A Comparative Study on Chemical Constituents and Biological Activities of *Curcuma caesia* Roxb. (Marlar)

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Abstract

Curcuma caesia (Marlar) is a wonder herb and contains the highest content of curcumin. It is prescribed for treating of piles, impotency menstrual disorders and epilepsy. In addition, leaves and rhizome of *Curcuma caesia*s were used in medical formulations. This paper included phytochemicals determination, minerals contents and antimicrobial activity screening of flowers and rhizome of *Curcuma caesia*. Phytochemicals were investigated by reported methods. Alkaloids, phenolic compounds, flavonoids, reducing sugar, tannins, saponin, glycosides, carbohydrates, and starch were present in both sample. Physiochemical parameters such as moisture content, fat content, ash content, protein content, fiber content and energy value of plant samples were determined by AOAC methods. According to experimental data, food energy of rhizome (338 Kcal/100g) was higher than that of flower (210Kcal/100g). Mineral contents were studied by AAS and EDXRF. In elemental analysis, the values of Cd content in rhizome (0.131 ppm) were higher than flower (0.096 ppm). Antimicrobial activity of plant samples were screened by agar well diffusion method. From the observation, water extract and EtOA extract of flower and EtOAc extract of rhizome had high potent antimicrobial activity.

Key words: *Curcuma caesia*, phytochemicals, minerals, antimicrobial activity

Introduction

Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive compounds. Developing countries still depend mainly on medicinal herbs due to their cheaper cost and their effectiveness in the treatment of various infectious diseases with lesser side effects. Traditional tropical medicinal plants could serve as a good supply of new dependable, biodegradable and renewable drugs for the healing of many diseases. Over the past 20 years, there has been an increased interest in the investigation of natural materials as source of new anti-bacterial agents. Traditional medicines by means of plant extracts continue to provide health coverage for over 80% of the world's population mainly in the developing countries. Black turmeric or *Curcuma caesia* is endangered medicinal plant that grows in the hilly areas of the country. It is fast dying out and thus needs to be conserved for future generations.

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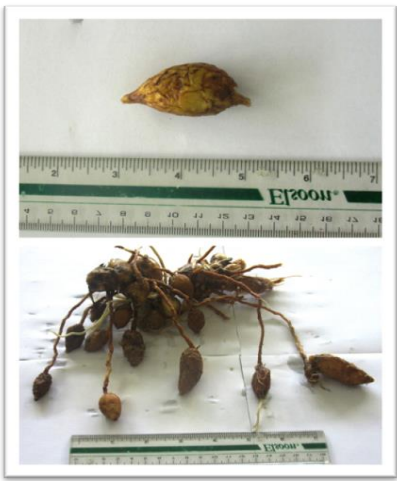
Botanical Aspects of Selected Medicinal Plant

Botanical name : *Curcuma caesia* Roxb.
Family name : Zingiberaceae (Ginger)
Genus : *Curcuma*
Species : *Curcuma caesia* Roxb.
Myanmar name : Mar-Lar
Common name : Black Turmeric
Part used : flower and rhizome
Synonym : *Curcuma kuchoorRoyle, nomennudu*

(a) plants

(b) Flower



(c) Rhizome

Figure 1. *Curcuma caesia* Roxb. (a) plants (b) flower (c) rhizome

Distribution of *Curcuma caesia Roxb*

Curcuma caesia known as black turmeric in English, is a perennial herb with bluish-black rhizome, native to North-East and Central India. It is also sparsely found in Papi Hills of East Godavari, West Godavari, and Khammam Districts of Andhra Pradesh. It is distributed mostly as wild and widely cultivated as a medicinal plant in Southeast Asia Countries like China, Indonesia, Myanmar and Thailand. It is also well-known as the Marlar in Myanmar, is best grown in the tropics or warm sub-tropical regions.

Description of *Curcuma caesia* Roxb

Curcuma caesia belongs to family *Zingiberaceae* (Ginger). The plant is normally erect with height ranging from 0.5 to 1.0 m. It is divided into underground large avoid tuberous rhizome often called rootstock and an erect aerial shoot along with leaves and reproductive part. The rhizome is bitter, hot taste with sweet odour, about 2–6 cm in diameter, the shape and size is often variable. The surface (cork) of rhizome is dark brown, bluish black, or buff in colour.

