INVESTIGATION OF CHEMICAL CONSTITUENTS AND SOME PHARMACOLOGICAL PROPERTIES OF *MELICOPE PTELEFOLIA* (THIT- KHA) LEAF

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Abstract

Chemical constituents of *Melicope ptelefolia* (Thit-Kha) leaf and some pharmacological properties were investigated. The preliminary phytochemical test was determined by test tube method. Dried leaf sample was found to consist of 6.39 % of moisture, 7.64 % of ash, 21.94 % of protein, 14.92 % of dietary fiber, 3.93 % of crude fat, 45.18 % of carbohydrate, and 303 kcal/100g of energy value, respectively. According to the EDXRF spectrum, the samples had relatively high Si, K and Ca. Total phenol and total flavonoid contents of *M. ptelefolia* (Thit-Kha) leaf by using Folin- Ciocalteu reagent (FCR) method and by using spectroscopic method. According to the observed data, total phenol content was very high in watery extract (273.78 μg GAE/mg) followed by ethanol extract (119.87 μg GAE/mg) and methanol extract (103.84 μg GAE/mg). In addition, the total flavonoid was also very high in watery extract (79.24 μg QE/mg) followed by ethanol extract (78.81 μg QE/mg) and methanol extract (60.09 μg QE/mg). The antioxidant activity of watery extract (IC_{50} = 15.44 μg/mL was found to be higher than those of ethanol extract (IC_{50} = 59.62 μg/mL) and methanol extract (IC_{50} = 45.79 μg/mL) determined by DPPH radical scavenging assay. Antimicrobial activity was also determined by agar well diffusion method. Ethyl acetate and methanol extracts exhibited the highest antimicrobial activities. The cytotoxicity of methanol extract was studied by MTT assay method. From the results, the concentration of methanol extract increased, the cell viability also decreased.

Keywords: *Melicope ptelefolis*, chemical constituents, total phenol, total flavonoid, antioxidant activity, antimicrobial activity, cytotoxicity

Introduction

Medicinal plants are important source of valuable therapeutic agents, both in modern and in traditional medicine. Many plants, particular medicinal plants, have been extensively studied for their antioxidant activity in recent years. The study of traditional medicinal plants and their therapeutic properties play a very important role in the health care system of the country. Indigenous medicine is widely practiced in Myanmar due to its long and deep rooted tradition and also due to the trust placed by the people in its therapeutic qualities. There are many traditional plants are being used in the traditional systems of medicine in many parts of the world, especially in rural communities, for the control, management and or treatment of a variety of human and animal ailments (Kumar and Pandey, 2013). Free radicals and other reactive oxygen species are being constantly produced in the human body and they are known to be responsible for various deadly diseases such as cancer, aging, atherosclerosis, immunodeficiency, and infections. On the other hand, synthetic drugs bring about various side effects such as gastrointestinal disturbances, hypoglycemia and liver disfunction (Rang *et al*., 2003). Free radical scavenger or antioxidant may play a major role in the prevention of a number of diseases, some forms of cancer, and may age related disorders. Among other medicinal plants, *Melicope ptelefolia* leaf was selected for this study since they are widely grown in Kayah State.

M. ptelefolia belonging to family Rutaceae is Thit-Kha in local name and this is the first research from the leaf of *M. ptelefolia* in Kayah State, Myanmar. Therefore, it was chosen to investigate its phytochemical constituents, nutritional values, mineral values, total phenol and total

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flavonoid contents, and some bioactivities including antioxidant and antimicrobial activities, and cytotoxicity.

Materials and Methods

Sample Collection and Characterization

M. ptelefolia leaf sample (Figure 1) was collected in December, 2018 from West Padaung, Demawso Township, Kayah State. The taxonomists of the Department of Botany, Loikaw University botanically identified and authenticated the plant.

Figure 1 Photograph of the leaf of *Melicope ptelefolia* (Thit-Kha)

Determination of Phytochemical Constituents and Nutritional values of *M. ptelefolia* Leaf

Ethanol, petroleum ether, ninhydrin, sulphuric acid, α-napthol, Mg turning, lead acetate, ferric chloride, acetic anhydride, gelatin solution, acetic acid, sodium picrate, Benedict's solution, mercuric chloride, iodine, picric acid, acetone and sodium carbonate were used to measure phytochemical tests. The leaf extracts of *M. ptelefolia* were determined for the presence of phytochemicals such as alkaloids, carbohydrates, saponins, proteins, phenolic compounds, flavonoids and glucosides according to the common phytochemical methods described by Harbone, 1998 and Sofowora, 1994. The mineral value was also determined by using Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometer. The nutritional values such as moisture, protein, fat, fiber, and carbohydrate of *M. ptelefolia* were determined at the SME Development Department, Yangon, Ministry of Industry.

