STUDY ON MORPHO-ANATOMICAL CHARACTERS, PHYTOCHEMICAL INVESTIGATION ON LEAVES OF *SANTALUM ALBUM* L. AND ITS ANTIMICROBIAL ACTIVITIES

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Abstract

Santalum album L. is belonging to the family Santalaceae which is known as Nant-thar-phyu in Myanmar and Sandalwood in English. The samples were collected from Neibainda Hill in Pyay Township during June, 2023 and February, 2024. In this study, morphological and anatomical characters, preliminary phytochemical tests ,elemental analysis and antimicrobial activity of *Santalum album* L. were carried out. In morphological study, the plant is semiparasitic evergreen small tree with slender drooping branches. Leaves were simple and opposite. Flowers were complete, bisexual, regular, actinomorphic, perigynous. Perianth-segment provided with a tuft of hairs behind the stamens. Stamens 4, epipetalous, filaments long, anther dithecous, adnate, introrse. One carpel, axile placentation, one ovule, pendulous, style filiform, stigma tri-lobed, disc present, ovary half-inferior. Fruits globose drupe, seeds hard, globose or obovoid. In histological study, the epidermal cells were thin-walled parenchymatous cells and papillose formed in lower epidermis. The stomata were paracytic type and vascular bundles were collateral type and exarch. Druses-shaped calcium oxalate crystals were abundantly found in collenchyma and rarely found in parenchyma. In the preliminary phytochemical investigation, alkaloids, carbohydrates, flavonoids, glycosides, phenol, reducing sugars, saponins, starch and tannins were present and α - amino acid was absent. In the antimicrobial test, ethyl acetate extract was showed the activity on all of six microorganisms. Among them, acetone extract was showed the best activity on *Staphylococcus aureus*. Ethyl acetate showed activity on all organisms. Watery extract was showed activity on only *Candida albicans*. From the research, the findings were significance and as an authentic information could be used to future research.

Keywords: *Santalum album*, paracytic

Introduction

Man's life needs food, clothes, shelters and medicines. All of these are natural resources from plants. In a tropical country like Myanmar, the population since ancient time has household traditional remedies, which constituted mostly herbal plants category. Myanmar has richness in varieties of medicine as well as aromatic plants due to the presence of different climate zones in the country.

Medicinal plants are abundant in Myanmar. The 85% of the population live in rural areas. Most of the people use the traditional medicinal plants for the treatments on the various diseases. Although synthetic drugs and antibiotic brought about a revolution in controlling diseases, plant occupy a very significant for some important drugs. (Medicinal plants of Myanmar, 2000).

The habitat of *Santalum album* L. is eastern Indian, sandalwood is cultivated in Southeast Asia for its wood and essential oil. The trees felled throughout the year. Sandalwood's aroma has been highly esteemed in China and India for thousands of years. The wood is often burned as increase and plays a part in Hindu ritual. The heartwood is most often used in perfumery, but it also been taken as a remedy in China since around 500CE (Chevallier and Fnimh, 2016).

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The sandalwood tree, from southern India yields a fragrant white timber suitable for fine carvings and carpentry, while sandal oil, used in eastern countries for anointing the body and in the manufacture of soap and perfumes, is distilled from the yellow heartwood and roots. Sandalwood is used as incense in Hindu, Buddhist, and Muslim ceremonies and uses as joss sticks (Heywood *et al*., 2007).

Sandalwood and its essential oil are used for their antiseptic properties in treating genitourinary conditions such as cystitis and gonorrhea. In Ayavedic medicine, a paste of the wood is held to be useful for chest and abdominal pain. The leaves and bark are effective in treatment of dandruff, lice, skin inflammation and sexually transmitted diseases (Chevallier and Fnimh, 2016).

Leaves, fruits and seeds contain santabolic acid, palmitic acid, oleic acid, linoleic acid and sugar. Fruits yield betalic acid and β-sitosterol and a fatty oil. Betalinic acid, β-sitosterol, glucose, fructose and sucrose from leaves. Heartwood yields essential oil which contain ά, ά and β-santalenes, santenol and teresantanol (Prajapati *et al*., 2003).

Nowadays, the Republish of the Union of Myanmar also encourages for development of scientific on herbal and traditional medicine. To contributes such health policy of country, this project attempted to demonstrate regarding the evaluation and investigation of effective plants.

