



RESEARCH ARTICLE

A COMPREHENSIVE STUDY ON THE BARK ANATOMY, PHYTOCONSTITUENT AND DETERMINATION OF ANTIMICROBIAL EFFICACY OF *ARAUCARIA COOKII*

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Abstract

Araucaria cookii, a peculiar ornamental exotic conifer found in India and prevalent in varied European countries and the United States. These have exhibited diverse therapeutic properties, but still evidently unclear. Bark anatomy has been contributed significantly in the analytical observation of its potential and found very scanty in published literatures. In present study, we studied the anatomical observation of the bark of *A. cookii*, and identify the anti-microbial activities of methanol bark extract of *A. cookii*. The bark of *A. cookii* was collected from the *A. cookii* from Nilgiri Hills of Ooty Tamil Nadu. The anatomical structures of the bark were elucidated, based on the pattern of the bark, macroscopically and microscopically by using the techniques such as tangential longitudinal section (TLS) and radial longitudinal section (RLS). The phytochemical examination of the methanol bark extract was done to confirm the presence of phytochemical components. The antimicrobial activity of the bark extract was also studied based on the zone of inhibition. The phytochemical constituents detected were presumed to be responsible factors for its medicinal features. Thus, our results showed that the bark of *A. cookii* could act as a possible antimicrobial natural source against probable infectious pathogens.

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Introduction:-

In our country, one of the popular traditional medicinal systems is Ayurveda, and still in use by the majority section of the rural populations (Banerjee & Ganguly, 2014). Based on the WHO Global report on Traditional and Complementary Medicine (2019), traditional medicine could be an option used by a people centered health system that manages ailments services with preventive care. Use of plant sourced drugs, for the management of common diseases has been found indiscriminate in the majority of the aged population. In India, several pharmacological researches involve clinical potentials of the plants and their realistic use in the health care sector. It has been reported that about 20,000 plants contain medicinal properties, among them 7000-7500 plants are being utilized by traditional medical practices (Samal, 2016).

Araucariaceae family is an exotic gymnosperm comprises dominant flora on the earth (Uniyal and Aswathi, 2000). The unique characteristic features of gymnosperms represent lack of vegetation, reproduction, by adopting cutting, layering and slow grower. Ovules are unshielded and absence of vessels in xylem and companion cells. *A. cookii* is popularly called as "Christmas tree", belongs to ornamental plants, reportedly grown in tropical lands, and a

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common evergreen conifer that grows about 7 feet height (Banerjee et al., 2014). It is an exotic species usually, found in New Zealand, South California, Mexico and Hawaii. While compared with other woody plants, only limited literature on the anatomy of bark has been described in published sources,. Therefore, the present study attempted to find the anatomy of the bark of *A. cookii*. Antimicrobial potentials help in preventing the effectiveness and examining virulent nature of the pathogenic organisms. Many of phytochemical studies reported, a wide spectrum of phytoconstituents responsible for antimicrobial ability with other desirable properties used to the human health (Bhaigybati et al, 2020, Idrees et al., 2016). Hence, the present study also attempted to determine the antimicrobial potential of methanol bark extract of *A. cookii* and its phytochemical constituents.

Fig 1A. cookie Tree

Materials And Methods:-

Bark Anatomy :

A well sized tree of *A. cookii* was chosen from Nilgiri hills of Ooty, Tamil Nadu, and bark specimen was peeled off from the opposite directions of tree stem, at breast height was collected. Immediately it was fixed in 5% glutaraldehyde in 0.05M phosphate buffer and fixed in an appropriate fixative for proper microtome sectioning. All the sections obtained were photographed with a digital camera, connected with a Leica Wet alar orthoplan Light microscope. Measurements were performed, based on Carlquist (2001). Sheath Parenchyma was considered and used to explain parenchyma which is closely associated with secretory canals (Sen et al., 2011). Bark tissues were also submerged in Glycol methacrylate (GMA), followed by the modification of Feder and O'Brien techniques. Transverse, tangential and radial bark sectioning of 5µm thickness were cut by adapting ultramicrotome. Obtained bark sections were well stained with toluidine blue, prior to mount in the Entellan.

Study on Phytochemical constituents

To find out the phytochemical constituents present in the bark methanol extract of *A. cookii*, Mbaebie et al. 2012 protocol was followed. The presence of Tannin, Saponins, Alkaloids, Flavonoids, Steroids, varied Protein Anthraquinones, and Phenol content was detected following the below procedure.