Preparation of Different Extracts

The air-dried powdered sample (20 g) was macerated with 100 mL of water in closed flask for twenty-four hours, during which they were frequently shaken six hours interval and allowed to stand for 18 h. Afterwards, they were filtered rapidly against loss of water and then 25 mL of filtrates were evaporated to dryness in a tarred flat-bottomed shallow dish. They were dried at 105 °C and weighed.

PE, MeOH, EtOAc, and EtOH extracts of leaf of *M. ptelefolia* powder sample was determined by the method given in "The British Pharmacopoeia" as described in "the preparation of water extract" by using 100 mL of respective solvents instead of water.

Determination of Total Phenol and Total Flavonoid Content

Total phenol content (TPC) in each extract was determined using the FC method described by McDonald *et al*. (2001). The calibration curve was established using gallic acid and the TPC value was expressed as mg gallic acid equivalents per gram of dried extract. The diluted extract or gallic acid (1.6 mL) was added to 0.2 mL FC reagent and mixed thoroughly for 3 minutes. Sodium carbonate 0.2 mL was added to the mixture and the mixture was allowed to stand for 30 minutes

at room temperature. The absorbance of the mixture was measured at 760 nm using UV-Visible spectrophotometer.

The total flavonoid content (TFC) of each extract was investigated using the aluminium chloride colorimetry method described by Chang *et al.* (2002). The calibration curve was prepared by diluting quercetin (2.0 mL) was mixed with 0.1 mL of aluminium chloride solution and 0.1 mL of potassium acetate solution. The mixture was kept at room temperature for 30 min. Then the maximum absorbance of the mixture was measured at 420 nm using UV-Visible spectrophotometer. Total flavonoid content (TFC) was expressed as mg quercetin equivalent per gram of dried extract.

Determination of Some Bioactivities of *M. ptelefolia* Leaf Extracts

The radical scavenging activity of the ethanol, methanol and watery extracts was investigated by using DPPH assay according to the spectrophotometric method. Test tubes, electric balance, magnetic stirrer, oven, water bath, glass rod, glass tube, cell (5 mL), vortex mixer, UV-Visible spectrophotometer (UV-7504), micropipette (3 mL and 5 mL) were used for the determination of antioxidant activity In this experiment, six different concentrations (10, 20, 40, 60, 80 and 100 µg/mL) of each crude extract in ethanol solvent were used. Determination of absorbance was carried out at wavelength 517 nm using UV visible spectrophotometer. Each experiment was done triplicate (Leea, 2002).

Screening of antimicrobial activity of crude extracts namely PE, MeOH, EtOAc, EtOH, MeOH and H_2O extracts from *M. ptelefolia* leaves sample was done by agar well diffusion method. In this investigation, the extracts were tested against six microorganisms: *Bacillus subtilis, Staphylococcus aureus, Preudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E. coli* species. The measurable zone diameter, including the filter paper showed the degree of antimicrobial activity (Cruickshan *et al.,* 1960).

The cytotoxicity of methanol extract of *M. ptelefolia* (Thit-Kha) leaf was studied by MTT assay method. HepG2 hepatocellular carcinoma cell line, culture media (RPMI-1640) contains 20 mM HEPES, MTT powder, Dimethyl sulfoxide were used for the determination of cytotoxicity of crude extract. MTT powder (500 mg) was dissolved in 10 mL phosphate buffer solution. The solution was stirred by a magnetic stirrer for about 1 h in the dark. The sterilized solution was filtered by 0.22 mm filter (Millipore, Ireland) and it is stored in10-mL aliquots (50 mg/mL) at -20 °C (van Meerloo *et al*., 2011). The working solution (5 mg/mL) will be prepared on the day of experiment by dilution.

Results and Discussion

Phytochemical Constituents, Nutritional Values and Mineral Values of *M. ptelefolia* Leaf

Preliminary phytochemical investigation was carried out to know the types of phyto-organic constituents present in the leaf of *M. ptelefolia.* According to these results, *M. ptelefolia* leaf sample showed the presence of alkaloids, glycosides, α-amino acids, carbohydrates, flavonoids, phenolic compounds, saponins, steroids, and terpenoids, however, reducing sugar, starch and tannins are absent.

For quality control assessment of medicinal plant materials, some nutritional value such as moisture, fat, ash, fiber, protein, carbohydrates and energy value of *M. ptelefolia* leaf was determined. The results for these contents are summarized in Table 1. The moisture and ash content of leaf were found to be 6.39 % and 7.64 %, respectively. The protein content of leaf was found to be 21.94 %. Fiber, fat and carbohydrate contents were respectively found to be 14.92 %, 3.93 % and 45.18 %, with energy value of 303.85 kcal/ 100 g of leaf extract.

