INVESTIGATION ON SOME PHYTOCHEMICAL CONSTITUENTS AND BIOACTIVITIES OF *MADHUCA LONGIFOLIA* L. (MYINTZU-THAKA-NATPAN) LEAVES

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Abstract

Some phytochemical constituents from the leaves of *Madhuca longifolia* L. (Myintzu-tha-ka-natpan) and its biological activities such as antioxidant and antimicrobial activities were investigated. Preliminary phytochemical tests have revealed that the presence of alkaloids, flavonoids, α-amino acids, organic acids, phenolic compounds, saponins, glycosides, cyanogenic glycosides, tannins, steroids and terpenoids in the sample. The leaves of sample was relatively high Ca, K and S according to EDXRF spectrum. *In vitro* antioxidant activities of 95 % EtOH and watery extracts from *M. longifolia* leaves were assessed by DPPH radical scavenging activity assay. IC_{50} values were found to be 6.64 µg/mL for H_2O extract and 3.62 µg/mL for 95 % EtOH extract of *M. longifolia* leaf. The *in vitro* antimicrobial activities of PE, EtOAc, $CHCl_3$, 95 % EtOH and H_2O extracts from *M. longifolia* leaves were screened by agar well diffusion method on six species of microorganisms, namely *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The inhibition zone diameters were found in the range between 14-19 mm for ethanol extract, 15-17 mm except *Pseudomonas aeruginosa* organisms for PE extract, 12-16 mm except *Escherichia coli* for chloroform extrat , 13-15 mm for ethyl acetate extract and 11-27mm except *Bacillus pumilus* organisms for water extract. Therefore , ethyl acetate extract exhibited the highest antimicrobial activities that followed by EtOH extract, PE extract, water extract and $CHCL_3$ extract. Therefore, on the basis of the results that have been observed in the present study, it can be inferred that the leaves may have therapeutic potential as remedy by virtue of its biochemical activities.

Keywords: Madhuca longifolia L, Myintzu-tha-ka-natpan, terpenoids antioxidant activity, antimicrobial activity

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Introduction

Traditional Medicine and Medicinal Plants

Medicinal herbs are moving from fringe to main stream use with a great number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed.

Traditional medicine is the sum total of the knowledge, skill and practices based on the theories, beliefs, and experiences, indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO, 2008).

The medicinal function of the plants are normally attributed to the rich reservoirs of secondary metabolites found in them. Knowledge on the chemical constituents of these medicinal plants found in the form of secondary metabolites is required not only for the discovery of newer therapeutic molecules but also will help in identifying the new sources of economic phytochemicals for the synthesis of complex and targeted drug systems. Madhuca is a medicinal system primarily practiced in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body and mind. The pharmacological treatment of disease began long ago with the use of herbs. Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. Similar to prescription drugs, a number of herbs are though to be likely to cause adverse effects (WHO, 2008).

Medicinal plants may be defined as a group of plants that possess some special properties or virtues that quality them as articles of drugs and therapeutic agents, and are used for medicinal purposes. Plants have formed the basis of sophisticated traditional medicine (TM) practices that have been used for thousands of years by people in China (WHO, 2015).

Scientific evidence from randomized clinical trials is only strong for many uses of traditional medicines, some herbal medicine and for some of the manual

therapies. Further research is needed to ascertain the efficacy and safety of several other practices and medicinal plants.

Botanical aspects of *Madhuca longifolia*

Family	- Sapotaceae
Botanical name	- *Madhuca longifolia*
Myanmar name	- Myintzu-thaka-natpan
Common name	- Madhuca

Description and distribution

M. Longfolic trees are normally 15-16 m high, with clustered leaves at the end of branches. The barks are brownish to yellowish grey in colour. Elliptic flowers are small, cream coloured and produced in clusters. *Madhuca longifolia* leaves are distributed in South India like Karnataka, Tamil Nadu, Andhra Pradesh and Kerala. These are cultivated in large deciduous dry tropical and such tropical climatic condition.

Chemical constituents of *Madhuca*

The *M. longifolia* leaves contain flavonoids and triterpenoids, vitamin A and vitamin C, tannins, phenols, saponins and cardinoloids.

