract. The extract also exhibited antibacterial activities on Bacillus cereus, B. polymyxa, B. stearothermophillus, Clostridium sporogenes, Klebsiella pneumoniae, Micrococcus luteus, Morganella sp., Proteus vulgaris, Providencia rettgeri, Salmonella typhimurium, and Staphylococcus aureus at varying degrees and concentrations. The study concluded that P. jeckeri is composed of bioactive substances with values that can be implicated in pharmaceutics for manufacturing drugs for the treatment of diseases caused by the tested susceptible bacterial species.

KEY WORDS*:* *lichen, alkaloids, phytochemicals, Punctelia jeckeri, antimicrobial resistance*

INTRODUCTION

Lichens are a composite organism comprising a fungus and a chlorophyllous partner that can be either green algae or cyanobacteria, or both, living together in a symbiotic association. The fungi provide suitable habitation for the algal partner which

in turn provides photosynthetically fixed carbon as an energy source for the association (Rashmi and Rajkumar, 2014; Ines *et al*., 2016). The vegetative bodies of lichens vary in colour, size, and growth forms, and some may live for several thousand years (Denton and Karlén, 1973). Some lichens have leaf shapes (foliose lichens), some cover the substrates where they grow like a crust (crustose lichens) while others appear as shrub-like or fibril (fruticose lichens) (Mustafa, 2012). Lichens are common in our surroundings and are found in almost all terrestrial environments, ranging from tropical to polar climatic precincts, and coastal to high-altitude habitats (Ines *et al.*, 2016). They grow on the surface of every form of substrate including plants, rocks, and bare soils, and have been found in marine intertidal zones and freshwater streams, and on many man-made material surfaces (Nash, 2008).

The "Doctrine of Signatures" which stated that, "a plant could treat a disease it most looked like", was reported to form the basis of phytotherapy in traditional systems of medicine and Western medical herbalism (Brown, 2001). Fascinating enough, the word "Lichen" derived from the Greek word "Leikhen" meaning "Leprous" formed the basis of the use of lichens for the treatment of skin diseases as a result of their peeling skin appearance (Brown, 2001; Malhotra *et al*., 2008). Historically, several infectious diseases had been treated with herbal medications (Salvet *et al*., 2001; Geyid *et al*., 2005), and even in modern times, plants continue to play significant roles in primary health care as healing remedies in many developing countries of the world (Zakaria, 1991; Abayomi *et al*., 2013). The discovery of therapeutic agents in different parts of the world is crucial to establishing new approaches toward the propagation of alternative medicinal plants offering enhanced economic and social benefits to man. For instance, controlling bacterial infections using plants has been effective since the invention of antibacterial medicines, although, there are challenges of antibacterial resistance to first-generation antibiotics by several pathogens. The increasing rate of antimicrobial resistance alongside the emergence of undesirable side effects of certain antibiotics led to the quest for new antibacterial sources (WHO, 2024).

In ancient times, lichens such as *Rocella, Evernia, Lobaria, Pertusaria, Peltigera, Parmelia, Physica, Cladonia, Usnea,* and *Xanthoria* were used for medicinal purposes (Perez-Llano, 1944). Some lichen species were even reported to be good for treating jaundice, rabies, coughs and restoring lost hair (Pereira, 1853). In fact, the medicinal use of lichens has been traced back to the 1700 – 1800 BC when the lichen *Evernia furfuracea* (L.) Mann was first reportedly used as a drug (Launert, 1981). Natural products are recommended as good sources of therapeutic alternatives to orthodox antimicrobial treatments (Nimri *et al*., 1999). The diverse activities of lichen

substances have also been recognised, but, their curative potentials have not yet been explored in greater detail and thus remains less pharmaceutically unexploited (Müller, 2002; Rashmi and Rajkumar, 2014). It is well known that pathogenic microbes pose stern risks to human health, and are also increasing in prevalence in established healthcare settings due to the rising resistance against antibiotics (Sharma *et al.*, 2024). New alternatives for tackling the spread of diseases caused by antibiotic-resistant microbes are therefore necessary for keeping pace with the evolution of superbug pathogens. The objective of this study was therefore to analyse the methanol extract of *Punctelia jeckeri* – a lichen, for its lichen chemical substances and test the antibacterial potential of the extract on selected bacteria species.

