Determination Of Antimicrobial Activities, Antioxidant Activities And Isolation Of Pure Organic Compounds From *Curcuma caesia* Roxb. (Sa-Nwin-Tain-Pyar)

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Abstract

In this study, one Myanmar indigenous medicinal plant *Curcuma caeia* Roxb. (Sa-nwin-tain-pyar) belonging to the family Zingeberaceae was collected from Tanaing Township, Kachin State.The rhizome of this plant was selected for phytochemical analysis, antioxidant activity and antimicrobial determinations, and isolation of compound. The phytochemical analysis gave rise to positive test for alkaloid, flavonoid, saponin, glycoside, tannin, polyphenol, terpene and reducing sugar respectively. The mineral contents of *Curcuma caesia* Roxb. (Sa-nwin-tain-pyar) were determined by EDXRF method. Moreover, the antimicrobial activities of the crude extract of the rhizome of Sa-nwin-tain-pyar on six microorganisms were determined by Agar-well diffusion method. The antioxidant activity of ethanol extract of the rhizome of *Curcuma caesia* Roxb. was determined by using (1-1-Diphenyl 2-picryl hydrazyl) DPPH Assay method. In addition, compound I and II were isolated from the rhizome of *Curcuma caesia* Roxb. (Sa-nwin-tain-pyar) by using Thin Layer and Column Chromatographic methods. Finally, the functional groups in isolated compounds were identified by FT-IR spectroscopic method.

Keyword:EDXRF, Agar-well diffusion, DPPH, FT-IR

Introduction

Curcuma caesia Roxb. also called black turmeric, it is perennial herb with bluish-black rhizome, native to south eastern Asia, North East and central India. The rhizome of black turmeric has a lot of economic importance owing to its putative medicinal properties. The cultivation and harvesting practices are similar to that of common turmeric which is used in recipes (Asem, *et al*., 2012).

Curcuma caesia Roxb. is used for curing of piles, wounds, pimples, allergies, raw paste of rhizomes is applied externally. For migraine, 2, 4 drops of fresh juice are poured in nose. For longevity, impotence, infertility, irregular menstrual flow, a spoonful powder from dried rhizomes are mixed with a spoonful of honey or a cup of milk is taken twice a day. For gastric troubles, a fresh piece of rhizome is chewed (Sotanaphum, 1993).

The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, terpene, flavonoids and phenolic compounds.

Myanmar is rich in varieties of medicinal plants due to the presence of different climate zones in the economy. About 7000 different known plants are growing in Myanmar and most of them have been recognized as medicinal plants. So, in this research work, one of Myanmar medicinal plants *Curcuma caesia* Roxb. are selected for chemical analysis, antioxidant activity, antimicrobial determination and isolation of pure compounds.

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Botanical description

Figure (1) The Plant, Flower and Rhizome of Sa-nwin-tain-payr.

Family name	-	Zingiberaceae
Genus	-	Curcuma
Botanical name	-	*Curcuma caesia* Roxb.
Myanmar name	-	Sa-nwin-tain-pyar
English name	-	Black Turmeric
Site of Collection	-	Tanaing Township, Kachin State

Materials and Methods

Sample collection

The rhizome of *Curcuma caesia* Roxb. (Sa-nwin-tain-pyar) was collected from Tanaing Township, KachinState. Firstly, this sample was dried in the shade and chopped into small pieces. And then, the raw material was kept in the glass bottle with stopper.

Preliminary Phytochemical Constituents of *Curcuma caesia* Roxb.

Phytochemical investigation on *Curcuma caesia* Roxb. was carried out according to the reported methods (Harbone, 1973).

Determination of Mineral Contents of the Rhizome of *Curcuma caesia* Roxb.

The mineral contents of the rhizome of *Curcuma caesia* Roxb. was measured by applying EDXRF (Energetic Dispersive X-ray fluorescence) spectrophotometer at Department of Chemistry, Monywa University.

Determination of Antimicrobial Activities of the Rhizome of *Curcuma caesia* Roxb.

The antimicrobial activities of the crude extract of the rhizome of *Curcuma caesia* Roxb. were tested in various solvent systems by using Agar-well diffusion method on six selected organisms in Central Research and Development Centre (CRDC), Insein, Yangon.

