

# Investigation of Phytochemical, Antioxidant Activity and Antimicrobial Activity of *Vitis repens* Wight & Arn.

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## Abstract

The present research deals with the phytochemical screening, elemental analysis, antioxidant activity and antimicrobial activity of *Vitis repens* Wight & Arn. Phytochemical screening of *Vitis repens* Wight & Arn. was performed to examine the different types of phytoconstituents present in the powder sample of *Vitis repens* Wight & Arn. It was observed that these tests show the presence of alkaloids, carbohydrate, glycosides, phenolic compounds, saponins, starch and tannins in this sample.  $\alpha$ -amino acids, flavonoids and reducing sugars were not observed in *Vitis repens* Wight & Arn.

Elemental analysis of the *Vitis repens* Wight & Arn. sample was characterized by EDXRF method. It was observed that Ca (76.8 %) and K (21.3 %) as major inorganic constituents and Ti (0.646 %), Mn (0.393 %), S (0.154%), Fe (0.142 %), Si (0.127 %), Mg (0.127 %) and P (0.108 %) as minor inorganic constituents. The antioxidant activity using DPPH assay method with Ascorbic acid standard, expressed as IC<sub>50</sub> value was observed 25.72 ppm. The antimicrobial activity of some crude extracts (ethanol and water) of *Vitis repens* Wight & Arn. was screened by agar-well diffusion method against six organisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli*. It was observed that *pseudomonas aeruginosa* showed both ethanol and water extract.

**Keywords:** Phytochemical, antioxidant activity, antimicrobial activity.

## Introduction

*Vitis repens* Wight & Arn., which belongs to the family vitaceae. There are 26 general and 350 species in Vitaceae [6]. It is tendrillar climber and grows in beach forest, vine thicket and well developed lowland and upland rain forest. Especially, it is widely distributed in Shan State.

Myanmar is abundant plant resources and Myanmar people have inherited their own traditional medicine to maintain their health and treat various ailments for over millennia of history. According to World Health Organization report, eighty percent of population in Asia and Africa countries relies on traditional medicine for their primary health care. Folk medication is mostly based on natural food sources like fruits, vegetables and herbs [3].

In Myanmar, *Vitis repens* Wight & Arn. is the effective antitumor herbal medicinal plant. The rhizome of *Vitis repens* Wight & Arn. is used for the treatment of sore, carbuncles, ulcers, hepatitis and jaundice, peptic ulcer, tumors and hypertension in Myanmar traditional medicine. Plants are sources of natural antioxidant and some of the compounds have significant anti-oxidative properties and health benefits [5].

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. The potential of the antioxidant constituents of plant materials for the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists and food manufacturers as consumers move toward functional foods with specific health effects [4].

The anti-oxidative effect is mainly due to phenolic compounds. Making health care and medicinal facilities to the people is now a major concern of a large number of countries [1]. Due to the toxic and adverse reactions of synthetic and chemical medicines being observed around the globe herbal medicine has made a comeback to improving the fulfillment of our present and future health needs. The multiple use of this important herb has led to its indiscriminate collection [5].

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal and human body are generally designated as medicinal plants. Our ancestors were forced to use any natural substances that they could find to ease their sufferings caused by acute and chronic illness and even terminal illness. Since that ancient time, plants with therapeutic properties have occupied an important plant in the disease treatment practices.

Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as important therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicines. They are rich sources of bioactive compounds and thus serve as important raw materials for drug production.

## Material and Methods

### Collection of leaves

*Vitis repens* Wight & Arn. sample was collected at Panglong Township, Loilem District, Southern Shan State, Myanmar. The sample was taken and then air dried in shade at room temperature. They were ground by electric blender. They were stored in a well-stopped bottle and used for chemical analysis.

### Chemicals

All chemicals used in this work were from British Drug House Chemical Ltd., Poole, England. All standard solutions and other diluted solutions throughout the experimental runs were prepared by using distilled water. In all the investigations the recommended methods and standard procedures involving both conventional and modern techniques were employed [7]. DPPH (2,2-diphenyl,1-picrylhydrazyl) radical, Gallic acid, ascorbic acid and Folin–Ciocalteu reagent were obtained from Sigma-Aldrich, USA. All other chemicals and reagents used were of analytical grade.

### Preliminary Phytochemical analysis

Qualitative phytochemical analyses were performed in filtrates of *Vitis repens* Wight & Arn. The phytochemical screening of *Vitis repens* Wight & Arn. revealed that the presence of alkaloids, glycosides, phenolic compounds, saponins, starch and tannins as described by standard procedure.

### Test organism

Screening of antimicrobial activity of various crude extracts such as 95% EtOH, and watery extract of *Vitis repens* Wight & Arn. sample was investigated by Agar Well Diffusion Methods. In the present work, the test microorganisms were *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli*.

### Preparation of inoculum

The microorganisms were inoculated into nutrient broth and Rose Bengal broth for bioassay and incubated for 24 and 48 h at 37°C. The turbidity of the medium indicates the growth of organisms.

### Antimicrobial studies

The agar well diffusion method was employed for the determination of antimicrobial activity of extracts. Lawn culture of *E.coli*, *Candida albicans*, *Bacillus pumilus*, *psrudomonus aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* were spread on nutrient agar and *A. niger* & *A. flavus* spread on Rose Bengal Agar using sterile cotton swabs.

The wells (6mm in diameter) were cut from the agar plates using a cork borer. 30μl of the extracts (7mg/ml) were poured into the well using a sterile micro pipette. The plates were incubated at 37±2°C for 24 hours for bacterial activity and 48 hours for fungal activity. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

### **DPPH (2, 2-Diphenyl-1-picryl-hydrazyl) radical scavenging activity**

The ability of the extract to scavenge DPPH radical was determined according to the method described by [2]. In this experiment, each sample was dissolved in ethanol to get 0.2 mg/mL concentration and then it was diluted with ethanol to obtain 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39μg/mL concentration.

After mixing with DPPH solution, the absorbance of each solution was measured at 517nm. The antioxidant potential of sample can be determined by IC<sub>50</sub> (50% inhibition concentration).

### **Results and Discussion**

The phytochemical screening of *Vitis repens* Wight & Arn. revealed that the presence of alkaloids, glycosides, phenolic compounds, saponins, starch and tannins. Elemental compositions of *Vitis repens* Wight & Arn. which was determined by EDXRF method. It can be observed that the sample contains Ca (76.8 %) and K (21.3 %) as major inorganic constituents and Ti (0.646 %), Mn (0.393 %), S (0.154%), Fe (0.142 %), Si (0.127 %), Mg (0.127 %) and P (0.108 %) as minor inorganic constituents. The result are shown in table 1 and figure 2.

Calcium element is required for bone development. Potassium element is very effective essential element for the human metabolisms. It helps to reduce the hypertensive effect. Other minor trace elements are important nutrients for human health's. The antioxidant activity using DPPH assay method with Ascorbic acid standard, expressed as IC<sub>50</sub> value was observed 25.72 ppm. The result are shown in table 2,3 and figure 3,4 and 5.

The antimicrobial activity of crude extract such as ethanol and water extracts was investigated by agar well diffusion method. The test microorganisms were *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli*.

In this study, water extract showed (14mm) and EtOH extract showed (15mm) inhibition against the *Pseudomonas aeruginosa* microorganisms. The result are shown in table 4 and figure 6.

### Conclusion

From the present work study on the phytochemical, elemental analysis, antioxidant activity and antimicrobial activity of *Vitis repens* Wight & Arn. sample, the following inferences could be deduced. The phytochemical screening, it was found that the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, saponins, starch and tannins in the *Vitis repens* Wight & Arn.

$\alpha$ -amino acids, flavonoids and reducing sugars were not observed in this sample. The present phytochemical compounds are very important for human nutrition and vital role. *Vitis repens* Wight & Arn. can be observed that the sample contain Ca (76.8 %) and K (21.3 %) as major inorganic constituents and Ti (0.646 %), Mn (0.393 %), S (0.154%), Fe (0.142 %), Si (0.127 %), Mg (0.127 %) and P (0.108 %) as minor inorganic constituents. Calcium element is required for bone development.

Potassium element is very effective essential element for human metabolisms. It helps to reduce the hypertensive effect. Other minor trace elements are important nutrient for human health's. Study of antioxidant activity expressed as IC<sub>50</sub> value was observed 25.72 ppm.

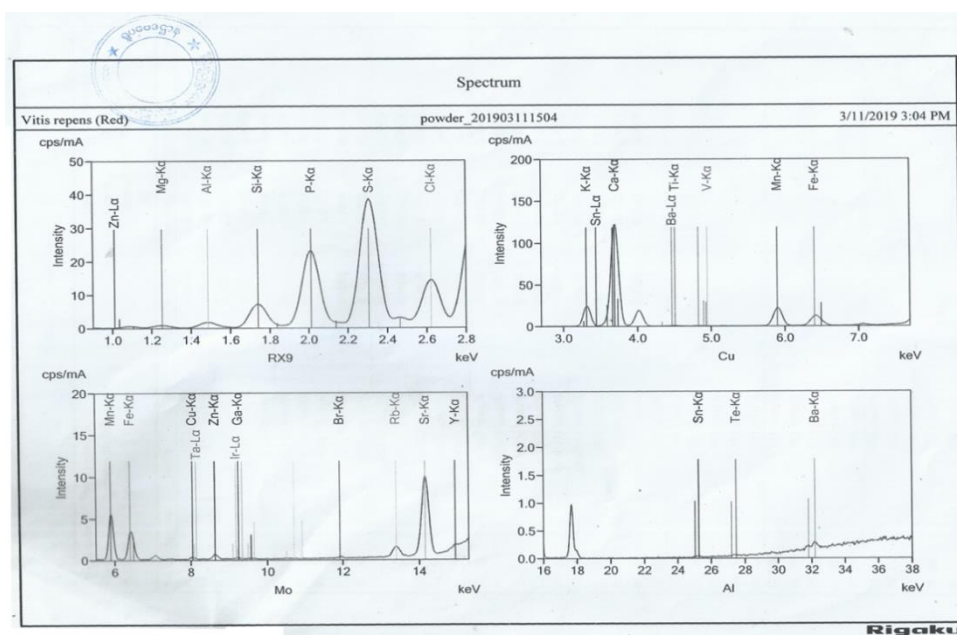
The lower the IC<sub>50</sub> value, the higher the antioxidant activity of the sample. Watery extract showed (14mm) and EtOH extract showed (15mm) inhibition against the *Pseudomonas aeruginosa* microorganisms.



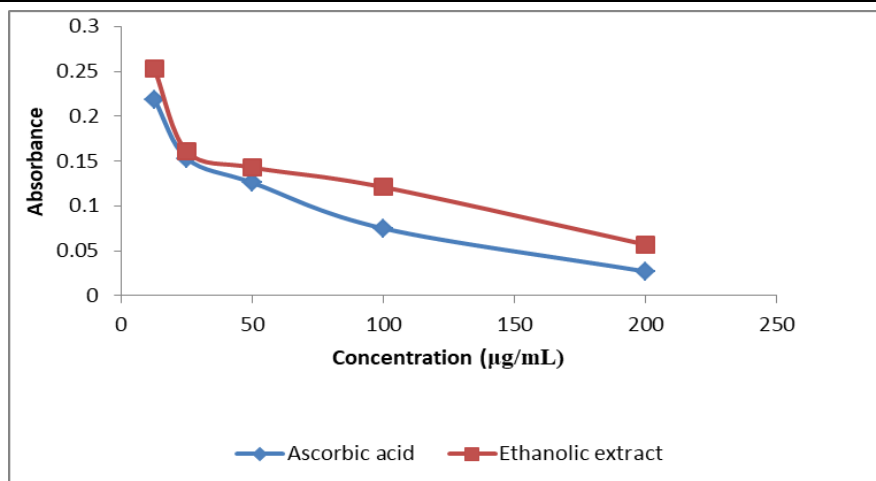
Figure 1. Plant of *Vitis repens* Wight & Arn

**Table 1. Elemental Analysis of *Vitis repens* Wight & Arn. by EDXRF Method**

No	Elements	Mass %
1	Ca	76.8
2	K	21.3
3	Ti	0.646
4	Mn	0.393
5	S	0.154
6	Fe	0.142
7	Si	0.127
8	Mg	0.127
9	P	0.108
10	Al	0.0631

**Figure 2. EDXRF spectrum of *Vitis repens* Wight & Arn.****Table 2. Absorbance of Standard Ascorbic Acid and Ethanol Extract of *Vitis repens* Wight & Arn. at 517 nm by UV Spectrophotometer**