Thus, the aim of this study is to examine the medicinal plant scientifically, which have effective medicinal values. To fulfillment this aim, the main objectives are to identify and study the morphological and histological characteristics of *Santalum album* L. to investigate the preliminary phytochemical analysis and to perform the antimicrobial activity from leaves of the plant.

Materials and Methods

Collection and Identification of *Santalum album* L.

The specimens used in this research were collected from from Neibainda Hill in Pyay Township, West of Bago Region during June, 2023 and February, 2024. Fresh specimens of the vegetative parts and floral leaves were studied. The taxonomic identification of the plant was made according to the method used in Department of Botany, Pyay University to obtain correct family, genus and species with the help of literatures such as Handley and Chit Ko Ko (1986), Kirtikar and Basu (1935), Backer and Brink (1963), Dassanayake (1999), Kress *et al*., (2003). The histological characters of the plant were identified by according to the references such as Esau (1965), Metcalfe and Chalk (1950), Lawrence and Cathy (1988), Pandey and Chadha (2000) and Trease and Evans (1999).

Preparation for histological Studies of *Santalum album* L.

The histological characters of fresh specimens were examined by preparing free hand sections with the razor blade. The specimens were cleared with chloral hydrate solution. The safranin solution was used for staining. Phloroglucinol and con. HCl were used for lignin test. The presence of calcium oxalate crystals was tested by using 80% sulphuric acid.

The collected specimens were washed and cut into small piece then air dried at room temperature. After that, these were homogenized by grinder to get powder and stored in air tight containers for histological, phytochemical studies, elemental analysis and antimicrobial test.

Preliminary phytochemical Analysis on leaves of *Santalum album* L.

The preliminary phytochemical investigation on the leaves had been undertaken. The experiment was carried out to determine the presence and absence of alkaloids, α-amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, starch and tannin according to the method of Marini Bettalo *et al.,* (1981), Central Council of research in Unani Medicine (1987) and Harbone (1989) at Department of Botany, Pyay University.

Antimicrobial activities of various solvent extracts from leaves of *Santalum album* L.

The various solvent extracts were tested on six pathogenic microorganisms. Antimicrobial activities were conducted by using paper disc diffusion method (Davis and Stout, 1971) at the Microbiology Laboratory, Botany Department, Pyay University. Test organisms and diseases were shown in Table (1).

Extraction and test organisms

The dried powdered sample was extracted with petroleum ether, chloroform, acetone, methanol, ethanol, ethyl acetate and water by percolation method. Crude extracts of various solvents of leaves were tested on six microorganisms such as *Bacillus pumilus, Candida albicans, Escherichia coli, Micrococcus luteus, Pseudomonas fluorescens,* and *Staphylococcus aureus*. The test organisms used in this study were obtained from the Microbiology Laboratory, Department of Botany, Pyay University and conducted for determination of the antimicrobial activities.

Preparation of sample for the test of antimicrobial activity

Nutrient agar was prepared according to the method described by Davis and Stout, 1971. Nutrient agar was used as basal cultural medium for the bacteria test organisms. Each test organism (50 uL) was added to 100 ml of assay medium and then poured into plate.

Shake well for homogeneously

After solidification, paper disc (6mm) impregnated with extracts (20µl paper disc) were applied on the test agar plate and incubated for a day. After that, clear zones (inhibitory zones) surrounding the disc were measured. These zones indicate the presence of antimicrobial metabolites which inhibit the growth of test organisms.

Table 1 Types of test organisms, their respective code numbers and diseases

No.	Test organisms	Code number	Diseases
1.	*Bacillus pumilus*	IFO 90571	Food poisoning
2.	*Candida albicans*	NITE 09542	Skin infection, virginal candidiasis
3.	*Escherichia coli*	AHU 5436	Cholera, diarrhea
4.	*Micrococcus luteus*	NITE 83297	Skin disease
5.	*Pseudomonas fluorescens*	IFO 94307	Urinary tract infection, dermatitis
6.	*Staphylococcus aureus*	ATCC 12877	Skin disease, food poison, wound infection

Results

Scientific name	-	*Santalum album* L.
Family	-	Santalaceae
Common name	-	Sandalwood
Myanmar name	-	Nant-thar-phyu

Outstanding Characters of *Santalum album* L.

