INVESTIGATION OF PHYTOCHEMICAL CONSTITUENTS, ANTIBACTERIAL ACTIVITY, ANTIOXIDANT ACTIVITY AND ANTI-INFLAMMATORY ACTIVITY FROM THE ROOT OF *PITTOSPORUM NAPAULENSIS* (DC.) REHDER &WILSON (MAYANIN)

Yanant Lay Hnin[1], Tin Myo Thant[2], Thinn Myat Nwe[3]

Abstract

In this research work, one of Myanmar medicinal plants namely, *Pittosporum napaulensis* (DC.) (Mayanin) was first chosen for chemical investigation. Phytochemical screening of the selected sample was carried out by using standard method. The sample contains alkaloids, flavonoids, glycosides, phenolic compounds, polyphenols, saponins, terpenes and tannins. Moreover, the antibacterial activity of various extracts of sample were tested by using Agar well diffusion method on six selected test organisms**.** According to the results, the *n*-hexane extract showed the highest activity against *Salmonella typhi.* Ethyl acetate extract and water extract were showed the highest activity on *Micrococcus luteus and Salmonella typhi.* Furthermore, antioxidant activity of various crude extracts of sample were carried out by 1,1-diphenyl-2-picrylhydrazyl (DPPH) essay method using UV-Visible spectrophotometer. According to the result, the antioxidant activity of ethyl acetate extract was more effective than *n*-hexane extract. Moreover, the results of anti-inflammatory for the treatment groups at different dosages indicate varying degrees of inhibition: 40 % at 0.75 mg/ear, 56 % at 0.59 mg/ear, 62 % at 0.45 mg/ear and 0.36 mg/ear.

Keywords*:* – *Pittosporum napaulensis,* phytochemical analysis, antibacterial activity, antioxidant activity, anti-inflammatory activity

Introduction

Medicinal plants are the primary life supporting natural resources for rural and tribal communities. About 80 % of the world population rely on the use of traditional medicine which is predominantly based on plant materials.There are many plants which are being used traditionally but not noted for their pharmacological activities in classical texts of Ayurveda or ayurvedic pharmacopoeia. *Pittosporum floribundum* Wight & Arn. Synonym *Pittosporum napaulense* (DC.) Rehder & Wilson (Family-Pittosporaceae), one among such plant is a small evergreen tree, found along the foot of outer Himalayas from Punjab eastwards to the hills of Assam and in the hills of peninsular India, ascending up to an altitude of 2400 m above mean sea level (msl) and also the Dun in shady places or ravines (Abhay *et al*., (2019).This species is distributed in Bangladesh, Bhutan, China, India, Nepal, Madagascar, Myanmar, Pakitan and Thailand (Rajeev, 2016).Single hand information about the ethnomedicinal, economical uses of the species of *Pittosporum floribundum* is still lacking. Despite its range of traditional medicinal uses, the phytochemistry and therapeutic potential of P. *floribundum* has not been extensively studied (Kishangiri *et al*., 2020). The bark has ginger -like smell when cut fresh, hence called "ginger tree". It can be used skin diseases and leprous affection, antidote to snake bite. Root paste applied to dropsical and rheumatic swellings (Rejeev *et al.*, 2009). Since ancient time, in various cultures worldwide, inflammatory disorders and related diseases have been treated with plants or plant derived formulations (Rathore *et al*, 2006). Inflammation plays on important role in various diseases (Hanada Yoshimra, 2002). The anti-inflammatory activity of several plant extracts and isolated compounds has already been scientifically demonstrated (Krishanswamy, 2008).

[1] Department of Chemistry, University of Mandalay
[2] Department of Chemistry, University of Mandalay
[3] Department of Chemistry, University of Mandalay

Botanical Description

Family Name - Pittosporaceae

Scientific Name - *Pittosporum napaulnesis*

Myanmar Name - Mayanin

English Name - Ginger tree

Part used - Root

Figure 1 The plant of *Pittosporum napaulenesis* (DC.) Rehder & Wilson (Mayanin)

Material and Methods

Sample Collection and Preparation

The root of *Pittosporum napaulenesis* (DC.) was collected from Mt.Popa National Park, Kyaukpadaung Township, Mandalay Region, on August 2023. Firstly, the root of sample was cleaned, then chopped into small pieces and allowed to air in the well-ventilated room for about two weeks. These air-dried pieces of sample were stored in a glass bottle with stopper and used throughout the experiment.