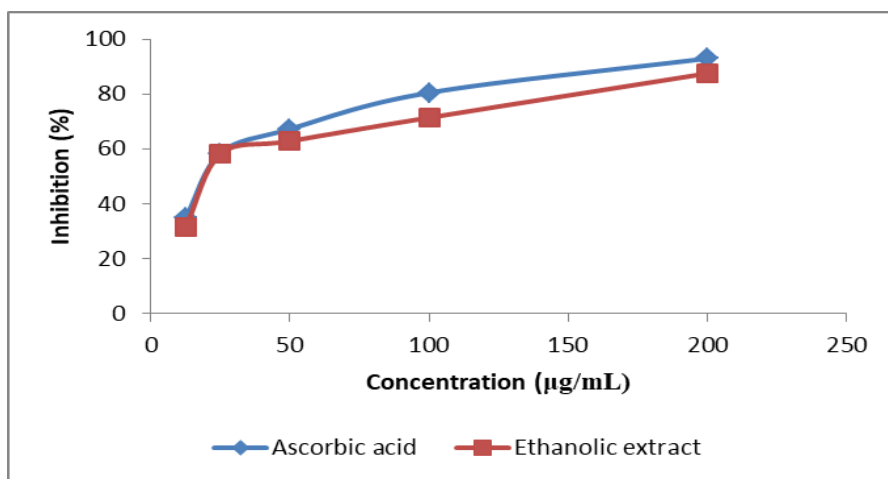
No.	Concentration (µg/mL)	Ascorbic acid (Standard)	Ab <sub>Sample</sub>
1	12.5	0.251	0.264
2	25	0.160	0.161
3	50	0.126	0.143
4	100	0.075	0.110
5	200	0.027	0.048



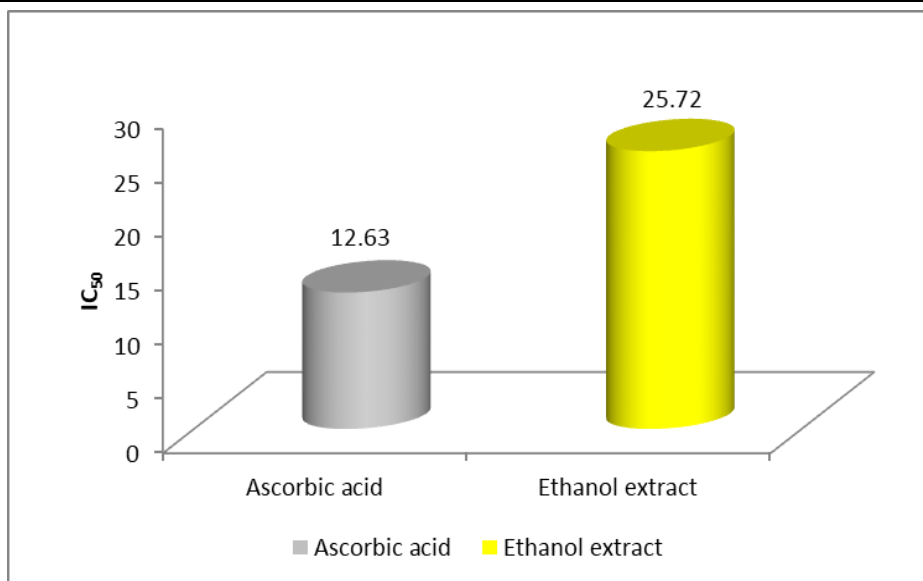
**Figure 3. Absorbance of standard ascorbic acid and *Vitis repens* ethanol extract with concentrations**

**Table 3. Percent Inhibition of Standard Ascorbic Acid and Ethanol Extract of *Vitis repens* (DPPH Scavenging Assay Method)**

No.	Concentration (µg/mL)	Ascorbic acid (Standard)	Abs <sub>sample</sub>
1	12.5	34.81	31.42
2	25	58.44	58.18
3	50	67.27	62.86
4	100	80.52	71.42
5	200	92.99	87.53



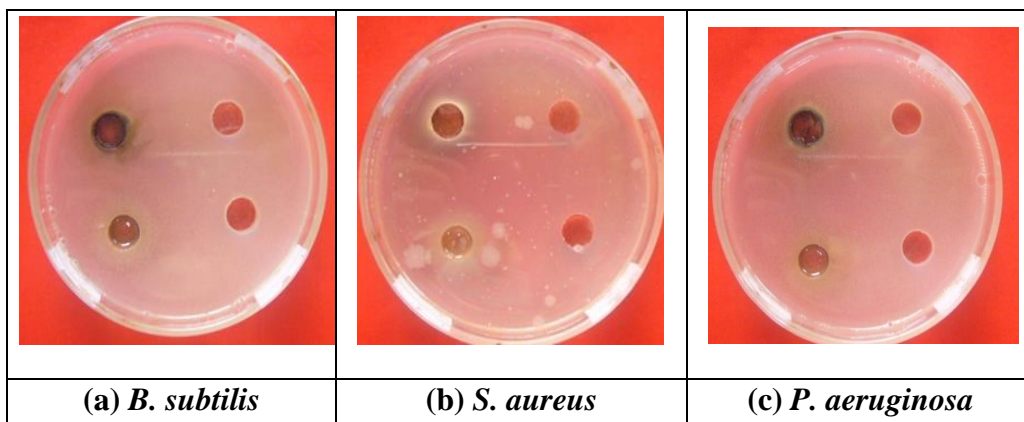
**Figure 4. Antioxidant activities of standard ascorbic acid and ethanol extracts with concentrations**



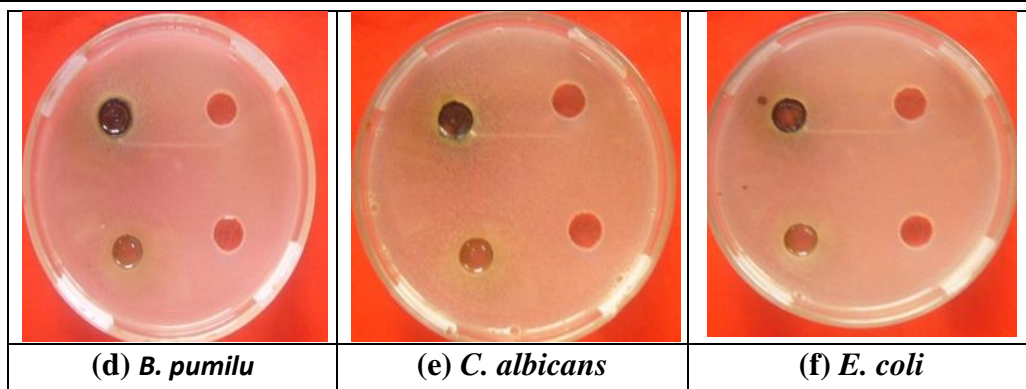
**Figure 5. IC<sub>50</sub> values of standard ascorbic acid and ethanol extract of *Vitis repens***

**Table 4. Diameter of Inhibition Zone of Crude Extracts of *Vitis repens* on Different Microorganisms**

No	Types of Microorganism	Diameter of inhibition zone (mm)	
		Water	Ethanol
1	<i>Bacillus subtilis</i>	-	-
2	<i>Staphylococcus aureus</i>	-	-
3	<i>Pseudomonas aeruginosa</i>	14 (+)	15 (+)
4	<i>Bacillus pumilus</i>	-	-
5	<i>Candida albicans</i>	-	-
6	<i>E. coli</i>	-	-







**Figure 6. Antimicrobial activities of crude extracts of *Vitis repens* Wight & Arn. by agar well diffusion method**

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## Isolation of Pure Bioactive Organic Compound from Nodule of *Boscia variabilis* Collett & Hemsley. (Thamon)

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### Abstract

In this research paper, one Myanmar indigenous medicinal plant, *Boscia variabilis* Collett & Hemsley. (Thamon) collected from Pakokku University campus, in Pakokku District, Magway Region, Myanmar, was selected for the chemical analysis. Phytochemical constituents of nodule of Thamon were determined by standard methods. Some phytochemical constituents such as alkaloid, steroid, terpene, glycoside, phenolic compound, reducing sugar and saponin were observed in the sample. The elemental content of the sample were also determined by using Energy Dispersive X-ray Fluorescence (EDXRF) spectrophotometer. The percentage of potassium was found the highest amount in the sample. The percentage of sulfur, silicon, calcium, phosphorus, iron, barium, manganese, copper and strontium were found in the range of 0.406 to 0.002%. The antimicrobial activities of the sample were tested by agar well diffusion method on six types of microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albican* and *Escherichia coli*. Ethyl acetate crude extract of nodule of Thamon gave high potent activity on all tested organisms. Furthermore, a pure compound was isolated from the nodule of Thamon as white crystal (0.01 g) by using thin layer and column chromatography. The yield percent was found to be (0.48 %) based upon the ethyl acetate crude extract. Moreover, antimicrobial activities of this pure compound were rechecked by using agar well diffusion method. The isolated pure compound responded to high potent activity on all tested organisms. Finally, the functional groups of isolated pure compound could be determined by FT-IR spectroscopic method. Present investigations indicate that locally available nodule of Thamon contains active components of great medicinal values.

**Keywords:** *Boscia variabilis*, antimicrobial activities, chromatography, FT-IR.

## Introduction

The world is rich with natural and unique medicinal plants are now getting more attention than ever because they have offered of many benefits to society or indeed to all human kind, especially in terms of medicinal and pharmacological constituents that produce definite physiological action on the human body. Large numbers of plants are constantly being screened for their possible pharmacological value (Singh, 2011). Many traditional medicines in use are derived from medicinal plants, minerals and organic matter (Grover *et al.*, 2002).

Medicinal Plants have been contributing for the management of cancer since time immemorial. Plants are sources of natural antioxidants, and some of the compounds have significant antioxidative properties and health benefits (Exarchou *et al.*, 1998). The cancer chemopreventive activities of naturally occurring phytochemicals are of great interest (Shahidi *et al.*, 1992). The *Boscia variabilis* genus belongs to the Cappariaceae family. Myanmar name is Thamon. The flowering period is between January and March and the fruiting period is between March and May.

It is a very useful plant and it plays a great part in the field of Myanmar indigenous medicine. The nodule possesses much medicinal value. It is bitter. It alerts the nervous system. It is useful in the treatment of coal and clammy, blurring of vision, heaviness, muscle ache and pain, tightness of chest and cancer. The leaves and flowers are used as salad and soup. It is a middle-sized tree and grows in India and Thailand (Ah Shin Nagathein, 1983). It grows in Magway, Mandalay and Sagaing Region, Myanmar.

In Myanmar, medicinal plants have always been a basic resource for human health. Appreciation for the preventative and therapeutic value of herbal remedy and the additional benefits of their low cost, the government encourage indigenous form of medicine rather than rely on imported drugs. On the other hand, there are strong moves to revive traditional cultures (Encyclopaedia, 2006). There are approximately 10 times as many bacteria cells as human cells in the human body, with large numbers of bacteria on the skin and in the digestive tract.

Pathogenic bacteria contribute to other globally important diseases, such as pneumonia, which can be caused by bacteria such as *Streptococcus* and *Pseudomonas* (Arthanriswaran *et al.*, 2012). Some organisms, such as *Staphylococcus* or *Streptococcus*, can cause skin infections, pneumonia, meningitis and even overwhelming sepsis, a systemic inflammatory response producing shock, and death (Fish, 2002). In addition, *Pseudomonas aeruginosa*, cause disease mainly in people suffering from cystic fibrosis (Heise, 1981).

The aim of this project is to be aware of the local people of the effective use of nodule of Thamon amply grown in Myanmar. In this research work, determination of phytochemical constituents, elemental content and antimicrobial activities of nodule of Thamon collected from Pakokku University campus were carried out. Furthermore, extraction and isolation of pure bioactive organic compound from nodule of Thamon were carried out.

## Materials and Methods

### Sample Collection

The nodules of *Boschia variabilis* Collett & Hemsley. (Thamon) were collected from Pakokku University campus, in Pakokku District, Magway Region, Myanmar. Photographs of the plant, nodule and powder of Thamon were shown in figure 1.

The collected samples were cleaned from dust, washed with water and dried at room temperature for one month. The dried materials were powdered by using grinding machine, and stored in airtight glass bottles until they were used.



**Figure 1. The plant, nodule and powder of Thamon**

### Preliminary Phytochemical Investigation of the Sample

The phytochemical constituents of the sample were determined by standard methods (Harborne, 1984). The results were shown in figure 2.

### Elemental Analysis of the Sample by EDXRF Spectrophotometer

The elemental content of the ground and sieved sample were examined by EDXRF spectrophotometer at Monywa University. The resulting data of the sample was shown in table 1.

**Antimicrobial Activities of the Crude Sample**

Four solvent extracts of the sample were sent to the Pharmaceutical Research Department, Ministry of Industries, Insein, Yangon for the measurement of antimicrobial activities.

In this experiment, antimicrobial activities of the sample were tested on six organisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albican* and *Escherichia coli* by using agar well diffusion method (Cruickshank, 1975). The results were described in table 2.

**Extraction and Isolation of Pure Compound from the Nodule of Thamon**

Air dried sample 250 g was percolated with 95% ethanol (450 ml) for about one month. Percolated solution was filtered and concentrated to yield residue. It was extracted with ethyl acetate and evaporated.

The ethyl acetate crude sample (2.1g) was obtained. It was separated by column chromatography over silica gel (70 - 230 mesh) eluted by various volume ratios of n-hexane and ethyl acetate from non-polar to polar. After the column separation, total (100) fractions were obtained.

Each fraction was checked by TLC. These fractions were combined according to same  $R_f$  value under UV lamp and iodine vapour. Twelve combined fractions were obtained. Finally, the fraction (D) gave one spot on TLC in ( $R_f = 0.4$ ) with n-hexane: ethyl acetate (3:2 v/v) and UV active.