MATERIALS AND METHODS

Collection of Lichen Sample. The plant material for this research work is a lichen species *Punctelia jeckeri* (Roum). Kalb. (Figure 1). The sample was collected at Olubuse Quarters, Ile-Ife, Osun state, Nigeria (Latitude: 7.51431 °N and Longitude: 4.56852 °E) from the bole of a *Tectona grandis* pole into a polythene bag, transported to the laboratory and identified at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The sample was air dried at the ambient temperature in the laboratory and then kept for further laboratory analysis.

FIG. 1. *Punctelia jeckeri* (Roum.) Kalb. Growing on the Bole of *Tectona grandis* Pole

Test Organisms. The test bacteria species used in the study included *Bacillus cereus* (NCIMB 532), *B. polymyxa, B. stearothermophillus* (NCIMB 8222), *B. subtilis* (NCIMB 3610), *Clostridium sporogenes* (NCIMB 532), *Klebsiella pneumoniae* (NCIMB 418), *Micrococcus luteus* (NCIMB 196), *Morganella* spp. (LIO), *Proteus vulgaris* (NCIMB 67), *Providencia rettgeri* (NTCT 1180), *Pseudomonas aeruginosa* (NCIMB 950), *Salmonella typhimurium* (LIO) ATCC14028, *Serratia marcescens* (NCIMB 1377) and *Staphylococcus aureus* (NCIMB 25923). The bacterial organisms were obtained from the Department of Microbiology, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria.

Extract Preparation. Extract of the lichen, *Punctelia jeckeri* was prepared by soaking the air-dried lichen sample (30 g) in 250 ml of methanol for 72 hours, after which the resulting suspension was filtered, using Whatman number 1 filter paper. The filtrate was concentrated in a rotary vacuum evaporator at 40℃ and evaporated to dryness in a glass Petri dish at room temperature to obtain the crude extract that was used for further laboratory analyses.

Qualitative Phytochemical Screening. Crude extract of *P. jeckeri* (50 mg) was dissolved in 5 ml methanol and sonicated for 45 minutes at 40 ℃ followed by centrifugation at 1,000 ×g for 10 minutes. The clear supernatant was collected and stored in an amber bottle for analysis (Suman *et al*., 2014). The presence of phytochemicals such as tannins, flavonoids, alkaloids, phenols, glycosides, resin, saponins, phlobatanins, carbohydrates, terpenoids, and sterols in the lichen methanol extract was tested using previously described standard methods (Abulude, 2007 and Tojola *et al*., 2019).

Determination of Total Phenol Content. The standard method by Singleton and Rossi (1965) as reported by the method of Gulcin *et al*. (2004) was used to determine the total phenolic content of the lichen extract. To twenty (20) ml of the sample and 86 ml of distilled water was added 40 ml of Folin-Ciocalteu's phenol reagent, after which the resulting mixture was vortexed and was allowed to stand for 5 minutes. A hundred (100) ml of 7% (w/v) NA_2C0_3 solution was added and the mixture was then distilled to 2.5 ml before incubating for 90 min at room temperature. The absorbance against a negative control containing 1 ml of water in place of the sample was then taken at 750 nm. The standard used was Gallic acid at 0.1 mg/ml to determine Gallic acid equivalent per dry lichen sample (GAE/g) based on a prepared standard calibration curve while distilled water was used as the blank.

Determination of Total Flavonoids Content. The total flavonoid content of the extract was determined based on the aluminium chloride colourimetric assay method (Miliauskas *et al.*, 2004; Suman *et al*., 2014) with slight modifications. Standard

quercetin with varying concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml was used as a standard in comparison to the sample extract. To 20 µl of extract and the standard was added 80 µl of distilled water. This was followed by 20 µl of 5% sodium nitrite. After 5 minutes, 20 µl of 10% aluminium chloride and 40 ml of sodium hydroxide were added and the solution was made up to 70 ml with distilled water. The absorbance of both the extract and the standard was measured against the blank at 510 nm. The total flavonoid content of the lichen extract was calculated from the calibration curve and expressed as mg quercetin equivalent per gram of the dried lichen extract (QE/g).

Determination of Total Alkaloid Content. To 1 ml of the plant extract (1 mg/ml), 1 ml of 2 N HCl was added and filtered. This filtrate was transferred to a separating funnel, and 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3, and 4 ml of chloroform by vigorous shaking, collected in a 10 ml volumetric flask, and then diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80, and 100 µg/ml) were prepared in the same manner as described above. The absorbance for test and standard solutions was determined against the reagent blank at 470 nm with a UV/visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract (Fazel *et al.*, 2008).