The leaves are usually present in the groups of 10–20; each leaf is broad oblong lanceolate and glabrous. The leaves have a deep violet-red patch which runs through the length of the lamina. Flowering bracts are green with a ferruginous tinge. Flower petals may be deep pink or red in color. Flowers are pale yellow colour with reddish border. Calyx is 10-15 mm long, obtuse and 3 toothed. Collar is long tubular, pale yellow lip-3 lobe semi-elliptic. Flowering starts towards the end of May and continues into September. Fruits are not found.

Medicinal Uses of *Curcuma caesia* Roxb

Curcuma caesia is a wonder herb and contains the highest content of curcumin among all curcuma or turmeric species, a chemical substance with many curative properties. It is perhaps one of the few medicinal plants that are used in herbal treatment. The herb is prescribed for treatment of piles, impotency, menstrual disorders and epilepsy. It is used both externally and internally. Externally, it is used in the treatment of wounds, white patches on the skin and leprosy sores. Some herbal doctors prescribe it for the treatment of piles and the enlargement of the spleen and certain types of tuberculosis. The rhizomes of the herb are often used for the treatment of pneumonia, cough, and cold in children, and for fever and asthma in adults. The rhizomes are claimed to have a property of acting against leukoderma, epilepsy, cancer and HIV/AIDS. Intake of small amount of rhizome paste is claimed to expel gases from, the stomach and cure menstrual disorders. The fresh and dried rhizomes of *Curcuma caesia* are used for treatment of various diseases.

Antimicrobial Activity

An antimicrobial activity is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungal are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called biostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis.The main classes of antimicrobial agents are disinfectants ("nonselective antimicrobials" such as bleach), which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics (which are applied to living tissue and help reduce infection during surgery), and antibiotics agents can be further subdivided into bactericidal agents, which kill bacteria, and bacteriostatic agents, which slow down or stall bacterial growth. Many antimicrobial agents exist, for use against a wide range of infectious diseases.

Materials and Methods

Collection and Preparation of samples

The flower and rhizome of *Curcuma caesia* were collected from Dawei University campus, Longlone Township, Thanitharyi Region, Myanmar, during June 2015. The

collected samples were identified by botany department, Dawei university. Samples were washed and air dried at room temperature for two weeks and then grounded into powder by using grinding machine. The dried powdered samples were separately stored in air-tight container.

Investigation of Phytochemical Constituents in *Curcuma* Samples

The phytochemical examination of plants is one of the important experiments because it provides classification of groups of chemical substances present in it. The preliminary phytochemical examination by test tube method indicated the presence of chemical constituents of *Curcuma* flower and rhizome samples.

Physicochemical Characterization of the *Curcuma* Samples

Physicochemical parameters such as water content or moisture %, the fat content, water soluble ash, acid-insoluble ash, protein content, fiber content, carbohydrate content and energy value of plant sample was determined by A.O.A.C methods at UMFCCI (Union of Myanmar Federation of Chambers of Commerce and Industry),Yangon.

Determination of Some Heavy Metals by Atomic Absorption Spectrophotometry (AAS)

Some heavy metals (Pb and Cd) in flower and rhizome of *Curcuma caesia* were measured by AAS method using Perkin Elma A Analyst 800 Spectrophotometer instrument at the Universities' Research Center (URC), Yangon.

Determination of Relative Composition of Some Elements by Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometry

Relative composition of some elements in flower and rhizome of *Curcuma caesia* were measured by EDXRF method using EDX-700 instrument at the Universities' Research Center (URC), Yangon.

Screening of Antimicrobial Activity of *Curcuma* Samples

In this Section, the screening of antimicrobial activity of various crude extracts such as PE, EtOAc, 70 % EtOH, MeOH extracts of flower and rhizome of *Curcuma caesia*were carried out by agar disc diffusion method at Fermentation Department, Development Center of Pharmaceutical and Food Technology (DCPFT), Ministry of Industry (1), Yangon, Myanmar. Six microorganisms namely *Bacillus subtilis* (JAP-022/215), *Staphylococcus aureus* (ATCC-12277), *Pseudomonas aeruginosa* (IFO-3080), *Bicilluspumilus* (IFO-12102), *Candida albicans* (IFO-1060) *and Escherichia coli* (ACCT-25922) were used for this test.

