RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume 59 Issue 12: 1941-1946 (December 2025)

Bark Anatomy of *Podocarpus elongatus* (Ait) L. Herit and Screening of Phytochemical Constituents with Antimicrobial Potential

Arunthathi[1], J. Valentina[1], T.V. Poonguzhali[1]

10.18805/IJARe.A-5780

ABSTRACT

Background: Conifer *Podocarpus elongatus* belongs to the family Podocarpaceae and some species of *Podocarpus* are used in traditional medicine. The present study was undertaken to study the anatomical features of the bark of *Podocarpus elongatus* (Ait) L.Herit, The study was also focused on the antimicrobial activity and phytochemicals present in the bark extract.

Methods: The bark was collected from the *P. elongatus* from Nilgiri Hills of Ooty Tamil Nadu. The anatomical structures of the bark were elucidated macroscopically and microscopically by using the sectioning techniques. The phytochemical examination was done to confirm the presence of phytochemical components in the methanol extract. The antimicrobial activity was also studied based on the zone of inhibition.

Result: The surface of the bark was highly distinguished from other related species, in the pattern of colour, stripes and thickness. Microscopical study envisaged that periderm is characterised by well distinct, continuous structures of the branchy sclereids with thick undulate, continuous segments that have been originated from a deeper cortex. TLS and RLS of the bark view elucidated that the phloem component appeared to be a vertical plane in the fibres. Phloem rays were found as uniseriate with wide sieve cells. Furthermore, antimicrobial activities of the bark exhibited potential effects against examined bacterial and fungal strains.

Key words: Antimicrobial potentials, Bark anatomy, Phytoconstituents, *Podocarpus elongatus*.

INTRODUCTION

Podocarpus elongatus, the Breede River yellowwood, belongs to conifers in the family Podocarpaceae. It can be identified by its relatively elongated, grey-blue leaves. *P. elongatus* normally grows approximately as wide as it is tall and its foliage ranges very low, similar to a hut in the habit. The primary branches characterised as pseudo-whorls around the trunk. There are about 17 genera and 125 species in this family (Chan, 1985) (Fig 1). Compared to wood, studies on the anatomy of bark are relatively scanty. Very few extensive studies have been made on the bark of gymnosperms. In recent decades, anatomical characteristics of the bark were described for the Cupressaceae family, native species of South America (De Magistris and Castro, 2001). Robinson and Grigor (1963) examined the origin of the periderm in some New Zealand plants including eight members of the Podocarpaceae family. Studies on the African species of *P. elongates* is scanty compared to those of New Zealand, Australia, China and Japan (Abdillahi *et al*., 2010; Kucera and Butterfield, 1977). Hence the present study investigates the anatomy of *P. elongatus* bark based on macroscopic and microscopic observations.

The global occurrence of diseases caused by bacteria is a foremost health problem. Plants are the potential source that has antimicrobial properties due to the presence of tannins, terpenoids, alkaloids and flavonoids. Crude plant extracts are traditionally used as herbal medicine for several human infectious diseases (Gonelimali *et al*., 2018; Canaparo *et al.,* 2019). Several studies on phytochemical constituents and biological activities on podocarpus genus, in specific Himalayan Conifer of Nepal was investigated (Gautam *et al.,* 2005). Hence, in addition to bark structure, the present study also aims to detect the phytochemical constituents and the antimicrobial potential that are present in the bark extract.

[1]Department of Botany, Queen Mary's College, Chennai-600 004, Tamil Nadu, India.

Corresponding Author: T.V. Poonguzhali, Department of Botany, Queen Mary's College, Chennai-600 004, Tamil Nadu, India. Email: pooqmc@gmail.com

How to cite this article: Arunthathi, Valentina, J. and Poonguzhali, T.V. (2025). Bark Anatomy of *Podocarpus elongatus* (Ait) L. Herit and Screening of Phytochemical Constituents with Antimicrobial Potential. *Indian Journal of Agricultural Research*. **59(12):** 1941-1946. doi: 10.18805/IJARe.A-5780.

Submitted: 27-03-2021 **Accepted:** 02-07-2021 **Online:** 16-08-2021

MATERIALS AND METHODS

Bark anatomy

The bark from the *P. elongatus* was collected from Nilgiri hills of Ooty Tamil Nadu and fresh bark collected from the top of the branch was kept in formalin-acetic and alcohol (FAA). The dry parts were dehydrated, before fix in FAA for 24 hrs. The study was conducted from September 2019 to March 2020, in the Department of Botany, Queen Mary's

College, Chennai, India. Using a rotary microtome, transverse, tangential and radial bark sections, measuring 15-30 µm thick were cut.

