Determination of Antioxidant Activities of Turmeric Oil, Curcumin and Ethanol Extract from *Curcuma longa* Linn. (Turmeric)

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***Abstract*: In this research, the antioxidant and antimicrobial activities of turmeric oil, curcumin and ethanol extract from *Curcuma longa* Linn. (Turmeric) were studied. The fresh rhizomes of turmeric were collected from Hanmyintmo Village, Kyaukse Township, Mandalay Region, Myanmar on September in 2015. Firstly, the turmeric oil was extracted from fresh rhizomes of turmeric by steam distillation method. And then the phytochemicals screening of turmeric rhizomes were done. The curcumin was extracted from turmeric oleoresin of turmeric rhizomes by selective solubility method. The isolated curcumin was identified by FT-IR spectroscopy and thin layer chromatography methods. The purity of curcumin was checked by melting point and Rf value of curcumin were determined by solvent system. The antioxidant activities of turmeric oil, curcumin and ethanol extract were investigated in *vitro* by DPPH (1,-1 diphenyl-2-picryl hydrazyl)radical scavenging assay.**

***Keywords*: Turmeric oil, curcumin, antioxidant activities, DPPH, Turmeric oleoresin**

I. INTRODUCTION

Medicinal plants have been used for mankind as source of medicines since a long time ago. More than 35,000 plant species have been reported to be used in various human cultures around the world for medical purposes. Plant oils and extracts have been used for a wide variety of purposes for thousands of years. One of their purposes is as a source of medicine as they contain organic compounds with therapeutic values. Majority of people depend on traditional medicine as their primary healthcare. About 80% of people in this world depend on herbs for health. In most developing countries, the use of medicinal plants which is a basis for maintenance of good health has been discovered [9].

Zingiberaceae family comprises of many genera of aromatic and medicinal plants such as Curcuma, Alpinia, Zingiber and Kaempferia and one of the plants that commonly found in Malaysia is *Curcuma Longa Linnaeus* or turmeric. *Curcuma Longa* is sometimes called *Curcuma domestica*as it is always used in the kitchen for preparing dishes. *Curcuma Longa* has been used for preparing traditional Indian curries for hundreds of years as a flavor, color, and preservative [9].

Turmeric (*Curcuma longa* L.) is a plant native to Southeast Asia that belongs to the family Zingiberaceae. The therapeutic properties of turmeric include insecticidal, antimicrobial, antifungal and antioxidant actions. Turmeric has been observed to be toxic to fungi involved in the deterioration of agricultural products, interfering with the development of mycelia [3].

Essential oils may be an alternative to syntheticpesticides for the control of food fungi and pests. The interest in their use has been increasing because they present lower risks for human health and the environment and do not leave residues in food, another growing concern of the population [2].

The present study is aimed to extract turmeric oil and curcumin and determine the antioxidant activitie of extracts from *Curcuma longa* Linn. (Turmeric). The turmeric oil was extracted with steam distillation method and curcumin was extracted from turmeric oleoresin of turmeric by selective solubility method.

Figure 1. Turmeric field in Hanmyintmo village

Figure 2. Rhizomes of Turmeric

II. Materials and Methods

Extraction of Essential Oil

Fresh rhizomes of *curcuma longa* Linn were collected from Hanmyintmo village, Kyaukse Township, Mandalay Region, Myanmar on September in 2015. The rhizomes were washed in running water, dried using a paper towel and grated.

5dm^3 of distilled water was poured into the still body until the water level was nearly reached below the base of the grill and perforated cone was put over it. 600 g of the cut sample was placed on the perforated cone of the still. It was heated by using a hot plate.

After heating for two hours, the steam which contains the essential oil is passed through a condenser to condense the steam, which forms a liquid and collected in the glass bottle. Turmeric oil and water were separated by using separating funnel. The filtrate (cohobation water) was stored for the use of next extraction. The turmeric oil was dried over anhydrous sodium sulphate and stored in a glass bottle.

Duration of distillation was six hours per day for two days. Distillation was considered as complete when the distillate collected showed no trace of essential oil. The above experiment was carried out for four times, each time 600 g of the dried rhizomes of *Curcuma longa* Linn. (Turmeric), 2.5 dm^3 of distilled water, and 2 dm^3 of cohobation water were used. Finally, the yield % of turmeric oil was calculated.