Determination of Total Tannins Content. The tannin content of the extract was determined by Folin-Ciocalteu's method (Miean and Mohamed, 2001; Vijay and Rajendra, 2014). The sample extract (0.1 ml) was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water, 5 ml of Folin-Ciocalteu's phenol reagent, and 1 ml 35% Na_2CO_3 solution, and then diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of Gallic acid (20, 40, 60, 80, and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for the test and the standard solutions were measured against the blank at 725 nm with a UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE/g of extract.

Screening for Anti-bacterial Activity. The prepared *P. jeckeri* crude extract was screened for antibacterial activity against the test bacteria using the agar well diffusion method (Akinpelu *et al*. 2015). The bacterial strains were grown in nutrient broth for 18 hours and standardized to 0.5 McFarland standard. Sterile Mueller-Hinton agar prepared in a McCartney bottle was allowed to cool to 45°C and then poured into a sterile Petri dish. This was allowed to set before inoculating with the standardised inocula of the test organism by spreading using a sterile swab stick. Wells were bored into the medium using a sterile 6 mm cork borer and plates were labelled appropriately.

With the aid of sterile pipette tips, the lichen extract (30 mg/ml) was transferred aseptically into the wells. Care was taken to avoid overflow of the extract onto the surface of the agar. The plates were left on the laboratory bench for one hour to allow proper diffusion of the extract through the medium. Then the plates were incubated in an upright position at 37°C for 24 hours after which the plates were observed for zones of inhibition. The diameter of the zone of inhibition was measured and recorded in millimetres. Streptomycin was used as the positive control at a concentration of 30 µg/ml while methanol was used as the negative control.

Determination of Minimum Inhibitory Concentration (MIC). The minimum inhibitory concentration (MIC) of *P. jeckeri* methanolic extract was determined as previously described (Akinpelu *et al.* 2015). Two-fold dilutions of the lichen extract were prepared and 2 ml of different concentrations of the solution was added to 18 ml of pre-sterilised molten nutrient agar in a McCartney bottle. The medium was then poured into sterile Petri dishes and left on the bench for 1 hour to set before streaking the standardised test organisms. The plates were incubated at 37°C for 48 hours after which the plates were examined for the presence or absence of growth. The lowest concentration preventing the growth of each susceptible bacterial strain was taken as the minimum inhibitory concentration (MIC) of the extract.

Determination of Minimum Bactericidal Concentration (MBC). The minimum bactericidal concentration (MBC) of *P. jeckeri* methanol extract was determined following the method of Wiley *et al.* (2004). Samples were taken from plates with no visible growth in the MIC assay plates and streaked on freshly prepared sterile nutrient agar plates without the extract and incubated at 37°C for 48 hours. The MBC was taken as the minimum concentration of the extract that prevents the growth of test bacterial strains.

RESULTS AND DISCUSSIONS

Qualitative Phytochemical Screening. The results of the qualitative phytochemical screening of the lichen, *P. jeckeri* methanol extract are presented in Table 1 below. Of the eleven phytochemicals assayed in the lichen, six were present. The results of the quantitative phytochemical analyses of phenol, flavonoid, alkaloid, and tannin contents of the extract are shown in Table 2.

Preliminary Sensitivity Test. The results of the preliminary sensitivity test of *P. jeckeri* methanol extract on the selected bacteria (Table 3) showed no activity by the extract against *B. subtilis* (NCIMB 3610), *P. aeruginosa* (NCIMB 950) and *S. marcescens* (NCIMB 1377) but was active against *B. cereus* (NCIMB 532), *B. polymyxa, B. stearothermophillus* (NCIMB 8222), *C. sporogenes* (NCIMB 532), *K. pneumoniae* (NCIMB 418), *M. luteus* (NCIMB 196), *Morganella*

spp (LIO), *P. vulgaris* (NCIMB 67), *P. rettgeri* (NTCT 1180), *S. typhimurium* (LIO) ATCC14028 and *S. aureus* (NCIMB 25923) at varied degrees.