The leaves of *M. longifolia* contained β–carotene and xanthophylls; exthrodiol, palmitic acid, myricetin and its 3-0-arabinoside and 3-0-L rhamnoside, quercetin and its 3-galactoside; 3β –caproxy and 3β – palmitoxy – olean – 12-en-28-ol, oleanolic acid, β – sitosterol and its 3-0-β. Dglucoside, stigmasterol, β – sitosteral - β – Dglucoside, n – hexacosanol, 3β – caproxyolcan – 12-en-38-oL, β – carotene, n – octacosanol, sitosterol, quercetin. (Krishnasamy *et. al.,* 2012). The structure of some chemical constituents of *M. longifolia* were shown in figure 2.

Medicinal uses

Vapors of boiling *madhuca* leaves are useful in relieving the pain of orchitis or the inflammation of testicles. The leaves of the tree are useful in the treatment of eczema. The leaves smeared with sesame oil warmed over a fire and bandaged on the affected parts provide relief. They should be changed after every 3 to 4 . The ash of the leaves, mixed with ghee, is often used as a

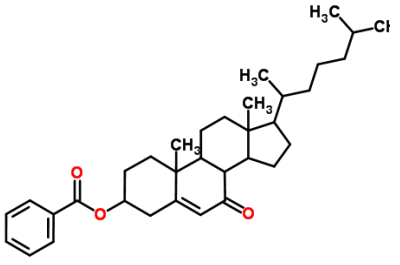
dressing for burns and scalds in the indigenous system of medicine. The aerial parts are used for treatment of inflammation. *M. longifolia* leaves are expectorant and also used for chronic bronchitis and cashing's disease.

The medicinal properties attributed to this plant are stimulant, demulcent, emollient heating and astringent. The traditional uses of *M. longifolia* leaves are Verminosis, gastropathy, consumption, dematopathy, rheumatism, cephalgia and hemorrhoids. Madhuca leaves are boiled and used for relief from orchitis. The photographs of *M. longifolia* were shown in figure 1.

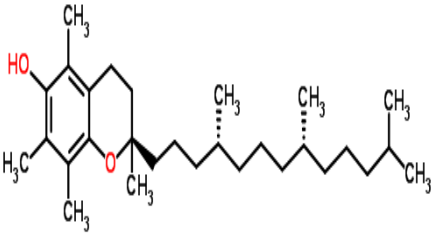
Leaves

Fruits

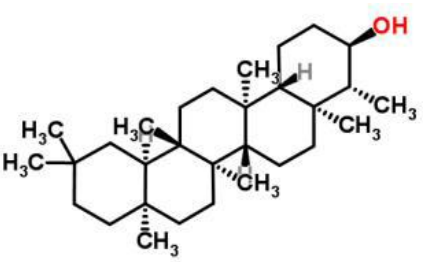
Figure 1: Photograph of *Madhuca longifolia*.



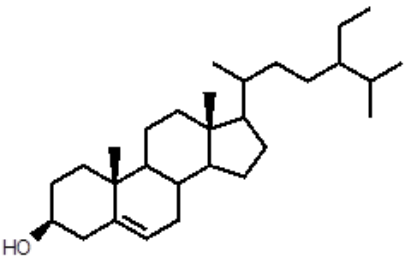
Steroid derivative



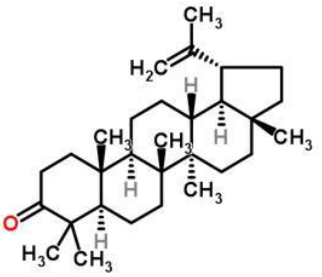
Methylated phenol



Terpenoid



β-Sitosterol



Terpenoid

Terpenoid

Figure 2: Structures of some chemical constituents of *M. longifolia*.

The main aim of this study is to investigate some phytochemical constituents from the *M. longifolia* (MYINTZU-THAKA-NATPAN) leaves and to study some of its biological activities such as antimicrobial activity and antioxidant activity.

Materials and Methods

Collection and Preparation of the Sample

The leaves samples of *Madhuca Longifolia* Linn. (Myint-zu-tha-ka-nat-pan) were collected from Zalun Township, Ayeyarwady Region in August, 2016. After being collected, the scientific name of the sample was identified by authorized botanists at Botany Department, Hinthada University. The fresh sample was cleaned by washing with water and air-dried. The dried sample was ground using grinding machine. This powdered sample was the

kept in the sealed air-tight container to prevent moisture changes and other contamination.

Qualitative Screening of the Phytochemicals

In order to find out the types of organic constituents present in the sample, preliminary phytochemical investigation was carried out according to the test tube methods.