Preliminary Phytochemical Screening of *Curcuma longa* Linn.

The plant may be considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oils, tannins, saponins and flavonoids etc. These compounds are termed as secondary metabolites and are responsible for therapeutic effects. The presence or absence of primary and secondary metabolites in *Curcuna longa* Linn. (Turmeric) were carried out [4].

Extraction of Turmeric Oleoresin by using Soxhlet Extraction Method

The powder of dried rhizomes of *Curcuma longa* Linn. (Turmeric) was extracted with 95%ethanol using a soxhiet extractor. 50 g of turmeric powder in a thimble was placed in extraction section of the soxhlet apparatus. 350 mL of 95%ethanol was added into the round bottom flask attached to a soxhiet extractor. Extraction was started by maintaining the temperature at 50 to 60 °C and continued until the solvent which fill the extraction unit is almost colorless. After complete extraction, the soxhlet was removed and the ethanol extract (turmeric oleoresin) was concentrated until all ethanol was ensure evaporated.

Isolation of Curcumin from Turmeric Oleoresin by Selective Solubility Method

Turmeric oleoresin was dissolved in mixture of 60 ml of methanol/ hexane mixture and was stirred using magnetic stirrer at ambient temperature until a homogenous suspension was achieved and then filtered. Filtration of the homogeneous suspension was performed and the powder was recovered from the filter. Then, the powder was washed with *95* % ethanol and evaporated. After evaporation, the powder was dried at 110°C for one hour, desiccated and finally weighed. The yellow-brown powder was obtained. The weight of powder was 0.37 g.

Then, the powder was repeated washed with mixture of methanol/ hexane, hexane and stirred until the orange yellow colour was obtained. Finally they are crystallized with 95 %ethanol. dried, desiccated, and weighed. Orange yellow crystalline powder (0.205 g) was formed. The melting point was checked for purity. Pure curcumin melts at 179 to 180 °C.

Figure 3. Isolated Curcumin from Turmeric Oleoresin

Determination of Antioxidant Activities of Turmeric Oil, Curcumin and Ethanol Extract

1,1-diphenyl -2-picryl hydrazyl (DPPH) radical scavenging assay was chosen to access the antioxidant activities[6].

Solution Prepared

60 μM DPPH Solution

2.364 mg of DPPH powder and l0 ml of 95%ethanol were thoroughly mixed by vortex mixer. This solution was freshly prepared in the brown colored flask. Then it must be stored in the fridge for no longer than 24 hours.

Control Solution

The control solution was prepared by mixing 1.5 ml of60 μM DPPH solution and 1 .5m1 of 95%ethanol with vortex mixer.

Test Sample Solution

0.2 g of test sample and l0mL of 50%ethanol were thoroughly mixed by vortex mixer. The mixture solution was filtered and the stock solution was obtained. The stock solution was diluted with 50% ethanol to obtain desired concentration.

Blank Solution

The blank solution was prepared by mixing 1.5mL test solution and 1.5 mL 50 % ethanol with vortex mixer. The stock solution was diluted with 50% ethanol to obtain desired concentration.

Sample Solution

The sample solutions were prepared by thoroughly mixing desired concentrations of stock solution and 1.5 ml each of 60 μM DPPH solution with vortex mixer.

Procedure

Measurement of DPPH radical scavenging activity was determined by spectrophotometric method. The control solution, blank solutions and sample solutions were allowed to stand at room temperature for 30 minutes. After 30 minutes, measurements of absorbance at 517 nm were made by using spectrophotometer. Absorbance measurements were done for each solution. Absorbance values so obtained were used to calculate % inhibition by following formula. Then IC_{50} value was calculated by Linear Regressive Excel Program.

$$\% \text{ Inhibition} = \frac{\text{DPPH alone} - (\text{Sample} - \text{Blank})}{\text{DPPH alone}}$$

Where,

% Inhibition = percent inhibition of test sample
DPPH alone = absorbance of DPPH solution
Sample = absorbance of sample solution
Blank = absorbance of blank solution

III. RESULTS AND DISCUSSION

Determination of Turmeric Oil Content by Stem Distillation Method

Turmeric oil of dried rhizomes of *Curcuma longa* Linn (Turmeric) was extracted by steam distillation method. The average percentage of turmeric oil content was found to be 0.184 %. The results were shown in table.

