Microscopical Characters, Phytochemical and FTIR Studies on rhizome of *Zingiber officinale* Rosc. (Gyin)

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Abstract

The plant, *Zingiber officinale* Rosc .is belongs to the family Zingiberaceae. It is abundantly found in Pyin Oo Lwin Township, Mandalay Division. The morphological, microscopic, phytochemical and Fourier Transform Infrared Spectroscopy (FTIR) studies of rhizomes were carried out. In the morphological study, the plant is an annual or biennial, horizontal creeping rhizomatous herbs and terminal spikes with pale yellowish flowers. In microscopical study, the rhizome was circular, oval or broadly elliptical in outline. The cork cells are arranged in radial rows. Vascular bundles are scattered throughout the cortex and occurred more numerously just beneath the endodermis and various sizes.The rhizome powder showed fragment of parenchymatous cells, vessel, fibre, crysal, oil droplet with simple or compound starch grains. The sensory characters of rhizome were found in whitish-brown in color and granular. Odour was strong aromatic and acrid. In phytochemical study, alkaloid, glycoside, phenol, tannin, saponin, reducing sugar, carbohydrate, lipophilic, flavonoid and polyphenol were observed . The FTIR spectrum analyses were carried out the presence of alcohol or phenol group, aliphatic hydrogen group, unsaturated carbonyl group, ethers and esters.

Keywords: morphological,microscopical,phytochemical and FTIR

Introduction

Zingiber officinale Rosc., one of the important plants belongs to family Zingiberaceae. Seventeen species of the genus *Zingiber* were found in Myanmar (Kress *et al.* 2003). *Zingiber officinale* was native to India and Asia, throughout the tropical regions. The plant was perennial herb with lanceolate leaves; flowers orange - yellow and ovate-oblong spike with fruit a capsule and arillate seeds (Chevallier 1996).The Zingiberaceae consisted of 40 genera and a thousand species (Dessanayake 1991). Zingiber officinale was originate from tropical Asia, widely cultivated in the tropics.

The plant is herbaceous with underground rhizome. The stem grows above ground and leaves are narrow, long, lanceolate with distinct venation patten and pointed apex. Flowers are white or yellowish-green, steaked with purple and fragrant .The rhizome of is one of the most widely used of species and common condiment for various foods and beverages. The plant is used in traditional medicine for the treatment of several diseases in different parts of the world and primary health care because of their efficacy, safety and lesser side effects. (Akhani 2004).

The rhizomes of cork tissue of *Zingiber officinale* had an outer and inner zone of cortical cells. Within the corks a broad cortex, differentiated into an outer and inner zones of parenchyma. The cortical cells contained abundant starch grains and were ovoid or sack shaped. Scattered in the cortex were numerous oil cells with yellowish brown content. The inner cortical zone contained closed collateral vascular bundles. Each bundle had a phloem and xylem composed of vessel with annular, spiral or reticulate thickening. The ground mass of the stele was composed of parenchyma and containing much starch with numerous oil cells (Kapoor 2001). The cortex of the rhizome was isodiametric and thin-walled parenchyma cells contained

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abundant starch granules and showing scattered secretion cells with yellowish brown content, endodermis thin walled cells, vascular bundles close collateral and reticulate, scalariform and spiral vessels, fibers with wide lumen and small oblique slit like pits (WHO 1999).

The powders ginger of *Zingiber officinale* was yellowish-white to yellowish-brown. Fragments of thin-walled parenchyma cells contained starch grains; thin-walled septate fibers with oblique slit-like pits; fragments of scalariform, reticulate, and spiral vessels; fragment of droplet with oil cells scattered in parenchyma cells (WHO 1999). The powders of *Zingiber officinale* was yellowish colour and contained abundant starch grains, mostly simple; fibers; vessels elements; thin-walled cork epidermal cells; abundant parenchymatous cortical cells filled with starch (Santra *et al.*1999).