A standard protocol was used for a light microscopic study based on Carlquist, 2000. The staining was made using Toluidine Blue for 1 minute, before a wash with water or 1:1 Alcian blue safranin mixer. The obtained sections were mounted properly in Euparol mounting fluid. The measurements of bark components were performed by employing the UTHSCSA Image tool Version 3 software package (Brent. All sections were photographed with a KY-F1030 digital camera, annexed with a Leitz Wetzlar Ophthoplan microscope. Bark fragments were also entrenched in glycol methacrylate (GMA) described by Feder and 'O Brien (1968) method with some modifications. Transverse, tangential and radial sections were cut with a thickness of 15 µm subsequently stained with toluidine blue before mounting in Entellan (Prodhan and Sarkar, 2002; Martin and Crist, 1970).

Phytochemical screening

The phytochemical constituent present in the methanol bark extracts was examined based on the solubility colour change and precipitation after the addition of specific reagents (Joseph *et al.* 2016). The phytochemicals such as Tannins, Saponins, Flavonoids, Alkaloids, Proteins, Steroids, Anthroquinones and Phenol were detected based on the method described by Valentina *et al.* (2015).

Tannin

1 mL of the sample was taken in a test tube and 0.1% Ferric chloride was added dropwise to yield brownish-green or blue colour, indicating the presence of tannin.

Saponin

1 mL of the sample was mixed with 2 mL of water and shaken well using a graduated cylinder for 15 min. A foam-like layer indicating the presence of Saponin.

Flavanoid

1 mL of the extract was added to NaOH solution to give yellow colour, subsequently turned to white colour when concentrated HCl was added.

Alkaloids

A few drops of dragandoff reagent was added to the extract and mixed to get a clear yellow precipitate indicating the result of the presence of Alkaloids.

Steroid

1 mL of the extract was added with 2 drops of 10% concentrated H_2SO_4 to yield brown colour and visualized.

Anthraquinones

1 mL of the sample was mixed with 3 mL of 10% Lead acetate solution a sudden transformation of colour was observed. The pink or red colour in the aqueous layer proves the occurrence of anthraquinones.

Phenol

4 ml of bark extract was mixed with 3 mL of 10% Lead Acetate solution, a visualized white precipitation was observed that indicates the presence of a phenolic component.

Antimicrobial activity of *P. elongatus* bark

In the present investigation, nutrient growth media was prepared with a pH of 6.8 and sterilized in an autoclave at 12°C for 20 min. Pure culture of bacteria such as *Staphylococcus aureus, Lactobacillus acidophilus, Vibrio alginolyticus* and *Salmonella typhii* and two fungal strains such as *C. albicans* and *Rizhopus* species were selected for the anti-microbial study.

The antibacterial ability of bark methanolic extract of *P. elongates* was performed, using the diffusion method. The prepared inoculum was inoculated on Muller Milton Agar Medium (MHM) was used for bacteria and SDA media was used for the fungal species. After placing the disc on the plated, 20 µl of bark extract with varied concentration such as 20 µg, 15 µg and 10 µg for 1000 µg/mL, 750 µg/mL and 500 µg/mL respectively were kept on the disc and incubated at 37 for 24 h. By measuring the diameter of the zone of inhibition, using a ruler, the antibacterial ability of extract was determined (Zaidan *et al.,* 2005).

RESULTS AND DISCUSSION

Bark anatomical observations

The bark anatomical structure (Microscopic) *of P. elongatus* revealed that the thickness of the bark was found to be 500 µm. The bark constitutes a deep-seated periderm, followed by a thick cortex and very hard secondary phloem. The periderm is characterized by a fairly thick, undulate and continuous segment and originated from the deeper of the cortex. Periderm involves 3-4 layers of phellem, which seems to be dark, attributable to the gathering of tannin was observed in *P. elongatus*. Consequently, phelloderm cells were found with four layers and were squarish in shape. In specific, a rectangular narrow layer, beneath the phellem was noticed. Usually, phelloderm cells are found 3-5 layers of phellem shown to be radially elongated along with dense



Fig 1: Podocarpus elongatus tree.

cell organelles. The thickness of the cortex was 100 µm and cortical cells showed a horizontally elongated and cylindrical shape. Moreover, a few cortical cells contain a dense accumulation of tannin was detected in *P. elongatus* bark (Fig 2).

