ANTIOXIDANT ACTIVITIES ON *Picrasma javanica* BL. (NANN-PAW-KYAWT) BY USING DPPH AND NITRIC OXIDE ASSAYS

Zin Thu Khaing[1], May Zin Phyo[2], Daw Hla Ngwe[3]

Abstract

Picrasma javanica Bl. (Nann-paw-kyawt) bark is one of the well known traditional medicinal plants. Since it has an invaluable medicine purpose, the bark of *P. javanica* was chosen for this research work. This study was designed to examine the phytochemicals, mineral contents, antioxidant activity and total phenol contents of *P. javanica* bark. In the present work, the preliminary phytochemical tests revealed that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, terpenoids and organic acids were present and cyanogenic glycosides were not found in *P. javanica* bark. The mineral contents (Ca, K, Si, Fe, S, Mn, Ti, Zn, Cu, Sr and Rb) of *P. javanica* bark powder sample were determined by EDXRF. Among them, the calcium content of this sample was the highest (66.904 %). A compound Des-4-methyl-19-hydroxyquassin (0.013 %, white solid) was isolated from chloroform extract of *P. javanica* bark. *In vitro* antioxidant activities of ethanol and watery extracts of *P. javanica* bark were also assessed by 2, 2 diphenyl-1-picrylhydrazyl (DPPH) and Nitric Oxide free radical scavenging (NO) assays. By using DPPH assay the IC_{50} values of ethanol and watery extracts of *P. javanica* bark were 23.28 μg/mL and 17.47 μg/mL respectively. And also IC_{50} values of ethanol and watery extracts of *P. javanica* bark were 271.80 μg/mL and 105.28 μg/mL by using nitric oxide assay. The total phenol contents were determined by Folin-Ciocalteu Reagent (FCR) method and watery extract (19.67 μg GAE/mg) was found to be higher than ethanol extract (18.97 μg GAE/mg).

Keywords*:* *Picrasma javanica* Bl., phytochemicals, antioxidant activity, total phenol content, authentic compound, free radical

Introduction

Traditional herbal medicines are naturally occurring, plant-derived substances with mineral or no industrial processing that have been used to treat illness within local or regional healing practices. Traditional herbal

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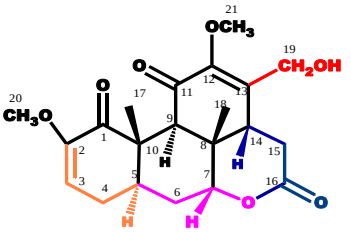
medicines are getting significant attention in global health debates. In China, traditional herbal medicine played a prominent role in the strategy to contain and treat severe acute respiratory syndrome. Many hope traditional herbal medicine will play a critical role in global health. China, India, Nigeria, the United States of America (USA) and WHO have all made substantial research investments in traditional herbal medicines (WHO, 2003).

Plants have long been serving mankind as sources of useful drugs, food, additives, flavoring agents, colorants, binders and lubricants (Falodun *et al.*, 2006). Medicinal plants are sources of important drugs used in the treatment of diseases either alone or in combination with other plants. Chemical substances found in plants include alkaloids, glycosides, essential oil, saponins, tannins, steroids, terpenoids, resins, flavonoids, proteins and others. These substances are potent bioactive compounds found in medicinal plant parts that can be used for therapeutic purposes (Nwachukwu, 2010). These inherent bioactive principles differ from plant to plant as a result of their biodiversity and they produced a definite physiological effect on human body. Several authors have screened different medicinal plants for the presence of these active principles. The knowledge of medicinal plants is important in pharmaceutical industry. *Picrasma javanica* Bl. (Nann-paw-kyawt) extract was chosen for evaluation of phytochemical test, antimicrobial activity, antioxidant activity, total phenol contents, antitumor activity. It has been found to be useful for treatment of malaria, antitumor, antiplasmodia, antibacterial, and skin diseases (Koike *et al*., 1991, Ohmoto *et al*., 1989).