Chemical constituents on rhizome of *Zingiber officinale* included volatile oil, zingiberene, oleoresin, gingerol and shogoals (Chevallier 1996). Ginger contained volatile oil, zingiberine, cineol, borneol, gingerol, oleoresin, starch and phenolic compound (Kapoor 2001). Purnomo *et al.* (2010) stated that the FTIR spectrum of the compound *Zingiber officinale* showed seven characteristic bands in the functional group region (OH stretching), (C-H stretching), (C=O stretching),(NH stretching), (CH stretching) (C=C) stretching,(C-O-C stretching)(CH_3)stretching. *Zingiber officinale* was used for heart disease, throat bronchitis, asthma, cough, digestant, laxative and mucolytic (WHO 1999; Pandy 2000). Chemical constituents produced in the living cells of plants are found to be distinctly effective on the physiological activities of man and animal, and valuable in the treatment of diseases (Padua 1999).

Drugs–yielding plants are mostly used in a crude condition even up to now adays. The advancement of pharmaceutical researches makes to increase exploitation for more medicinal plant resources. Drugs containing glycoside, esters and essential oils are usually less stable than those containing alkaloids and tannins (Siemonsma 1994).

The plants were used as such or the drugs were prepared in the crude form and used as pastes, decoction, etc., but with the advance of science and technology, the active principles of several drugs have been isolated for use. Complex chemical substances present in different plant tissues are responsible for the curative properties of drugs and enhance the chemical value of resource plants. The most important of these chemical substances are alkaloid, glycosides, steroids, essential oils, etc. (Sen 1996).

The plant is used in traditional medicine for thetreatment of several ailments in different parts of the world. Such ailments includes: Rheumatism,stomach disorder, diabetes, wounds, snake bite,baldness, toothache, respiratory disorders,arthritis, bleeding. The non-volatile extracts of the plant are known to possess antimicrobial and anti inflammatory activities. Its efficacy is the treatment of wounds.The toxicological properties of *Zingiber officinale* in rats and reported that a safe herbal medicine (Lantz 2007). *Zingiber Officinale* is a commocondiment for various foods and beverages and a long history of important traditional Medicine herb for the treatment of stomach disorders**.** The constituents present in ginger have potent antioxidant and anti-inflammatory activities. Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy,safety and lesser side effects. (Jantan 2005). Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects. India despite its rich traditional knowledge, heritage of herbal medicines and large biodiversity has a dismal share of the world market due to export of crude extracts and drugs (Shalansky 2007). The rhizome can be used in the form of tea. These plant parts were crushed and reduced to a coarse powder form and kept in a tea-strainer. Hot water is poured over it. Then liquor, adequate quantity of milk and sugar or salt as per taste can be added. The fresh tea is prepared and given to the patient at the seasonal time (WHO 1990).

The present study of this research is to identify with microscopical, phytochemical and Fourier Transform Infrared Spectroscopy (FTIR) of *Zingiber officinale* Rosc.

Materials and Methods

The plants were collected from Pyin-Oo Lwin, Township, Mandalay Division. The specimens were identified by taxonomic description with the literatures of Backer (1965), Dassanayake (1991) and Chevallier (1996). After the collection, the fresh specimens of rhizome were preserved in 50% ethyl alcohol solution for further study. Then, the dehydration, infiltration, staining and mounting were made according to Johansen's method of 1940. The specimens were macerated with studied by Jeffery's 1917 method. Finally, photomicrographs of these sections have been prepared and presented. According to Wallis (1967) & Trease and Evans (1978) stated that the collected samples were washed with water to remove impurities. After washing the samples, they were air dried and ground to get powder and stored in air tight containers for further uses. Then, powder sample was examined under the microscope with the help of a digital camera. For phytochemical tests, the air-dried powders of the rhizome were used. Test for alkaloids, glycosides, phenols, tannins, saponins, reducing sugars, carbohydrates, lipophilic compounds, flavonoids and polyphenols were carried out by using the extracts obtained from various solvents.The experimental procedure was prepared according to Harborne (1984). FTIR analysis were carried out by using KBr method with Fourier transform infrared spectrophotometer (FTIR) - 8400 S in Ministry of industry (1), Myanmar Spirulina Factory, Sagaing. The spectral data were studied according to Silverstein *et al.* (1998).