The pure compound, white crystal (0.01 g), was obtained. The yield percent was found to be (0.48%) based upon the ethyl acetate crude extract.

**Antimicrobial Activities of Pure Compound**

Antimicrobial activities of pure compound were rechecked by using agar well diffusion method with six organisms. The results were shown in figure 4.

**Spectroscopic Study of Pure Compound**

Pure compound was subjected to analysis by FT-IR spectrophotometer at the Department of Chemistry, Monywa University. The results were described in table 3.

**Results and Discussion**

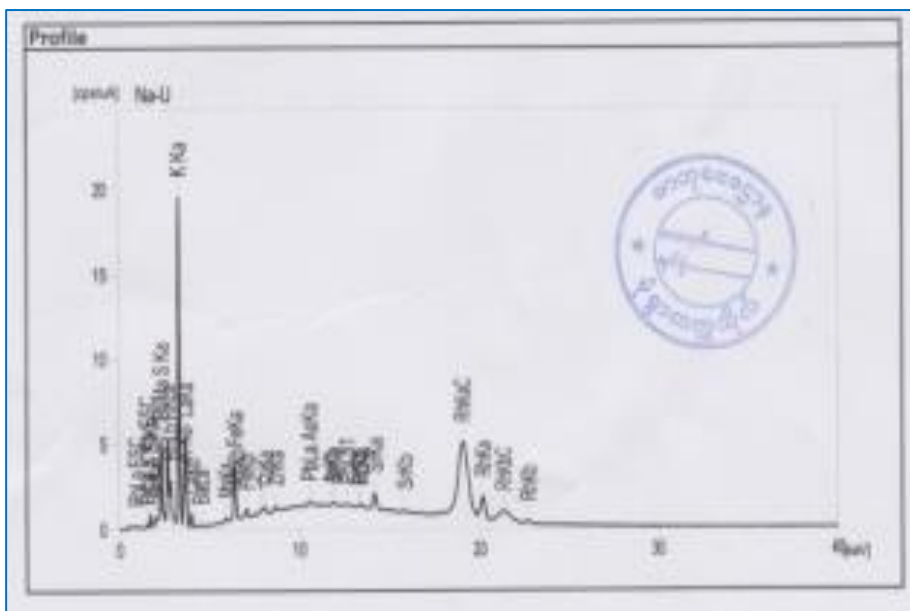
The results obtained from phytochemical screening of nodule of Thamon were described in figure 2.



**Figure 2. Photochemical test of nodule of Thamon**

According to the experimental result, the nodule of Thamon contains alkaloid, steroid, terpene, glycoside, phenolic compound, reducing sugar and saponin respectively.

The results of the nodule of Thamon by EDXRF spectrophotometer were shown in figure 3.



**Figure 3. EDXRF spectrum of nodule of Thamon**

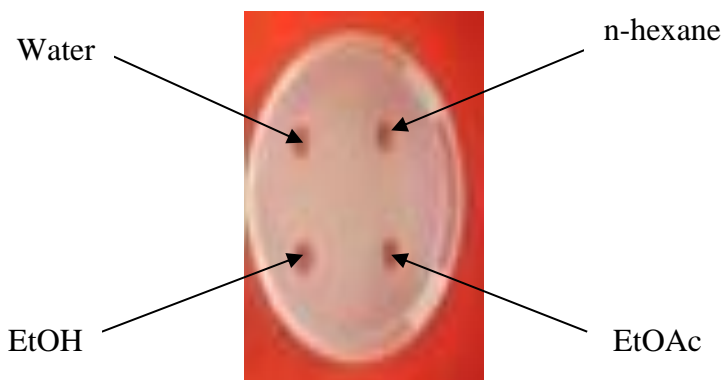
**Table 1. Mineral Content of Nodule of Thamon from EDXRF Analysis**

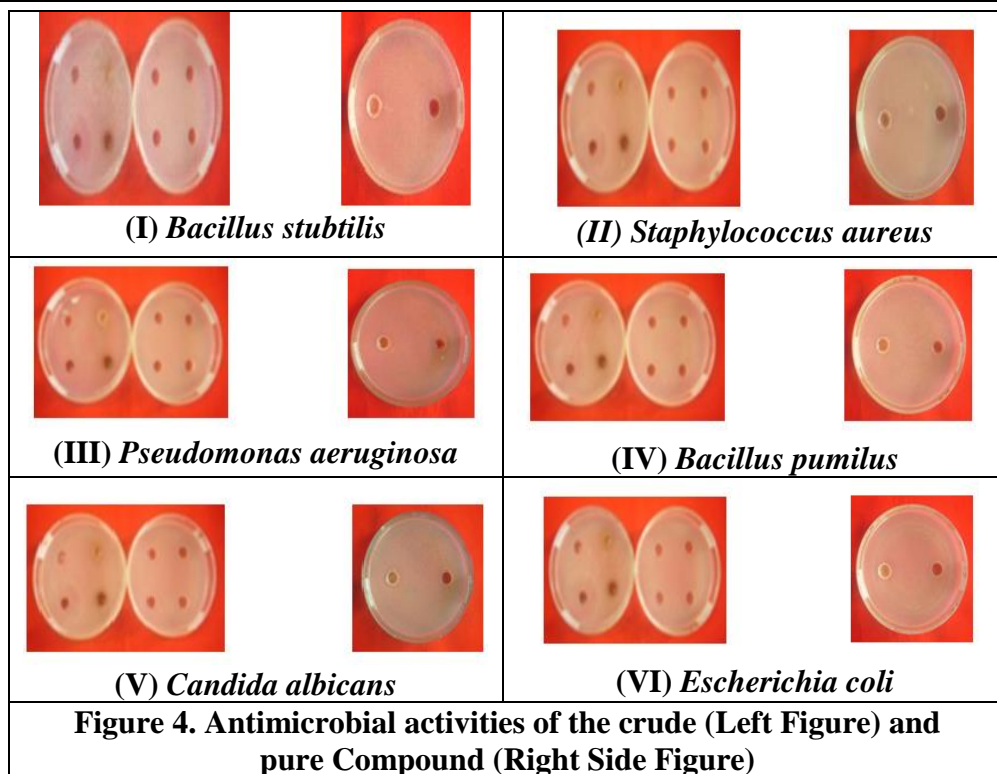
No.	Element	Amount(%)
1.	Potassium (K)	1.193
2.	Sulfur (S)	0.406
3.	Silicon (Si)	0.337
4.	Calcium (Ca)	0.214
5.	Phosphorus (P)	0.102
6.	Iron (Fe)	0.020
7.	Barium (Ba)	0.006
8.	Manganese (Mn)	0.002
9.	Copper (Cu)	0.002
10.	Strontium (Sr)	0.002
11.	Lead (Pb)	0.001
12.	Zinc (Zn)	0.001
13.	Hydrocarbon(CH)	97.713

According to the EDXRF spectral data, the percentage of potassium was found the highest amount in the sample. The percentage of sulfur, silicon, calcium, phosphorus, iron, barium, manganese, copper and strontium were found in the range of 0.406 to 0.002%.

As a result, potassium in the samples assists for the proper function of all cells, tissues and organs in the human body.

The results of antimicrobial activities of the crude sample and pure compound were shown in figure 4.





The results of antimicrobial activities of the crude sample and pure compound were compared in table 2.

**Table 2. Antimicrobial Activities of the Crude Sample and Pure Compound**

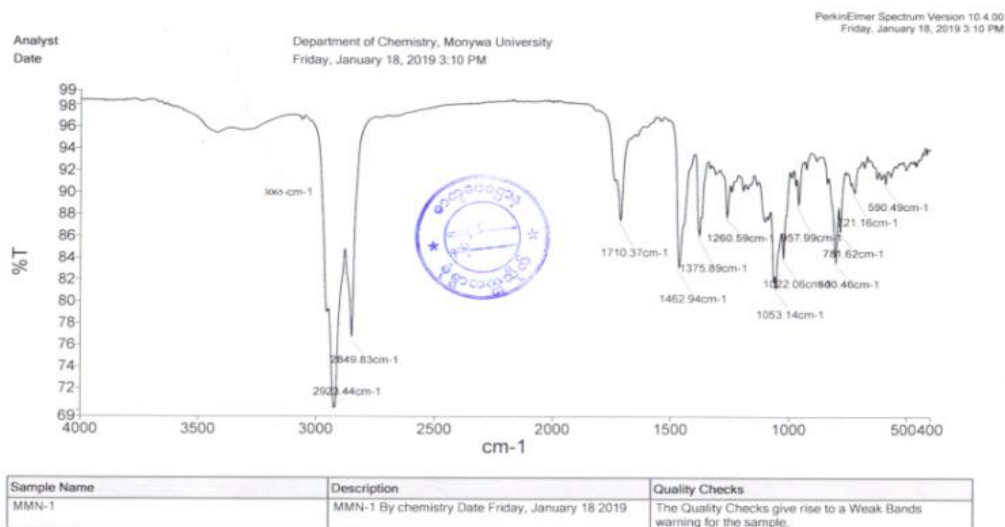
Samples	Solvent	Organisms					
		I	II	III	IV	V	VI
<b>Thamon (crude)</b>	n-hexane	-	11mm (+)	-	-	12mm (+)	-
	EtOAc	23mm (+++)	20mm (+++)	30mm (+++)	22mm (+++)	22mm (+++)	28mm (+++)
	EtOH	14mm (+)	13mm (+)	12mm (+)	14mm (+)	14mm (+)	14mm (+)
	Water	-	11mm (+)	-	11mm (+)	-	-
<b>Pure Compound</b>	EtOH	21mm (+++)	20mm (+++)	24mm (+++)	20mm (+++)	20mm (+++)	22mm (+++)
<b>Organisms</b>				<b>Agar well ~ 10 mm</b>			



I	- <i>Bacillus subtilis</i> (N.C.T.C-8236)	10 mm ~ 14 mm(+)
II	- <i>Staphylococcus aureus</i> (N.C.P.C-6371)	15 mm ~ 19 mm (++)
III	- <i>Pseudomonas aeruginosa</i> (6749)	20 mm above (+++)
IV	- <i>Bacillus pumilus</i> (N.C.I.B-8982)	
V	- <i>Candida albican</i>	
VI	- <i>Escherichia coli</i> (N.C.I.B-8134)	

According to these data, ethyl acetate crude extract of nodule of *Thamnon* responds to high potent activity on all tested organisms and ethanol extract gives low activity on all tested organism. Moreover, pure compound responds to high potent activity on all tested organisms.

FT-IR spectrum of the pure compound was shown in figure 5.



**Figure 5. FT-IR spectrum of pure compound**

In the spectrum of pure compound, the band which appears at  $3065\text{cm}^{-1}$  should be C=C-H stretching vibration of  $\text{sp}^2$  hydrocarbons. The band which appears at  $2923.44\text{cm}^{-1}$  and  $2849.83\text{cm}^{-1}$  are due to asymmetrical and symmetrical C-H stretching vibration of  $\text{sp}^3$  hydrocarbons. The band at  $1710.37\text{cm}^{-1}$  should be (C=O) stretching vibration of carbonyl group. The band at  $1462.94\text{cm}^{-1}$  is due to C=C stretching vibration of alkenic group. The band at  $1375.89\text{cm}^{-1}$  should be C-H bending vibration of  $\text{sp}^3$  hydrocarbons. The band at  $1260.59\text{cm}^{-1}$  is due to (C-C-O) stretching vibration of ester group. The two bands at  $1053.14$  and  $1022.06\text{cm}^{-1}$  are indicated (C-CO-O) stretching vibration of ester group. The band at  $957.99\text{cm}^{-1}$  should be C-H out of plane bending vibration of E or trans alkene.

The band at  $800.46\text{ cm}^{-1}$  should be C=C-H out of plane bending vibration of cis or Z alkenic group. The two bands at  $781.62$  and  $721.16\text{ cm}^{-1}$  are indicated C-H out of plane bending vibration of Z or cis alkene.

The functional groups observed in FT-IR spectrum are tabulated in table 3.

**Table 3. FT-IR Assignment of Pure Compound**

	<b>Absorption band (<math>\text{cm}^{-1}</math>)</b>	<b>Assignments</b>
1	3065	C=C-H stretching vibration of $\text{sp}^2$ hydrocarbons
2	2923.44, 2849.83	asymmetrical and symmetrical C-H stretching vibration of $\text{sp}^3$ hydrocarbons
3	1710.37	C=O stretching vibration of carbonyl group
4	1462.94	C=C stretching vibration of alkenic group
5	1375.89	C-H bending vibration of $\text{sp}^3$ hydrocarbons
6	1260.54	C-C-O stretching vibration of ester group
7	1053.14, 1022.06	C-CO-O stretching vibration of ester group
8	973.82	C-H out of plane bending vibration of E or trans alkene
9	800.46	C=C-H out of plane bending vibration of cis or Z alkenic group
10	781.62, 721.16	C-H out of plane bending vibration of Z or cis alkene

## Conclusion

In this research, nodule of Thamon selected for chemical analysis was based on its traditional health, local availability and distribution. The nodules of Thamon were collected from Pakokku University campus. Qualitative phytochemical screening showed the presence of alkaloid, steroid, terpene, glycoside, phenolic compound, reducing sugar and saponin in the sample. In the elemental analysis, the percentage of potassium was found the highest amount in the sample. As a result, the sample helps to generate muscles, nerves and skin cells in human body. In contrast, trace amount of lead was found in the sample. Antimicrobial activities of four solvent extracts of the sample were tested by agar well diffusion method on six types of microorganisms.