Picrasma javanica (Nann-paw-kyawt) (Figure 1) is known as bitter plant and from Java (Christophe, 2006). In Myanmar, it is commonly known as Nya-bo-jaw (Poe Kayin) and Nann-paw-kyawt (Sagaw Kayin). It is a rainforest tree which grows to a height of 24 m and has a girth of 150 cm. The plant is found spanning Southeast Asia, Papua New Guinea and the Solomon Island. In Myanmar, it is only found in the border area of Kayin and Thailand, Myaing-Gyi-Ngu Special Region. The bowl is fluted, and the bark is dark, smooth, and brittle with bitter, with the inner bark, yellowish with brownish spa-wood. Leaves: compound, stipule and fragrant. The stipules are leafy. The blade comprises of 5-7 medium-sized and very thin follicles attached to 7 mm long petioles. The flowers are four and numerous and

whitish, and comprise of a thick nectar disc and few free carpel, each containing a single ovule. The fruits are green, red or blue drupes (Phokaew, 2005). The aim of this research is to study on the Myanmar traditional herbs that play a very important role in the development of new drugs. The objective of this research is to find out the elemental contents and antioxidant activity of *Picrasma javanica* (Nann-paw-kyawt). The isolation of new compound Des-4-methyl-19-hydroxyquassin from *Picrasma javanica* (Nann-paw-kyawt) bark and its anti-malaria activity had been explored in Department of Chemistry, University of Yangon (Yi Yi Win,2004).



Des-4-methyl-19-hydroxyquassin

Botanical Description of *Picrasma javanica* BL. (Nann-paw-kyawt) Bark

Kingdom	:	Plantae
Phylum	:	Tracheophyta
Class	:	Magnolipsida
Family	:	Simaroubaceae
Genus	:	*Picrasma*
Species	:	*P.javanica*
Myanmar Name	:	Nann-paw-kyawt

Figure 1:Photographs of *Picrasma javanica* BL. (Nann-paw-kyawt)

Materials and Methods

Sample Collection and Preparation of *P. javanica* BL. (Nann-paw-kyawt)

Bark of *P. javanica* Bl**.** (Nann-paw-kyawt) was collected from Myaing-Gyi-Ngu Special Region on October; 2016. The sample was identified at the Department of Botany, University of Yangon. After cleaning the sample, it was dried at room temperature followed by making into powder and stored in an air-tight container.

Preliminary Phytochemical Investigation of *P. javanica* Bl. (Nann-paw-kyawt) Bark

In order to find out the types of phyto-organic constituents such as alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, and terpenoids in the sample, preliminary phytochemical tests were carried out according to the appropriate methods. Various crude extracts (PE, EtOH, EtOAc) of *P. javanica* were prepared for TLC investigation, which were loaded on the precoated TLC silica gel plate and the chromatography was carried out by using an appropriate standard solvent system for *P. javanica*. The developed chromatograms were first inspected under UV-254 nm and 365 nm light and then sprayed with detecting reagents to classify the compounds present and their functional groups.

Qualitative Elemental Analysis by Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometry

The relative abundance of elements present in Nann-paw-kyawt bark was determined by (EDXRF) Spectrometer (Shimadzu-EDX-700) Department of Chemistry, West Yangon University. Each sample was run for a counting time of 100 seconds and the spectrum obtained was stored using EDX-700 software (Griken and Markowitch, 1993).

Isolation of Bioactive Organic Constituent from Bark of *P. javanica* (Nann-paw-kyawt)

The dried powder sample (300 g) was percolated with 95% ethanol (1000 mL) one week and filtered. This procedure was repeated for three times. The combined filtrate containing plant constituents were evaporated under reduced pressure by means of a rotary evaporator. Consequently, 95% ethanol soluble extract was obtained. The 95% ethanol extract was then partitioned with pet-ether (60-80 °C) (1000 mL) by using separatory funnel. The pet-ether fraction was removed under reduced pressure in a rotary evaporator. The pet-ether extract was obtained. The defatted alcohol soluble portion was then partitioned between chloroform and water by using separatory funnel. The solvent of chloroform fraction was removed under reduced pressure in a rotary evaporator. The chloroform extract was obtained.

Isolation and Purification of Bioactive Organic Constituents from Chloroform Extract of *P. javanica* (Nann-paw-kyawt) Bark

The chloroform extract (3 g) was dissolved in a volume of 10 mL of $CHCl_3$ and made slurry with silica gel. The resulting slurry was separated by column chromatography on a silica gel column (45 cm × 1.5 cm) using silica gel 40-60 μ m as stationary phase and $CHCl_3$ and MeOH (gradient elution) as mobile phase. The fractions were monitored by TLC. The fractions gave the similar appearance on TLC were combined and finally 4 main fractions FI to FIV were collected. Fraction II (0.580 g) was chromatographed by using precoated TLC silica gel with toluene: chloroform: ethyl acetate (1:1:1, v/v) solvent system. The seven detectable bands were collected by scraping from the TLC chromatogram after being checked under UV_{254}. Then the individual

component was eluted from gel by eluting with $CHCl_3$: MeOH (7:3) as eluent. The compound "A" was collected as white solid residue after evaporating the solvent.