Result

Morphological Characters

Scientific Name - *Zingiber officinale* Rosc. Trans. Linn. Soc. 8. 348. 1807.
Family - Zingiberaceae
Myanmar Name - Gyin-sein
English Name - Ginger
Part –used - Rhizome

Annual or biennial, horizontal creeping rhizomatous herbs. Leaves simple, alternate; blades oblong-lanceolate, acute at the apex, entire along the margin obtuse at the base. Inflorescences terminal spikes. Flowers bisexual, zygomorphic, pale yellow. Calyx 3-lobed, split down on one side, white. Corolla infundibuliform, pale yellow, upper lobe concave, lip shorter than the corolla-tube. Stamen 1, dark- purple, as long as the lip; filament filiform; anther dithecous, oblongoid, spur absent; lateral staminodes absent; two basal staminodes linear. Ovary inferior, globoid, trilocular with 2 rows of ovules in each locule on the axile placentae; style filiform; stigma subgloboid. Fruits not seen. (Figure. 1, A)

Microscopical studies

Microscopical characters of *Zingiber officinale* Rosc.

The macroscopical characters of the rhizome of *Zingiber officinale*, its microscopical characters and diagnostic characters of the powder are describer.

Macroscopical Characters

The rhizome was fleshy and flattened with small and large branches. Base of the main rhizome to tip of the uppermost branch was 7 to 15 cm long and 3 to 8 cm wide. The nodes were distinct with parts of scale leaves. The fresh rhizome was light yellowish brown and the

dry one deep yellowish brown with longitudinal folds. The cut surface of the rhizome was showed a central ring and yellowish in colour, deeper in the central. (Figure 1, C)

Microscopical Characters

Rhizome

In transverse section of the rhizome was circular, oval or broadly elliptical in outline. The epidermis was one layer and made up of parenchymatous cells. The cells were rectangular, oval or irregular in shape. Internal to the epidermal layer was periderm which composed of 5 to 7 layers of thin-walled cork cells. The cork cells are arranged in radial rows. Cortex zone is interior to the cork. Few secretory cells are scattered in the cortex. Some of the cortical cells were contained many starch grains. The cortex consisted of collateral closed vascular bundles and fibres. Each vascular bundle consisted of phloem and xylem, composed of 2 to 6 vessels with spiral or scalariform thickening. The bundles are surrounded by a bundle sheath and associated with a group of fibres. The cortical region is divided into outer and inner parts by a layer of thin-walled endodermis. Pericycle was lied under the endodermis. Vascular bundles are scattered throughout the cortex and occurred more numerously just beneath the endodermis and various sizes. (Figure 2, A-F)

Diagnonostic Characters of Powder Rhizome of *Zingiber officinale* Rosc.

The microscopical study of powdered drug showed yellowing matter containing cells, oil droplets, fragments of parenchymatous cells, vessel elements with spiral thickening, fibers. The starch grains were simple, oval or oblong or rounded with a triangular or stellate hilum. (Figure 3, A-F)

Measurement of cells, starch grain, crystals and oil droplets of the rhizome of Zingiber officinale Rosc. (Figure 3, D)

Measurement of cells were shown in (Table 1)

Sensory characters of rhizome powder of *Zingiber officinale* Rosc.

Colour - pale yellowish brown
Texture - Granular and fibrous
Odour - Aromatic
Taste - Hot and Acrid

Table 1. Measurement of cells, starch grain, crystals and oil droplets of the rhizome of *Zingiber officinale* Rosc.