Results and Discussion

Phytochemical Constituents in *Curcuma* Samples

Preliminary phytochemical investigation of flower and rhizome of *Curcuma caesia* were carried out according to the reported methods. From the results, alkaloids, phenolic compounds, flavonoids, reducing sugar, tannins, saponin, glycosides, carbohydrates, and starch were present in both sample but α-amino acids was found to be absent in both sample.

Table 1. Phytochemical Constituents in *Curcuma* Samples

No.	Test	Extract	Test Reagents	Observation	(a)	(b)
1	Alkaloids	1% HCl	Wagner's reagent Mayer's reagent	Brown ppt White ppt	+ +	+ +
2	Phenolic compounds	H_2O	1% $FeCl_3$	Deep blue	+	+
3	Flavonoids	EtOH	Mg tunning/conc: HCl	Pink	+	+
4	Reducing sugars	H_2O	Benedict's solution	red colour	+	+
5	Tannins	H_2O	1% Gelatin	White ppt	+	+
6	Saponins	H_2O	Distilled water	Frothing	+	+
7	Glycosides	H_2O	10% lead acetate	White ppt	+	+
8	Carbohydrates	H_2O	10% α- naphthol&H_2SO_4	Red ring	+	+
9	Starch	H_2O	1% Iodine solution	Deep blue	+	+
10	α-Amino acids	H_2O	Ninhydrin reagent	No purple spot	-	-

(+) = Present (-) = Absent (ppt) = Precipitate (a) = Flower (b) = Rhizome

Determination of Nutritional Values of *Curcuma caesia*

The determination of nutrient values from *Curcuma caesia* samples such as protein, crude fiber, fat, carbohydrate, moisture and ash contents were carried out according to the AOAC methods. The moisture content was determined by oven drying method. The moisture content of *Curcuma caesia* flowers and rhizome were found to be 15.39% and 10.20 %. The ash contents of samples were measured by ashing in muffle furnace. The samples were heated in muffle furnace at the temperature of about 600 ºC for two hours. The ash content of flowers and rhizome were 11.12% and 3.90%.

Protein content (nitrogen content × 6.25) was measured by Macro Kjeldahl Method. The values of protein content were 9.75% in flower and 6.33% in rhizome. Fiber content of flower and rhizome samples were determined by Fiber cap method and it was found to be 24.03% and 3.48%. Fat content was measured by Soxhlet extraction method. The results of 1.86% in flower and 1.82% in rhizome were obtained.

Carbohydrate content of sample was obtained by subtracting the sum of the percentage of moisture, ash, protein, fiber and fat contents from 100. The value of carbohydrate content in *Curcuma caesia* flowers and rhizome were 37.85 % and 74.27 %. In this study, carbohydrate content of flower and rhizome were higher percentage compared to its ash, fat, fiber and protein contents. Food energy of *Curcuma caesia* flower and rhizome were 210 kcal/100g and 338 kcal/100g.

Table 2. Nutritional Values of *Curcuma caesia* Roxb.

No.	Test Parameter	Percent Content (%)	
		Flower	Rhizome
1.	Protein	9.75	6.33
2.	Crude Fiber	24.03	3.48
3.	Fat	1.86	1.82
4.	Carbohydrate	37.85	74.27
5.	Moisture	15.39	10.20
6.	Ash	11.12	3.90
7.	Energy Value	210	338

Determination of Some Heavy Metals by Atomic Absorption Spectrophotometry

In the present work, content of Pb and Cd in *Curcuma caesia* were determined by AAS. The values of Pb content were 0.384 ppm in flower and 0.675 ppm in rhizome. The values of Cd content were 0.096 ppm in flower and 0.131 ppm in rhizome.

Table 3. Some Heavy Metals in *Curcuma caesia* Roxb.

Sr. No.	Part used	Content (ppm)	
		Pb	Cd
1.	Flower	0.384	0.096
2.	Rhizome	0.675	0.131

Determination of Relative Composition of Some Elements by EDXRF Spectrometry

In this work, relative composition of elements in *Curcuma caesia* Roxb was determined by EDXRF spectrometer applying fundamental parameter method. It was found that K, S, Mn, Ca, Fe, Zn and Ti were contained in flower and rhizome. Among these elements, the potassium contents were 3.394% in flower and 1.131% in rhizome as major constituents.