Tannin:

To detect the presence of tannin, 1mL of extract sample was taken in a glass tube and added a limited quantity of one drop of 0.1% ferric chloride to provide brownish green colour visually and affirmed the occurrence of tannin.

Saponin:

Added 1ml of plant bark extract, with 2 mL of water and shaken well, using graduated cylinder for 15 minutes, resulting in foam like structure or layer evolved, indicating the occurrence of saponin.

Alkaloids:

With a drop of test solution few drops of drangorndolt reagent was added, yellow colour precipitation was formed indicating the presence of alkaloids.

Flavonoid Test:

4mL bark extract was mixed with NaOH to get yellow colour, and while we mixed with 1mL of concentrated HCL progressively turned into white.

Steroid Test:

1mL of bark methanolic extract was mixed with 2 little drops of concentrated H₂ SO₄ to evolve visualized brown colour, indicating the presence of steroids.

Protein test:

1mL of extract mixed with two drops of Bradford reagent poured, a blue colour was formed, confirming the occurrence of protein in the tested sample.

Anthraquinone analysis:

1mL of test solution was added with 3mL of 10% lead acetate, a change in colour was observed as pink or red colour in aqueous layer, indicating the presence of Anthraquinones.

Phenol content:

When 1mL of test solution was blended with 8mL of 10% lead acetate solution, a white colour precipitation was formed indicates the presence of phenol.

Antimicrobial Determination**Test Microorganisms:**

The clinical pathogens such as *Staphylococcus aureus*, *Lactobacillus acidophilus*, *Vibrio alginolyticus* and *Salmonella typhi* were examined. Further, Fungal strains such as *Candida albicans* and Rhizopus were also experimented.

Agar Disc diffusion method

Antibacterial efficacy of methanol extract of *A. cookii* bark was examined using Agar disc diffusion method. Mueller Hinton Agar medium (MHA) was prepared and previously prepared inoculums were inoculated over on the plate. The disc were kept on the MHA plates, and 20µL of bark extract in a varied concentrations such as 1000µg, 750µg and 500µg were added in the discs. This experimental set up was incubated at 37°C for a 24 hours. Diameter of Zone of inhibition was measured using a ruled scale and determined the antimicrobial ability.

Anti-fungal activity

Stock culture was kept going at 4°C on Sabouraud Dextrose agar slant carefully. Selected active culture was transferred from stock solution to glass tubes, possessing sabouraud dextrose broth and set up was kept on going at 48 hours at normal room temperature. The Fungal activities were examined using disc diffusion technique. Amphotericin-B served as a positive control. Antimicrobial activity was examined using the procedure explained above

Results And Discussions:-**Anatomical observations of A. cookii Bark**

The bark specimen was observed macroscopically and microscopically for visualized characteristics.

Macroscopic observations

The outer view of the bark has shown to be irregular shallow ridges and dark green in color (Fig. 1), whereas the inner side of the specimen showed, light yellow in color along with many vertical striation parallel directions. Visible tiny circular pits were found on the vertical striations (Fig. 2).

Fig 2:- External morphology of *A. cookii*

Anatomical (Microscopic) observations

The bark constitutes a well-structured Periderm, continued with thick, cylinder chopped brachy sclereids (Fig.3&4).

Fig 3 (1) T.S of bark showing periderm and inner layer of brachy sclereids, (2) Cortical zone with tannin bodies, (3) Secondary phloem zone (Co: Cortex; Pe: Periderm; Sph: Secondary phloem; PhE: Phloem Elements; PhR: Phloem Ray; Scl: Sclereids; Ta: Tannin).

The visible periderm comprises of several homocellular phellem, covering about 11 layers of rectangular squarish cells, arranged compactly in radial files (Fig. 4). Furthermore, brachy sclereids were reported and shape was seen angular to circular along with lignified walls and a fair wide lumen. The immediate successive layer of periderm was brachysclereids.

Fig 4 (1) TS of periderm and brachy sclereids (2) Cortical zone (Co: Cortical cells; Phm: Phellem; Scl: Sclereids; Ta: Tannin)

Secondary phloem:

The microscopic observations on the secondary phloem were found significantly thick, compact. This constitutes small sized, rectangular shaped cells textured in vertical lines. The phloem rays were big and wide rectangular shaped thin walled cells. There were sporadically dispersed, circular brachy sclereids observed (Fig.5).