Furthermore, pure compound, white crystal (0.02 g, 4.54%) based upon the ethyl acetate crude extract, could be isolated from the ethyl acetate extract of nodule of Thamon by using thin layer and column chromatography. The antimicrobial activity of this pure compound responds to high potent activity on all tested

organisms. In addition, functional groups of the pure compound could be determined by using FT-IR spectral data.

Present investigations indicate that locally available nodule of Thamon contains active components of great medicinal values. Therefore nodule of Thamon could be considered as “a gift of nature”. More research on the study of isolation and identification of pure bioactive organic compounds from other parts of Thamon can be done.

### Acknowledgements

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# Investigation of Phytochemical Screening and Some Chemical Constituents of Leafy Extract from *Piper betle* Linn. (Betel vine)

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## Abstract

*Piper betle* Linn leaf is a well-known plant in ancient medical sciences. It has reported that *Piper betle* Linn extract showed anti-diabetic and anti-inflammatory effects showed antipyretic effect, leaves extracts showed antioxidant effect. In this study, *Piper betle* Linn leaf was extracted by using different solvents such as PE, CHCl<sub>3</sub>, EtOAc and MeOH. The preliminary phytochemical screening of powdered *Piper betle* Linn leaf was carried out. It was revealed the presence of alkaloids, steroids, terpenoids, flavonoids, phenolic compounds, glycosides, saponins,  $\alpha$ -amino acids and carbohydrates. In the examination of qualitative phytoconstituents, various phytoconstituents like phytosterol, phenolic compounds, flavonoids and alkaloids were present in *Piper betle* Linn leaf by the chromatographic separation technique (TLC). In the determination of soluble extractive parameter, it was found that petroleum ether soluble extractive (12 %), chloroform soluble extractive (8.5 %), ethyl acetate soluble extractive (10.2 %) and methanol soluble extractive (17.5 %). In the determination of nutritional value, moisture (11.61 %), fat (1.01 %), ash (13.89 %), fiber (14.74 %), protein (19.52 %), carbohydrate (32.23 %) and energy value (245Kcal/100g) were observed.

**Keywords:** *Piper betle* Linn., TLC, Phytoconstituents.

## Introduction

*Piper betle* Linn. (Family–Piperaceae) is a well-known plant in ancient medical sciences. It has reported that *Piper betle* Linn. Leaves extract showed anti-diabetic and anti-inflammatory effects showed antipyretic effect, leaves extracts showed antioxidant effect. Herbs contain some complicated mixtures of organic chemicals that may vary depending upon many factors related to the growth, production, and processing of the herbal product [5] Antipyretics; from the Greek anti, *against*, and pyreticus, (*pertaining to fever*) are drugs or herbs that reduce fever [2].

Body temperature rises due to derangement of heat regulating mechanism in the brain. The rise in body temperature above 99°F is called fever. Fever generally

occurs due to the infections by virus, bacteria, protozoa and other microorganisms that produce pyrogens. These pyrogens act on WBC which in turn produced endogenous toxins. They act on the anterior hypothalamus and the body temperature is elevated causing fever. Fever leads to the disturbance of metabolism and it increases blood pressure, pulse rate, cardiac output, respiration rate, etc. The antipyretic agents treat these symptoms and completely eliminate fever [6, 10].

Most antipyretic medications have other purposes. The most common antipyretics are ibuprofen and aspirin, which are used primarily as pain relievers. Non-steroidal anti-inflammatory drugs (NSAIDs) are antipyretic, anti-inflammatory, and pain relievers [2, 10].

Various medicinal plants are used as an antipyretic agent from the ancient time. Many traditional formulating medicines are available here for treating different types of diseases like Hepatitis, Cancer, ulcer etc. from generation after generation. In nature there is so many types of plants which has some antipyretic activity are available. It is believed that the herbal medicine is the best one because it has no side effect in our body [9]. plant is stout glabrous climber with leaves of 5-20 cm long broadly ovate, slightly cordate and unequal at the base, shortly acuminate with yellowish or bright green shining on the both size. The stem are dichotomous, articulate, swollen and rooted at nodes 3mm in diameter, woody and with 2.5 to 4 cm long internodes. Stem stout with pinkish-stripe along node dilated and rooting. The fruits are drupaceous, orange, and 3mm in diameter.

The chemical constituents of leaf have been reported to contain of alkaloids, steroids, terpenoids, flavonoids, phenolic compounds, glycosides, saponins,  $\alpha$ -amino acids, carbohydrates, fat, protein, fiber and moisture of them, the important constituent which determines the value of the leaf is the essential oil (eugenol). The essential oil of leaf is bright yellow to dark-brown liquid. It has an aromatic somewhat creosote like odor with a pungent sharp taste. *Piper betle* Linn leaf has been used from ancient times as an aromatic stimulant and ant flatulent. It is useful in arresting secretion or bleeding and is an aphrodisiac.

Its leaf is used in several common household remedies. *Piper betle* Linn leaf juice is credited with diuretic properties. Its juice, mixed with dilute milk and sweetened slightly, helps in easing urination. Leaves are beneficial in the treatment of nervous pains, nervous exhaustion and debility. The juice of a few *Piper betle* Linn leaves, with a teaspoon of honey, will serve as a good tonic. A teaspoon of this can be taken twice a day. *Piper betle* Linn leaf has analgesic and cooling properties [7, 8].

It can be applied with beneficial results over the painful area to relieve intense headache. *Piper betle* Linn leaves are useful in pulmonary affection in childhood and age. The leaves, soaked in mustard oil and warmed, may be applied to the chest to relieve cough and difficulty in breathing. Applied locally, *Piper betle* Linn leaves are beneficial in the treatment of inflammation such as arthritis and archivist, that is inflammation of the tastes. *Piper betle* Linn leaves can be used to heal wounds.

The juice of a few leaves should be extracted and applied on the wound. Then, Kun leaf should be wrapped over and bandaged. The wound will heal up with a single application within 2 days. The herb is also on effective remedy for boils. A leaf is gently warmed till it gets softened, and is then coated with a layer of castor oil. The oiled leaf is spread over the inflamed part. This leaf has to be replaced, every few hours. After a few applications, the boil will rupture draining all the purulent matter. The application can be made at night and removed in the morning. A hot poultice of the leaves or their juice mixed with some bland oil such as refined coconut oil can be applied to the loins with beneficial results in lumbago [3].

*Piper betle* Linn leaf juice, mixed with honey, helps in the case of indigestion, flatulence, fever aliment etc, in children and mixed with milk, helps in whooping cough for women. It is used for eye injury and infection as a baby lotion for the new born. It can also use in treatment of pulmonary infection in children and old age , dry cough, productive cough, irritable cough, cough due to bronchitis, bronchial asthma, malaria, nervous pains, exhaustion, debility [9].

*Piper betle* Linn leaf have many biological activity. They are anti-oxidant activity, anti-fungal activity, anti-ulcer genic activity, anti-platelet activity, antimicrobial activity, anti-inflammatory activity and radio-protective activity [4].

## Materials and Methods

### Collection and Preparation of Sample

*Piper betle* Linn leaf were collected from Ton-Tay Township, Yangon Division, Myanmar. The collected plant was identified and confirmed as *Piper betle* Linn. at Botany Department, Dagon University.

The collected plant samples were washed thoroughly with water. After cleaning, the sample were cut into small pieces and air dried at room temperature for three weeks. The dried samples were powdered by using grinding machine and stored in air- tight container and labeled systematically to prevent moisture changes and other contamination.

### **Chemicals**

All chemicals used in this work were from British Drug House Chemical Ltd., Poole, England. All standard solutions and other diluted solutions throughout the experimental runs were prepared by using distilled water. In all the investigations the recommended methods and standard procedures involving both conventional and modern techniques were employed. All other chemicals and reagents used were of analytical grade.

### **Preliminary Phytochemical analysis**

Qualitative phytochemical analyses were performed in Preliminary Phytochemical analysis were performed in extraction of *Piper betle* Linn. Leaf. Preliminary phytochemical test were carried out according to determine the presence of phytochemicals the alkaloids, steroids, terpenoids, flavonoids, phenolic compounds, glycosides, saponins,  $\alpha$ -amino acids and carbohydrates were found to be present but reducing sugar was absent in *Piper betle* Linn leaf sample as described by standard procedure.

### **Qualitative Determination of Phytoconstituents of Various Crude Extracts by TLC Method**

Dried powdered plant material namely *Piper betle* Linn. Leaf was extract with 50 mL methanol, petroleum ether, ethyl acetate and chloroform for TLC investigation. Their extract were loaded on the percolated TLC silica gel plate and the chromatography was carried out using an appropriate standard solvent system for *piper betle* Linn.

The developed chromatograms were first inspected under UV-254 nm and 365nm light and sprayed with detecting reagents to classify the compounds present and their functional groups.

### **Moisture Content**

The moisture content of *Piper betle* Linn. Leaf was determined by AOAC (2000) Method No. 934-01 accordingly. 10 g sample was dried in hot air oven (Model: DO-1-30/02, PCSIR, Pakistan) at a temperature of  $105 \pm 5$  °C for the duration until weight was constant.

### **Crude Protein**

The Kjeltch Apparatus (Model: D-40599, Behr Labor Technics, Gumby-Germany) was used for the determination of nitrogen percent in garlic using AOAC (2000) Method No. 984-13. Accordingly, *Piper betle* Linn. Leaf was digested with concentrated  $H_2SO_4$  by using digestion mixture ( $K_2SO_4:FeSO_4:CuSO_4$  i.e. 100:5:10) until the color was light greenish.



The digested material was diluted up to 250 mL in volumetric flask. 10 mL of 40% NaOH as well as 10 mL of digested sample was taken in distillation apparatus where liberated ammonia was collected in beaker containing 4% boric acid solution using methyl red as an indicator. This resulted in formation of ammonium borate that was used for nitrogen determination in sample. Thus percentage of nitrogen in sample is assessed by titrating distillate against 0.1N H<sub>2</sub>SO<sub>4</sub> solution till color is light golden. Crude protein content was estimated by multiplying nitrogen percent (N %) with factor (6.25).

### Crude Fat

The crude fat content in *Piper betle* Linn. Leaf sample was estimated following guidelines of Method No 920-39 in AOAC (2000). Dried sample (3 g) was refluxed in soxhlet apparatus (Model: H-2 1045 Extraction Unit, Hoganas, Sweden) using n-hexane as a solvent.

### Crude fiber

The *Piper betle* Linn. Leaf sample was subjected to crude fiber content by elaborating Method No. 978-10 outlined in AOAC (2000). Fat free sample was digested with 1.25% H<sub>2</sub>SO<sub>4</sub> followed by 1.25% NaOH solution in Labconco Fibertech apparatus (Labconco Corporation Kansas, USA).

After filtration and washing with distilled water remaining residues was weighed and ignited in muffle furnace at temperature of 550-650°C till grey or white ash was obtained. The crude fiber percentage was estimated according to the expression given below.

### Total ash

The ash content of peel was estimated according to the procedure mentioned in AOAC (2000) Method No. 942-05. For which, 5 g sample was directly charred on flame in crucible until there was no fumes coming out. Afterwards sample was ignited in muffle furnace (MF-1/02, PCSIR, Pakistan) at 550-600°C for 5-6 hours or until grayish white residues were obtained.

### Results and Discussion

Phytochemical test in the *Piper betle* Linn. leaf indicated the presence of glycosides, carbohydrates,  $\alpha$ -amino acids, flavonoids, terpenoids, phenolic compounds, alkaloids, steroids and saponins were found to be present. The results of phytochemical tests are summarized in Table 1. They are of great medicinal value and have been extensively used in drug and pharmaceutical industry. The physical constant evaluation of the drug is an important parameter in detecting improper hand of drugs.

The medical plants are rich in secondary metabolites which include alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, tannins and reducing sugar,  $\alpha$ -Amino acid were found to be present.

In the determination of nutritional values were detected moisture (11.61 %), fat (1.01 %), ash (13.89 %), fiber (14.74 %), protein (19.52 %), and carbohydrate (39.23 %). The data are shown in Table 2.

Composition and nutrient contents (ash, fat and carbohydrates content) of *Piper betle* Linn. were investigated by standard methods. A number of developing solvent systems were tried, but the satisfactory resolution was obtained in the solvent systems mentioned in table 4, 5, 6, 7 and 8.

After development of plates, they were air-dried and numbers of spots were visualized by spraying with various spraying reagents to find different compounds present in the extract. Liebermann-Burchard reagent for steroids, terpenoids, saponins and essential oils, 1% aluminium chloride reagent for flavonoid, 10% sulphuric acid reagent for steroids and terpenoids, 5 % ferric chloride for phenolic compound.

### Conclusion

Preliminary phytochemical investigation on the *Piper betle* Linn. Leaf indicated the presence of glycosides, carbohydrates,  $\alpha$ -amino acids, flavonoids, terpenoids, phenolic compounds, steroids, alkaloids and saponins. Moisture, fat, ash, fiber, protein contents were found to be of (11.61 %), (1.01%), (13.89 %), (14.74 %) and (19.52 %), respectively. The carbohydrate content was 39.23 %.