Secondary phloem

occurs with 400 µm thickness and it was considered to be the thickest region of the bark. The zone of collapsed phloem constituted and represented with dilated rays, undilated, masses of sclereids, phloem fibres and dilated parenchyma cells of phloem with collapsed sieve cells. The undilated rays have shown to be isolated uniseriate and are being radically elongated, rectangular, with numerous dilated rays thick cells. The cells were angular, oblong, isolated and randomly distributed branchy sclereids were found abundant in the collapsed phloem segment *of P. elongatus*. The fibres of phloem were varied in shape and size. Few fibres were rectangular including an elliptical lumen, the rest of the cells were small and a polygonal shape was observed (Fig 2).

Zone of non-collapsed phloem

The microscopical observation of the zone of non-collapsed phloem envisaged that it consisted of the thick radial segment covering phloem fibres and appeared in regular horizontal lines. Subsequent cells of the fibre were known to be sieve cells, which seems to be rectangular in size and shape and distinguishable based on the size. The fibres appeared 20-30 µm in the tangential plane and 15 µm in radial plane respectively, whereas sieve cells were found 40 µm in the horizontal plane and 15 µm in radial plane respectively. The parenchyma cells appeared in polyhedral or squarish shape (Fig 3).

Crystal dispersement

The phloem and periderm were found, abundantly distributed with calcium oxalate crystals, which are tiny and glandular. Crystals were noticed in a thin line along the radial walls of phloem parenchyma, sieve cells and fibres obviously and they extended their appearance as thick bands, along the tangential walls in the periderm tissues (Fig 3).

Observations on the tangential longitudinal section (TLS)

On the view of the longitudinal section of *P. elongatus,* it was important to note that, phloem elements appeared to be in a vertical position as mentioned and shown to have fibres with lignified walls as depicted in the slide. Phloem rays represented as non-storied and specifically, uniseriate and heterocellular. The ray cells contain squarish cells in the middle, while margins constitute triangular cells. The range of ray cells includes 3-20 cells in height. They were measured as 60-220 µm height and 25-30 µm thick respectively.

The specified ray cells were not lignified. Phloem parenchyma cells have been found in narrow and vertical strands. The other cells visualized as darkly stained amorphous bodies (Sieve cells were appeared wide rather than parenchyma cells and covered profound sieve areas on the lateral side of the walls consequently, sieve cells showed oblique end walls.

The radial longitudinal section (RLS) of *P. elongatus* showed that phloem rays were found as thick ribbon or horizontal thick body. Ray cells were seen thick,