Fig 5 (1) TS of outer secondary phloem (2) TS of inner secondary phloem (ISph: inner secondary phloem; Osph: Outer secondary phloem; PhR:PhloemRay;Scl: Sclereids; Ta: Tannin)

Consequently, there were numerous compact files of cells located in parallel, covering phloem parenchyma, sieve cells and interestingly albuminous cells appeared. Sieve cells appeared to be wide, relatively with thick lateral walls and abundant sieve areas. Albuminous cells were found to be small, squarish and filled with a thick cytoplasmic matrix. The cells of phloem parenchyma appeared as wide, four angled and smooth walled.

Tangential longitudinal section (TLS)

In the TLS view of the bark section exhibited, phloem rays appear with spindle shaped, lie in a vertical plane. The rays appeared to be thin walled and less projecting, uni and multi seriate, non-storied, hetero cellular, characterized with middle procumbent cells with terminal upright cells. The rays were measured as 750µm in length and 90µm in thickness. The sieve cells were also found in vertical plane and they appeared wide and long Lateral walls seems to be modulated, which were attributable to the occurrence of lateral wall sieve areas (Fig. 6&7).

Fig 6 (1) TLS of secondary phloem (2) TLS of secondary phloem further enlarged. (Php;Phloem parenchyma ,PhR: Phloem Ray; SC;Sieve cells)

Fig7 (1)TLS of long thick phloem rays. (2)TLS of short narrow phloem rays. PhR; Phloem Ray, PRc;Procumbent cells; SC;Sieve cells; URC;Upright cells) Radial longitudinal section

In the view of, Radial Longitudinal sections, as shown in the Fig.8 & 9, Phloem rays were exhibited as horizontal layers, similar to the ribbon arranged one on the above other. They were horizontally elongated and rectangular in shape. Walls appears to be thick and possess dense sieve areas.

Fig 8 (1) RLS of Phloem Ray-Ray cells in horizontal layers, (2) Phloem ray cells-Ray cells are thick and darkly stained. (Php;Phloem parenchyma; PhR;Phloem Ray).

Many literature favored that in the Conifer family, constituents of secondary phloems, sclerenchyma, calcium oxalate crystal were shared in a relatively varied amount (Hudgins and Franceschi, 2004). The layer of Sieve cells observed in the bark of *A. cookii* may be attributable for mechanical protection against pathogenic organisms invasion. Further, invariably, minute crystals appeared in radial walls of sieve cells, parenchyma cells and fibres were also well studied in Libocedrus species extensively in earlier. Phelloderm cells developed into sclereids, and a new phellogen has been developing inside the phloem and was detected in *L. bidwilli* (Chan, 1985). Similar observation was also depicted in the present study.

Fig 9 (1) RLS of phloem ray. (2) RLS of phloem showing sieve cells.

A study conducted by Evert (2006), reported that albuminous cells were distinct with other phloem parenchyma cells showed a link with sieve cells pores on the sieve cell and the albuminous cell. This unique feature was documented in our study, in the microscopical characteristics. A clear description on the presence of albuminous cells was still unclear, as these cells may be considered to be ordinary ray cells that showed slightly upstanding. Similar to other study, described by Franceschi et al (2005), Cortex has been formed at the primary development of stem and sustained for a long period as secondary growth. Moreover, Sclerenchyma and calcium oxalate crystals were also seen within the cortex in the microscopically observations. It was presumed that the cortex served as a defensive natural barrier, during the primary development of the stem. It was in good agreement with the previous study.

Phytochemical compounds detected in *A. cookii*

The detected phytochemicals were tannins, flavonoids, protein, steroid, whereas, saponins, alkaloids, phenol and anthraquinones observed as negative results. In the present investigation, the detected phytochemicals were described in many earlier research, on gymnosperms and angiosperms and it was well determined, as they were potential for varied biological characteristics (Ncube et al., 2011).

Fig 10 The Phytochemical studies of *A. cookii*

Table No. 1:- Detection of Phytochemical compounds.