The yield percent (%) of PE extract is (2.035), ethyl acetate extract is (3.78), chloroform extract is (8.75) and methanol extract is (13.14) were detected. *Piper betle* Linn. Leaf can be used as medicinal purpose because of their phytochemical constituents activities. After development of TLC plates, they were air-dried and numbers of spots were visualized by spraying with various spraying reagents to find different compounds present in the extract. Using Liebermann-Burchard reagent was observed steroids, terpenoids, saponins and essential oils. And then using 1 % aluminium chloride reagent was observed flavonoid. Using 10% sulphuric acid reagent was observed steroids and terpenoids. 5% ferric chloride solution using was observed phenolic compound.

For the purpose of quality control, assessment of purity and identification of any sample, standardization is much essential. The physical constant evaluation of the drug is an important parameter in detecting improper handling of drugs.

The medical plants are rich in secondary metabolites which include alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, tannins and reducing sugar,  $\alpha$ -Amino acid were found to be present. They are of great medicinal value and have been extensively used in drug and pharmaceutical industry.



**Figure 1. Plant of *Piper betle* Linn. (Betel vine)**

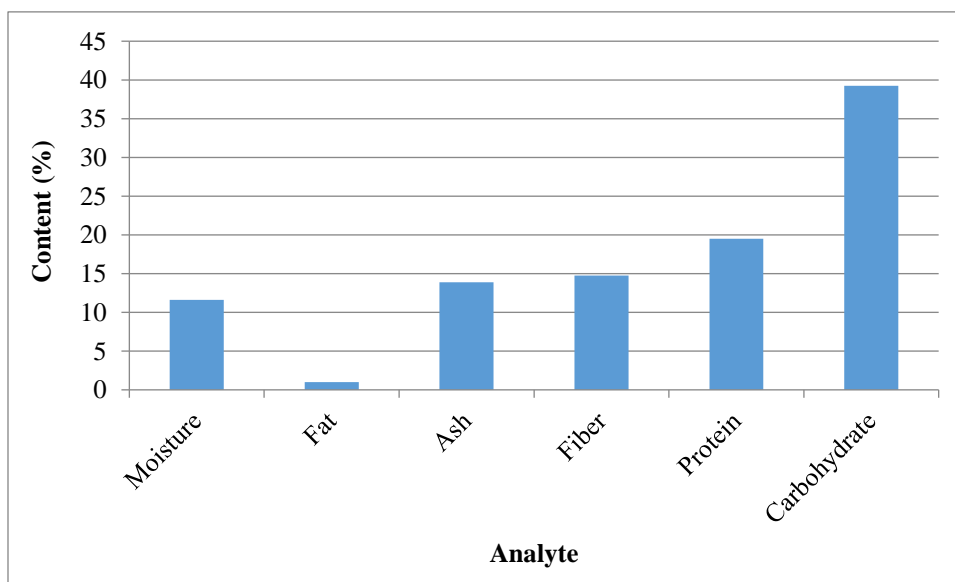
**Table 1. Results of Preliminary Phytochemical Tests on the *Piper betle* Linn. leaf**

S. No	Types of compounds	Extract	Test reagents	Observation	Remark
1	Alkaloids	1 % HCl	Mayer' reagent	Pale yellow ppt	+
			Dragendorff' reagent	Orange ppt	+
			Wagner' reagent	Brown ppt	+
2	Glycosides	H <sub>2</sub> O	10% lead acetate	White ppt	+
3	Carbohydrates	H <sub>2</sub> O	10% naphthol and conc: H <sub>2</sub> SO <sub>4</sub>	Red ring	+
4	$\alpha$ -amino acids	H <sub>2</sub> O	Ninhydrin reagent	Pink spot	+
5	Flavonoids	95% EtOH	Mg turning and conc: HCl	Pink color	+
6	Steroids/ Terpenoids	PE /CHCl <sub>3</sub>	Acetic anhydride and conc: H <sub>2</sub> SO <sub>4</sub>	Greenish blue	+
7	Phenolic compounds	H <sub>2</sub> O	5% Ferric chloride	Deep blue	+

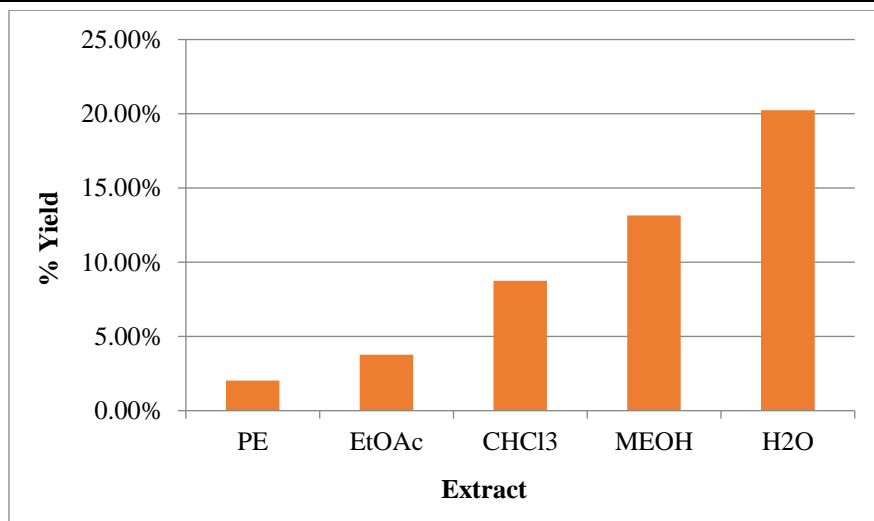
8	Tannins	H <sub>2</sub> O	Gelatin solution	no.ppt	-
9	Saponins	H <sub>2</sub> O	Distilled water	Frothing	+
10	Reducing Sugars	H <sub>2</sub> SO <sub>4</sub> (dil)	NaOH (dil) and Benedict's solution	no ppt	-
(+) = present and (-) = absent					

**Table 2. Results of Nutritional Values of *Piper betle* Linn. Leaf**

No	Analyte	Content (%)
1	Moisture	11.61
2	Fat	1.01
3	Ash	13.89
4	Fiber	14.74
5.	Protein	19.52
6.	Carbohydrate	39.23

**Figure 2. Nutritional value of *Piper betle* Linn. Leaf****Table 3. Results of Yield (%) *Piper betle* Linn. Leaf Crude Extracts**

No	Extract	% Yield
1	PE	2.035 %
2	EtOAc	3.78 %
3	CHCl <sub>3</sub>	8.75 %
4	MEOH	13.14 %
5	H <sub>2</sub> O	20.235%



**Figure 3. Results of Yield (%) *Piper betle* Linn. Leaf Crude Extracts**

**Table 4. Qualitative Determination of *Piper betle* Linn Crude Extracts by Libermann reagent**

Test Extract	Solvent System	Number of Spots	R <sub>f</sub> values					
			1	2	3	4	5	6
PE	PE : EA 2 : 1	6	0.36	0.44	0.56	0.72	0.84	0.96
EtOAc	PE : EA 2 : 1	6	0.36	0.64	0.76	0.86	0.96	1.02
CHCl <sub>3</sub>	PE : EA 2 : 1	6	0.36	0.64	0.76	0.86	0.96	1.02
MeOH	PE : EA 2 : 1	2	0.44	0.62	-	-	-	-

**Table 5. Qualitative Determination of *Piper betle* Linn Crude Extracts by 10 % H<sub>2</sub>SO<sub>4</sub> reagent**

Test Extract	Solvent System	Number of Spots	R <sub>f</sub> values				
			1	2	3	4	5
PE	PE : EA 2 : 1	5	0.38	0.45	0.73	0.53	0.87
EtOAc	PE : EA 2 : 1	5	0.53	0.64	0.74	0.83	0.94
CHCl <sub>3</sub>	PE : EA 2 : 1	5	0.47	0.64	0.74	0.83	0.94
MeOH	PE : EA 2 : 1	1	0.56	-	-	-	-

**Table 6. Qualitative Determination of *Piper betle* Linn Crude Extracts by 5 % FeCl<sub>3</sub> reagent**

Test Extract	Solvent System	Number of Spots	R <sub>f</sub> values		
			1	2	3
PE	PE : EA 2 : 1	3	0.43	0.79	0.96
EtOAc	PE : EA 2 : 1	5	0.77	-	-
CHCl <sub>3</sub>	PE : EA 2 : 1	5	0.60	-	-
MeOH	PE : EA 2 : 1	1	0.58	-	-

**Table 7. Qualitative Determination of *Piper betle* Linn Crude Extracts by 1% AlCl<sub>3</sub> reagent under UV (365nm)**

Test Extract	Solvent System	Number of Spots	R <sub>f</sub> values		
			1	2	3
PE	PE : EA 2 : 1	-	-	-	-
EtOAc	PE : EA 2 : 1	-	-	-	-
CHCl <sub>3</sub>	PE : EA 2 : 1	-	-	-	-
MeOH	PE : EA 2 : 1	1	0.6	-	-

**Table 8. Qualitative Determination of *Piper betle* Linn Crude Extracts by Dragendorff reagent**

Test Extract	Solvent System	Number of Spots	R <sub>f</sub> values			
			1	2	3	4
PE	PE : EA 2 : 1	5	0.38	0.51	0.85	0.95
EtOAc	PE : EA 2 : 1	5	0.49	0.79	0.97	-
CHCl <sub>3</sub>	PE : EA 2 : 1	5	0.50	0.87	0.74	-
MeOH	PE : EA 2 : 1	1	0.15	0.89	-	-

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# Knowledge, Attitudes and Practices among Medical Officers on Screening for Tuberculosis: A Case Study in Kalutara District, Sri Lanka

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## Abstract

Worldwide Tuberculosis (TB) still remains one of the top 10 leading causes of death. Nearly one third of the world population is infected with *Mycobacterium tuberculosis* and bearing the risk of developing disease. Annually 6000 new Tuberculosis (TB) cases are reported in Sri Lanka. Underutilization of microscopic Centres (MCs), poor contact tracing and inadequate detection of new cases have been identified as major weaknesses in the TB control program in Sri Lanka. Objective of this study was to assess the knowledge, attitudes and practices among Medical Officers (MO) on screening for TB in selected hospitals in Kalutara district, Sri Lanka. Descriptive cross-sectional study was carried out from January to March in 2017. Total study population was 202 and respondent rate was 83.6% (n=169). Pretested, self-administered questionnaire was used for data collection. Practices were assessed using clinical vignettes. The cutoff score for satisfactory knowledge was 68, a favourable attitude was 36.1 and satisfactory practice was 66.7. Majority of MOs (86.9%, n=147) had satisfactory knowledge and favorable attitudes (87%, n=147) on screening for TB. Less than half of MOs (46.7%, n=79) had favorable attitudes to refer for microscopic examinations of patients with more than 2 weeks of cough. Less than 40% of MOs are (n=68) performing CXR on suspected pulmonary TB (PTB). Less than 40% of the MOs (n=62) had satisfactory practices for TB screening. By analyzing two clinical vignettes it was noticed that the antibiotics seem to be prescribed by majority of MOs to treat PTB suspected patients.

**Keywords:** Microscopic centers, screening of tuberculosis, Knowledge, attitude, Practice.



## 1. Introduction

Worldwide Tuberculosis (TB) still remains one of the top 10 leading causes of death. Nearly one third of the world population is infected with *Mycobacterium tuberculosis* and bearing the risk of developing disease. In 2015, although the estimated incidence of TB in the world was 10.4 million, only 6.1 million cases have been identified. This revealed a gap of 4.1 million between the estimated and identified incidents. India, China and Indonesia, account for 45% of the total incidence in the world. In 2015, the estimated total deaths due to TB was 1.4 million and additionally, 0.4 million deaths occurred due to TB among HIV infected patients (WHO, 2016).

At end of 2015, WHO introduced eliminating strategies for TB for the period 2016 -2030 which is called “End TB Strategy”. Ultimately this was incorporated with the sustainable development goals introduced by United Nations for the same period. The “End TB Strategy” is based on three pillars of TB controlling strategies. The first pillar is “Integrated, patient-centered care and prevention” which includes early diagnosis of TB with universal drug-susceptibility testing, systematic screening of high-risk groups and contacts, treatment of all patients, supporting patients, collaborative HIV/TB activities, management of co-morbidities, preventive treatment of persons at high risk, and vaccination against TB.

The second pillar is “bold policies and supportive systems” which includes political commitment for TB care with adequate resources, engagement of communities, civil society organizations, and public and private care providers, universal health coverage, policy regulatory frameworks, rational use of medicine, vital registration, social protection, poverty alleviation and actions on other determinants of TB. The third pillar is “intensified research and innovation” which includes discovery, development and rapid uptake of new tools, interventions and strategies, research for optimizing implementation, impact, and promote innovations (Global TB report 2016).