The plant *Santalum album* L. is semiparasitic evergreen small tree with slender drooping branches, reaching up to 18cm in height. Leaves were simple and opposite, leaf blade elliptic-lanceolate, glabarous, apice acuminate, base acute, margin undulate, petiolate, exstipulate. Inflorescence axillary and terminal, short paniculate cyme. Flowers bracteate, ebracteolate, pedicellate, complete, bisexual, regular, actinomorphic, perigynous. Tepals (4), synsepalous, valvate, campanulate, tepaloid (dark brownish red), adnate to the base of the ovary. Perianth-segment provided with a tuft of hairs behind the stamens. Stamens 4, epipetalous, opposite to the tepals, filaments long, anther dithecous, adnate, introrse, longitudinal dehiscence. Carpels (3), tricarpellary, syncarpous, axile placentation, ovules 2-3, pendulous, style filiform, stigma tri-lobed, disc present, ovary half-inferior. Fruits globose drupe, purple black with ribbed endocarp and seeds hard, globose or obovoid.

Flowering and fruiting period from December to March.

Histological characters of *Santalum album* L.

Lamina

The lamina of *Santalum album* L. was typically dorsiventral, venation reticulate, distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System

Composed of epidermal cells, there are stomata and trichome in epidermal tissue system. In surface view, the cells of both surfaces were parenchymatous, thin and straight-walled. The upper epidermal cells were rectangular in shaped. The lower epidermal cells were barrel in shaped. Paracytic type of stomata were present on lower surfaces and papillose were formed. The guard cells were reniformed in shape and contained abundant chloroplasts. In cuticle layer, the upper is thicker than the lower. These are shown in figure 2.

Ground Tissue System

The mesophyll consisted of palisade and spongy parenchyma. The palisade mesophyll was made up of 3 layers. Vertically cylindrical cells, which are closely packed with one another. They contain numerous chloroplasts. The spongy mesophyll composed of 3-5 layers. They were rounded in shape and loosely arranged. Intercellular spaces were present among them. The oil cells were embedded in mesophyll tissues. These were shown in figure 2.

Vascular Tissue System

Vascular bundles were embedded in mesophyll. They were very small in size, ovate to elongate in shape, collateral type and exarch, the phloem tissues were very small in size. The xylem tissues were made up of 3-5 layers. These are hexagonal in shape. The xylem consisted of fiber and spiral tracheid. These were shown in figure 2.

Midrib

In transverse section, slightly convex in the upper surface and very concave in the lower surface, distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System

In surface view, the cells were irregular in shaped. In transverse section, the epidermal cells were barrel in shape single layer. The cuticle layers of both surfaces were thin. They were shown in figure 3.

Ground Tissue System

The cortex was made up of collenchyma and thin-walled parenchyma cells. In the above of vascular bundle, thin-walled parenchymatous cells were composed of 3-8 layers and collenchymatous cells were composed of 3-5 layers. In the below of vascular bundle, the collenchymatous cells were found in three patches and 2-9 layers in each. The parenchymatous cells were round in shape and 3-10 layers. The rosette crystals were abundant in collenchymatous cells and rarely found in parenchymatous cells. They were shown in figure 3.

Vascular Tissue System

The vascular bundle was crescent shaped, collateral and close type. The phloem tissues were small. The xylem tissues were exarch and made up of 3-9 layers and 20-22 rays. These were hexagonal in shape. The xylem composed of fiber, spiral tracheid and pitted vessels. They were shown in figure 3 and 5.

Petiole

In transverse section, flat in upper surface and two horns in laterals, very concave in lower and distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System

In surface view, the cells were parenchymatous, thin-walled and irregular shaped along the length of the petiole. In transverse section, the epidermal cells were barrel in shape. The cuticle layer was thin-walled. They were shown in figure 4.

Ground Tissue System

The cortex was made-up of two different types of tissues, parenchyma and collenchyma. In the above of vascular bundle, the parenchyma tissues were made up of 5-10 layers and the collenchyma tissues were made up of 3 paths and 2-4 layers in each. In the below of vascular bundle, the parenchyma tissues were made up of 3-7 layers, the collenchyma tissues were found in under of lower epidermal cells and made up of 2 layers and 3 paths of 3-9 layers in below the vascular bundle. The rosette-shaped calcium oxalate crystals were abundant in parenchyma tissues and rarely found in collenchyma. They were shown in figure 4.

Vascular Tissue System

The vascular bundle was crescent shaped in outline, collateral and close type. The phloem cells were small size. The xylem tissues were exarch, made up of 5-11 layers and 25-27 rays. The xylem consisted of fiber and spiral tracheid pitted vessels. They were shown in figure 4 and 5.