Characterization and Identification of the Isolated Compound "A"

The isolated compound "A" was characterized by determination of some physical properties such as R_f value and melting point. The isolated compound A was subjected to TLC analysis and the R_f value of spot was determined. GF_{254} silica gel pre-coated aluminium plate (Merck) was employed and the chromatogram was developed in the appropriate solvent for the isolated compound. After the plate was dried, the R_f value of isolated compound was determined. Localization of spots was made by viewing directly under UV (254-365) or using spraying agents. For the identification of isolated compound, its ultra violet absorption spectrum was also recorded and examined by using UV-visible spectrophotometer at Universities' Research Centre, Yangon University. The infrared spectrum of the compound "A" was recorded by Shimadzu Perkin Elmer GX FT IR spectrophotometer at Chemistry Department, Yangon University.

Determination of Antioxidant Activity of Crude Extracts of *P. javanica* (Nann-paw-kyawt Bark) by DPPH Free Radical Scavenging Assay

The free radical scavenging activity of crude extracts of *P. javanica* (Nann-paw-kyawt) bark was measured by using DPPH free radicals scavenging assay. The activities of ethanol and water extracts of *P. javanica* (Nann-paw-kyawt) bark were determined by using UV-visible spectrophotometer (GENESYS 10S UV-Vis) (Marinova and Batchvarov, 2011). The control solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of ethanol in the brown bottle. The sample solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of test sample solution. These bottles were incubated at room temperature and were shaken on a shaker for 30 min. After 30 min, the absorbance values of these solutions were measured at 517 nm and the percentage of radical scavenging activity (% RSA) was calculated by the following equation.

$$\% \text{ RSA} = [\{(A_{DPPH} - A_{sample}) - A_{blank}\} / A_{DPPH}] \times 100$$

Where, %RSA = %radical scavenging activity of test sample

A_{DPPH} = absorbance of DPPH in EtOH solution

A_{sample} = absorbance of sample+ DPPH solution

A_{blank} = absorbance of sample + EtOH solution

The antioxidant power (IC_{50}) is expressed as the test substance concentration (μ g/mL) that results in a 50 % radical scavenging property of the sample. The standard deviation was also calculated by the following equation.

$$\textbf{Standard Deviation (SD)} = \sqrt{\frac{(\bar{x}-x_1)^2+(\bar{x}-x_2)^2+\ldots(\bar{x}-x_n)^2}{(n-1)}}$$

$\bar{x}$ = Average % Inhibition,

n = number of times

$x_1, x_2, \ldots, x_n$ = % inhibition of test sample solution

Determination of Antioxidant Activity of Crude Extracts of *P. javanica* (Nann-paw kyawt) Bark by Nitric Oxide Assay

The free radical scavenging activity of crude extracts of *P. javanica* (Nann-paw-kyawt) bark was measured by using Nitric Oxide method. Nitric oxide generated from sodium nitroprusside interacts with oxygen to produce nitrite ions which was measured by the Griess reaction (Marcocci *et al*., 1994). The reaction mixture containing 3.0 mL of 3 mM sodium nitroprusside in phosphate buffered saline (pH 7.4) and various concentrations of (400, 200, 100, 50, 25 μ g/mL) were incubated at 25 °C for 180 min. The NO° radical thus generated interacted with oxygen to produce the nitrite radical (NO°) which was assayed at 30 min intervals by mixing 1.0 mL of incubation mixture with an equal amount of Griess reagent (1% sulphnilamide in 5 % phosphoric acid and 0.1 % naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulphanilamide and subsequent coupling with naphthyl ethylene diaminedihydrochloride was measured at 546 nm. Each experiment was carried out at least three times and the data presented as an average of three independent determinations. The percentage of NO° radical

scavenging activity (% RSA) was calculated by the similar formula as mention in DPPH free radical rcavenging assay.

Determination of Total Phenolic Content of *P. javanica* (Nann-paw-kyawt) Bark by FCR Method

One of the anti-oxidative factors, total phenolic content (TPC) was measured by spectrophotometrically according to the Folin-Ciocalteu method. A 0.2 mL of each sample solution was mixed with 1.5 mL of Folin-Ciocalteu Reagent in a test tube covered with aluminum foil. After 5 min, 1.5 mL of 10 % Na_2CO_2 was added to each test tube. The sample was then incubated for 90 min at room temperature. The absorbance was measured at 765 nm spectophotometrically (KWF UV-7504). A standard curve of Gallic acid solutions (ranged from 0 - 250 μg mL^{-1}) was used for calibration. The experiment was done in triplicate. Concentrations of Gallic acid equivalent (GAE) in the plant extracts were calculated from the linear regression equation explored from standard curve construction for Gallic acid. TPC in the plant samples were expressed as (μg GAE/mg) (Reynertson, 2007).