Table 1. Yield of Turmeric Oil

No. of Expt	Wt. of sample (g)	Wt. of oil (g)	Yield
1	600	1.115	0.186
2	600	1.090	0.182
3	600	1.110	0.185
4	600	1.105	0.148
		Mean value	0.184

Preliminary Phytochemical Screening of *Curcuma longa* Linn.

Preliminary phytochemical screening of *Curcuma long* Linn. (Turmeric) was qualitative identification tests. The obtained results from the phytochemical screening showed that the *Curcuma longa* Linn. (Turmeric) contains phenols, polyphenols, flavonoids, alkaloids, tannins, saponins, terpenes and proteins but reducing sugars are absent. The results were shown in Table 2.

Table 2. Preliminary Phytochemical Tests of *Curcuma longa* Linn. (Turmeric)

No.	Constituents	Extract	Test reagent	Observation	Inference
1	Phenols	Ethanol	10 % $FeC1_3$	purple	+
2	Polyphenols	Ethanol	l%$FeC1_3$, 1% K_3 [$Fe(CN)_6$]	blue green	+
3	Flavonoids	Ethanol	Conc.HC1, Mg	deep red	+
4	Saponins	Distilled water	water	frothing	+
5	Tannins	Distilled water	10 % lead acetate	white precipitate	+
6	Reducing Sugars	Distilled water	Benedict's solution	no brick red precipitate	-
7	Proteins	Distilled water	Conc.HNO_3	yellow color	+
8	Alkaloids	1.5 %HC1	Wagner's reagent	brown precipitate	+
9	Terpenes	Ethanol	acetic anhydride, $CHC1_3$, conc: H_2SO_4	red colour solution	+

+ = Presence
- =Absence

Curcuma longa Linn. (Turmeric) is a well known kind of medicinal plant. According to the results, the *Curcuma longa* Linn. (Turmeric) contain the bioactive compounds such as phenols, polyphenols, flavonoids (antioxidant activity) and alkaloids and saponins (antimicrobial activity). So it may be the source of antioxidant and have antimicrobial activity.

Identification of Functional Groups in Curcumin by FT-IR Spectroscopy Method

The functional groups in curcumin were identified by FT-IR spectroscopy method. The FT-IR data of curcumin were shown in Figure (4). In the FT-IR spectrum of curcumin the absorption band at 3412.19 cm^{-1} is due to the O-H stretching vibration of phenolic group. The absorption band at 3007.12 cm^{-1} is due to the C-H stretching vibration of aromatic ring. The asymmetrical and symmetrical C-H stretching vibrations of sp^3 hydrocarbon observe at 2920.32 cm^{-1} and 2848.96 cm^{-1}. The CO stretching vibration of β-diketons group appears at 1627.97 cm^{-1}. The C=C stretching vibrations of aromatic ring represents at 1597.11 cm^{-1}, 1508.38 cm^{-1} and 1429.30 cm^{-1}. The absorption bands at 1278.85 cm^{-1}, 1205.55 cm^{-1} and 1028.09 cm^{-1} are due to the asymmetrical and symmetrical C–O–C stretching vibrations of ether group. The C–C–O stretching vibration of phenolic group indicates at 1153.97 cm^{-1} and out of plane bending vibrations of C-H shows at 960.58 and 812.06 cm^{-1}.