Preliminary Phytochemical Screening of the Root of *Pittsporum napaulensis* (DC.)

The preliminary phytochemical screening of the root sample for alkaloid, flavonoid, glycoside, phenolic compound, polyphenol, saponin, steroid, reducing sugar, terpene and tannin were carried out by standard method (Harbornes, 1993).

Antibacterial Activity of the Root of *Pittosporum napaulensis* (DC.)

The study of antibacterial activity was performed in four different solvent systems by Agar-well diffusion method on six microorganisms at Department of Chemistry, University of Mandalay. Six microorganisms such as *Agrobacterium tumerfaciens, Bacillus subtilis, Salmonella typhi, Pseudomonous aeruginosa, Micrococcus luteus* and *E-coli* are used in this research work.

Screening of Antioxidant Activity by DPPH Free Radical Scavenging Assay Method

Screening of antioxidant activity of the crude extracts (ethyl acetate and n-hexane) of *Pittosporum napaulensis* (DC.) were carried out by DPPH free radical scavenging assay using UV spectroscopic method (Brand-Williams *et al*., 1995). Absorbance measurements were done for each sample solutions. Absorbance values obtained were used to calculate % inhibition, 50% inhibitory concentration (IC_{50}). Lower the IC_{50} values indicates higher the free radical scavenging activity. Ascorbic acid was used as standard compound and the antioxidant activities were calculated from ascorbic acid standard curve.

Anti-inflammatory Activity by the Croton Oil-Induced Ear Edema Assay Method

Anti- inflammatory activity of selected sample was determined in ethanol extract by using croton oil-induced ear edema assay method at the Department of Biotechnology, Mandalay Technological University, Patheingyi Township, Mandalay Region, Myanmar.

The internal surface of the right ear of five groups of mice with weight of 30 g was treated with 20 µL of 5 % croton oil as an irritant to induce skin inflammation. The left ear remained untreated. The experimental mice were topically applied 0.75 mg/ear, 1.55 mg/ear, 3.0 mg/ear and 4.5 mg/ear of ethanol extract prior to 30 min croton oil. The positive control group was received 0.50 mg/ear aspirin. Ear thickness was evaluated with clipper 6 hours after edema induction.

The mice were randomized into five groups, each of which included five mice.

1. Negative control group: 5 % croton oil only.
2. Positive control group: Received aspirin (0.50 mg/ear).
3. Group A, Received 0.75 mg/ear of ethanol extract of test sample
4. Group B, Received 1.15 mg/ear of ethanol extract of test sample
5. Group C, Received 3.0 mg/ear of ethanol extract of test sample
6. Group D, Received 4.5 mg/ ear of ethanol extract of test sample

The percentage of edema inhibition was defined in relation to the control group, which received the croton oil solution, according to the following formula:

Inhibition % = (DControl - DTreated / DControl) × 100

where DControl is the difference in edema thickness in the control group and DTreatment is the difference in edema thickness for the treated groups.

Result and Discussion

Preliminary Phytochemical Test

According to the phytochemical study, the root of *Pittosporum napaulnese* (DC.) consists of alkaloids, flavonoids, glycosides, phenolic compounds, polyphenols, saponins, steroids, terpenes and tannin.

Table 1 Phytochemical Screening of the Extract of *Pittosporum napaulensis* (DC.) Rehder & Wilson

No.	Test for Compounds	Extract Solvent	Test Reagent	Observation	Remark
1	Alkaloids	1 % HCl	Dragendorff's reagent	Orange ppt	+
			Wagner's reagent	Brown ppt	+
2	Flavonoids	95 % EtOH	Small pieces of Mg ribbon, conc; HCl	Pink colour solution	+
3	Glycosides	Distilled water	10 % lead acetate solution	White ppt	+
4	Phenolic compounds	Distilled water	10 % ferric chloride solution	Dark green colour solution	+