Results and Discussion

Phytochemical Constituents of *P. javanica* (Nann-paw-kyawt) Bark

According to the phytochemical tests in order to know their types present in the selected sample, alkaloids, α-amino acids, carbohydrate, flavonoids, glycosides, phenolic compound, reducing sugars, saponins, starch, steroids, tannins, terpenoids, and organic acids were found to be present, however, cyanogenic glycosides was absent.

Elements Present in *P. javanica* (Nann-paw-kyawt) Bark by EDXRF Method

X-ray spectroscopy permits simultaneous analysis of light elements to heavy elements. In this work, relative abundance of elements present in the bark of *P. javanica* was determined by EDXRF spectrometer. It was observed that Ca and K are the major elements present in the barks of Nann-paw-kyawt. In addition the trace elements such as Si, Fe, S, Mn, Ti, Zn, Cu, Sr and Rb were also found in Nann-paw-kyawt bark. Among them, the

calcium content was the highest (66.90 %) in this bark (Table 1). Calcium is a mineral that is an essential part of bones and teeth. The heart, nerves and blood-clotting systems also need calcium work.

Table 1: Relative Abundance of Elements in *P. javanica* (Nann-paw-kyawt) Bark

Elements	Relative Abundance (%)
K	24.239
Si	3.413
Fe	3.183
S	1.272
Mn	0.294
Ti	0.227
Zn	0.178
Cu	0.105
Sr	0.080
Rb	0.050

Separation, Isolation and Purification of Compound "A" from Chloroform Extract of *P.javanica* (Nann-paw-kyawt) Bark

The chloroform extract (3 g)of Nann-paw-kyawt bark was separated column chromatographically on silica gel GF_{254} adsorbent by increasing the polarity of eluent, chloroform, chloroform : methanol (20:1, 15:1, 10:1 v/v) solvents, followed by TLC chromatographic separation. It was purified by washing with PE followed by crystallization from PE and EtOAc to give compound "A" 0.013 % as a white solide. After purification, compound "A" was stored for further pharmacological investigation.

The melting point of compound "A" was found to be 148 °C. Its R_f value was 0.17 (PE: EtOAc = 1:3 v/v). A purple spot on TLC after spraying and heating with 10 % H_2SO_4 reagent confirmed that the isolated compound A was a terpenoid compound. These physical properties of compound "A" are shown in Table 2. The melting point and R_f value of compound "A" were consistent to those of authentic Des-4-methyl-19-hydroxyquassin (148 °C). It is soluble in moderately polar solvents. It is UV active under 254 nm. It was

also inferred that compound "A" may not include phenolic group because no coloration was observed when it was treated with 5 % $FeCl_3$ solution. In the UV spectrum of compound "A", (Figure 2), the absorption maxima at 259 nm and 346 nm in MeOH indicates the presence of π→π* and n→π* transitions. It also agreed with the presence of double bond conjugation. conjugated with carbonyl group in compound A (Table 3). In FT IR spectrum of compound "A" (Figure 3), the –OH stretching of aliphatic alcoholic group appeared at 3533, 3479 and 3433 cm^{-1}. The strong =CH stretching band at 3070 cm^{-1} and =CH out of plane bending at 800 cm^{-1} indicated the presence of alkenic group. The absorption bands at 2970, 2931 and 2850 cm^{-1} indicated the presence of CH_3- and -CH_2- groups. The C = O stretching absorption band at 1735 cm^{-1} and C-O-C stretching band at 1190 cm^{-1} for ester. The stretching of α, β-unsaturated carbonyl group at 1690 cm^{-1} and C-O-C stretching of alcohol at 1034 cm^{-1} were also observed (Table 3). According to spectral data, melting point and Co-TLC chromatogram of isolated compound "A" and authentic Des-4-methyl-19-hydroxyquassin, the isolated compound "A" can be assigned as Des-4-methyl-19-hydroxyquassin.