Qualitative Elemental Analysis of Leaves Sample by EDXRF Spectrometry

Elements in the leaves of *M. longifolia* were detected by Energy dispersive X – ray fluorescence Spectrometer.

Preparation of Crude Extracts for Bioactivities Tests

Preparation of aqueous extract

A 20 g of the fine powder sample was boiled with 100 mL of distilled water for six hours and filtered. It was repeated three times and the filtrates were combined followed by evaporated on water bath and the resulted extract was dried in oven. After getting the constant weight, each extract was stored in refrigerator for the screening of biological activities.

Preparation of 95% ethanol extract

A 20 g of the fine powder sample was percolated with 95% ethanol 100 mL, shaking frequently and allowed to stand for one week and filtered. This procedure was repeated for three times. The filtered filtrates were concentrated by rotary evaporator to get 95% ethanol extract and the resulted extract was dried in oven. After getting the constant weight, each extract was stored in refrigerator.

Preparation of pet-ether extract

A 20 g of the dried sample was extracted with pet- ether (60-8 0 °C) in similar manner mentioned in above procedure to yield the pet-ether soluble extracts.

Preparation of ethyl acetate extract

A 20 g of the dried sample was extracted with ethyl acetate in similar manner mentioned in above procedure to yield the ethyl acetate extract.

Preparation of chloroform, extract

A 20 g of the dried powder sample was extracted with chloroform in similar manner mentioned in above procedure to yield the chloroform extract.

Screening of Antioxidant Activity of Crude Extracts from *M. longifolia* Leaves by

DPPH Assay

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of leaves extracts. This assay has been widely used to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenols in food system (Leea *et al*, 2002). In this experiment, the antioxidant activity was studied on 95 % ethanol extract, and aqueous extracts from selected leaves sample by DPPH free radical scavenging assay. (Yamaguchi *et al*, 1998)

***In vitro* Screening of Antimicrobial Activity**

The antimicrobial activities of different crude extracts such as pet ether, chloroform, ethyl acetate, ethanol and water extracts were determined against six microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Eshcherichia* species by employing agar well diffusion method at Central Research and Development Centre, Ministry of Industry, Yangon. (Finegold, *et al*, 1978).

Results and Discussion

Phytochemical constituents of *M. longifolia* Leaves

The preliminary phytochemical investigation was carried out for the leaves with a view to determine the presence or absence the types of phytochemical constituents according to test tube method. The results are shown in Table 1. From these results, it was observed that α-amino acids, alkaloids, cyanogenic glycosides, flavonoids glycosides, organic acids, saponins, steroids, terpenoids, phenolic compounds, and tannins are present in the sample but reducing sugar, starch, carbohydrates are absent.

Table 1: Results of Phytochemical Investigation on *M. longifolia* leaves

Sr. No.	Tests	Extract	Test Reagents	Observation	Remark
1.	α-Amino acids	H_2O	Ninhydrin reagent	pink colour	+
2.	Alkaloids	1 % HCl	Mayer's reagent Dragendorff's reagent Wagner's reagent Sodium picrate	white ppt orange ppt brown ppt yellow ppt	+ + + +
3.	Cyanogenic glycosides	H_2O	Sodium picrate solution	brick red	+
4.	Carbohydrate	H_2O	10 % α-naphthol & H_2SO_4	red ring	-
5.	Flavonoids	EtOH	Mg ribbon & conc.HCl	pink colour	+
6.	Glycosides	EtOH	10 % lead acetate	white ppt	+
7.	Organic acids	H_2O	Bromocresol green	blue colour	+
8.	Phenolic compounds	EtOH	1 % $[K_4Fe(CN)_6]$ & 5% $FeCl_3$	Deep blue colour	+
9.	Reducing sugars	H_2O	Benedict's solution	no brick-red ppt	-
10.	Starch	H_2O	Iodine solution	Blue colour	-
11.	Saponins	H_2O	Distilled water	frothing	+
12.	Steroids	PE	Acetic anhydride & conc. H_2SO_4	green colour	+
13.	Tannins	H_2O	Gelatin & 1 % $FeCl_3$	white ppt	+
14.	Terpenoids	$CHCl_3$	Acetic anhydride & conc. H_2SO_4	pink colour	+

(+) presence (-) absence

Some Elements Present in *M. longifolia* Leaves by EDXRF Spectrometry

The relative abundance of elements present in *M. longifolia* leaves was determined by EDXRF spectrometer. It can be seen that calcium, potassium and sulphur were found to be present as major elements and phosphorous, iron, manganese, zinc, copper and rubidium were as trace elements. The results were shown in Table 2.