Determination of Antioxidant Activity of Rhizome of *Curcuma caesia* Roxb. By DPPH Assay

The antioxidant activity of the rhizome of sensitive plants was done by using DPPH assay method at Department of Chemistry, University of Mandalay. In this experiment, 1, 1-diphenyl-2-picryl hydrazyl (DPPH) powder was used as a stable free radical. Ascorbic acid was used as standard antioxidant. Ethanol (Analar grade) was also used as solvent. The absorbance was determined at 517 nm wavelength. (Stjepan and Bozidar, 2006) (Lovo, 2010)

Preparation of Plant Extract

The dried sample (150g) was percolated with ethanol 700mL for 2 months. And then the extracted solution was filtered with filter paper and the residue was washed with small amount of ethanol and filtered again. The filtrate was concentrated at room temperature and crude extract was obtained. 10.5 g of ethanol extract of the rhizome of Sa-nwin-tain-pyar was accurately weighed and placed in 250 mL beaker and extracted with 50 mL of ethyl acetate. The resulting ethyl acetate solution was evaporated at room temperature. Finally, 3 g of ethyl acetate crude extract was obtained.

Isolation of Pure Compound I and II

The ethylacetate extract (3 g) was chromatographed on silica gel column as eluting was various ratios of n-hexane and ethyl acetate which gave rise to the fractions. Each fraction was checked by TLC and then fractions of the same $R_f$values were combined. Nine combined fractions were obtained. The combined fractions II and VI have found to be the main portions. These fractions have shown only one spot on TLC. Combined fraction IIgave compound I and combined fractions VI gave compound II.

Results and Discussion

Preliminary Phytochemical Examination of *Curcuma caesia* Roxb.

Phyochemical tests were carried out to detect the presence of organic constituents in the rhizome of *Curcuma caesia* Roxb. The results were obtained in Table (1).

Table (1) Preliminary Phytochemical Examination of *Curcuma caesia* Roxb.

No	Test	Extract	Reagents	Observation	Remark
1	Alkaloids	10%HCl	i. Wagner's reagent ii.Dragendorff's reagent	Reddish brown ppt Orange red ppt	+ +
2	Flavonoid	95%EtOH	Mg turning, conc:HCl	Green	+
3	Glycoside	DW	10 % lead acetate	White ppt	+
4	Saponin	DW	DW	Forth	+
5	Tannin	DW	10 % lead acetate Dilute H_2SO_4	Yellowish brown ppt	+
6	Steroid	95%EtOH	$(CH_3CO)_2O$, Conc:H_2SO_4	Green color	-
7	Phenolic	DW	10%$FeCl_3$	Orange ppt	+
8	Terpene	95%EtOH	$(CH_3CO)_2O$, Conc:H_2SO_4,$CHCl_3$	Reddish brown ppt (or) Pink	+
9	Polyphenol	95%EtOH	10% $FeCl_3$,$K_3[Fe(CN)_6]$	Greenish blue color	+
10	Reducing Sugar	DW	Benedict's Solution	Orange red ppt	+

(+) = presence (-) = absence ppt = precipitate

According to the results, the rhizome of *Curcuma caesia* Roxb. extract consists of alkaloid, flavonoid, glycoside, terpene, saponin, reducing sugar, polyphenol, phenol and tannin respectively.

Mineral Contents of the Rhizome of *Curcuma caesia* Roxb. by EDXRF Method

The elemental compositions of the rhizome of *Curcuma caesia*Roxb.were determined and the results were shown in Table (2).

Table (2) Mineral compositions of *Curcuma caesia* Roxb.

No	Symbol	Element	Relative advancenes (%)
1	K	Potassium	1.032 %
2	Si	Silicon	0.674 %
3	P	Phosphorus	0.270 %
4	Al	Aluminium	0.187 %
5	S	Sulfur	0.141 %
6	Ca	Calcium	0.106 %
7	Mn	Manganese	0.053 %
8	Fe	Iron	0.023 %
9	Zn	Zinc	0.010 %
10	Ti	Titanium	0.004 %
11	Cu	Copper	0.002 %
12	Rb	Rubidium	0.002 %

From Table (2), it was found that the amount of potassium is the highest. Potassium is health in sure as it keeps the heart beating maintain normal body growth and build protein. The amount of silicon and phosphorus of the sample is higher than the others. Silicon is used for skin healing and for treating sprains and strains, as well as improving hair and nail quality and also digestive system disorders. We also need phosphorus for many functions such as filtering waste and repairing tissue and cells and to keep bones strong and healthy and to move muscle.

Determination of Antimicrobial Activities of the Rhizome of *Curcuma caesia* Roxb.

The results of antimicrobial activities of crude extract sample were shown in Table (3).

Table (3) Antimicrobial Activities of *Curcuma caesia* Roxb.