Mortality due to TB in Sri Lanka is 5.9 per 100,000 populations (NPTCCD, 2015). Around 300,000 labor migrants leave Sri Lanka annually and 93% of them work in the region of Eastern Mediterranean Region where 37% of the global TB burden exists (Wickramage *et al.*, 2013). Although the current prevalence is comparatively low Sri Lanka still has a high potential of re-emergence of TB. Annually only around 11,000 new cases are reported in Sri Lanka out of its estimated 17,000 TB cases. This creates a gap of 6,000 undetected cases. Out of all the patients presenting with respiratory symptoms, 1-2% are considered as infected with TB.

In order to early detection of TB cases, National Program for Tuberculosis and Chest Disease (NPTCCD) emphasizes to improve referrals of suspected TB cases to MCs for sputum smear from all the Out Patient departments (OPD) and other units. But referrals for screening remains remarkably low and MCs are underutilized island wide (Ministry of Health, 2016). Poor contact tracing and inadequate detection of new cases have been identified as major weaknesses in TB control program in the western province by a study conducted in 2016 (Dolamulla and Samaraweera, 2016).

Objective of this study was to assess the knowledge, attitudes and practices among Medical Officers (MO) on screening for TB in selected hospitals in Kalutara district, Sri Lanka.

## 2. Methods

A hospital based descriptive cross sectional study was conducted during April to May 2017 in seven selected Government Hospitals in Kalutara district. Out of those of BH Panadura, Horana, District hospital(DH) Bulathsinhala, Mathugama, Ingiriya and Beruwala have microscopic examination facilities. District General Hospital (DGH) Nagoda has the district chest clinic which receives all suspicious patients for further evaluation and treatment.

All the Medical Officers (n=202) working at Outpatient department (OPD), Preliminary care unit(PCU)/Emergency treatment unit (ETU), Medical wards, Pediatric wards and Medical Officers working on common working rosters in the DH were included in the population. All Consultants, all Medical Officers on long term leave, released for training and not attending the duty during the period of study were excluded. Pre-tested, self-administered questionnaire with 4 sections was used for data collection.

Part 1 comprised of Socio-demographic, personal and carrier information of participant. Part 2 and part 3 assessed the knowledge and attitudes among MOs on screening for TB respectively. Part 4 measured the practices among MOs on screening for TB using clinical vignettes (case scenarios) which is a simple tool to measure the practices of clinicians (Peabody *et al.*, 2004). Questionnaire was developed with the assistance of Consultant Chest Physician (CCP), District Tuberculosis and Chest disease Officer (DTCO) and experts in the field such as Public Health Laboratory Technicians (PHLTs). WHO, National TB manuals, guidelines and the questionnaires used in previous studies were used to obtain content validity (Gutsfeld *et al.*, 2014, LoBue *et al.*, 2001, Blanc *et al.*, 2004, Getahun *et al.*, 2015, Alwis *et al.*, 2011, Sarukkali *et al.*, 2008, Gutsfeld *et al.*, 2014, LoBue *et al.*, 2001).

## **2.1 Knowledge**

Most of the questions were stem framed. Some questions were weighted with 3 marks according to gold standards available in the guidelines developed by NPTCCD and expert opinion. Rest of the questions was given 1 mark per each item. Each wrong answer, “Do Not Know” answer and missing values were given zero mark. The total cumulative knowledge score for each person was expressed as a percentage form total score.

All the questions necessarily considered as important for screening a Pulmonary tuberculosis (PTB) suspects (weighted score questions) were selected and their cumulative score percentage came as 68%. Below 68, the “unsatisfactory category” was defined and the satisfactory category was set above that value.

## **2.2 Attitudes**

A six point likert scale was used to assess attitudes and the MOs were asked to choose single response 6 to 1 marks and 1 - 6 marks respectively for positively phrased and negatively phrased questions. An individual was expected to at least slightly agree for positively phrased statements and slightly disagree for negatively phrased statements which carried 4 marks at each.

Therefore minimum score is set by multiplying 4 marks into the number of questions and the minimum attitudinal score was 36 for the 9 questions. The cumulative attitude score is calculated accordingly. Below 36, ‘unsatisfactory level of Attitudes’ was defined and above 36.1 ‘satisfactory level of attitudes’ was defined.

## **2.3 Practices**

Three clinical vignettes (Peabody et al., 2004) were used to assess practices. Each correct answer of question was given one (1) mark. When the correct answer is not known or incorrect, zero (0) mark was given.

The aggregate score of important or “must do” practices was 66.7%. This was decided with CCP guided with TB management guidelines.

The scores below 66.7% were categorized as “unsatisfactory level of practices” and above 68% were categorized as satisfactory level of practices. The significant level of knowledge, attitudes and practices among selected variables were analyzed using Fisher's Exact Test and Chi Square test. Ethical clearance was obtained by Post graduate institute of Medicine University of Colombo and administrative approvals were obtained by Provincial Director of health services western province and the Director of Nagoda hospital prior to conduct the study.

### 3. Results

Total study population was 202 and respondent rate was 83.6% (n=169) Majority of MOs were females (59.8%, n=101) and their mean age was 36.84 (SD = 9.01). About twenty five percent of MOs was belong to 25- 29 year age group (26.6%, n=45) and 2.4% (n=4) was belong to 50-54 year age group.

Less than 20% of MOs had post graduate qualifications (16.6%, n=28). Majority of MOs did not have experience in NPTCCD or chest clinic (CC) (76.9%, n=130) or any training during their career on TB (77.5%, n=131).

#### 3.1 Knowledge

**Table 1. Knowledge, Attitudes and Practices Levels of respondents**

Level of Performance	Variable		
	Knowledge	Attitudes	Practices
Unsatisfactory (Un favorable)	n=22,13%	n=22,13%	n=107,63.3%
Satisfactory (Favorable)	n=147,87%	n=147,87%	n=62,36.7%

The lowest recorded knowledge score was 45.45% and the highest score was 100%. The mean knowledge score was 78.45 (SD=12.50). Majority of MOs had satisfactory knowledge on screening of TB (87%, n=147). Only small proportion of MOs shows unsatisfactory knowledge (13%, n=22).

**Table 2. Frequency distribution of MOs on different aspects of knowledge on TB Screening (N=169)**

Question No	Questions	Correctly answered	
		No	%
Q1	TB transmission is by air droplets	167	98.8
Q2	High risk people are		
	a) Prisoners	161	95.3
	b) Elderly homes	148	87.6
	c) Patients from slums	148	87.6
Q3	Risk of EPT <sup>4</sup> is high when age<5 years	67	39.6
Q4	TB screening criteria are		
	a) Cough more than 2 weeks	155	91.7
	b) Uncontrolled DM with cough	124	73.4
	c) Pregnancy with cough and poor	117	69.2

	weight gain		
	d) Malnutrition & respiratory signs	157	92.9
	e) All contact children <5years old	99	58.6
Q5	Mantoux positivity is $\geq 10\text{mm}$	139	82.2
Q6	Essential Investigations for PTB diagnosis are		
	a) CXR	130	76.9
	b) Sputum AFB	163	96.4
	c) Mantoux test <sup>1</sup>	49	29
	d) FBC <sup>1</sup>	71	42
	e) ESR <sup>1</sup>	36	21.3
Q7	Diagnostic of PTB		
	a) $\geq 2$ Sputum AFB positivity	151	89.3
	b) 1 Sputum AFB positive with CXR abnormalities	146	86.4
	c) Only CXR abnormalities <sup>2</sup>	120	71
	d) Only by Mantoux test positivity <sup>2</sup>	142	84
Q8	Investigations done in MC		
	a) Sputum smear for AFB <sup>3</sup>	165	97.6
	b) Mantoux tests	104	61.5
	c) PCR for tuberculosis	75	44.4
Q9	For PTB screening, 3 sputum samples are examined initially	156	92.3
<sup>1</sup> Mantoux, FBC and ESR are not essential investigations for diagnosing PTB			
<sup>2</sup> PTB diagnosis is not made only with CXR abnormalities and Mantoux			
<sup>3</sup> Only Sputum smear for AFB (SSAFB) done at MC for TB diagnosis <sup>4</sup> EPT(Extra Pulmonary TB)			

Majority of MOs correctly answered to knowledge questions on transmission and risk factors (92.32%, n=158) but only about 40% (n=67) knew that the children below 5 year of age are vulnerable for extra pulmonary TB. Majority of MOs had knowledge on duration of cough in diagnosing PTB (91.7%, n=155) and relationship between malnutrition and TB (92.9%, n=157).

Majority knew the relationship between diabetes & TB (73.4%, n=124) and the relationship between pregnancy and TB (69.2%, n=117) which is comparatively low. Only 58.6% (n=99) MOs knew that all children less than 5 years with contact history should be subjected for screening.

Majority (86.65%, n=146.5) knew CXR and sputum AFB as essential investigations for diagnosing PTB. Only few MOs knew that there Mantoux test, FBC and ESR were not essential for making diagnosis of PTB (29%, (n=49), 42% (n=71), 21.3% (n=36) respectively). Majority Of the participants, (82.68%, n=148.5) knew the correct combinations of investigations in diagnosis of PTB. More than 95% of the participants (n=165) knew that the sputum AFB is done at MCs but only about half (n=89) knew that Mantoux test and PCR for TB are not performed at MCs. However, 92.3% (n=156) MOs knew that 3 sputum sample are examined initially in order to make the diagnosis of PTB (Table 2).

**Table 3. Distribution level of attitudes on screening for TB among MOs (n=169)**

Question No	Question	Satisfactory Attitudes
Q1*	Screening is unnecessary as tuberculosis incidence is very low in SL at present	n=155,91.7%
Q2	Sputum examination is an effective method in screening for tuberculosis	n=151, 89.3%
Q3*	PTB is unlikely although the patient has cough more than 2 weeks	n=79,46.7%
Q4	Always screen for TB with CXR in those who have cough > 2 weeks	n=101, 59.8%
Q5*	Filling up TB forms and registers are not my work	n=128,75.7%
Q6	Microscopic centers should be planned in all hospitals	n=140, 82.8%
Q7	Microscopic centers are effective resource for tuberculosis control in SL	n=151,91.1%
Q8	Willing to attend training programs on TB if selected	n=150, 88.8%
Q9	Guidelines on TB screening are very useful for a Medical Officer	n=166,98.2%
*Questions are negatively worded.		

### 3.2 Attitude

Mean attitude score of the study population was 41.6 (SD±4.8), minimum score was 27 and maximum is 54. Majority of MOs have satisfactory level of attitudes towards screening of PTB (87%, n=147) (Table 1). More than 80% of subjects had favorable attitudes on all the questions in different aspects of TB screening except third and fourth. About half of subjects (n=79) had favorable attitudes to suspect PTB and about 60% (n=101) had favorable attitude in doing a CXR when

a patient present with cough more than 2 weeks (Question no 3 and 4 respectively). Ninety eight percent (n=48) of MOs less than 30 years of age had favorable attitude where as 82.5% (n=99) of MOs above 30 years had favorable attitude. This difference of percentages of attitude was significant ( $P= 0.005$ ).

Ninety six percent MOs (n=31) below five year experience had favorable attitudes where as 75.6% MOs (n=59) more than five year experience had favorable attitude. This difference of percentages of attitude was significant with  $P= 0.007$ . Higher percentage of MOs working in Base hospital (BH) or above (n=124, 89.9%) had favorable attitude than MOs working in a District hospital (DH) (n=23, 74.2%).

This difference was also significant with  $P=0.02$ . Higher percentage of MOs working in medical and pediatric ward (n=54, 94.7%) had favorable attitude than MOs working in a different unit (n=93, 83%). This difference was significant with  $P=0.05$ .

### 3.3 Practices

The mean practices score was 61.8 ( $SD \pm 21.8$ ). Minimum score was 11.11 and maximum score was 100. The practices among majority of subjects (63.3%, n=107) on screening for TB was unsatisfactory (Table 1).

**Table 4. Distribution of correct practices among MOs on screening for TB (N=169)**

Screening Practice	Correct Practice
Case scenario -1	
You encounter a 10 year old child with poor weight gain and cough for 1 week. His BCG scar is absent.	
a) Refer him for sputum AFB & CXR	n=92, 54.4%
b) Refer him to chest clinic	n=109, 64.5%
c) Treat him with antibiotics and revive in 1 week*	n=69, 40.8%
Case scenario -2	
You encounter a 42 year old poorly controlled type 1 diabetic patient with	
a cough for 10 days with no fever	
a) Ask his socio economic conditions	n=139, 82.2%
b) Send for CXR , sputum AFB & treat him with antibiotics	n=116, 68.6%
c) Send for FBC & Treat him with antibiotics	n=35, 20.7%

and bronchodilators*	
Case scenario -3	
35 year old garment factory worker complaints of cough for 14 days and weight loss	
a)Treat with ciprofloxacin or levofloxacin*	n=132,78.1%
b)Treat with co-amoxyclave and send for CXR	n=92,54.4%
c)Refer for microscopic center for sputum examination	n=151,89.3%

The clinical vignettes (case scenario) and responses are described below

### 3.3.1 Case scenario-1

Once a TB suspected young child presents, only 54.4% (n=92) of MOs send sputum AFB and CXR which are two essential investigations to diagnose TB and 64.5% (n=109) MOs refer him to (CC). Only about 40% MOs (n=69) do not treat with antibiotics which is the expected practice in screening process.