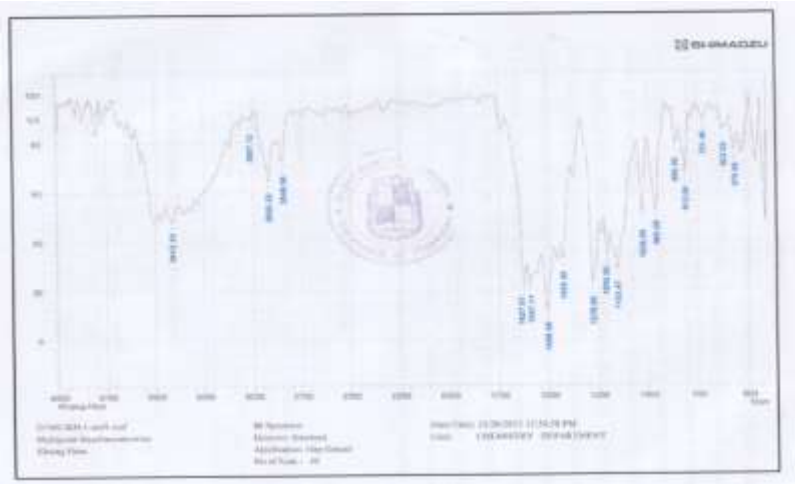


Figure 4. FT-IR Spectrum of Curcumin

Determination of Antioxidant Activities of Turmeric Oil, Curcumin and Ethanol Extract by DPPH Test

Table 3 % Inhibition Effect of Turmeric Oil

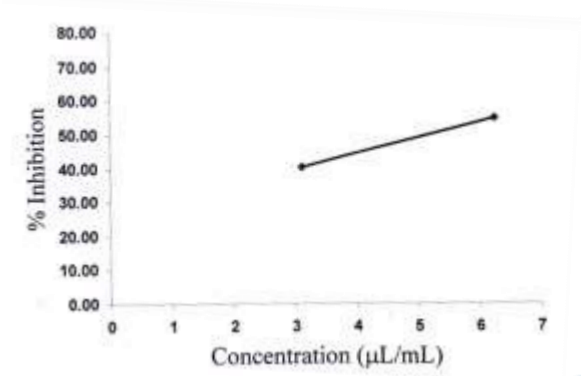
Turmeric oil	Concentration	3.125 µL/mL	6.25µL/mL	12.5 µL/mL	25 µL/mL	50 µL/mL	IC_{50}
	Absorbance	0.182	0.129	0.052	0.027	0.007	5.22
	% Inhibition	40.36	54.74	79.58	87.91	97.71	

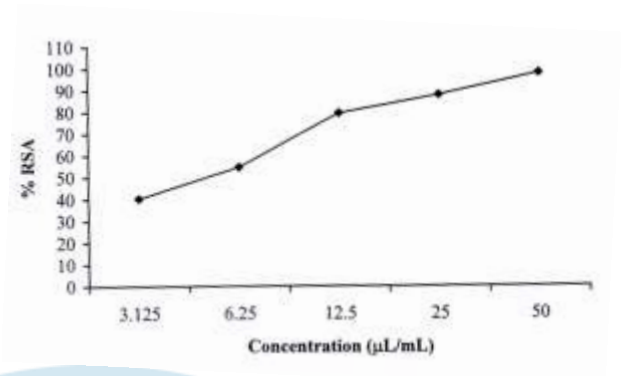
Table 4. % Inhibition Effect of Ethanol Extract

Ethanol extract	Concentration	3.125 µL/mL	6.25µL/mL	12.5 µL/mL	25 µL/mL	50 µL/mL	IC_{50}
	Absorbance	0.303	0.279	0.247	0.179	0.096	28.47
	% Inhibition	10.30	14.03	26.57	46.72	70.45	

Table 5. % Inhibition Effect of Curcumin

Curcumin	Concentration	1.25 µL/mL	2.5µL/mL	5 µL/mL	10 µL/mL	20 µL/mL	IC_{50}
	Absorbance	0.258	0.23	0.218	0.147	0.089	11.21
	% Inhibition	7.65	13.33	21.85	47.04	71.48	