No.	Test for Compounds	Extract Solvent	Test Reagent	Observation	Remark
5	Polyphenols	95 % EtOH	1% $FeCl_3$, 1 % K_3 [$(Fe(CN_6)]$	Dark blue color solution	+
6	Saponins	Distilled water	Distilled water	Frothing	+
7	Steroids	Pet-ether	Acetic anhydride, conc; H_2SO_4, chloroform	Green colour solution	+
8	Reducing sugars	Distilled water	Benedict's reagent	No brick red ppt	-
9	Terpenes	95 % EtOH	Acetic anhydride, chloroform, conc; H_2SO_4	Pink colour solution	+
10	Tannins	95 % EtOH	10 % $FeCl_3$ (2 drops), dil; H_2SO_4 solution	White ppt	+

Antibacterial Activity of the Root of *Pittosporim napaulensis* (DC.)

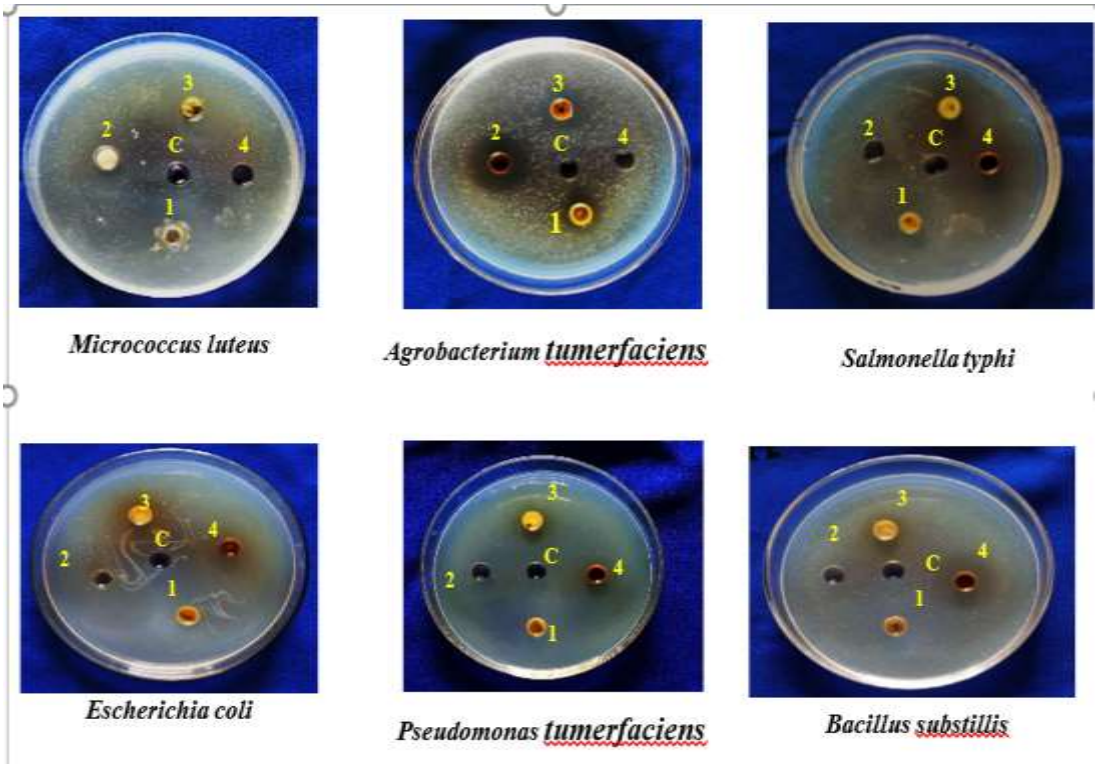
Antibacterial activity of the root of *Pittosporim napaulensis* (DC.) was measured by Agar well diffusion method at Department of Chemistry, University of Mandalay. The results are shown in Table 1 and Figure 2.

Table 2 The Results of Antibacterial Activity of the Root of *Pittosporum napaulensis* (DC.)