TABLE 1. Qualitative Phytochemical Screening of *Punctelia jeckeri* Methanol Extract

Phytochemical		**Inference**
Tannins		+
Alkaloids		+
Phenols		+
Glycosides		-
Resin		-
Saponins		+
Phlobatanins		-
Carbohydrates		+
Terpenoids		-
Sterols		-
Flavonoids	Alkaline reagent test	+
	Lead acetate test	+

KEY: + = Presence of phytochemical; - = Absence of phytochemical

TABLE 2. Quantitative Phytochemical Analysis of *Punctelia jeckeri*

Phytochemicals	**Concentration**
Phenols	54.596 ± 0.274 mg GAE/g
Flavonoids	54.907 ± 2.589 mg QUE/g
Alkaloids	51.348 ± 0.489 mg AE/g
Tannins	29.836 ± 1.550 mg GAE/g

Values are expressed as the mean of three replicates (n=3) ± standard error

KEY: AE = Atropine equivalent; GAE = Gallic acid equivalent; QUE = Quercetin equivalent

TABLE 3. Preliminary sensitivity test of *P. jeckeri* methanol extract on selected bacterial species

		Zone of Inhibition ± Standard Deviation (mm)	
SN	Bacteria	Extract (30 mg/mL)	Streptomycin (30 µg/mL)
1	*B. cereus* (NCIMB 532)	18.0 ± 0.0	13 ± 1.0
2	*B. polymyxa*	18.5 ± 0.5	14.5 ± 0.5
3	*B. stearothermophillus* (NCIMB 8222)	14.5 ± 0.5	16.0 ± 0.0
4	*B. subtilis* (NCIMB 3610)	0.0 ± 0.0	0.0 ± 0.0
5	*C. sporogenes* (NCIMB 532)	19.0 ± 1.0	12.5 ± 0.5
6	*K. pneumoniae* (NCIMB 418)	12.0 ± 0.0	0.0 ± 0.0
7	*M. luteus* (NCIMB 196)	12 ± 0.0	13.5 ± 0.5
8	*Morganella* spp (LIO)	15.5 ± 1.5	13.5 ± 0.5
9	*P. vulgaris* (NCIMB 67)	13.0 ± 1.0	0.0 ± 0.0
10	*P. rettgeri* (NTCT 1180)	15.5 ± 0.5	13.5 ± 0.5
11	*P. aeruginosa* (NCIMB 950)	0.0 ± 0.0	0.0 ± 0.0
12	*S.* Typhimurium (LIO) ATCC14028	12.0 ± 0.0	13.5 ± 0.5
13	*S. marcescens* (NCIMB 1377)	0.0 ± 0.0	0.0 ± 0.0
14	*S. aureus* (NCIMB 25923)	16.5 ± 0.5	12.5 ± 0.5

Values are expressed the as mean of three replicates (n=3) ± standard error

KEY: LIO = Locally Isolated Organism, NCIMB = National Collection of Industrial, Food and Marine Bacteria, NTCT = National Collection of Type Cultures

Minimum Inhibitory Concentration. The results of the minimum inhibitory concentration test of *P. jeckeri* methanol extract on the selected bacteria are presented in Table 4.

TABLE 4. Minimum inhibitory concentration of *Punctelia jeckeri* methanol extract on tested bacterial species

		Minimum Inhibitory Concentration	
SN	Bacteria	Extract (mg/mL)	Streptomycin (µg/mL)
1	*B. cereus* (NCIMB 532)	0.31	3.75
2	*B. polymyxa*	0.31	3.75
3	*B. stearothermophillus* (NCIMB 8222)	0.31	3.75
4	*B. subtilis* (NCIMB 3610)	-	-
5	*C. sporogenes* (NCIMB 532)	0.31	3.75
6	*K. pneumoniae* (NCIMB 418)	5.00	-
7	*M. luteus* (NCIMB 196)	0.63	3.75
8	*Morganella* spp (LIO)	0.31	3.75
9	*P. vulgaris* (NCIMB 67)	5.00	-
10	*P. rettgeri* (NTCT 1180)	0.63	3.75
11	*P. aeruginosa* (NCIMB 950)	-	-
12	*S.* Typhimurium (LIO) ATCC14028	1.25	7.50
13	*S. marcescens* (NCIMB 1377)	-	-
14	*S. aureus* (NCIMB 25923)	0.63	15.00

KEY: LIO = Locally Isolated Organism, NCIMB = National Collection of Industrial, Food and Marine Bacteria, NTCT = National Collection of Type Cultures**,** - =Not Determined

TABLE 5. Minimum Bactericidal Concentration (MBC) of *P. jeckeri* methanol extract on selected bacteria