Table 2: Relative Abundance of Some Elements in *M. longifolia* leaves (By EDXRF)

No	Elements	Relative Abundance (%)
1	Calcium (Ca)	1.020
2	Potassium (K)	0.929
3	Sulphur (S)	0.299
4	Phosphorous (P)	0.074
5	Iron (Fe)	0.013
6	Manganese (Mn)	0.008
7	Zinc (Zn)	0.001
8	Copper (Cu)	0.001
9	Rubidium (Rb)	0.001
10	COH	97.654

Antioxidant Activity

The antioxidant activity of the leaves sample was determined by DPPH assay. The results obtained are shown in Table 3 and figure 3. From the observation, the activity of ethanol extract (IC_{50} = 3.62 μg/mL) was found to be more potent than that of aqueous extract (IC_{50} = 6.64 μg/mL)

Table 3: % Oxidative Inhibition and IC_{50} Values of 95 % EtOH and Aqueous Extracts of *M. longifolia* Leaves and Standard Ascorbic Acid

Samples	% Inhibition (mean ± SD) in different concentrations (µg/mL)						IC_{50} (µg/mL)
	0.625	1.25	2.5	5	10	20	
EtOH extract	14.95 ± 1.15	26.63 ± 2.31	43.21 ± 0.96	57.34 ± 2.69	61.96 ± 1.54	64.13 ± 1.15	3.62
Aqueous extract	18.83 ± 0.15	24.48 ± 0.59	31.01 ± 0.15	45.60 ± 0.15	59.00 ± 1.63	60.39 ± 0.89	6.64
Ascorbic acid	14.04 ± 2.09	54.83 ± 2.48	72.44 ± 3.83	81.13 ± 1.47	87.40 ± 2.37	91.21 ± 0.48	1.17

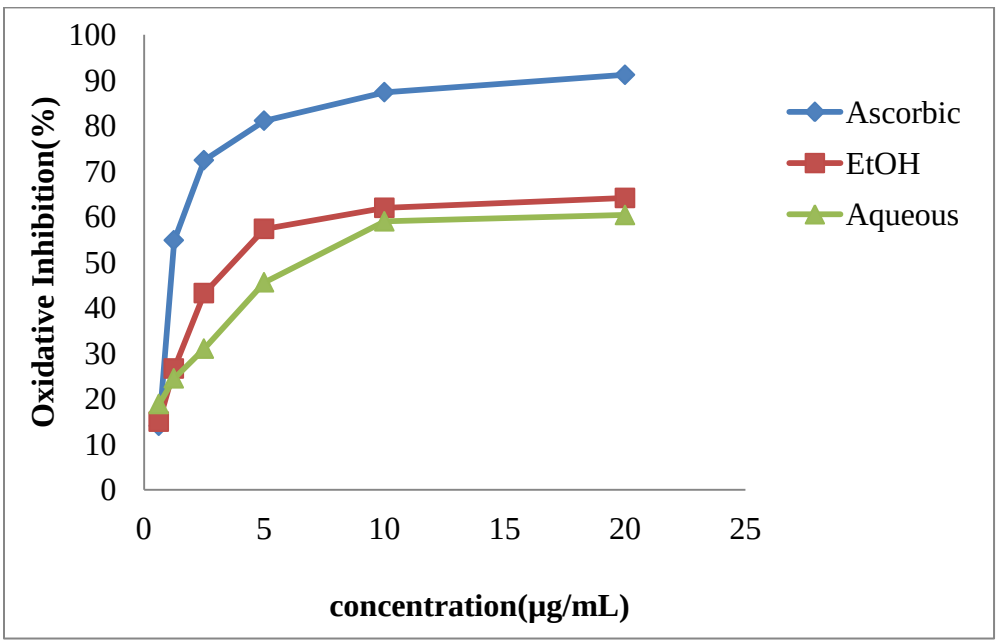


Figure 3: Plot of % oxidative inhibition Vs concentration (µg/mL) of ethanol and aqueous extracts of *M. longifolia* leaves and standard ascorbic acid