S.No.	Test	Detection
1.	Tannins	+
2.	Saponins	-
3.	Flavonoids	++
4.	Alkaloids	-
5.	Proteins	+
6.	Steroids	+
7.	Phenol	-
8.	Anthraquinones	-

Antimicrobial Activity

Table-2 shows the zone and percentage of inhibition of the examined bacterial and fungal strains, resulting in profound antibacterial and fungal efficiency. The results showed that *S.typhii* exhibited highest inhibitory potential when compared to other tested bacteria. Since the zone of inhibition although not close to the control value, among assayed bacterial strains, *S. typhii* showed higher inhibitory potential (Fig 11). The zone formed for *S. typhii* was measured as maximum as 9mm, 8mm and 7mm to the respective 1000µg, 750µg and 500µg concentrations

Table No. 2:- Anti-bacterial activity of *A. cookii*.

S.No.	Bacterial strains	Zone of inhibition (mm)			Antibiotic 1mg/mL
		1000	750	500	
1.	<i>S. aureus</i>	9	8	7	29
2.	<i>L. acidophilus</i>	8	7	7	17
3.	<i>Vibrio alginolyticus</i>	7	7	7	17
4.	<i>Salmonella typhi</i>	9	8	7	22

On the other part, the examination of bark extract potential against human pathogenic fungi *Candida albicans* and *Rizopus* species, results revealed that bark extract has shown to be effective against *Rizopus* species, rather than *C. albicans*. In the current investigation, it is evident that *A. cookii* contains moderate efficacy of antimicrobial potential against examined micro-organisms.

Fig 11 Antibacterial activity of *A. cookie* (a) *Staphylococcus aureus* (b) *Salmonella typhi* (c) *Lactobacillus acidophilus* (d) *Vibrio alginolyticus*

Similarly, against popular fungal strains examined, The effect on *Rizopus* species was significant and recorded as 16µm, 13µm in 1000µg, 750µg concentration whereas in 500µg it was recorded as 2µm, wherein positive control showed 27µm zone of inhibition (Fig 12). This inferred that inhibitory effects of bark extract required high concentration gradients against fungal pathogens. The similar findings with Zone of inhibition of 7µm was found by Fontoura et al. 2015.

Fig 12:- Antifungal activity of *A. cookie* (a) *Candida albicans* (b) *Rizopus***Table No.3:-** Anti-fungal activities of *A. cookii*.

S.No.	Fungal Strain	Zone of inhibition (mm)			Antibiotic 1mg/mL
		1000	750	500	
1.	<i>Candida albicans</i>	14	12	11	23
2.	<i>Rizopus Sp</i>	16	13	2	27

The phytochemical analysis of bark methanol extract elucidated that presence of flavonoids, alkaloids and tannin may have played a pivotal role in promoting anti-bacterial activity in the present investigation. Therefore it was inferred that active principles found in the bark *A. cookii* may be used in crucial therapeutic approaches in clinical settings for varied ailments with intensive further validation.

Salient Anatomical Diagnostic Features

1. Upper surface of the bark-dark green wide numerous small dots.
2. Lower surface light yellow with vertical thin lines.

3. Periderm includes only phellem which is superficial simple and homogenous.
4. Brachy sclereids cylinder thick continuous and occur immediately beneath the phellem.
5. Cortical zone parenchymatous allow with wide air-chambers.
6. Secondary phloem includes outer zone of collapsed elements and dilated parenchyma cells.
7. Inner zone of secondary phloem has sieve cells albuminous cells and parenchyma cells.
8. Phloem rays are uniseriate-short or multi seriate and long.
9. The rays are non-storied, hetero cellular and their walled.