Sample	Solvents	Inhibition zone					
		I	II	III	IV	V	VI
Sa-nwintain-pyar	n-hexane	13mm (+)	12mm(+)	12mm(+)	13mm(+)	13mm(+)	14mm(+)
	EtOAc	22mm (+++)	24mm(+++)	30mm(+++)	22mm(+++)	22mm(+++)	18mm(++)
	EtOH	16mm (++)	16mm(++)	15mm(++)	16mm(++)	17mm(++)	15mm(++)

Agar well ~ 10mm
10mm ~ 14mm (+) = low activity
15mm ~ 19mm (++) = medium activity
20mm ~ above (+++) = high activity

Organisms
I = *Bacillus subtilis*
II = *Staphylococcus aureus*
III = *Pseudomonas aeruginosa*
IV = *Bacillus pumilus*
V = *Candida albicans*
VI = *E coli*

According to Table (3), the ethyl acetate crude extract of *Curcuma caesia* Roxb. (Sa-nwin-tain-pyar) responds high activities on all tested organisms except *E-coli*. Ethanol crude extract gives medium activities on all tested organisms and n-hexane crude extract also responds low activities on all tested organisms.

Determination of Antioxidant Activity of the Standard Ascorbic Acid

Table (4) % Inhibition of Various Concentration of Standard Ascorbic Acid

Sample Concentration (µg/ml)	Mean inhibition	Mean % inhibition	IC_{50} (µg/ml)
200	0.136	79	12.11
160	0.157	75.77	
120	0.176	72.83	
80	0.198	69.44	
60	0.218	66.35	
20	0.392	39.5	

IC_{50} value was calculated by using linear regressive equation.

Determination of Antioxidant Activity of the Rhizome of *Curcuma caesia* Roxb.

Table (5) % Inhibition of Various Concentration of Sample

Sample Concentration (μg/ml)	Mean Absorbance	Mean % inhibition	IC_{50} (μg/ml)
20	0.243	60.38	16.97
16	0.345	44.88	
12	0.418	33.22	
8	0.44	29.71	
4	0.56	10.63	

IC_{50} value was calculated by linear regressive equation

According to results of measuring antioxidant activity, it can be known that IC_{50} value of the ascorbic acid is 12.11 μg/mL, and the selected sample is 16.97 μg/mL.

Isolation of Pure Compound from the Rhizome of *Curcuma caesia* Roxb.
Thin Layer Chromatography Checked for Compound I and Compound II

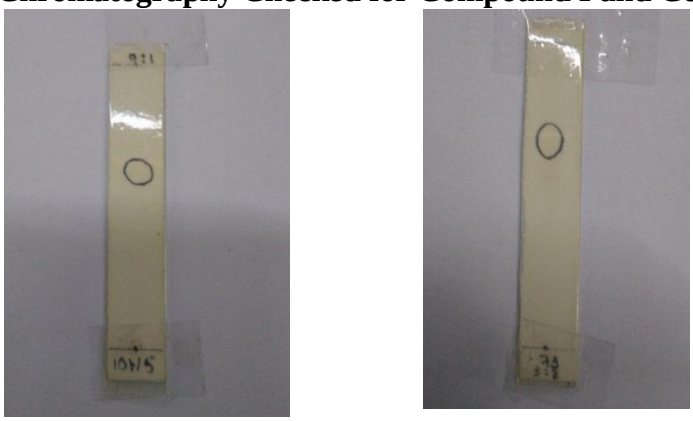

Figure (2) TLC Checked for Compound I and Compound II

Conformational Test of Terpene for Compound I

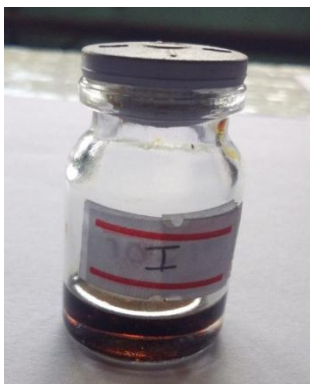

Figure (3) Terpene Test for Compound I

FT-IR Assignments of Isolated Pure Compound I and Compound II

FT-IR spectrums of isolated pure compound I and II were measured the Department of Chemistry, MonywaUniversit. The resutls are shown in Table (6 and 7)