### 3.3.2 Case scenario-2

Once strongly TB suspected middle aged patient presents with uncontrolled DM, majority takes a relevant history (82.2%, n=139) and only 68.6% (n=116) order CXR and sputum AFB. About 80% (n=134) of MOs send FBC and treat with antibiotics and bronchodilators which is not an expected practice.

### 3.3.3 Case scenario-3

When a middle aged patient presents with the typical history of TB, majority (78.2%, n=132) of MOs doesn't treat with ciprofloxacin and levofloxacin which has a masking effect on TB. More than half of (54.4%, n=92) of MOs treat the patient with co-amoxyclave and send for CXR. Majority (89.3%, n=151) of MOs refer to MCs for sputum examination.

## 4. Discussion

Majority of MOs do not have any post graduate qualification (83%, n=141), experience in NPTCCD or CC (76.9%, n=130) or any training during their career on TB (77.5%, n=131). This was identified gap which has to be addressed through higher education, training or continuous professional development. Only small proportion of MOs didn't have satisfactory knowledge (13%, n=22).

Most of them had good knowledge on transmission and risk factors, relationship between malnutrition and TB diabetes & TB and pregnancy and TB. Knowledge on TB and screening in patients less than five years of age was remarkably low among the subjects of study group.



Majority knew essential TB diagnostic correct combination of investigations (CXR; n=130,76.9%), (SSAFB; n=163, 96.4%). This was similar to the study findings in San Diego Country USA where 78% of physicians had correct knowledge about SSAFB (LoBue *et al.*, 2001). But according to a study findings in India (Singla *et al.*, 1998) among 204 private practitioners 89.5% physicians identified only CXR as diagnostic, 12% identified only sputum examination as diagnostic and only 11% identified both combinations as diagnostic. Poor knowledge on combination of CXR and sputum AFB may be associated due to non-probability convenient sampling method or their lack of knowledge in TB screening.

The overall attitudes among MOs were favorable (87%; n=147) (Table 1) on TB screening. But majority (50.9%, n=90) MOs had unfavorable attitudes in screening when a patient presented with cough more than 2 weeks. This is a major criteria on screening and 39.2 % (n=68) have unfavorable attitudes to do CXR on such patients (Table 4). When compared to a similar study done in Germany 81.1% physicians have favorable attitudes on newer screening method, immunodiagnostic techniques (IGRAs) for testing LTBI in risk groups (Gutsfeld *et al.*, 2014). This difference in attitudes can be explained by the well-established continues professional developments (CPD) and revalidation process in most of the European countries.

Age less than 30 year ( $P= 0.005$ ) and less than 5 year experience ( $P= 0.007$ ) MOs have significant favorable attitudes than others possibly due to updated knowledge among them. MOs working in medical and pediatric wards ( $p=0.05$ ) and working in a base hospital or DGH ( $p=0.02$ ) have significant favorable attitudes toward TB screening than the others. This could be due to their frequent updating knowledge, professional development and awareness.

### **Practices among Medical Officers on screening for tuberculosis**

The practices were measured using clinical vignettes (Table 4) which gave natural case presentations to simulate real situation of consultation (Peabody *et al.*, 2004). Majority of MOs have unsatisfactory level of practices (63.3%, n=107). According to 1<sup>st</sup> clinical vignette, once a TB suspected young child present, 54.4 % (n=92) MOs send sputum for AFB and CXR and 64.5 % (n=109) refer him to CC. Those can be considered as healthier practices. Simultaneously majority of MOs treat with antibiotics (59.2%, n=100) for TB suspected patients, which is a wrong practice. Again this was confirmed in 2<sup>nd</sup> clinical vignette which presented a TB suspect with uncontrolled DM. for that patient about 80% of MOs (n=134) treat with antibiotics, bronchodilators and sending nonspecific FBC. The 3<sup>rd</sup> clinical vignette explained a typical presentation of PTB.

The stem “treating with co-amoxyclav and sending a CXR” was the correct answer and majority accepted it (54.4%, n=92). The adaptation to guidelines, circulars and standard management protocols are essential in achieving expected outcome. Standard continuous medical education, continuous professional development and revalidations process are tools necessary to adopt for the betterment. Policy makers, Health care managers and relevant professional bodies need to address this situation adequately.

## 5. Conclusions and Recommendations

TB is identified as top ten leading cause to death worldwide. Globally and locally under identification is the major constrain in successful management. In Sri Lanka one out of three cases were not detected. Knowledge, attitudes and practices of the operational level health staff are essentially important to achieve successful outcome.

This study revealed the overall knowledge and attitudes were satisfactory among MOs but lack of knowledge and poor attitudes noticed on some areas.

Over half of the MOs (50.9%) had poor attitudes in referring patients with more than 2 weeks of cough to a MCs, and more than one third (39.2%) of MOs had unfavorable attitudes to perform CXR on PTB suspects. Unfavorable attitudes were significantly higher among MOs more than 30 year age ( $P=0.005$ ), more than 5 years' experience ( $P=0.007$ ) and working in a different unit other than medical and pediatric ward ( $p=0.05$ ).

Majority (63.3%) of MOs had unsatisfactory of practices towards screening of TB. By analyzing two clinical vignettes it was noticed that the antibiotics seem to be prescribed by majority of MOs to treat PTB suspected patients. At the same time majority of MOs hardly identified comparatively atypical patient presentations compared to typical presentations and investigate with non-specific investigations such as FBC and ESR.

Implementation of TB suspect Register (TBSR) at institutional level is one of the recommendations to improve patient referrals by MOs. Figures of TB suspects, extracted from TBSRs should be incorporated in OPD returns and IMMR. Continuous awareness programs about TBSRs should be held.

Importance of referrals at review meetings and in-service training programs (ISTP) must be emphasized in all levels of physicians and nurses. Continuous advocacy at review meetings must be held for physicians and guidelines must be made available for reference.

Compulsory ISTP should be implemented through protocols for OPD doctors and doctors working in DHs to train at NPTCCD before take up duties at OPD or DH. Identifications of different case presentations, importance of adherence to TBSR should be incorporated at training schedule.

## 6. Acknowledgement

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# Knowledge, Attitude and Practice of Neonatal Jaundice among Healthcare Workers in Bingham University Teaching Hospital, Jos, Nigeria

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## Abstract

Neonatal jaundice is still a serious health problem in Nigeria today which is often times associated with debilitating consequences. The knowledge, attitude and practice of healthcare workers could act as a two- edged sword either by posing risks to patients or providing help when needed. **Aim:** The study aims to assess the knowledge, attitude and practices of healthcare workers in Bingham University Teaching Hospital on Neonatal Jaundice. **Method:** This was a descriptive cross-sectional study which was carried out among Healthcare workers in Bingham University Teaching Hospital from April to June 2019. This was a proportionate purposive sampling of doctors, nurses and nursing aids that consented to the study. The knowledge, attitude and practice of Neonatal Jaundice was assessed using a pretested questionnaire which was analyzed using SPSS version 20. **Results:** The results showed 89% of respondents were able to correctly identify jaundice. About 80% of respondents recognized blood group incompatibility as a cause of jaundice, 80% of respondents encouraged patient to seek help within 24 hours after the appearance of jaundice and 88% of respondents recognize phototherapy as a modality of treatment for neonatal Jaundice. This shows an overall good knowledge, attitude and practice towards neonatal jaundice among tertiary healthcare workers in Bingham University Teaching Hospital.

**Keywords:** Attitude, Knowledge, Practice, Neonatal Jaundice, Healthcare workers, Jos.

## Introduction

Neonatal morbidity and mortality remain very high in the developing countries of sub-Saharan Africa, Asia and Latin America (Owa and Osinaike, 1998) and one of the important contributors to this is neonatal jaundice (NNJ) (Ogunfowora and

Daniel, 2006). Neonatal jaundice refers to the yellowish discoloration of the skin and sclera of a newborn by bilirubin (Zupan, 2005) it is a transitional phenomenon affecting most newborns with largely benign consequences in the first week of life. It typically resolves within 3 to 5 days without significant complications in the absence of comorbid prematurity, sepsis, or hemolytic disorders.

In some infants, NNJ may become severe enough to put them at risk for bilirubin-induced mortality or long-term neurodevelopmental impairments necessitating effective evaluation and treatment (Bolajoko *et al.*, 2016). Jaundice due to unconjugated hyperbilirubinemia is also the most common clinical problem in the neonatal period in many parts of the world (Ogunfowora and Daniel, 2006). Neonatal Jaundice is still a problem in Nigeria today and is known to have debilitating consequences including death (Parkash and Das, 2005).

The risk of severe NNJ is highest between 3 and 6 postnatal days when the plasma or serum bilirubin level reaches its peak in most infants. NNJ was estimated to account for 8 under-5 deaths per 100000 (95% uncertainty interval [UI]: 7–9) in 2016 globally. It ranked 16<sup>th</sup> from >100 possible causes of under-5 mortality consistently since 1990 (GBD 2016 Mortality Collaborators, 2016).

The mortality rankings among the 10 countries that frequently account for the largest number of neonatal deaths worldwide shows half of the countries (Nigeria, the Democratic Republic of the Congo, Ethiopia, Angola, and Kenya) are in Sub-Saharan Africa, 3 (India, Pakistan, and Bangladesh) are in South Asia, and 2 (China and Indonesia) are in East or Southeast Asia (UNICEF 2016).

Bilirubin-induced mortality was consistently among the top 15 causes of neonatal mortality in these 10 countries and among the top 20 causes of under-5 mortality in all but Indonesia, Angola, and Kenya (Liu 2016). Timely detection, monitoring, and treatment within this window is effective in preventing most bilirubin-induced mortality (Bolajoko *et al.*, 2016).

The healthcare worker has a huge role to play to educate and correct misconceptions about NNJ for the general population. Every health care worker irrespective of their cadre has a role to play in educating the population on neonatal jaundice whenever the need arises.

## Methodology

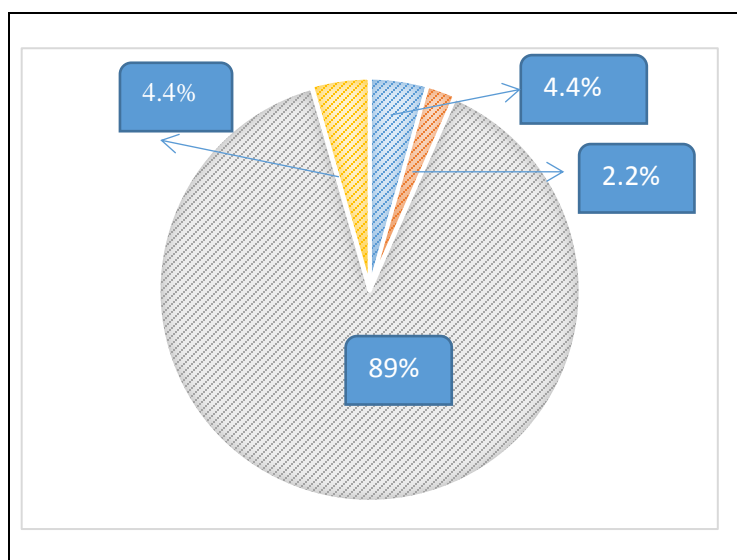
This was a descriptive cross- sectional study carried out at Bingham University Teaching Hospital, Jos, which is a tertiary healthcare centre.

Jos is the state capital of Plateau State located in the North Central Zone of Nigeria and home about 900,000 people according to the 2006 census. Jos also has four tertiary health centres as well as a good number of private hospitals and Primary health centres. The study enrolled a total of 113 respondents who are staff of the teaching hospital, representing doctors, nurses and nursing aides from all departments of hospital.

The doctors included consultants, residents, medical officers, postgraduate trainee. These respondents were grouped together as healthcare workers for the purpose of the study. The instrument used for data collection was a pre-tested self-administered questionnaire. A proportionate purposeful sampling technique method was used. The questionnaire was distributed to respondents and collected upon completion.

The questionnaire assessed the socio-demographic characteristics, knowledge and practice of Neonatal Jaundice among the respondents. The data was entered and analysed using SPSS v 20.0 statistical software (SPSS Inc., Chicago, Illinois, USA). The frequency was analyzed for each variable and results were presented in tables and figures. Ethical consideration taken for this study was reviewed and approved by Bingham University Teaching Hospital Ethical Committee and for each respondent, consent was obtained and confidentiality was ensured using serial numbers in place of names.

## Results



**Figure 1. Knowledge of healthcare workers on Neonatal Juandice**



89% of respondents correctly knew that NNJ is the yellowish discoloration of the sclera and skin, 4.4% described it as yellowish discoloration of the sclera only, another 4.4% do not know and 2.2% described it as yellowish discoloration of the skin only.

**Table 1. Source of Information on Neonatal Jaundice**

Source of Information	Frequency	Percent (%)
Healthcare Worker	68	60.1
Lectures/ Seminar	43	38.1
Relatives/Friends	2	1.8
Total	113	100

60% of the respondents' source of information on neonatal jaundice is from a healthcare worker, while about 1.8% got their information was from family and friends.