Table 2: Some Physicochemical Properties of the Isolated Compound "A" from *P.javanica* (Nann-paw-kyawt) Bark

Experiments	Observations	Remarks
Melting point	148 °C	Similar to 148 °C (authentic Des-4-methyl-19-hydroxyquassin)
R_f value	0.17 (PE:EtOAc), (1:3 v/v)	Similar to R_f (authentic Des-4-methyl-19-hydroxyquassin
10 % H_2SO_4, Δ	Brown on TLC	-
H_2SO_4-Anisaldehyde, Δ	Purple	Terpenoid
H_2SO_4-Valine, Δ	Blue	Terpenoid

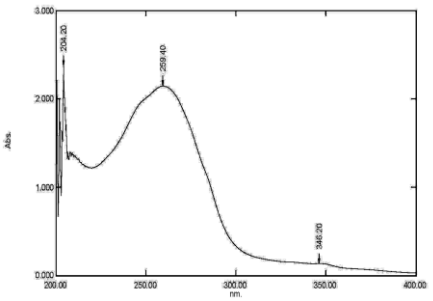


Figure 2: The UV spectrum of the isolated compound A (MeOH)

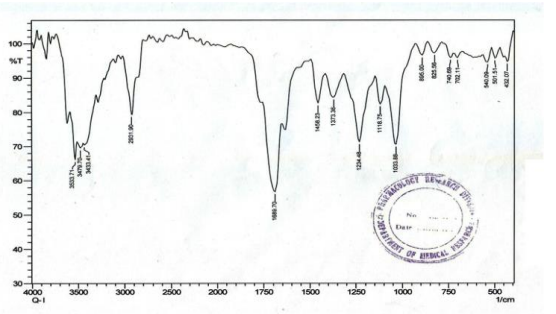


Figure 3: The FT IR spectrum of the isolated compound A (KBr)

Table 3: The FT IR Spectrum of the Isolated Compound "A"

Wave number (cm^{-1})	**Band Assignment**
3533,3479, 3433	υ_{OH} of OH group
3070, 3085	$\upsilon_{=C-H}$ of alkenic group
2970, 2931, 2850	$\upsilon_{C-H\ asym}$ and $\upsilon_{C-H\ sym}$ of CH_2 and CH_3 group
1735	$\upsilon_{C=O}$ of lactone ring
1690	$\upsilon_{C=O}$ of α , β -unsaturated C=O
1620	$\upsilon_{C=C}$ of alkenic group
1450	δ_{C-H} of CH_2 group
1380	δ_{C-H} of CH_3 group
1230	υ_{-C-O} of = C-O group
1190	υ_{C-O} of –C-O-C group
1034	υ_{C-O} of CH_2-OH group
800	δ_{oop} =C-H of alkenic group

Antioxidant Activity of *P.javanica* (Nann-paw-kyawt) Bark by DPPH Method

Antioxidants are essential and important for plants and animals' sustenance that protect cells from the damage caused by unstable molecules known as free radicals. The antioxidant activity of water and ethanol extracts of Nann-paw-kyawt bark were evaluated by DPPH free radical scavenging assay (Marinova and Batchvarov, 2011) and ascorbic acid was used as standard. The absorbance values of different concentrations (40, 20, 10, 5, 2.5, 0.625μg/mL) of tested samples were measured at wavelength of maximum absorption 517 nm by using UV-7504 spectrometer. It shows percent inhibition increased with increasing the concentration of crude extracts. The IC_{50} values of ethanol and water extracts were 23.28 μg/mL and 17.47 μg/mL respectively (Table 4 and Figure 4). So water extract was more potent in antioxidant activity than ethanol extract. However, antioxidant potency of both extracts were very weak when comparing with that of standard ascorbic acid (IC_{50} = 2.25 μg/mL).

Table 4 Oxidative Percent Inhibitions and IC_{50} Values of Crude Extracts of *P.javanica* (Nann-paw-kyawt) Bark by DPPH Method

Test samples	**% Inhibition of different concentrations (mean ±SD) (□g/mL)**						**IC_{50}(□g/mL)**
	1.25	**2.5**	**5**	**10**	**20**	**40**	
EtOH	10.32 ± 0.00	14.09 ± 0.01	15.83 ± 0.03	33.39 ± 0.03	47.78 ± 0.03	61.29 ± 0.00	23.28
H_2O	13.99 ± 0.01	20.46 ± 0.01	29.63 ± 0.01	39.19 ± 0.03	51.67 ± 0.01	59.56 ± 0.00	17.47
Ascorbic Acid	34.36 ± 0.00	53.86 ± 0.00	62.38 ± 0.00	66.87 ± 0.00	72.75 ± 0.01	82.5 ± 0.00	2.25