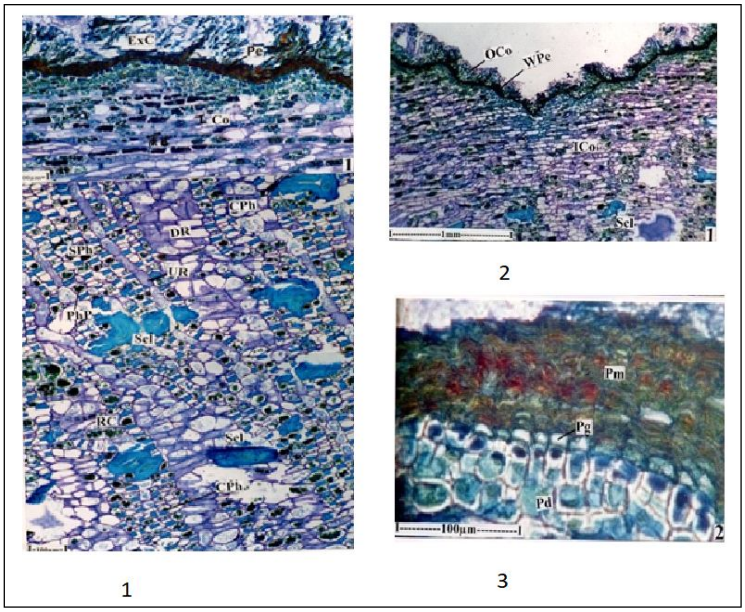

Fig 2: (1) T.S of bark entire view showing Periderm, cortex and inner bark, namely Secondary Phloem. (Co: Cortex (Inner); Cph: Collapsed phloem; SPh: Secondary Phloem; PhP: Phloem Parenchyma; Scl: Sclereids; Pe: Periderm; UR: Undilated Ray; TC: Tanniniferrous Cells. (2) T.S of bark showing periderm and cortical zone. (3) Periderm enlarged showing in three regions phellem, phellogen and phelloderm (ICo: Inner Cortex; OCo: Outer Cortex; Pd: Phelloderm; Pg: Phellogen; Pm: Phellem; WPe: Wavy Periderm).

cylindrical and thick-walled. It was noteworthy that no distinguishable found between procumbent and upright cells and were heterocellular. Sclerenchyma elements, fibres and sclereids were oriented vertically while parenchyma cells found in vertical strands. They contain few dark inclusions. The orsclereids have found to be large, rectangular, wedge-shaped squarish in shape (Fig 4).

Phytochemical constituents and antimicrobial activities

Phytochemical screening of the bark extract envisaged that the plant possesses tannins, flavonoids, proteins and

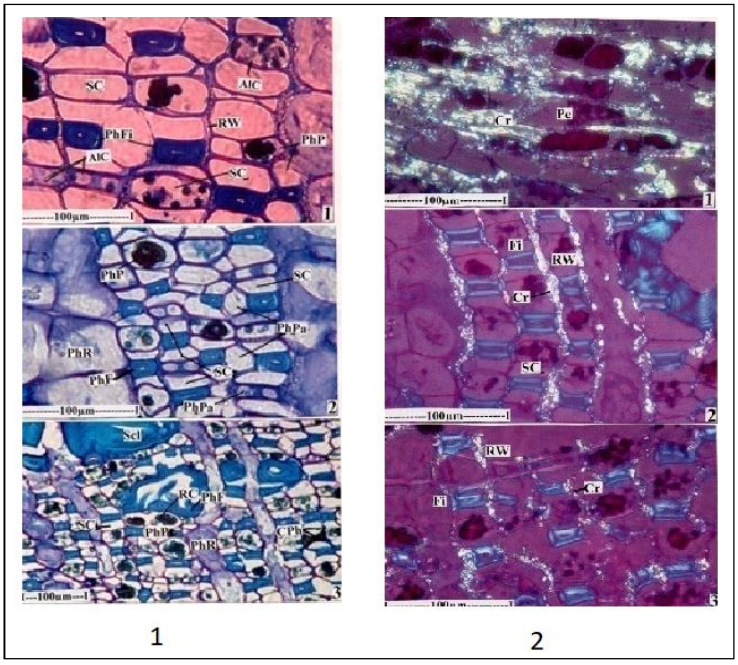

Fig 3: (1) Non-collapsed phloem elements, Dilated ray and radial belt of non-collapsed phloem, Collapsed phloem showing crushed Sieve cells, reduced fibres and slightly dilated Rays. (CPh: Collapsed Phloem; PhF: Phloem Fibres; PhP: Phloem Parenchyma; PhR: Phloem Ray; RW: Radial Wall; SCe: Sieve cells; Scl: Sclereid) (2) Crystal distribution in the periderm, Crystal distribution along the radial walls of phloem elements. (Cr: Crystal; Fi: Fibre; Pe: Periderm; RW: Radial Walls).