Antimicrobial Activity of *M. longifolia* Leaves

In vitro antimicrobial activity of various curde extracts was investigated by employing agar well diffusion method against six microorganisms. From the results, it was observed that all extracts of *M. longifolia* leaves except PE extract exhibited inhibition zone diameters between (12-27 mm) against *Pseudomonas aeruginosa.* PE, $CHCl_3$, EtOAc, EtOH and water extracts of *M. longifolia* leaves showed antimicrobial activity against *Bacillus subtilis* ranging the inhibition zone diameter 11-16 mm. PE, $CHCL_3$ EtOAc and EtOH extracts of *M. longifolia* leaves except water extract showed inhibition zone diameters about 14-15 mm against *Bacillus pumilus* but all extracts of *M. longifolia* leaves exhibited the inhibition zone diameters about 13-18 mm against *Staphylococcus aureus.* All extracts except $CHCL_3$ extract exhibited inhibition zone diameters between (14-18 mm) against *E.coli* and all extracts showed antimicrobial activity against *Candida albicans* (inhibition zone diameter = 11-19 mm). Therefore these extracts may have broad spectrum activity and ethanol extract exhibited the highest antimicrobial activities that are followed by EtOAc extract, PE extract, water extract and $CHCl_3$ extract. The results are shown in Table 4.

Table 4: Inhibition Zone Diameters of Crude Extracts of *M. longifolia* Leaves against Six Species of Microorganisms

Microorganisms	Types of Microorganisms	Inhibition Zone Diameters (mm)				
		PE	$CHCl_3$	EtOAc	EtOH	H_2O
Bacillus pumilus	Gram (+) ve	15	14	14	15	-
Bacillus subtilis	Gram (+) ve	15	15	15	16	11
Candida albicans	Fungi	17	16	15	19	11
Escherichia coli	Gram (–) ve	16	-	15	18	14
Pseudomonas aeruginosa	Gram (–) ve	-	12	13	14	27
Staphylococcus aureus	Gram (+) ve	15	15	13	18	14