Table 4. Relative Composition of Some Elements in *Curcuma caesia* Roxb.

Sr. No.	Elements	Relative composition (%)	
		Flower	Rhizome
1	K	3.394	1.131
2	S	0.230	0.116
3	Mn	0.097	0.089
4	Ca	0.214	0.057
5	Fe	0.089	0.052
6	Zn	0.004	0.007
7	Ti	0.015	0.007
8	P	0.235	-
9	Si	0.180	-
10	Rb	0.005	-
11	Cu	0.001	-
12	COH	95.534	98.541

Antimicrobial activity of *Curcuma caesia*

Invitro antimicrobial activities of two different portions of *Curcuma caesia* were studied on crude extracts such as PE, EtOAc, 95% EtOH and MeOH extracts by agar well diffusion method on six species of microorganisms such as *Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas aureginosa and Staphylococcus aureus*. According to these results, water extract of flower had more potent activity than other flower crude extracts. MeOH extract of flower was showed *B pumilus* and EtOH extract did not inhibited *B sub* and *Pseudomonas.* EtOAc extract of rhizome had more active activity than other rhizome extracts. Among the all crude extracts, water of flower and EtOAc extracts of flower and rhizome were more potent antimicrobial activity.

Table 5 Antimicrobial activities of two different portions of *Curcuma caesia*

Sample	Solvent	Organsims					
		B-sub	*S-aureus*	*Pseudo-monas*	*B-pumilus*	*Candida*	*E-coli*
Flower	P.E	12 mm (+)	12 mm (+)	-	-	-	-
	MeOH	30 mm (+++)	23 mm (+++)	30 mm (+++)	-	12 mm (+)	12 mm (+)
	E.A	15 mm (++)	18 mm (++)	26 mm (+++)	18 mm (++)	18 mm (++)	18 mm (++)
	EtOH	-	11 mm (+)	-	12 mm (+)	12 mm (+)	11 mm (+)
	DW	20 mm (+++)	20 mm (+++)	16 mm (++)	12 mm (+)	20 mm (+++)	13 mm (+)
Rhizome	P.E	16 mm (++)	17 mm (++)	17 mm (++)	15 mm (++)	17 mm (++)	16 mm (++)
	MeOH	12 mm (+)	11 mm (+)	-	12 mm (+)	12 mm (+)	12 mm (+)
	E.A	18 mm (++)	17 mm (++)	21 mm (+++)	17 mm (++)	17 mm (++)	12 mm (+)
	EtOH	13 mm (+)	13 mm (+)	14 mm (+)	13 mm (+)	12 mm (+)	12 mm (+)
	DW	-	-	-	-	-	-
Control	P.E	-	-	-	-	-	-
	MeOH	-	-	-	-	-	-
	E.A	-	-	-	-	-	-
	EtOH	-	-	-	-	-	-
	DW	-	-	-	-	-	-

Organisms

Agar well – 10mm
10mm ~ 14 mm (+)
15mm ~ 19 mm (++)
20mm above (+++)

(1) *Bacillus sublilis*(NCTC-8236)
(2) *Staphylococcus aureus*(NCPC-6371)
(3) *Pseudomonas aeruginosa* (6749)
(4) *Bacillus pumilus*(NCIB-8982)
(5) *Candidaalbican*
(6) *E-coli* (NCIB-8134)