No.	Test organisms Names	EtOAC extract	EtOH Extract	H_2O extract	Hexane extract
1.	*Pseudomonas fluorescens*	-	-	10.00	10.00
2.	*Agrobacterium tumerfaciens*	11.00	19.00	14.00	-
3.	*Esherichia coli*	-	-	15.00	14.00
4.	*Bacillus substilis*	18.01	-	12.00	19.00
5.	*Salmonella typhi*	18.00	-	23.05	24.01
6.	*Micrococcus luteus*	23.00	12.00	20	-

Well size – 8 mm
No activity (<12 mm) low activity (between 12-15 mm)
high activity (between16-20 mm) very high activity (>20 mm)

1.



EtOAc 2. Ethanol 3. Watery 4. *n*-Hexane C. control

Figure 2 The antibacterial activity of the extracts of *Pittosporum napaulensis* (DC.)

Determination of Antioxidant Activity of Crude Extracts of *Pittosporum napaulensis* (DC.)

The antioxidant activity of ethyl acetate extract and *n*-hexane extract were determined by DPPH free radical scavenging assay method. The result of antioxidant activity of crude extracts and ascorbic acid were shown in Table 2, 3, 4 and Figure 3, 4, 5.

Table 3 % Inhibition in Various Concentration of Standard Ascorbic acid and IC_{50} Value of Standard Ascrobic Acid

Sample	Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)
Ascorbic acid	1	0.228	34.34	4.412
	2	0.166	40.91	
	4	0.129	49.74	
	8	0.109	65.62	
	16	0.086	92.91	

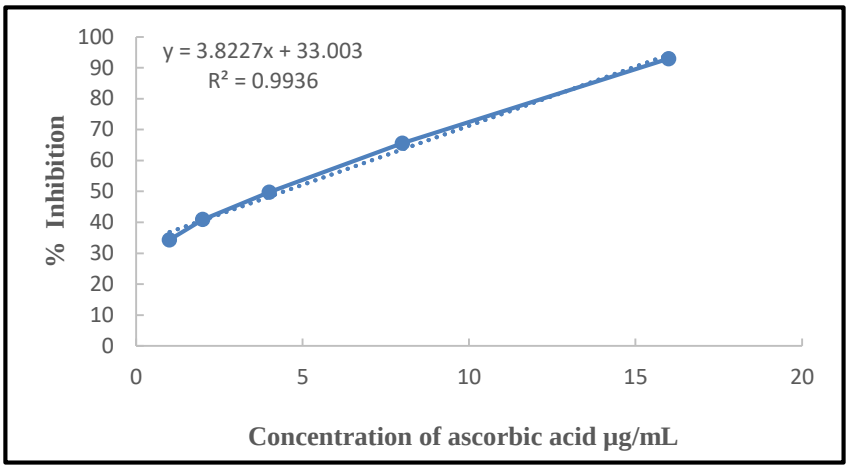


Figure 3 The plot of % inhibition Vs different concentration of ascorbic acid

Table 4 % Inhibition of various Concentration and IC_{50} Value of Ethyl Acetate of the Root of *Pittosporum napaulensis* (DC.)

Sample	Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)
Ethyl Acetate	25	0.801	41.125	63.618
	50	0.543	49.559	
	100	0.456	58.941	
	200	0.389	64.400	
	400	0.222	86.205	

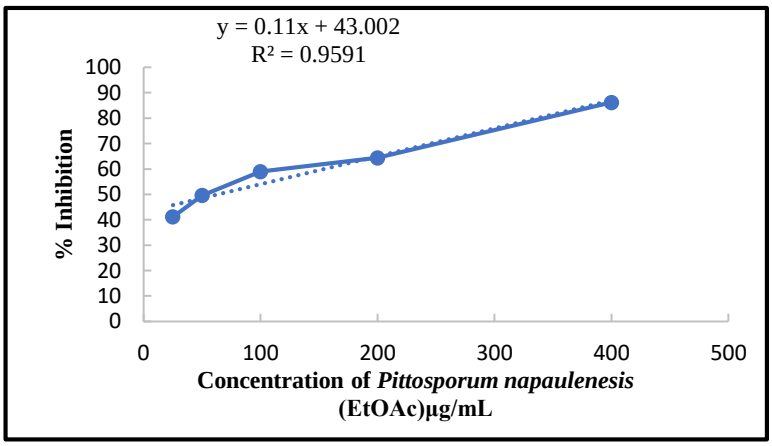


Figure 4 The plot of % inhibition Vs different concentration of ethyl acetate extract of the root of *Pittosporum napaulensis* (DC.)