**Table 2. Causes of Neonatal Jaundice**

Causes of Neonatal Jaundice	Frequency	Percent (%)
Blood group incompatibility	83	73.5
Haematologic/ Hereditary Disease	52	46
Breast milk jaundice	45	39.8
Napthalene balls	41	36.3
Infectious Diseases	50	44.2
Medications given during labour	17	15.0
G6PD Deficiency	49	43.4
I don't know	2	1.8
Prematurity	54	47.8
Delayed breastfeeding	45	39.8

The table shows knowledge of respondents on causes of neonatal jaundice. 1.8% of respondents do not know the cause of neonatal jaundice.

However, 73.5% of respondents identified blood group incompatibility as the cause of neonatal jaundice.

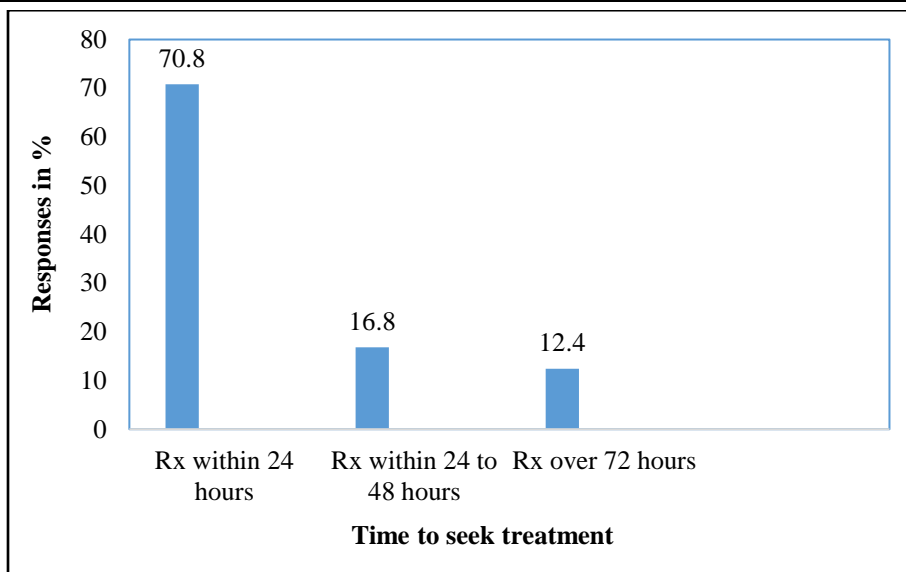


Figure 2. 70.8% of respondents encourage parents to seek treatment for NNJ within 24hours, 16.8% encourage parents to seek treatment within 24 to 48 hours while 12.4% of respondents encourage patients to seek treatment over 72hours.

**Table 3. Sites to look for jaundice**

Site	Frequency	Percent (%)
Eyes	99	87.6
Skin	88	77.9
Soles/Palms	36	31.9
Colour of stool/urine	27	23.9

The table shows respondents knowledge on sites to look for jaundice. 87.6% of respondents identified the eyes as common site to check for jaundice.

**Table 4. Danger signs of Neonatal Jaundice**

Danger signs	Frequency	Percent (%)
Fever	64	56.6
High pitch cry	59	52.2
Weakness	43	38.1
Upward rolling of the eye	33	29.2
Refusal to feed	62	54.9
Convulsions	42	37.2
Arching of the back	26	23.0
Fast Breathing	26	23.0

Table 4 shows the respondents knowledge on the danger signs of jaundice. Fever was the commonest known danger sign with 56.6%, the least known danger sign was arching of the back and fast breathing with 23% each.

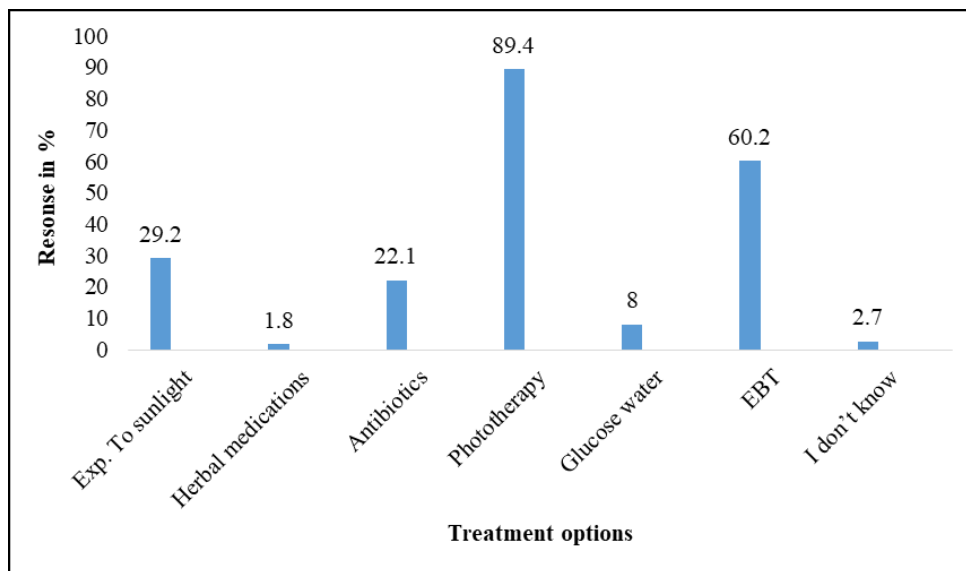


Figure 3 Shows that 88.4% of respondents recognize phototherapy as a modality of treatment for neonatal jaundice

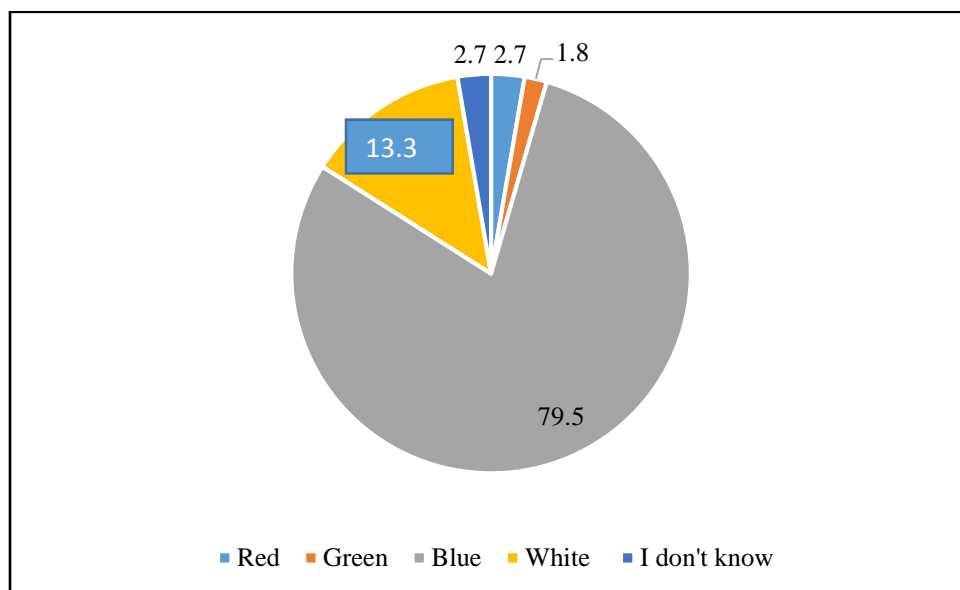


Figure 4 shows respondents answers on effective phototherapy. 79.5% of respondents agree that blue light is effective phototherapy. 13.3% of respondents chose white light, 2.7% chose red light and another 2.7% did not know.

**Table 5. Complications of NNJ**

<b>Complications of NNJ</b>	<b>Frequency</b>	<b>Percent (%)</b>
Blindness/Deafness	25	22.1
Delayed developmental Milestones	74	65.5
Death	54	47.8
Cerebral Palsy	63	55.8
Seizure disorder	52	46
I don't know	2	1.8

Table 5 shows complications of NNJ. 65.5% of respondents identified delayed developmental milestone as a complication of NNJ. This was followed by cerebral palsy with 55.8%.

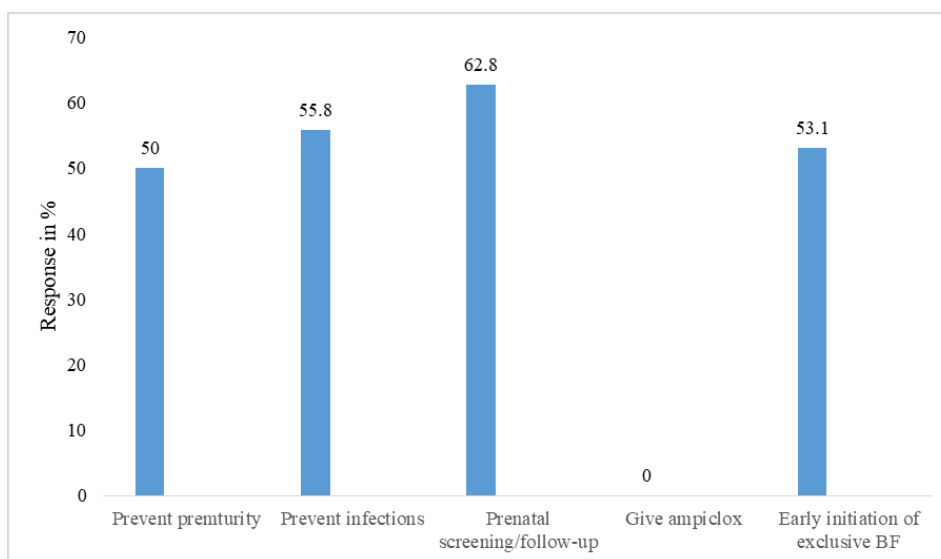


Figure 5 shows respondents answers to prevention of jaundice. No respondent gave the use of ampiclox as a means to prevent jaundice. while 62.8% of respondents gave prenatal screening and follow up as a means to preventing jaundice.

**Table 6. Peoples' belief on the cause of Jaundice**

<b>Cause of Jaundice</b>	<b>Frequency</b>	<b>Percent (%)</b>
Bad breast milk	45	39.8
Bad blood	42	37.2
Evil eye	28	24.8
Eating yellow colored food substances	10	8.8
Change in weather	12	10.6

The table shows respondents opinions on peoples' belief on the cause of jaundice. 39.8% of respondents think that people believe the cause of jaundice is bad breast milk.

### **Discussion**

As part of the diagnosis for NNJ is the ability to recognize jaundice as yellowish discoloration of the skin and sclera. Recognition of this usually paves the way for treatment. Participants were assessed on their knowledge of what neonatal jaundice is and various options were available. 89% of participants were able to identify jaundice as yellowish discoloration of the skin and sclera, while 4.4% of participants recognized it as yellowish discoloration of the sclera only, 2.2% of respondents recognized it as discoloration of the skin while 4.4% of the respondents did not know. The ability to recognize jaundice is significant in the management, as well as the parts of the body in which the jaundice was first noticed as this could give an idea on extent of the jaundice as well as progression of the jaundice. This finding is in contrast to another study conducted by Adeola and Adebola 2017 among Community Health Workers at PHCs in Ibadan where it was found that only 5.2% of participants correctly identified jaundice as yellowish discoloration of the skin and sclera. Another study also done among Community health workers by Olusoga and Olusoji in 2006 had 51.5% of respondents' correctly defining jaundice as yellowish discoloration of the skin and sclera.

The reason for this disparity could stem from the fact that tertiary health centers are usually the facility where NNJ is treated in this clime. Exposure to patients with NNJ, access to phototherapy, frequent lectures and seminars can contribute to better knowledge of NNJ by healthcare workers in tertiary health institutions. It would be safe to say that healthcare workers in the tertiary health centers have good knowledge of neonatal jaundice.

Source of respondents' information on NNJ was mostly from a healthcare worker. This accounted for 60.8% of participants. This was followed by seminars/ lectures which made up 38.1% of respondents and 1.1% from relatives and friends. Explanation for this could be that healthcare workers in tertiary health facilities have frequent lectures and seminars on various topics of interest. Formal learning and informal learning thrive in tertiary health facilities comparing with primary health centers.

Respondents also looked at the possible causes of jaundice from the study, 73.5% of respondents chose blood group incompatibility as a cause of jaundice, and this was followed by infections having 44.2% and G6PD deficiency accounting for 43.4%.

However, the leading causes of NNJ from studies that conducted appropriate clinical and laboratory investigations among infants detected with TSB  $\geq 10$  mg/dL (170  $\mu$ mol/L) were G6PD deficiency, ABO incompatibility, sepsis and prematurity/low birth weight, singly or in combination (Olusanya *et al.*, 2016). Respondents were also able to correctly identify causes of jaundice. A study done amongst community health workers showed 92% of respondents identifying malaria in pregnancy as the cause of NNJ. This is in contrast to respondents who work in a tertiary health center. For attitude, 70.8% of respondents encourage parents to seek for medical attention within 24 hours. This is also a fair assessment of the healthcare workers as time is a crucial factor in the management of NNJ. 88.4% of respondents correctly identified phototherapy as a modality of treatment for NNJ. Whereas a study done among community health workers, 67.1% of respondents recognized using glucose water as treatment of jaundice.

### Conclusion

The findings suggest that knowledge attitude and practice of health care workers on NNJ at the tertiary institution is good, however there is still room for improvement. We would encourage more lectures and seminars on the topic as well sensitization to even community health workers and the public at large.

### Acknowledgement

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