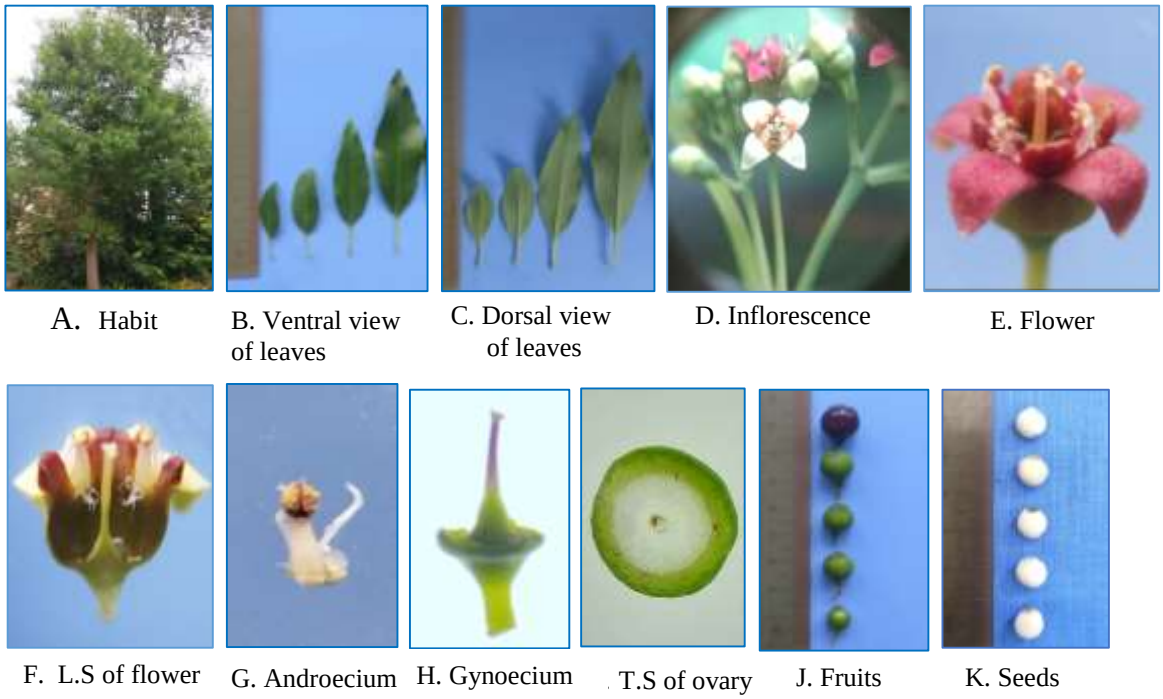


Figure 1 Morphological features of *Santalum album* L.

Figure 2 Histological Characters of lamina of *Santalum album* L.