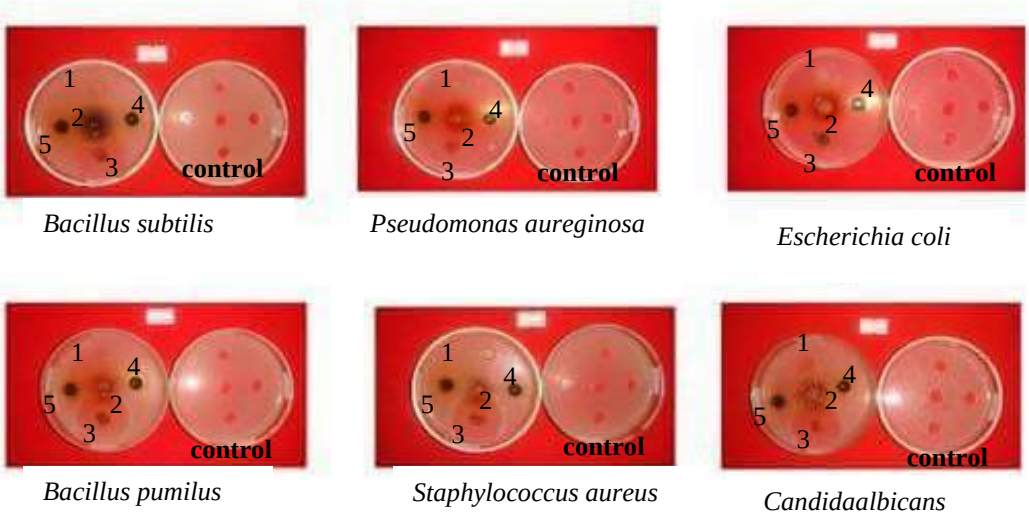
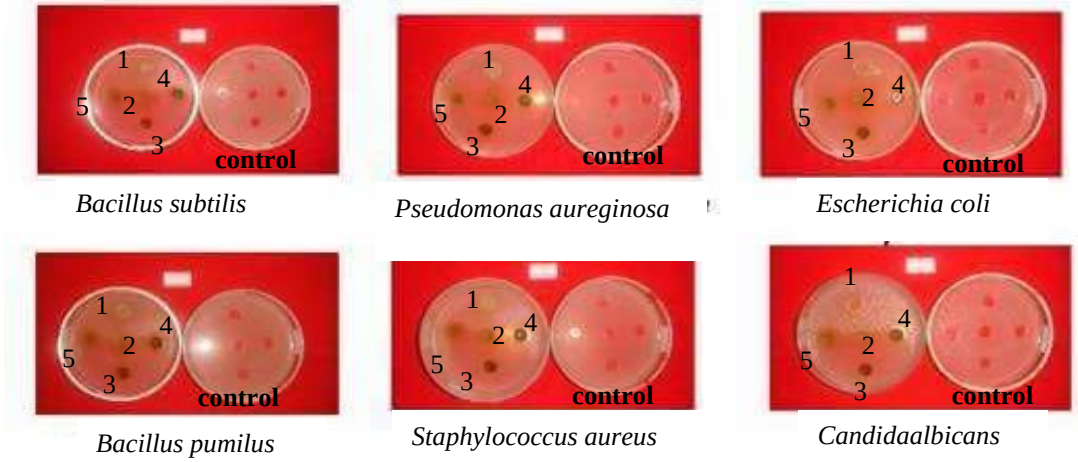


Figure 2. Inhibition well diameter of *Curcuma caesia* flower



(1) PE extract (2) H_2O extract (3) EtOAc extract (4) 95% EtOH extract (5) MeOH

Figure 3. Inhibition well diameter of *Curcuma caesia* rhizome

Conclusion

From the overall assessment of the present study, the following inferences can be concluded.

Preliminary phytochemical investigation of flower and rhizome of *Curcuma caesia* were found that alkaloids, phenolic compounds, flavonoids, reducing sugar , tannins, saponin, glycosides, carbohydrates, and starch were present in both sample but α-amino acids was found to be absent in both sample.

Nutritional values of *Curcuma caesia* samples were carried out by AOAC methods.The moisture content of flowers and rhizome samples were found to be 15.39% and 10.20 % andthe ash content were 11.12% and 3.90%.The values of protein content were

9.75% in flower and 6.33% in rhizome. Fiber contents of flower and rhizome samples were found to be 24.03% and 3.48%. The results of Fat content were 1.86% in flower and 1.82% in rhizome .The value of carbohydrate content in *Curcuma caesia* flowers and rhizome were 37.85 % and 74.27 %. In this study, carbohydrate content of flower and rhizome were higher percentage compared to its ash, fat, fiber and protein contents. Food energy of *Curcuma caesia* flower and rhizome were 210 kcal/100g and 338 kcal/100g.

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