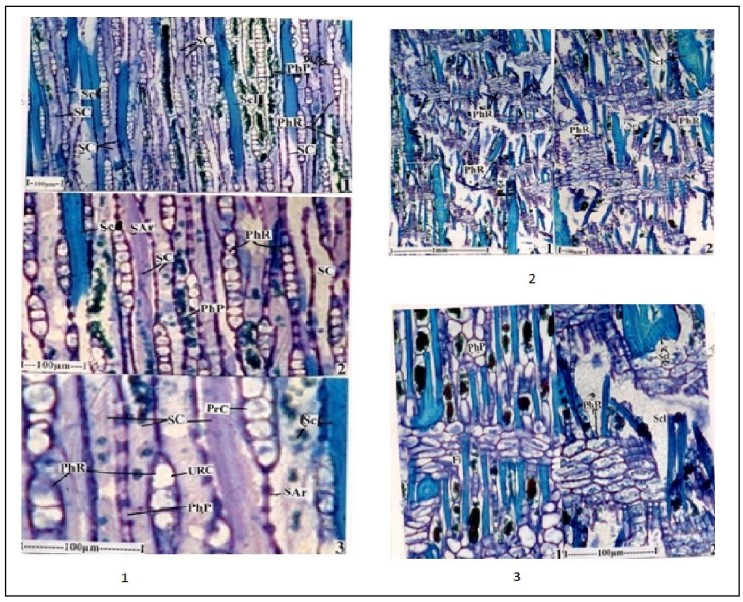

Fig 4: (1) TLS view of phloem showing phloem rays, Phloem fibres, parenchyma and sieve cells, Phloem rays and, parenchyma and sieve cells enlarged (SAr: SieveArea; SCe: SieveCells; PhP: PhloemParenchyma; PhR: PhloemRay; Sc: Sclerenchyma (Fibres); TaC: Tanniniferous Cells). (2) RLS of phloem showing horizontal Rays vertical fibres and other vertical cells (PhR: Phloem Ray; PSc: Phloem Sclerenchyma). (3) RLS view of Phloem (PhP: Phloem Parenchyma; PhR: Phloem Rays; Fi: Fibres; Scl: Sclereids).

Table 1: Phytochemical screening of the bark of *P. elongatus.*

Sample	Test						
P. elongates	Tannins	Saponins	Flavonoids	Alkaloids	Steroids	Anthraquinones	Phenol
	+	-	+/+	-	+	-	-

(+) = Positive, (-) = Negative.

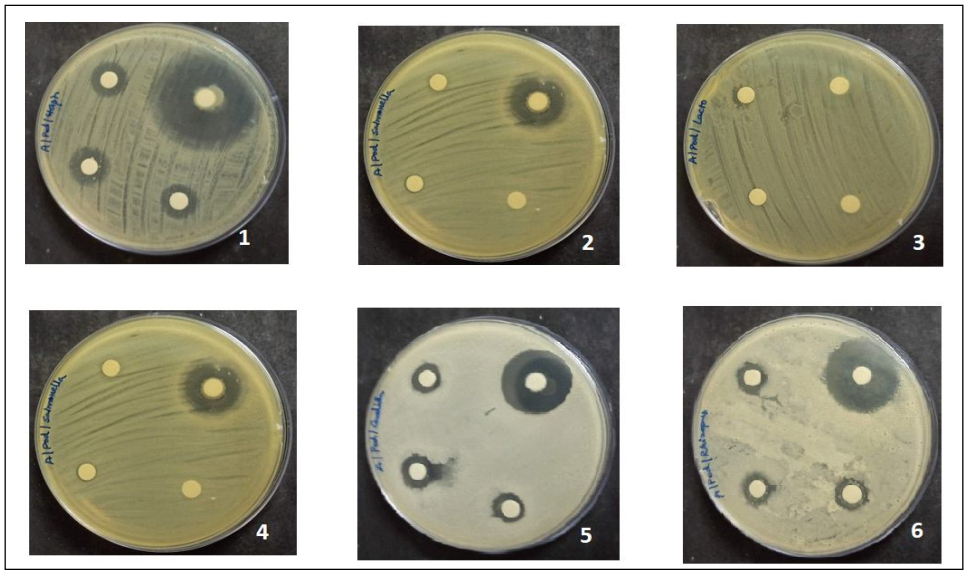

Fig 5: Antimicrobial activity of *P. elongatus*. (1) *Staphylococcus aureus* (2) *Salmonella Typhi* (3) *Lactobacillus acidophilus* (4) *Vibrio alginolyticus* (5) *Candida albicans* (6) *Rizhopus.*

steroids (Table 1). Negative results were detected for saponin, anthraquinones and phenols. The detected active biomolecules that occurred in the present investigation exhibited antimicrobial activity against all selected pure bacterial and fungal strains. Many studies emphasized that alkaloid, tannins and glycoside have been exhibited and they are the potential for anti-microbial actions (Banso, 2009; Gentallan *et al.,* 2019).

However, in the present investigation, tannin and flavanoids were excess and might have been responsible for antimicrobial efficiency. Further, Recent studies have deciphered that presence of Tannins and flavonoids has been reportedly inhibiting the development of microorganisms, targeting the microbial protein to fulfil their unavailability (Rani *et al.,* 2017). Hence, it has been presumed that active phytochemical present in the methanol bark extract, helps to exhibit a moderate antimicrobial potential which also suggested by Bagheri *et al.,* 2020.