Relative abundance of elements present in different varieties of *M. ptelefolia* leaf sample was determined by EDXRF spectrometer and the result of this sample is shown in Table 2. The relative abundant of the silicon was found 1.469 % in *M. ptelefolia* leaf.

Table 1 Some Nutritional Value of *M. ptelefolia* Leaf

No.	Parameters	Contents
1	Moisture (%)	6.39
2.	Carbohydrates (%)	45.18
3.	Fat (%)	3.93
4.	Protein (%)	21.94
5.	Ash (%)	7.64
6.	Crude fiber (%)	14.92
7.	Energy value (kcal/100g)	303

Table 2 Relative Abundance of Element in *M. ptelefolia* Leaf (EDXRF)

No.	Element	Results (%)
1.	Si	1.469
2.	K	0.748
3.	Ca	0.395
4.	S	0.236
5.	Mn	0.021
6.	Fe	0.008
7.	P	0.003
8.	Cu	0.002
9.	Zn	0.001
10	Br	0.001
11.	Rb	0.000
12.	C H	97.115

Total Phenol and Total Flavonoid Contents of *M. ptelefolia* Leaf

From the results given in Table 3, total phenol content (TPC) was expressed as microgram of gallic acid equivalent (GAE) per milligram of crude extract (µg GAE/ mg). The total phenol content of watery extract (273.78 ± 0.02 µg GAE/ mg) was found to be higher than ethanol extract (119.87 ± 0.01 µg GAE/ mg) and methanol extract (103.84 ± 0.01 µg GAE/ mg). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds. They possessed biological properties such as antiaging, antcarcinogen and antiflammation, radical scavenging activity and α-amylase inhibition activity (Zaino *et al*., 2003 and Chen *et al*., 2007).

Table 3 Total Phenol Content (µg GAE/mg) of *M. ptelefolia* Leaf Extracts

Extracts	Total phenol content (µg GAE/mg)
Watery	273.78 ± 0.02
Ethanol	119.87 ± 0.01
Methanol	103.84± 0.01

Total Flavonoid Content

Total flavonoid contents (TFC) was expressed as microgram of quercetin equivalents (QE) per milligram of crude extract (µg QE/ mg).The total flavonoids content of watery extract (79.24 ± 0.02 µg QE/ mg) was found to be higher than ethanol (78.81 ± 0.03 µg QE/ mg) and methanol extract (60.09 ± 0.02 µg QE/ mg) Table 4. The level of flavonoids in plants is vital for the resistance of external threats. Flavonoids rely on different phytochemicals which gives flavonoids the ability to defend the cells from oxidants.

Table 4 Total Flavonoid Content (µg QE/mg) of *M. ptelefolia* Leaf Extracts

Extracts	Total flavonoid content (µg QE/mg)
Watery	79.24 ± 0.02
Ethanol	78.81 ± 0.03
Methanol	60.09 ± 0.02

Some Bioactivities of *M. ptelefolia* Leaf Extracts

Antioxidant Activity

The percent oxidative inhibition values of watery, methanol and ethanol extracts measured at different concentrations and the results are summarized in Table 5. From these experimental results, it was found that as the concentrations increased, the radical scavenging activity of crude extracts usually expressed in term of % inhibition increased. From the average values of % inhibition, IC_{50} (50 % inhibition concentration) values in µg/mL were calculated by linear regressive excel program.

The antioxidant activity, IC_{50} values of watery, ethanol and methanol extracts were 15.44 µg/mL, 59.62 µg/mL and 45.79 µg/mL, respectively. Therefore, the watery extract possesses higher antioxidant potency than ethanol and methanol extract. The results showed that watery exhibited the optimal solvent to extract the bioactive components from *M. ptelefolia*. This extract contained the highest level of phenolic and flavonoids. Those compounds possess powerful antioxidant activity and consequently protect the human body against oxidative damage through scavenging diverse reactive oxygen species, including hydroxyl radicals, peroxide radicals, peroxynitrite and superoxide anions (Chao, 2014).