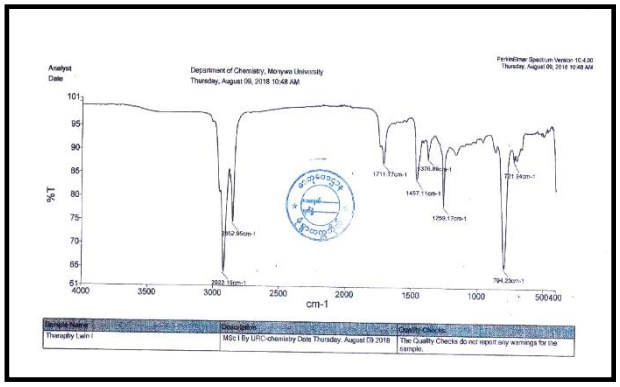

Figure (4) Fourier Transform Infrared Spectrum of Compound I

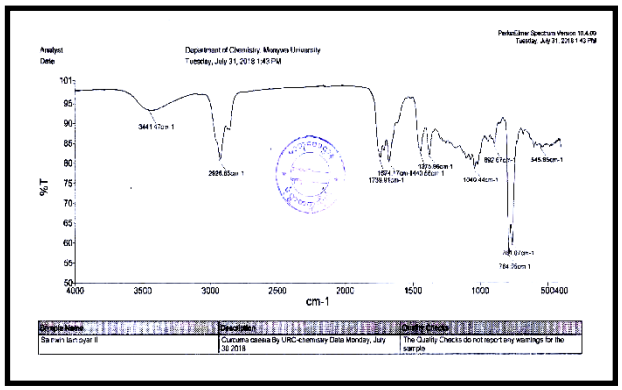

Figure (5) Fourier Transform Infrared Spectrum of Compound II

Table (6) Fourier Transform Infrared Spectrum of Compound I

No.	Wave Number (cm^{-1})	Functional groups
1.	2922.19, 2852.95	Asymmetric and symmetric C-H stretching sp^3 hydrocarbon
2.	1711.77	C=O stretching vibration of carbonyl group
3.	1457.11	C-H in plane bending vibration of methyl group
4.	1376.89	C-H in plane bending vibration of gem dimethyl group
5.	1259.17	C-C-O stretching vibration of carbonyl group
6.	781.54	C-H out of plane bending vibration

Table (7) Fourier Transform Infrared Spectrum of Compound II

No.	Wave Number (cm-1)	Functional groups
1.	3441.47	O-H stretching vibration of alcohol group
2.	2926.65	Asymmetric and symmetric C-H stretching vibration of sp^3 hydrocarbon
3.	1739.91, 1674.17	C=O stretching vibration of carbonyl group
4.	1440.56	C-H bending vibration of allylic hydrocarbon
5.	1375.96	C-H bending vibration of methyl group
6.	1040.44	C-O-C stretching vibration of ether group
7.	892.67,784.05, 761.07	C-H out of plane bending vibration of trans (or) E alkene group

Conclusion

One of Myanmar medicinal plants, Sa-nwin-tain-pyar has been widely used in traditional medicine. In this study, phytochemical analysis revealed that alkaloid, flavonoid, saponin, glycoside, tannin, polyphenol, phenol, terpene and reducing sugar were present.

According to the results of EDXRF method, the mineral contents of potassium, silicon, phosphorus, aluminium, sulfur, calcium, manganese, iron, zinc, titanium, copper and rubidium were observed in the sample. The amount of potassium, silicon and phosphorus is higher than other minerals. Potassium is important for a person's muscles to work effectively, including heart. Potassium also has a role in regulating blood pressure. They are important factors in maintaining physiological processes. Moreover, determination of antimicrobial activities, the crude ethyl acetate extract showed high activities on *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus*and *Candida albicans* and medium activity on *E-coil* organisms. Ethanol crude extract gave medium activities on all tested organisms and n-hexane crude extract also responds low activities on all tested organisms.

In addition, percent inhibition of standard ascorbic acid and IC_{50} value of ethanolic extract of the rhizome of *Curcuma caesia*Roxb.were determined by using DPPH radical scavenging assay. It was found that ethanol extract of sample (IC_{50}= 16.97 μg/mL) and standard ascorbic acid (IC_{50}= 12.11μg/mL). So, the sample extract has nearly antioxidant activity of standard ascorbic acid. Therefore, the sample has good antioxidant activity.

And then, Compound I and Compound II were isolated from the rhizome of *Curcuma caesia*Roxb.by using Column and Thin Layer Chromatographic separation methods. The R_f value of compound I is 0.6 (n-hexane: EtOAc, 4:1 v/v) and the yield percent is (0.056%) (1.7mg) based upon the ethyl acetate sample extract. Furthermore, the R_f value of compound II is 0.7 (n-hexane: EtOAc, 3:2 v/v) and the yield percent is 0.034 % (1.02 mg) based upon the ethyl acetate extract.

According to FT-IR assignments, the isolated compound I consists of sp^3 hydrocarbon, carbonyl group, methyl group, gem dimethyl group, and C-H out of plane bending vibration respectively.

According to FT-IR assignments, the isolated compound II consists of O-H functional group, sp^3 hydrocarbon, carbonyl group, allylic hydrocarbon, methyl group, ether group and trans (or) E alkene group respectively.

Finally, confirmational test for terpene, comparison of R_f value of compound I with literature data and FT-IR spectral data of compound I indicate that compound I may be camphor.

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