Figures:-

Fig 1:-



Fig 2:-



Fig 3:-

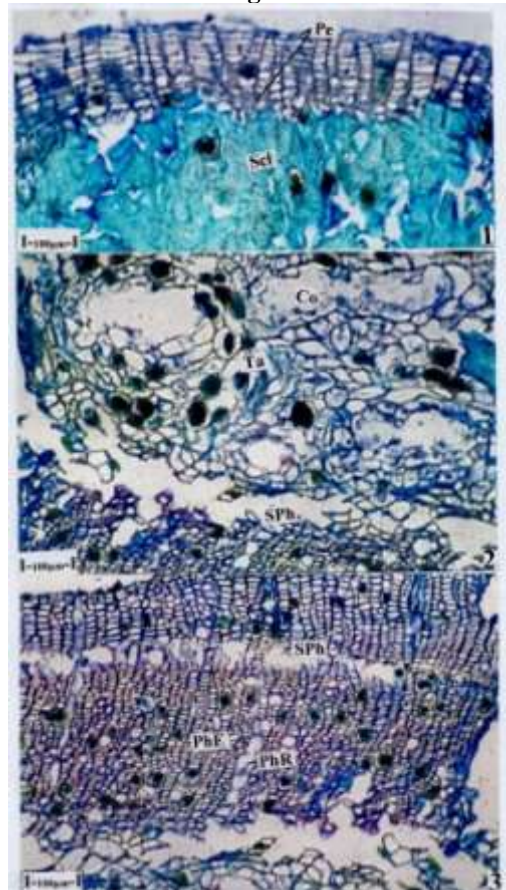


Fig 4:-

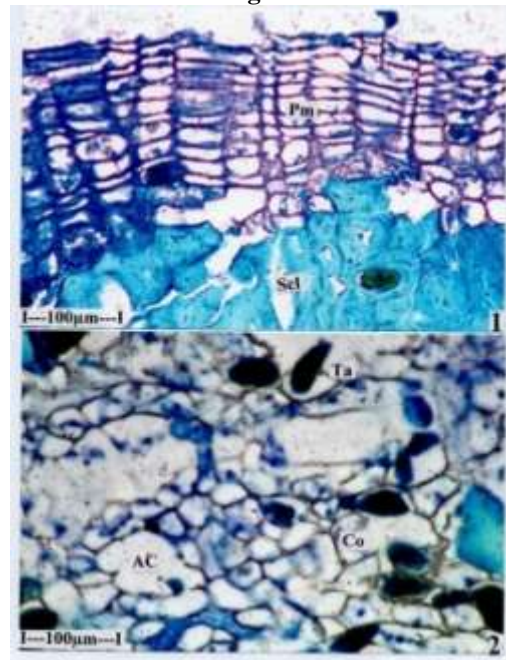


Fig 5:-

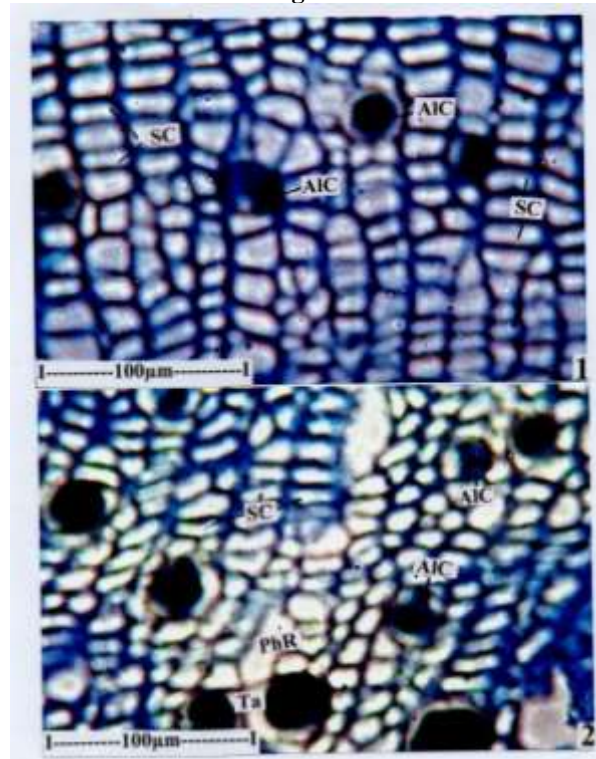


Fig 6:-

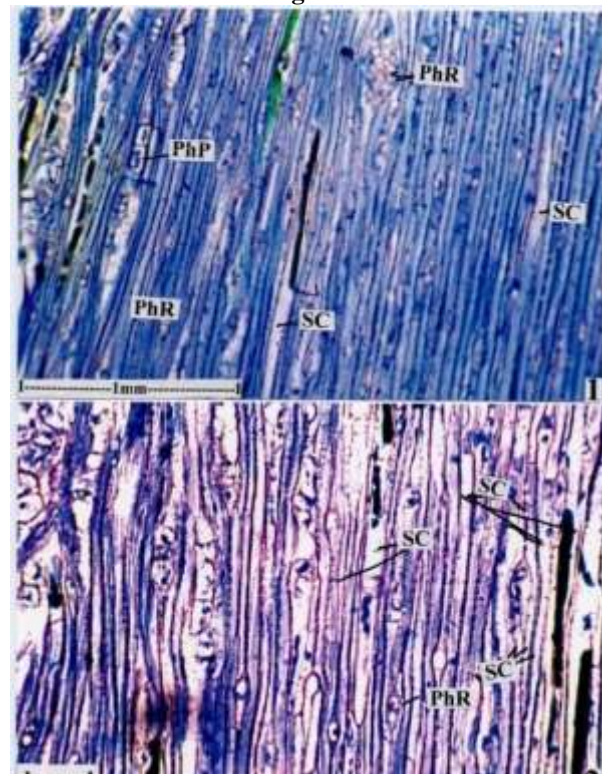


Fig 7:-

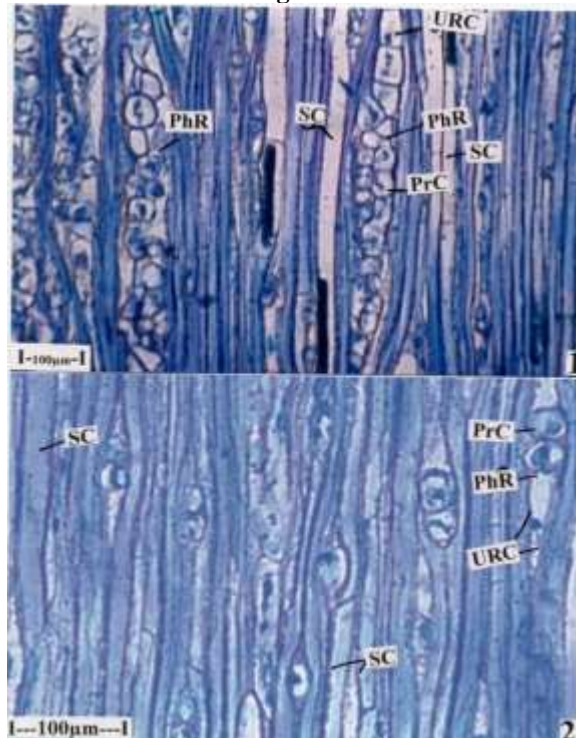


Fig 8:-



Fig 9:-

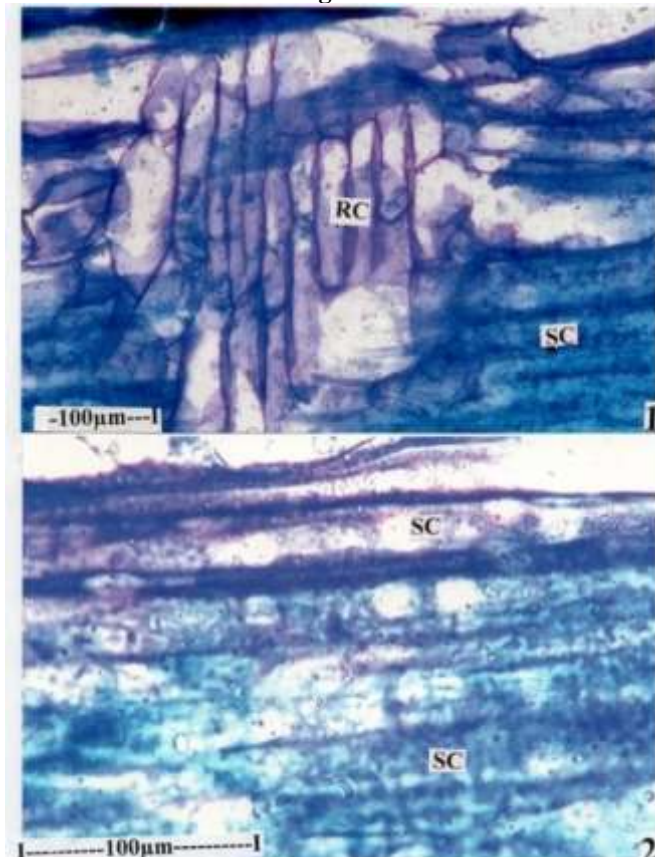


Fig 10:-



Fig 11:-



Staphylococcus aureus



Salmonella



Lactobacillus acidophilus



Vibrio alginolyticus

Fig 12:-



Candida albicans



Rizhopus

Conclusion:-

The present study focused on the anatomical observation of the bark of *A. cookii* and determined the anti-microbial activities of methanol bark extract of *A. cookii*. Our results proposed a report on the *A. cookii* as a possible antimicrobial agent against probable infectious pathogen. The present study can be extended to isolate the active compound from the bark extract that responsible for antimicrobial activity.

Conflict Of Interest

The authors declare no conflicts of interest

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