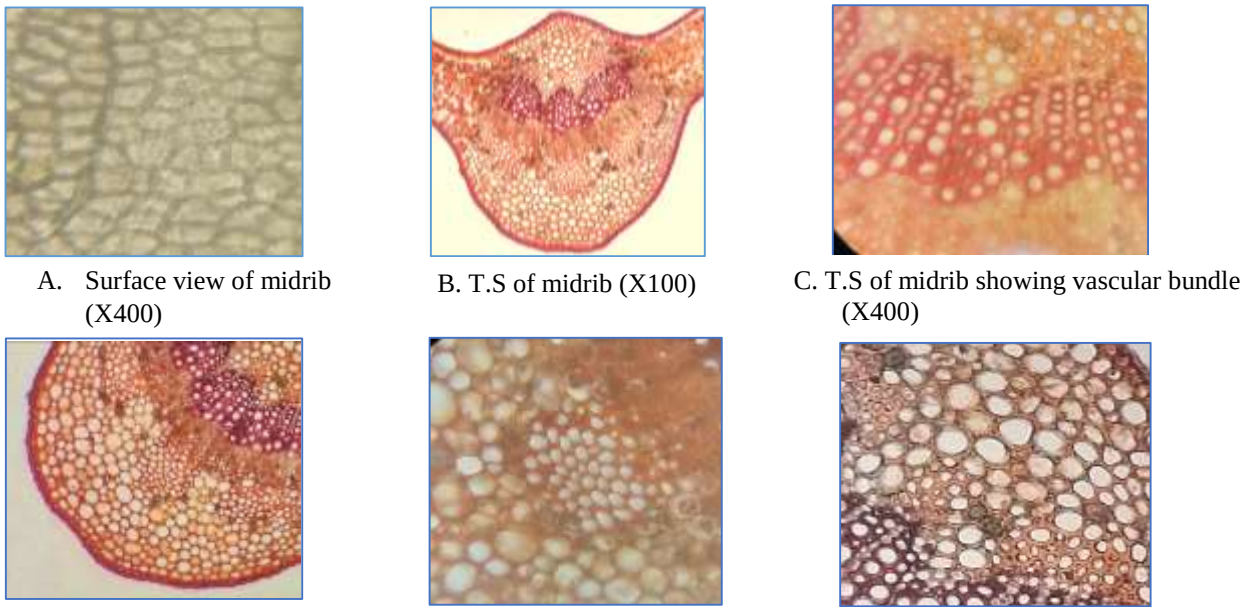
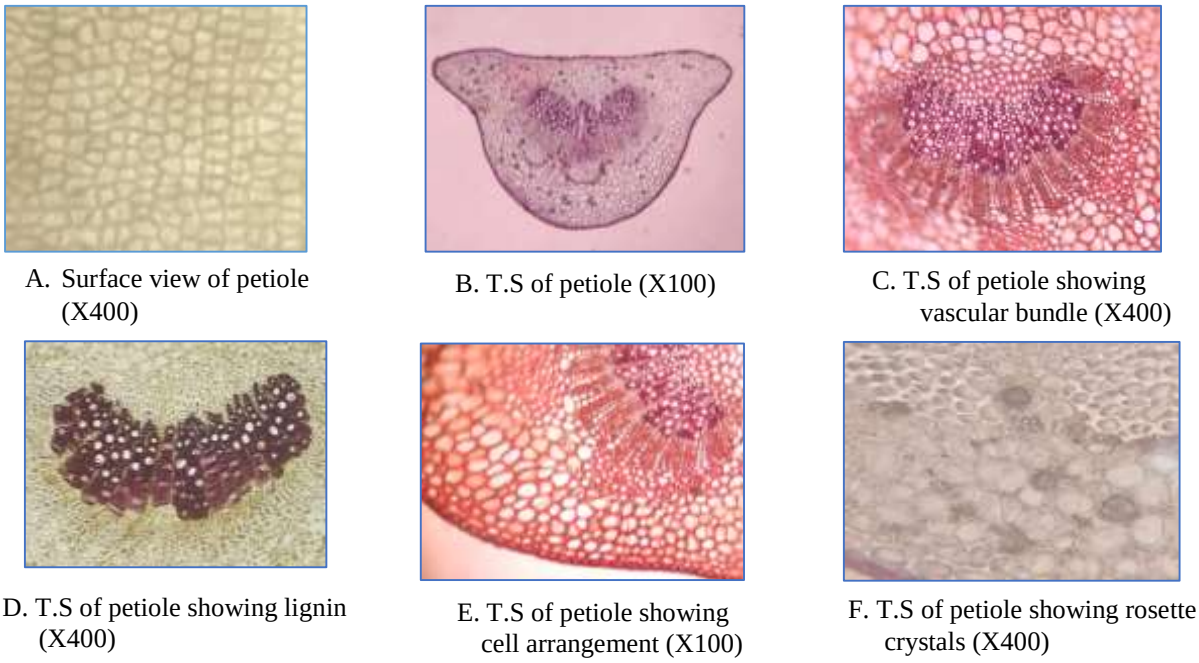


Figure 3 Histological characters of midrib of *Santalum album* L.



A. Surface view of petiole (X400)

B. T.S of petiole (X100)

C. T.S of petiole showing vascular bundle (X400)

D. T.S of petiole showing lignin (X400)

E. T.S of petiole showing cell arrangement (X100)

F. T.S of petiole showing rosette crystals (X400)

Figure 4 Histological characters of petiole of *Santalum album* L.