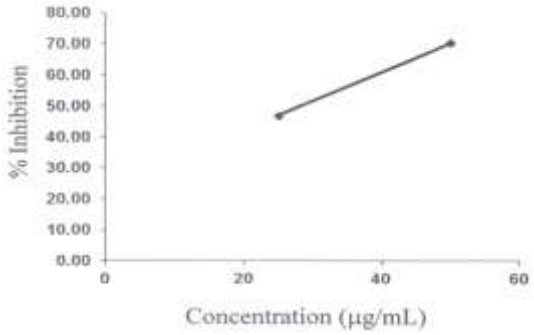


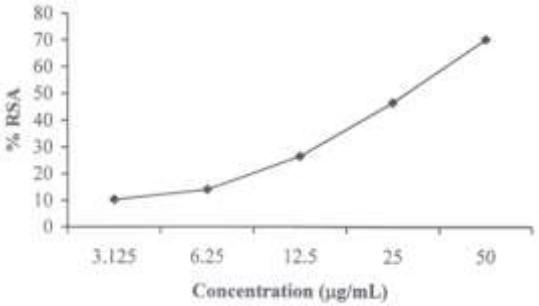


RSA= radical scavenging activity

Figure 5. Plot of % Inhibition Vs concentration for Turmeric OilTurmericOil

Figure 6.Plot of % RSA Vs Concentration for TurmericOil

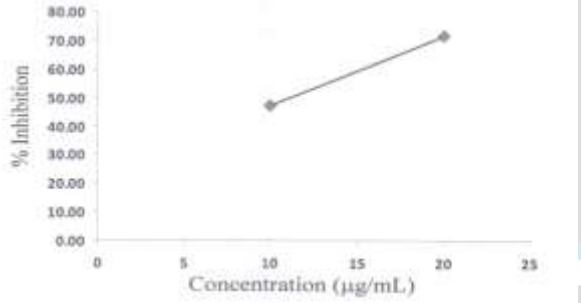


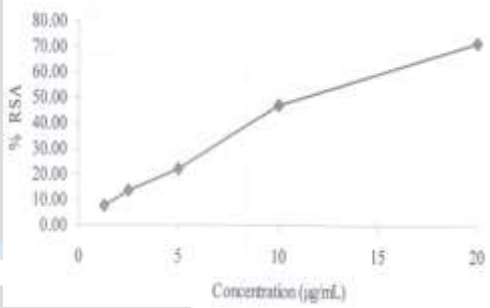


RSA= radical scavenging activity

Figure 7. Plot of % Inhibition Vs Concentration for Ethanol Ethanol ExtractEthanol Extract

Figure 8. Plot of % RSA Vs Concentration for Ethanol Extract





RSA= radical scavenging activity

Figure 9.Plot of % Inhibition Vs Concentration for Curcumin

Figure 10.Plot of % RSA Vs Concentration for Curcumin

IV. CONCLUSION

The dried rhizomes of *Curcuma longa* Linn. (Turmeric) were collected from Hanmyintmo Village, Kyaukse Township, Mandalay Region, Myanmar on September in 2015. Turmeric oil was extracted from fresh rhizomes of turmeric by steam distillation method. The yield percent of turmeric oil was found to be 0.184 **%.** This result was a little deviation from the literature value. It may be due to the climatic condition, locality, maturity of rhizomes, species, and instrumentation. According to the phytochemical results turmeric contains polyphenols, phenols, flavonoids (antioxidant activity) and alkaloids, saponins, terpenes, proteins, tannins, but reducing sugars are absent. In the extraction of curcumin from turmeric oleoresin by selective solubility method, the yield percent of curcumin based on raw turmeric was obtained at 0.4 **%.** This value was consistent with the literature value and the melting point of curcumin was also obtained in the range 176-180°C. The R_f value of curcumin was found to be 0.45 in pet ether: ethyl acetate (1:1) solvent system. It was in accordance with the literature value. The antioxidant activities of turmeric oil, curcumin, and ethanol extract were determined in *vitro* by DPPH test. It was found that the IC_{50} values of turmeric oil, curcumin, and ethanol

extract were 5.22 µL/mL, 11.21 µg/mL, and 28.47 µg/mL. According to the results, the turmeric oil exhibits the highest antioxidant activity.

Since *Curcuma longa* Linn. (Turmeric) contains the bioactive compounds such as turmeric oil and curcumin, it can help to prevent diseases due to free radicals cause (cancer, age-related, cardiovascular diseases etc.) and kill or hinder the growth of bacteria, viruses, fungi, and protozoa.

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