IC_{50} = 50 % Inhibition Concentration

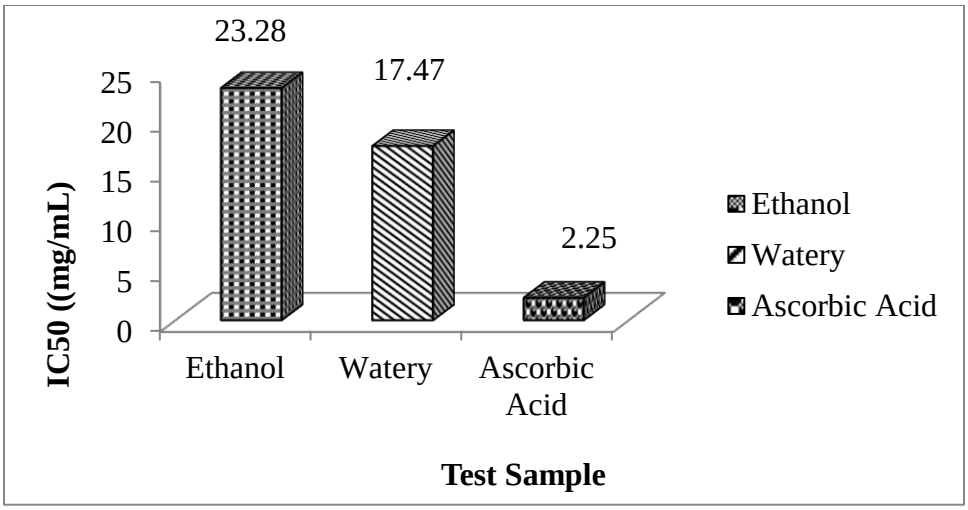


Figure 4: A bar graph of IC_{50} values of water and ethanol extracts of *P.javanica* (Nann-paw-kyawt) bark and standard ascorbic acid by DPPH method

Antioxidant Activity of *P. javanica* (Nann-paw-kyawt) Bark by Nitric Oxide Method

In this study the antioxidant activity of ethanol and watery extracts of (Nann-paw-kyawt) bark was evaluated by nitric oxide radical scavenging assay (Marcocci *et al*., 1994). Nitric oxide (NO°) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological process (Lata and Ahuga 2003). NO° is generated in biological tissue by specific nitric oxide synthesis (NOSs), which metabolizes arginine to citralline with the formation of NO° via a five electron oxidative reaction (Ross, 1993). NO° scavenging capacity is determined by the decrease in the absorbance at 550 nm, induced by antioxidant. The absorbance values of different concentrations (400, 200, 100, 50, 25μ g/mL) of tested samples were measured at 550 nm by using UV-7504 spectrometer. The NO° scavenging activity of crude extracts are expressed in terms of % RSA and IC_{50} (50 % inhibitory concentration) and these values are calculated by linear progressive excel program. The IC_{50} values of ethanol and watery extracts were 271.80μ g/mL and 105.28μ g/mL respectively (Table 5 and Figure 5). So the antioxidant activity of watery extract possessed higher

potency than ethanol extract. Their antioxidant activity were very weak when comparing with that of ascorbic acid (IC_{50} = 34.51 μ g/mL).

Table 5 Oxidative Percent Inhibitions and IC_{50} Values of Crude Extracts of *P.javanica* Nann-paw-kyawt Bark by Nitric Oxide Method

Test samples	**% inhibition(mean ± SD) of different concentrations (μg/mL)**					**IC_{50} (μg/mL)**
	25	**50**	**100**	**200**	**400**	
EtOH	33.42 ± 0.04	42.83 ± 0.00	44.17 ± 0.00	48.33 ± 0.02	53 ± 0.02	271.80
H_2O	34.95 ± 0.01	42.39 ± 0.01	49.37 ± 0.02	61.12 ± 0.01	65.28 ± 0.00	105.28
Ascorbic acid	44.61 ± 0.01	58.77 ± 0.06	61.27 ± 0.06	62.65 ± 0.00	63.90 ± 0.03	34.51