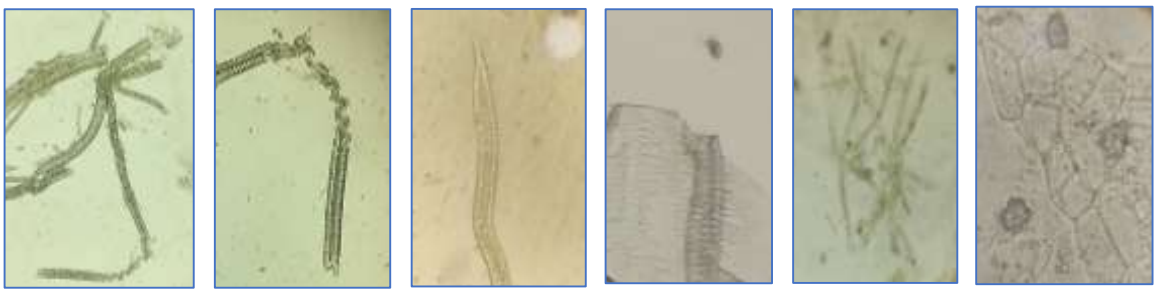
Diagnostic features of leaves powder of *Santalum album* L.

Sensory characters

Colour	Odour	Taste	Texture
Yellowish green	Astringent	Astringency	Fibrous

Microscopical characters of powdered leaves of *Santalum album* L.

Vessels, tracheids and fragments of sample were present in powdered leaves of *Santalum album* L. Vessels were found as pitted and tracheids were found as fibre, and spiral thickening. Fragments of sample were found as rosette crystals.



A. Group of tracheids (X100)

B. Sprial tracheids (X100)

C. Scalariform of tracheid (X400)

D. Pitted vessel (X400)

E. Group of fibers (X100)

F. Rosette crystals (X400)

Figure 6 Microscopical Characters of Powdered Leaves of *Santalum album* L.

Preliminary phytochemical study of the dried powdered leaves of *Santalum album* L.

The preliminary phytochemical tests of the dried powdered leaves of *Santalum album* L. were indicated that the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenol, reducing sugars, saponins, starch and tannins. α - amino acid, was absent. The results were shown in table 2.

Table 2 Preliminary phytochemical tests of the leaves of *Santalum album* L.

No	Test	Extract	Test reagent	Observation	Results
1	Alkaloids	D/W	1.Dragendroff's reagent	Orange ppt	+
			2.Mayer's reagent	Cream ppt	-
			3.Wagner's reagent	Reddish brown ppt	+
2	α - amino acid	D/W	Ninhydrin reagent	No color change	-
3	Carbohydrates	D/W	10% α-naphthol + H_2SO_4 (conc:)	Red ring or pink colour	+
4	Flavonoids	D/W	Mg /HCl (conc:)	Pink colour	+
5	Glycosides	D/W	10% lead acetate solution	White ppt	+
6	Phenol	D/W	10%$FeCL_3$, solution	Deep blue ppt	+
7	Reducing Sugars	D/W	Benedict's solution	Yellow/red ppt	+
8	Saponins	D/W	Distilled water	Frothing	+
9	Starch	D/W	I_2 solution	Blue black colour	+
10	Tannins	D/W	1%$FeCL_3$ solution	Yellowish brown ppt	+

(+) Present, (-) Absent, (ppt) Precipitate

Antimicrobial activities of different solvent extracts from leaves of *Santalum album* L.

Screening of antimicrobial activities of various extracts were carried out by using seven solvents such as acetone, ethyl acetate, ethanol, methanol, pet ether, chloroform and water. The results were shown in table 2 and figure 8.

Table 4. Inhibition zone exhibited by different extracts from leaves of *Santalum album* L. against six microorganisms

Solvent	**Organisms**					
	Bacillus pumilus	*Candida albicans*	*Escherichia coli*	*Micrococcus luteus*	*Pseudomonas fluorescens*	*Staphylococcus aureus*
Acetone	12.1 mm	15.3 mm	-	15.1mm	14.7 mm	**18.8** mm
Ethyl acetate	12.0 mm	14.8 mm	10.1 mm	12.1 mm	15.3 mm	15.3 mm
Ethanol	10.8 mm	11.8 mm	-	13.0 mm	-	15.4 mm
Methanol	10.4 mm	13.0 mm	-	12.0 mm	-	14.7 mm
Pet ether	8.8 mm	13.6 mm	-	10.6 mm	12.0 mm	15.8 mm
Chloroform	-	13.5 mm	-	9.7 mm	-	8.4 mm
Water	-	9.6 mm	-	-	-	-

Paper disc – 6mm

According to this experiment, the leaves extract with ethyl acetate showed effective antimicrobial activity on all different microorganisms but watery extract showed activity on only *Candida albicans*. Among them, acetone extract showed the highest activity on *Staphylococcus aureus*.