		Minimum Bactericidal Concentration	
SN	Bacteria	Extract (mg/mL)	Streptomycin (µg/mL)
1	*B. cereus* (NCIMB 532)	NBE	7.3
2	*B. polymyxa*	5.00	3.75
3	*B. stearothermophillus* (NCIMB 8222)	0.63	3.75
4	*B. subtilis* (NCIMB 3610)	-	-
5	*C. sporogenes* (NCIMB 532)	5.00	3.75
6	*K. pneumoniae* (NCIMB 418)	NBE	-
7	*M. luteus* (NCIMB 196)	2.50	15
8	*Morganella* spp (LIO)	NBE	3.75
9	*P. vulgaris* (NCIMB 67)	NBE	-
10	*P. rettgeri* (NTCT 1180)	2.50	3.75
11	*P. aeruginosa* (NCIMB 950)	-	-
12	*S.* Typhimurium (LIO) ATCC14028	5.00	15
13	*S. marcescens* (NCIMB 1377)	-	-
14	*S. aureus* (NCIMB 25923)	5.00	15

KEY: LIO = Locally Isolated Organism, NCIMB = National Collection of Industrial, Food and Marine Bacteria, NTCT = National Collection of Type Cultures**,** - = Not determined; NBE = No bactericidal effect recorded

Minimum Bactericidal Concentration (MBC). The results of the minimum bactericidal concentration test of *P. jeckeri* methanol extract on the selected bacteria are

presented in Table 5. The minimum bactericidal concentration recorded varied from one organism to the other and ranged between 0.625 mg/mL on *B. polymyxa* to 5 mg/mL on *S. aureus* and *S. typhimurium.*

In this study, the presence of phenols, flavonoids, alkaloids, and tannins in *P. jeckeri*, and its activity on selected pathogenic bacteria species were revealed. The recorded antibacterial activity could be attributed to the presence of the bioactive compound in *P. jeckeri* (Mustafa, 2012). Historically, humans have utilised plants and plant-derived compounds to cure ailments and treat infections, and there has been extensive research into the therapeutic potential of various secondary chemicals found in plants in recent years (Najmun *et al*., 2022). Several plant species have been evaluated for naturally occurring compounds with beneficial therapeutic functions to humans (Gan *et al*., 2018; Jan and Abbas, 2018). However, less extensive work has been conducted on lichens in this regard. In this study, *P. jeckeri*, a species in the family *Parmeliaceae* was screened to qualitatively and quantitatively determine the composition of its secondary substances, and as well test its antibacterial activity, as a strategy to assess its therapeutic potential. Secondary metabolites, including tannins, flavonoids, alkaloids, phenols, and saponins present in *P. jeckeri* in this study have been reported to play crucial roles in producing therapeutic effects in humans. (Mutha *et al*., 2021). Alkaloids are analgesic, and saponins are implicated in the treatment of human cardiovascular diseases and in reducing blood cholesterol (Abulude, 2007). The presence of phenolic compounds including the flavonoids in the methanol extract of *P. jeckeri* in this study is an indication of its potential as a good source of antioxidants, anti-inflammatory and neuroprotective agents (Al-Mamari, 2022; Mutha *et al*., 2021).

The antimicrobial properties of the methanolic extract of *P. jeckeri* evaluated by the disc diffusion method expressed broad-spectrum antimicrobial activities against the tested bacterial strains. The antibacterial effect of the extract on the tested bacteria indicates the applicability of *P. jeckeri*'s bioactive compounds in the treatment of infections caused by the tested bacteria pathogens. This can be timely because the use of antimicrobials in modern medicine has led to the development of resistance to an array of hitherto potent antimicrobial agents, thereby putting antimicrobial resistance on the front burner as a threat to public and global health (WHO, 2024). The inhibitory and bactericidal effects exhibited by the methanol extract of *P. jeckeri* against the tested bacterial species in this study compared well with those of the ethanolic extracts of notable plant materials that had been reported (Hemeg *et al*., 2020). Using only methanolic extracts of *P. jeckeri* may have limited the chance of comparing the efficacy of methanolic extraction with other extraction solvents. However, the study of Ghanimi

et al. (2022) on *Lavandula mairei* Humbert showed that flavonoids and phenolic contents were significantly higher in the methanolic extract of *P. jeckeri*. Nevertheless, further studies are needed to ascertain the relative suitability of different extraction solvents for the characterisation of other possible bioactive compounds in *P. jeckeri*.

CONCLUSIONS

This study revealed the presence of bioactive compounds which included alkaloids, saponins, phenols, tannins, and flavonoids in *P. jeckeri*. These detected lichen substances can be implicated in the production of potent medicinal drugs. The antibacterial activities exhibited by the extract against the tested bacterial species further confirmed the inherent potential of *P. jeckeri* for the manufacture of antimicrobials for the treatment of various diseases attributed to each of the tested and susceptible bacterial species.

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