Agar well diameter = 10 mm

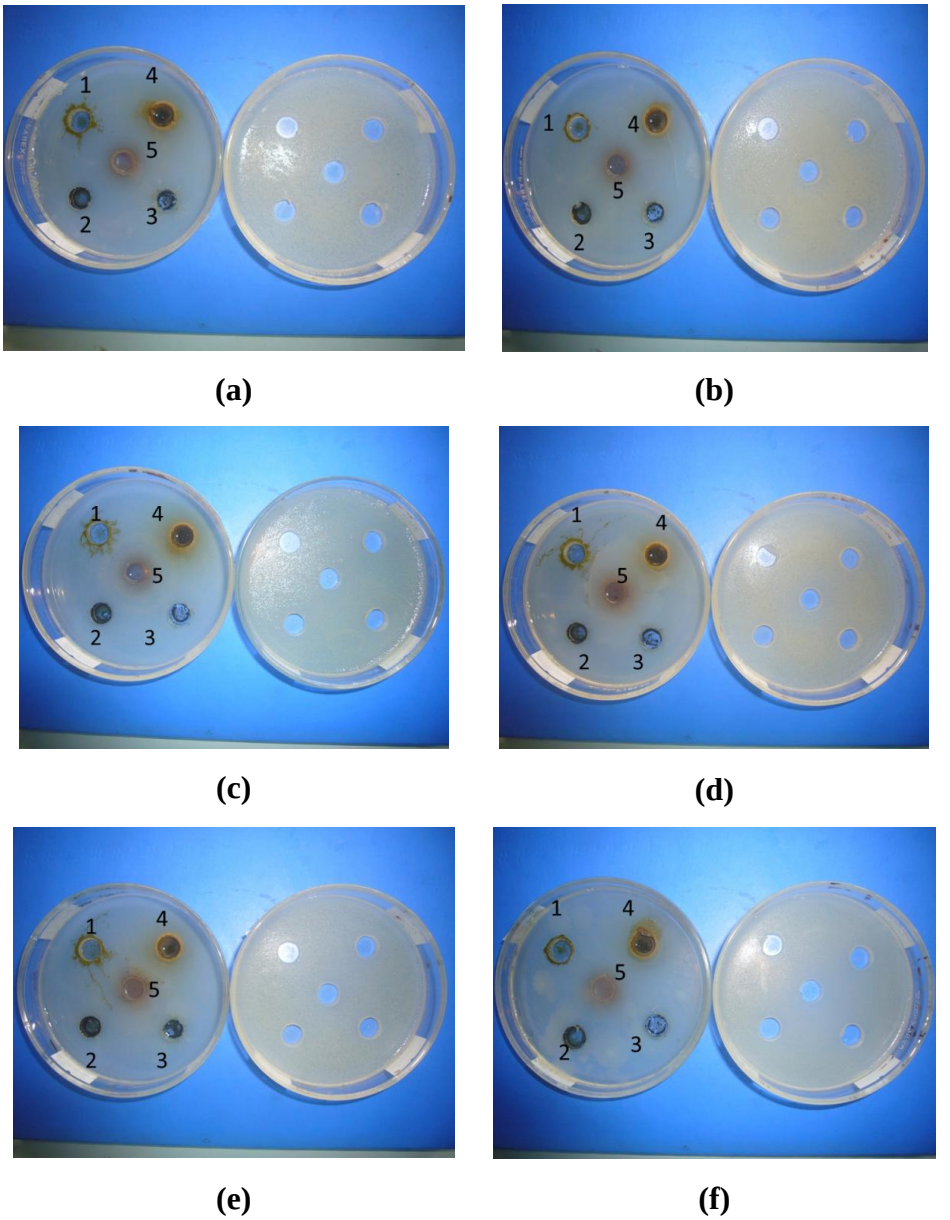


Figure 4: Inhibition zones of various crude extracts against (a) *Bacillus subtilis* (b) *Staphylococcus aureus* (c) *Pseudomonas aeruginosa* (d) *Bacillus pumilus* (e) *Candida albicans* (f) *Escherichia coli*

Conclusion

From overall assessment of the present work concerning with investigation of phytochemical constituents from *Madhuca longifolia* L. (Myintzu-thaka-natpan) leaves and some of its biological activities such as antioxidant and antimicrobial activities, the following inferences could be deduced.

The preliminary phytochemical tests on *M. longifolia* leaves revealed the presence of α-amino acids, alkaloids, cyanogenic glycosides, glycosides, phenolic compounds, saponins, steroids, organic acids, tannins, terpenoids, and but the absence of starch, reducing sugar, carbohydrates. Consequently, the leaves were good remedy for some diseases e.g., chronic bronchitis, cushing diseases.

From EDXRF elemental analysis of *M. longifolia* leaves, Ca, K and S were as major constituents.

According to antioxidant activity screening of ethanol and aqueous extracts from *M. longifolia* leaves using DPPH assay according to the spectrophotometric methods, the order of antioxidant activity was as ethanol extract (IC_{50} = 3.62 μg/mL) > aqueous extract (IC_{50} = 6.64 μg/mL). From these observations, the radical scavenging activity of ethanol extract of *M. longifolia* (Myitzu-thaka-natpan) leaves was found to be more effective than aqueous extract in antioxidant activity. Consequently, from these results, it can be inferred that *M. longifolia* leave possess antioxidant properties.

Screening of antimicrobial activities of various crude extracts such as PE, EtOAc, $CHCl_3$, EtOH and H_2O extracts from leaves sample were also investigated by employing agar well diffusion method against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E. coli* species. It was observed that EtOAc and EtOH extracts of *M. longifolia* leaves exhibited inhibition zone diameters between (13-19 mm) against both of the Gram positive and Gram negative microorganisms tested.

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References

Finegold, S, Martin, M., & Scort, W.J (1978). *E.G Diagnostic Microbiology* London: 5th Ed., mosby Co.Ltd., pp . 586-588.

Krichnasamy, T., Manian, S & Paulsamy, S. (2012). *Preliminary Phytochemical Screening from different parts.*

Leea, E.L.,Vitaly, I.V., Myung-Woo, B. and Cherl-Ho. L. (2002).“Detection of Free Radicals in Gamma-Irradiated Soybean Paste and Model System by Electron Spin Resonance Spectroscopy.” *Radiation Physics and Chemistry*, vol.64, pp.61-66

Vaghasiga, Y . & Chanda, S. (2009) . “Screening of some traditionally used India plants for antibacterial activity against Klebsiellapneumonia.” *Journal of herbal medicine and toxicology*; Vol 3 (2), pp.161-164

WHO.(2008). *Traditional Medicine, Health Topics*: Definitions. Geneva: World Health Organization.

WHO.(2015). *Traditional Medicine, Health Topics*. www.who.int/topics/traditional medicine/en (Accessed 6 September, 2016)

Yamaguchi, T., Takamura, H., Matoba T, and Terao. J. (1998). “HPLC Method for Evaluation of the Free Radical Scavenging Activity of Foods by Using 1,1-Diphenyl-2-picrylhydrazyl”. *Biochemistry*, vol.62(6), pp.1201-1204