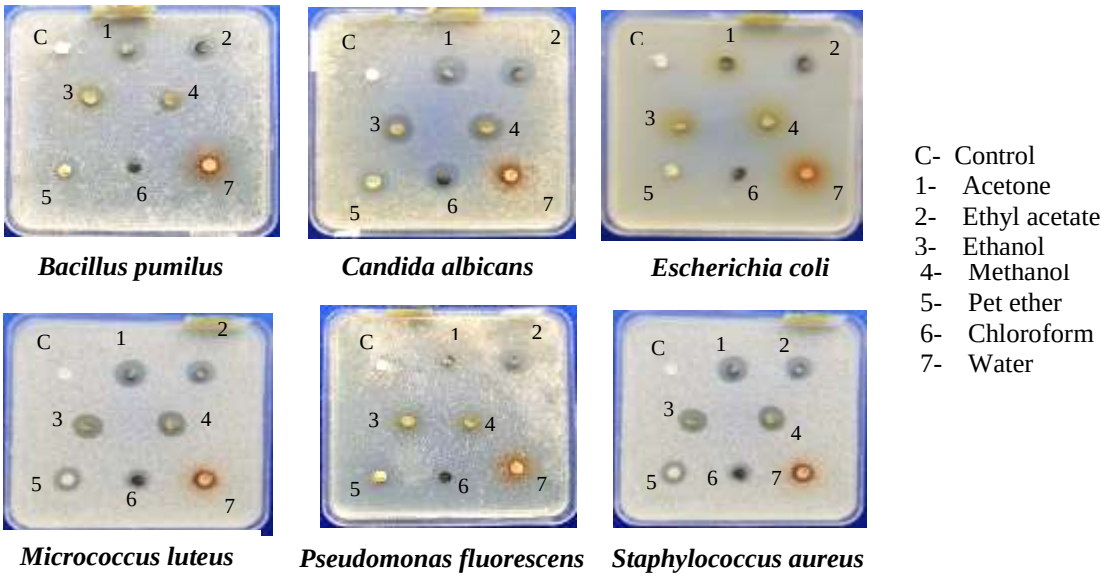


Figure 9 Antimicrobial activity from leaves extracts of *Santalum album* L. on six

Discussion

In this research work, morphological, histological characters, preliminary phytochemical investigation, elemental analysis and antimicrobial activities from leaves of *Santalum album* L. have been undertaken.

The morphological characters of *Santalum album* L. are agreement with mentioned by Kirtikar and Basu (1935), Handley and Chit Ko Ko (1986), Dassanayake (1999), Kress *et al*., (2003) except ovule characters.

Although Backer and Brink (1963) stated that 2-3 ovules and Dassanayake (1999) stated that 2-4 ovules, only 1 ovule observed in this study. Style filiform, stigma tri-lobed, disc present, ovary half-inferior. Fruits are globose drupe, purple black and seeds are hard, globose or obovoid. These are agreement with Kirtikar and Basu (1935), Handley and Chit Ko Ko (1986), Dassanayake (1999), Kress *et al*., (2003).

In histological studies, the characters of lamina, midrib and petiole are agreed with mentioned by Metcalfe and Chalk (1950).

In Preliminary phytochemical studies, carbohydrates, flavonoids, glycosides, phenol, reducing sugars, saponins, starch and tannins were present. In alkaloid test, showed the reaction with Dragendroff's reagent and Wagner's reagent, not showed with Mayer's reagent. α - amino acid was absent.

In the antimicrobial activities, the leaves extract with ethyl acetate showed effective on all different microorganisms but watery extract showed activity on only *Candida albicans*. Among them acetone extract showed the highest activity on *Staphylococcus aureus*. Anyhow, all the different solvent extracts showed activities on *Candida albicans*.

Conclusion

From the results, it can be concluding that leaves of *Santalum album* L. showed the presence of many secondary metabolites and phytochemical constituents, which could be used as a source of traditional medicine. It can help to local people such as this plant has medicinal value for curing the various diseases and could be utilized the efficiency for authentication of Myanmar traditional medicine systematically.

Acknowledgements

We wish to express our sincere thanks to Dr Hla Hla Win, Professor and head, Department of Botany, Pyay University for her permission to submit this paper. We would like to convey our thanks to Professor Dr Myat Myat Moe and Professor Dr Khin Min Min Phyo, Department of Botany, Pyay University for their kind suggestion for this research.

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