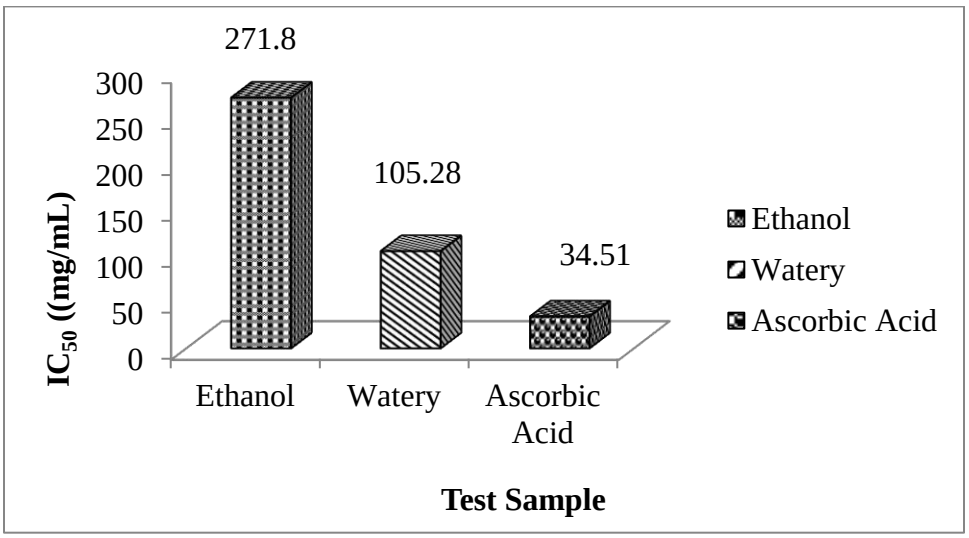


Figure 5: IC_{50} Values of standard ascorbic acid, EtOH and H_2O crude extracts of *P.javanica* (Nann-paw-kyawt) bark by using NO method

Total Phenolic Contents of Ethanol and Watery Extracts of *P.javanica* (Nann-paw-kyawt) Bark

In this study, the total phenolic content of *P.javanica* (Nann-paw-kyawt) bark was estimated by Folin-Ciocalteu method. Phenols react with an oxidizing agent phosphomolybdate in F-C reagent under alkaline conditions and result in the formation of blue coloured complex, the molybdenum blue which is measured at 765 nm colorimetrically. According to the results, the total phenolic content (TPC) (µg GAE/mg) of watery extract (19.67±1.96) was higher than that of ethanol extract (18.97±1.25). The greater the total phenolic content, the higher the antioxidant activity. Therefore, watery extract of *P.javanica* (Nann-paw-kyawt) bark has more antioxidant activity than ethanol extract. These results are reported in Table 6 and Figure 6.

Table 6: Total Phenolic Content of Ethanol and Water Extracts of *P.javanica* (Nann-paw-kyawt) Bark

No.	Test Samples	TPC (µg GAE/mg ± SD)
1.	Watery	19.67± 1.96
2.	Ethanol	18.97± 1.25

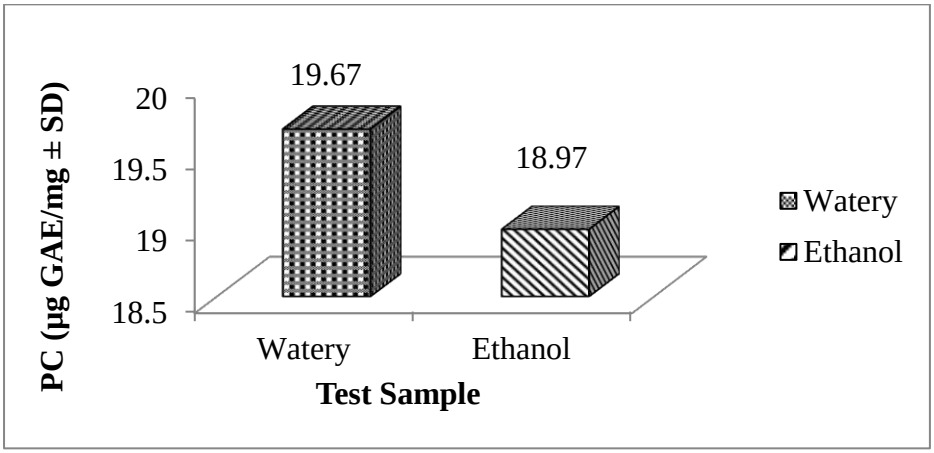


Figure 7: A bar graph of total phenolic contents of ethanol and watery extracts of *P.javanica* (Nann-paw-kyawt) bark

Conclusion

This research was intended to study some phytochemical constituents and antioxidant activities of *P.javanica* (Nann-paw-kyawt) bark.

Preliminary phytochemical tests showed the presence of the secondary metabolites such as α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compound, reducing sugar, saponins, starch, steroids, tannins, and terpenoids in *P. javanica* bark however cyanogenic glycosides were absent. Some elements such as Ca, K, Si, Fe, S, Mn, Ti, Zn, Cu, Sr and Rb were observed in the bark of *P. javanica* bark determined by EDXRF method. Among them, the calcium content of this sample was the highest (66.904 %).

A terpenoid, Des-A- methyl–19 – hydroxyquassin (0.013 % , based on chloroform extract, white solid , mpt. 148 °C) was isolated from chloroform extract.