The zone of inhibition indexed the ability of active principles of the plant to elevate the activity on the concentration dependant manner as illustrated by the value of the diameter of the zone of inhibition (Arora and Onsare, 2014). Table 2 indicated the antimicrobial activity of methanol extract of *P. elongatus* bark. The analysis revealed that methanol bark extract showed significant inhibition against *S. aureus* at the concentration of 1000 µg /mL and the zone of inhibition was recorded as 13 mm and 12 mm and 11 mm at the concentration of 1000 µg/mL, 750 µg/mL, 500 µg/mL respectively (Fig 5). However, positive control (antibiotic) was shown to be 29 µm. It was noteworthy that inhibition effects were observed relatively less against *S. typhii*, in varied concentration and not close to the effects of control, whereas against *L. acidophillus* and *V. alginolyticus* exhibited a moderate inhibition effect and rate of inhibition was maximum of 10 mm was observed in both strains at 1000 µg/mL. Arora and Mahajan, 2018 screened the antimicrobial activity of aqueous extract from the bark of wild Himalayan cherry (*Prunus cerasoides*) which showed the potential activity against various pathogenic microorganisms with inhibition zone ranging from 19 to 24 mm. Similarly, *S. Albicans* showed better effects rather than *Rizhopus* species. It was recorded as 14, 12 and 11 mm zone of inhibition against *C. albicans* with a concentration of 1000, 750 and 500 µg/mL and it exhibited 60.8% whereas *Rizhopus is* shown to have possessed 58% of MIC (Table 3).

Some species of Podocarpus are used in systems of traditional medicine for fever, coughs, arthritis, sexually transmitted disease and canine distemper (Abdillahi *et al*., 2011). This could be due to the presence of different phytochemical constituent and their potential activity. The bark studies also revealed the presence of calcium oxalate crystals and resin canals which are the characteristics of *Podocarpus elongatus* bark. Madivoli *et al.* (2019) studied the antimicrobial activity of *Prunus africana* and *Harrisonia abyssinic*a bark and revealed that the methanolic extracts of both plants showed moderate antibacterial activity.

Table 2: Anti-bacterial activity of *Podocarpus elongatus* bark.

Organisms	Zone of Inhibition (mm) Concentration (µg/mL)			Antibiotic (1 mg/ml)
	1000	750	500	
Staphylococcus aureus	13	12	11	29
Lactobacillus acidophilus	10	8	7	18
Vibrio alginolyticus	10	9	8	19
Salmonella Typhi	8	8	7	22

Table 3: Antifungal activity of *Podocarpus elongates.*

Organisms	Zone of Inhibition (mm) Concentration (µg/mL)			Antibiotic (1 mg/mL)
	1000	750	500	
Candida albicans	14	12	11	23
Rizhopus	16	13	2	27

CONCLUSION

P. elongatus bark extract exhibited an efficient antimicrobial activity against tested organism including bacteria and fungi. The present study has inferred that anatomical structure deciphered for a better understanding and it can be used as a reference for the bark anatomical studies of various species phylogenetically relating to the *Podocarpus elongatus*.

Conflict of interest

All authors declared that there is no conflict of interest.

REFERENCES

Abdillahi, H.S., Finnie, J.F. and Van Staden, J. (2011). Anti-inflammatory, antioxidant, anti-tyrosinase and phenolic contents of four Podocarpus species used in traditional medicine in South Africa. *Journal of ethnopharmacology*. **136(3):** 496-503.

Abdillahi, H.S., Stafford, G.I., Finnie, J.F. and Van Staden, J. (2010). Ethnobotany, phytochemistry and pharmacology of *Podocarpus sensu latissimo* (sl). *South African Journal of Botany*. **76(1):** 1-24.

Arora, D.S. and Mahajan, H. (2018). *In vitro* evaluation and statistical optimization of antimicrobial activity of *Prunus cerasoides* stem bark. *Applied Biochemistry and Biotechnology*. **184(3):** 821-837.

Arora, D.S. and Onsare, J.G. (2014). *In vitro* antimicrobial potential, biosafety and bioactive phytoconstituents of *Moringa oleifera* stem bark. *World J. Pharm Res*. **3:** 2772-2788.