Table 5 % Inhibition of Various Concentration and IC_{50} value of *n*-Hexane Extract of the Root of *Pittosporum napaulensis* (DC.)

Sample	Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)
n-Hexane	25	0.281	33.882	114.459
	50	0.246	42.118	
	100	0.219	48.471	
	200	0.151	64.471	
	400	0.039	90.824	

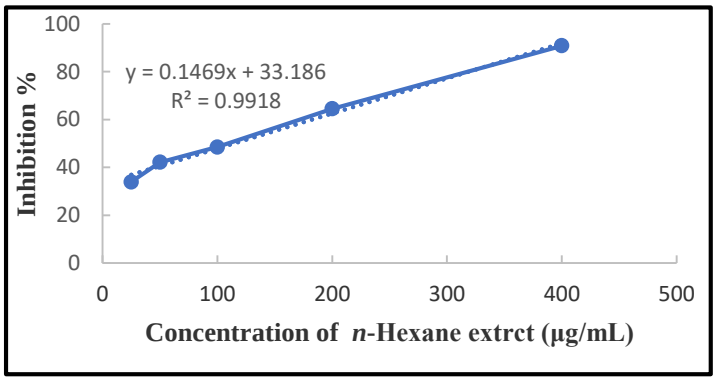


Figure 5 The plot of % inhibition Vs different concentration of *n*-Hexane extract of the root of *Pittosporum napaulensis* (DC.)

The antioxidant activity of ethyl acetate and *n*-hexane extracts were determined by DPPH scavenging assay method. IC_{50} value of standard ascorbic acid is 4.27 µg/mL, IC_{50} value of the ethyl acetate extract is 63.618 µg/mL and IC_{50} value of *n*-Hexane extract is 114.459 µg/mL. It was found that antioxidant activity of ethyl acetate extract is more effective than *n*-hexane extract.

Determination of Anti-inflammatory Activity of Ethanol Extract of *Pittosporum napaulensis* (DC.)

The anti-inflammatory activity of ethanol extract was determined by Croton Oil-Induced Ear Edema assay method. The result of anti-inflammatory activity of ethanol extract was shown in Table 6. In this table, the data reveals that the positive control group exhibited a remarkable inhibition rate of 86 % for evaluating the efficacy of the treatment groups. The results for the treatment groups at different dosages indicate varying degrees of inhibition: 40 % at 0.75 mg/ear, 56 % at 1.15 mg/ear, and 62 % at 2.25 mg/ear. This variation suggests that the treatments have some level of effectiveness, but the extent of that effectiveness is dose-dependent.

Table 6 Result of Percent Inhibition of Ethanol Extract of *Pittosporum napaulensis* (DC.)

Treatment	Dose	Differences (Thickness)	Inhibition %
Negative Control (Croton oil only)	20 μL	1.352 mm (D_C)	-
Aspirin (Positive Control)	0.5 mg/ear	0.194 mm (D_T)	86
Group A	0.75 mg/ear	0.816 mm (D_T)	40
Group B	1.15 mg/ear	0.590 mm (D_T)	56
Group C	3.0 mg/ear	0.466 mm (D_T)	66
Group D	4.5 mg/ ear	0.356 mm (D_T)	74