Table 5 Percent Oxidative Inhibition and IC_{50} Values of Watery, MeOH and EtOH Extracts from *M. ptelefolia* Leaf

Extracts	% Inhibition (mean ±SD) in different concentrations (µg/mL)						IC_{50} (µg/mL)
	10	20	40	60	80	100	
Watery	37.85 ±0.93	60.15 ±0.70	70.73 ±1.86	71.72 ±1.05	71.65 ±0.58	81.84 ±0.83	15.44
Ethanol	0.63 ±8.10	5.18 ±1.31	21.37 ±2.53	50.57 ±1.53	61.69 ±1.36	84.32 ±5.95	59.62
Methanol	6.05 ±3.09	24.59 ±0.49	43.47 ±0.62	65.97 ±0.76	63.58 ±0.99	61.02 ±0.38	45.79

Antimicrobial Activity

From the results given in Table 6, it was observed that EtOAC extract of *M. ptelefolia* leaves exhibit inhibition zone diameters between (12-20 mm) against *P. aeruginosa, B. pumilus, E. coli,* and *C. albicans* of Gram positive and Gram negative microorganisms tested and PE extract did not show any antimicrobial activity. MeOH extract showed inhibition zone diameters between (12-18 mm) against *S. aureus, B. pumilus, E. coli,* and *C. albicans.* EtOH extract show the activity against *S. aureus, B. pumilus, E. coli* and $CHCl_3$ extract show the activity against *S. aureus, B. pumilus,* and *P. aeruginosa.* EtOAc extract showed inhibition zone diameters between (12-16 mm) against *P. aeruginosa, B. pumilus, E. coli,* and *C. albicans.* Among the extracts, MeOH extract possesses the highest antimicrobial activity.

Table 6 Microbial Inhibition Zone Diameters of Crude Extracts from *M. ptelefolia* Leaf by Agar Well Diffusion Method (Disc diameter = 10 mm)

No.	Microorganism	Type	Diameter of Inhibition Zone (mm)					
			PE	$CHCl_3$	EtOAc	EtOH	H_2O	MeOH
1.	*B. subtilis*	Gram(+)	-	-	-	-	-	-
2.	*S. aureus*	Gram(+)	-	15	-	16	15	12
3.	*P. aeruginosa*	Gram(-)	-	11	12	-	-	-
4.	*B. pumilus*	Gram(+)	-	11	16	14	-	14
5.	*E. coli*	Gram(+)	-	-	16	14	-	15
6.	*C. albicans*	Fungi	-	-	15	-	-	18

Cytotoxicity

An MTT assay is a colorimetric assay based on assessing the cell metabolic activity. The sample was evaluated for cytotoxicity on human hepatoma carcinoma cell lines (HepG2), the results are shown in Table 7. In the present study, *M. ptelefolia* was found to show a potent inhibitory effect on HepG2 cell survival. Firstly, the toxicity of *M. ptelefolia* was examined on liver cancer HepG2 cells. The calculated IC_{50} (47.22 ± 0.27 μg/mL) implied the promising inhibitory effect against these cancer cells.

Table 7 Cytotoxicity of Different Concentration of Methanol Extract of *M. ptelefolia* Leaf against HepG2 Cell Line

Extracts	Cell viability (mean ± SD) in different concentration (μg/mL)							IC_{50} (μg/mL)
	3.125	6.25	12.5	25	50	100	200	
MeOH	0.98 ±0.25	0.73 ±0.11	0.67 ±0.05	0.66 ±0.33	0.48 ±0.22	0.42 ±0.26	0.17 ±0.03	47.22 ±0.27

Conclusion

From the overall assessment concerning with the chemical constituents and some biological activity investigation on *M. ptelefolia* (Thit-Kha) leaf, the following inferences could be deduced. *M. ptelefolia* leaf is a good remedy for some disease due to the presence of important phytoconstituents such as phenolic compounds, flavonoids, alkaloids and steroids, etc. Moreover, Si, K and Ca were found as a major constituent in leaf samples. Higher content of carbohydrate, protein and fiber as good source of nutrients was observed in leaf sample. According to data, the amounts of total phenol and total flavonoid were very high in watery extract, than methanol and ethanol extracts. The knowledge of the phenolic compound profile, occurring in *M. ptelefolia* holds great significance from the pharmaceutical point of view. The level of flavonoids in plants is vital for the resistance of external threats and the ability to defend the cells from oxidants. The radical scavenging activity of watery extract was found to be more effective than ethanol and methanol extracts. Moreover, these are natural antioxidants, so we should use *M. ptelefolia* leaf instead of synthetic antioxidants that have shown potential health risks and toxicity. EtOAC and MeOH extracts exhibited the highest antimicrobial activities. Therefore, these extracts except PE may have mild broad spectrum activity and would be helpful in testing diseases caused by infection of these microorganisms. From the results of cytotoxic activity of methanol extract, the concentration of methanol extract increased, the cell viability also decreased. Therefore, methanol extract of

M. ptelefolia leaf can protect liver cancer cell. According to these experimental results, *M. ptelefolia* leaf possess the some medicinal properties and may be used as potential sources for the treatment of diseases related to oxidative stress, cytotoxic and microbial infections.

Acknowledgements

The authors would like to express their profound gratitude to the Myanmar Academy of Arts and Science for giving permission to submit this paper.

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