Bagheri, G., Ayatollahi, S.A., Ramírez-Alarcón, K., Salehi, B., Mohammadi, R., Rahmani, A, Amarowicz, R. (2020). Phytochemical screening of *Alstonia venenata* leaf and bark extracts and their antimicrobial activities. *Cellular and Molecular Biology*. **66(4):** 224-231.

Banso, A. (2009). Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. *Journal of Medicinal Plants Research*. **3(2):** 82-85.

Canaparo, R., Foglietta, F., Giuntini, F., Della Pepa, C., Dosio, F. and Serpe, L. (2019). Recent developments in antibacterial therapy: Focus on stimuli-responsive drug-delivery systems and therapeutic nanoparticles. *Molecules*. **24(10):** 1991.

Carlquist, S. (2012). Wood anatomy of Gnetales in a functional, ecological and evolutionary context. *Aliso: A Journal of Systematic and Evolutionary Botany*. **30(1):** 33-47.

Chan, L.L. (1985). The anatomy of the bark of Libocedrus in New Zealand. *IAWA Journal*. **6(1):** 23-34.

De Magistris, A.A. and Castro, M.A. (2001). Bark anatomy of southern South American Cupressaceae. *IAWA Journal*. **22(4):** 367-383.

Feder, N.E.D. and O'brien, T.P. (1968). Plant microtechnique: some principles and new methods. *American Journal of Botany*. **55(1):** 123-142.

Gautam, L.M., Shrestha, S.L., Wagle, P. and Tamrakar, B.M. (2008). Chemical constituents from *Vitex negundo* (Linn.) of Nepalese origin. *Scientific World*. **6(6):** 27-32.

Gentallan, J.R., Altoveros, N.C., Borromeo, T.H. and Macabecha, C.G.A. (2019). Antimicrobial and Phytochemical Properties of *Atuna racemosa* Raf. Kernel Extract. *Indian Journal of Agricultural Research*. **53(6):** 733-736. doi: 10.18805/IJARe.A-415.

Gonelimali, F.D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M. and Hatab, S.R. (2018). Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Frontiers in Microbiology*. **9:** 1639.

Joseph, N., Anjum, N. and Tripathi, Y.C. (2016). Phytochemical screening and evaluation of polyphenols, Flavonoids and Antioxidant Activity of *Prunus cerasoides* D. Don Leaves. *Journal of Pharmacy Research*. **10(7):** 502-508.

Kucera, L.J. and Butterfield, B.G. (1977). Resin canals in the bark of Phyllocladus species indigenous to New Zealand. *New Zealand Journal of Botany*. **15(4):** 657-663.

Madivoli, E.S., Maina, E.G., Kairigo, P.K., Murigi, M.K., Ogilo, J.K., Nyangau, J.O. and Kipyegon, C. (2018). *In vitro* antioxidant and antimicrobial activity of *Prunus africana* (Hook. f.) *Kalkman* (bark extracts) and *Harrisonia abyssinica* Oliv. extracts (bark extracts): A comparative study. *Journal of Medicinal Plants for Economic Development.* **2(1):** 9.

Martin, R.E. and Crist, J.B. (1970). Elements of bark structure and terminology. *Wood and Fiber Science*. **2(3):** 269-279.

Prodhan, A.K.M. and Sarkar, D.N. (2002). Root and stem anatomy of *Sesbania rostrata*. *Indian Journal of Agricultural Research*. **36(1):** 1-9.

Rani, J., Kapoor, M. and Kaur, R. (2017). In-vitro anti-bacterial activity and phytochemical screening of crude extracts of *Catharanthus roseus* L. (G.) Don. *Agricultural Science Digest.* **37(2):** 106-111. doi: 10.18805/asd.v37i2.7983.

Robinson, D.E. and Grigor, J.K. (1963). The Origin of Periderm in Some Mew Zealand Plants.

Valentina, J., Poonguzhali, T.V. and Josmin, L.N.L.L. (2015). Phytochemical analysis of selected seaweeds collected from Mandapam Coast in Rameshwaram, Tamil Nadu, India. *International Journal of Advanced Research*. **3(7):** 972-976.

Zaidan, M.R., Noor Rain, A., Badrul, A.R., Adlin, A., Norazah, A. and Zakiah, I. (2005). In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Trop Biomed*. **22(2):** 165-170.