Conclusion

The root of *Pittosporum napaulenesis* (DC.) one of Myanmar medicinal plants was selected to investigate the phytochemical constituents, antibacterial activity, antioxidant activity, anti-inflammatory activity According to the phytochemical results, the root of *Pittosporum napaulenesis* contains alkaloids, saponins, flavonoids, glycosides, and polyphenols, steroids, terpenes and tannins. The antibacterial activity of crude extracts was tested by using Agar-well diffusion method on six selected test organisms: *Agrobacterium tumerfaciens*, *Pseudomonas fluorescens*, *Escherichia coli, Bacillus Substilis*, *Salmonella typhi, Micrococcus luteus*. Among the tested extracts, the *n*-hexane extract showed the highest activity (24 mm inhibition zone) against *Salmonella typhi*. The ethyl acetate and aqueous (water) extracts also demonstrated notable activity against *Micrococcus luteus* and *Salmonella typhi*. Other extracts exhibited moderate activity against *Agrobacterium tumefaciens*, *Esherichia coli*, and *Bacillus subtilis*. Antioxidant activity of the ethyl acetate and *n*-hexane extracts was assessed using the DPPH radical scavenging assay, indicating significant free radical scavenging potential. In addition, the anti-inflammatory effects were evaluated, revealing that the tested treatments exhibited significant anti-inflammatory activity in a dose-dependent manner. Results were compared to a positive control (86 % for 0.5 mg/ear) with groups A, B, C and D showing 40 % inhibition at 0.75 mg/ear, 56 % at 1.15 mg/ear, 66 % at 3.0 mg/ear and 74 % at 4.5 mg/ear respectively. These findings indicates that the root of *Pittosporum napaulense* possesses notable phytochemical, antibacterial, antioxidant, and anti-inflammatory properties.

Acknowledgements

The authors are thankful to Department of Chemistry, University of Mandalay for providing the research and analytical instrument facilities. Thanks to express their profound gratitude to the Department of Higher Education, Naypyitaw, Myanmar, for provision of opportunity to do this research.

References

Abhay, J. G., V. J. Shukla and R. N. Acharya., (2019). "Qualitative, Quantitative Screening and Antifungal Study of Pittosporum floribundum Wight & Arn.". *The Journal of Phytopharmacology*. Vol. 8(6), pp. 299-302

Brand, W., M. E. Cuvelier and C. L. W. T. Berset., (1995). "Use of Free Radical Method to evaluate Antioxidant Activity". *LWT-Food Sci. Technology*., vol. 28, pp. 25-30

Harborne, J B., (1993). *Phytochemical Methods: A Guide to Modern Techniques of Analysis*. London, 5th Ed., Chapman and Hall Ltd., vol. 3, pp. 54-84

Hanada, T., and A. Yoshimura., (2002). "Regulation of Cytokine Signaling and Inflammation". *Cytokine and Growth factor Reviews.* Vol 13(4-5), pp. 413-421

Kishangiri, K G., J. G. Abhay, A. Rabinarayan and V. J. Shukla., (2020). "Ethnomedical Uses, Phytochemistry and Pharmacological Activities of *Pittosporum floribundum* Wight. & Arn. A Review". *Europan Journal of Medicinal Plants*, India, vol. 31(3), pp. 48-55

Krishnaswamy, K., (2008). "Traditional India Species and their Health Significance'. *Asia Pacific Journal of Clinical Nutrition.* Vol.17 (S1), pp. 265-268

Manga, HM., D. Brkic, DEP. Marie and J. Quetin-Leclercq., (2004). In vivo anti-inflammatory activity of

Alchornea cordifolia (Schumach. & Thonn.) Mull. Arg. (Euphorbiaceae). J Ethnopharmacol. vol.92,pp.209–214

Rajeev, K. S., (2016). "Request for a binding decision on whether *Pittosporum napaulense (DC.)* Rehder & E.H. Wilson and *P. napalinese* Sherff *(Pittosporaceae)* are sufficiently alike to be confuded". *Journal of Request for Binding Decisions on Application of the Code.*, vol. 65(2), pp. 402

Rathore, B., A., A. Mahdr., and K.S. Das., (2007). "Indian Harbal Medicines; Possible Potent Therapeutic Agents for Rheumatoid Arthritis". *Journal of Clinical Biochemistry and Nutrition*. Vol.41 (1), pp.12-17

Rajeev, K S., P.G Diwakar., (2009). "Notes on the Identity and Taxonomic Status of Some Species and Inferaspecific Taxa of Genus *Pittosporum* Banks Ex Soland. *(Pittosporaceae)*". *Journal of Non-Timber Forest Products*, Vol. 16(3), pp. 211-214

Rathore, B., A. Mahdr., and K. S. Das., (2007). "Indian Harbal Medicines; Possible Potent Therapeutic Agents for Rheumatoid Arthritis". *Journal of Clinical Biochemistry and Nutrition*. Vol.41 (1), pp.12-17