DPPH assay method showed the antioxidant activity of ethanol and wateryextracts of *P. javanica* bark as 23.28 μg/mL and 17.47μg/mL of IC_{50} values, respectively. In addition, ethanol and watery extracts of *P. javanica* bark also respectively showed the antioxidant activity in 271.80 μg/mL and 105.28 μg/mL of IC_{50} values assessed by Nitric Oxide radical scavenging assay. Their antioxidant potency was concluded to be very weak when comparing with the potency of standard ascorbic acid (IC_{50}= 2.25 μ g/mL for DPPH assay and IC_{50} = 34.51 μ g/mL for NO° assay).

In the determination of the total phenol contents (TPC) of ethanol and watery crude extracts, it was observed that ethanol extract (52.31 ± 8.70 μg GAE/mg) was found to be higher than watery extract (16.92 ± 0.20 μg GAE/mg). It is a positive correlation between the total phenolic content and antioxidant activity in the selected plant sample. The results indicated that high phenolic content provided more potent antioxidant activity.

The findings from the present work will contribute to the scientific development of Myanmar traditional medicine, specifically in the areas concerned with oxidative stress.

The bark of *P. javanica* (Nann-paw-kyawt) could be applied as the local health remedy for the treatment of the diseases due to the oxidative stress.

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References

Christophe, W. (2006). *Medicinal Plants of the Asia-pacific Drugs for the Future?* Singapore: 1st Ed., World Science Publishing Co. Pte. Ltd., pp. 357 – 447

Falodun, A., Usifoh, C.O. and Nworgu, Z. A. M. (2006). "Smooth Muscle Relaxant Activity of 3-carbomethoxylpridine from *Pyrenucantha Staudtii* Leaf on Isolated Rat Uterus". *African Journal of Biotechnology*, vol 5(12), pp. 1271 – 1283

Griken, R. E. and Markowictz, A. A. (1993). Quantification in XRF Analysis of Intermediate Thickness Samples, New York: 1st Ed., Marcel Dekker Inc., pp 339 – 358

Koike, K., Ishii, K. Mitsunaga, K. and Ohmoto,T. (1991). "New Quassinoids from *Picrasma javanica*.Structures of Javananicins U, V, W, X and Y", *Chem. Pharm. Bull*., vol 39(8), pp. 2021 – 2023

Lata, H. and Ahuja, G. K. (2003). "Role of Free Radicals in Health and Disease" *Ind. J. Physiol. Allied Sci.*, vol 57, pp. 124 – 128

Marcocci, L., Maguire, J. J., Droy-Lefaix, MT. and Packer, L. (1994). "The Nitric Oxide Scavenging Properties of *Ginkgo biloba* Extract EGb 761", *Biochem. , Biophys. , Res. Commun*., vol 201, pp. 505 – 515

Marinova, G. and Batchvarov,V. (2011). "Evaluation of the Methods for Determination of the Free Radical Scavenging Activity by DPPH". *Bulgarian Journal of Agricultural Science*, vol 17, pp. 11 – 24

Nwachukwu , C.U., Umeh, C.N. and Kalu , I.G. (2010). "Identification and Traditional Uses of Some Common Medicinal Plants in Ezinihitte Mbaise L. G. A., of Imo State, Nigeria", Rep. Opinion , vol 2 (6), pp. 1-8

Ohmoto,T., Koike, K., Mitsunaga, K., Fukuda, H. and Kagei, K. (1989). "Studies on some Constituents of Indonesian *Picrasma javanica* III. Structures of New Quassinoids, Javanicins A, C and D", *Chem. Pharm. Bull*., vol 37(11), pp. 2991 – 2994

Phokaew, S. (2005). "Diversity Degrade Vulnerability of Cultural and Natural Diversity in Northern Karen State, Burma". *Karen Environmental and Social Action Network, Thailand*, pp. 65

Reynertson, K. A. (2007). "*Phytochemical Analysis of Bioactive Constiuents from Edible Myrtaceal Fruits*". PhD (Dissertation), New York, The City University, pp. 98

Ross, R. (1993). "The Pathogenesis of Atherosclerosis". *Free Rad. Res*., vol 362, pp. 801 – 804

Thomas, J., Joy, P. and Skaria, P. (1998). *Medicinal Plants. India:* 1st Ed., Kerala, pp. 3-4

WHO. (2003). "Clinical Trials on Treatment using a combination of Traditional Chinese Medicine and Western Medicine". *Traditional Medicine Strategy*, pp. 53 – 61

Yi Yi Win. (2014). *Antimalarial Activity of Stem Barks of Picrasma javanica Bl. (nann-paw-kyawt) and Alstonia scholaris L. (taung-mayoe) and Isolation and Identification of Active Compounds*. Phd (Dissertation), Myanmar: Department of Chemistry, University of Yangon