Characters	Length (µm)	Width (µm)
Epidermal cells	15.0 - 35.0 (mean 28.0)	12.0 -30.0 (mean 25.5)
Cortical cells	7.5 - 25.0 (mean 16.5)	10.0 - 30.0 (mean 26.5)
Endodermal cells	5.0 - 10.0 (mean 7.5)	5.0 -7.5(mean 6.0)
Pericyclic cells	10.0 - 15.0 (mean 12.0)	7.5 - 12.5 (mean 10.0)
Vascular bundle	70.0 - 130.0 (mean 110.0)	7.5 - 12.5 (mean 10.0)
Secretory cells	30.0 - 60.0 (mean 45.0)	20.0 - 45.0 (mean 35.0)
Starch grain	5.0 - 15.0 (mean 12.0)	5.0 - 13.0 (mean 10.0)
Crystal	3.0 - 5.0 (mean 2.0)	0.8 - 1.5 (mean 1.0)
Oil droplet	10.0 - 7.0 (mean 8.5)	2.5 - 7.5 (mean 5.0)

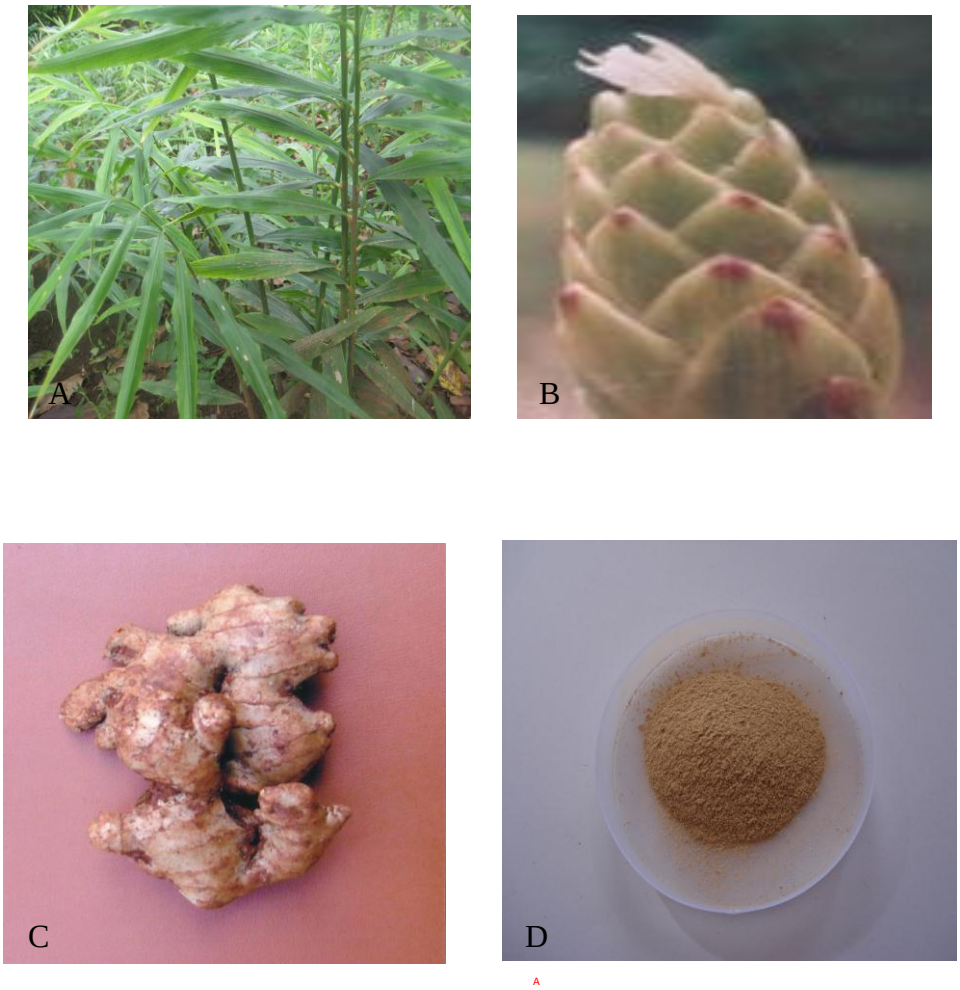

Fig. (1) Morphological characters of *Zingiber officinale* Rosc.
A. Plant in natural habit
B. Close up view of flower
C. Fresh rhizome
D. Dry powder

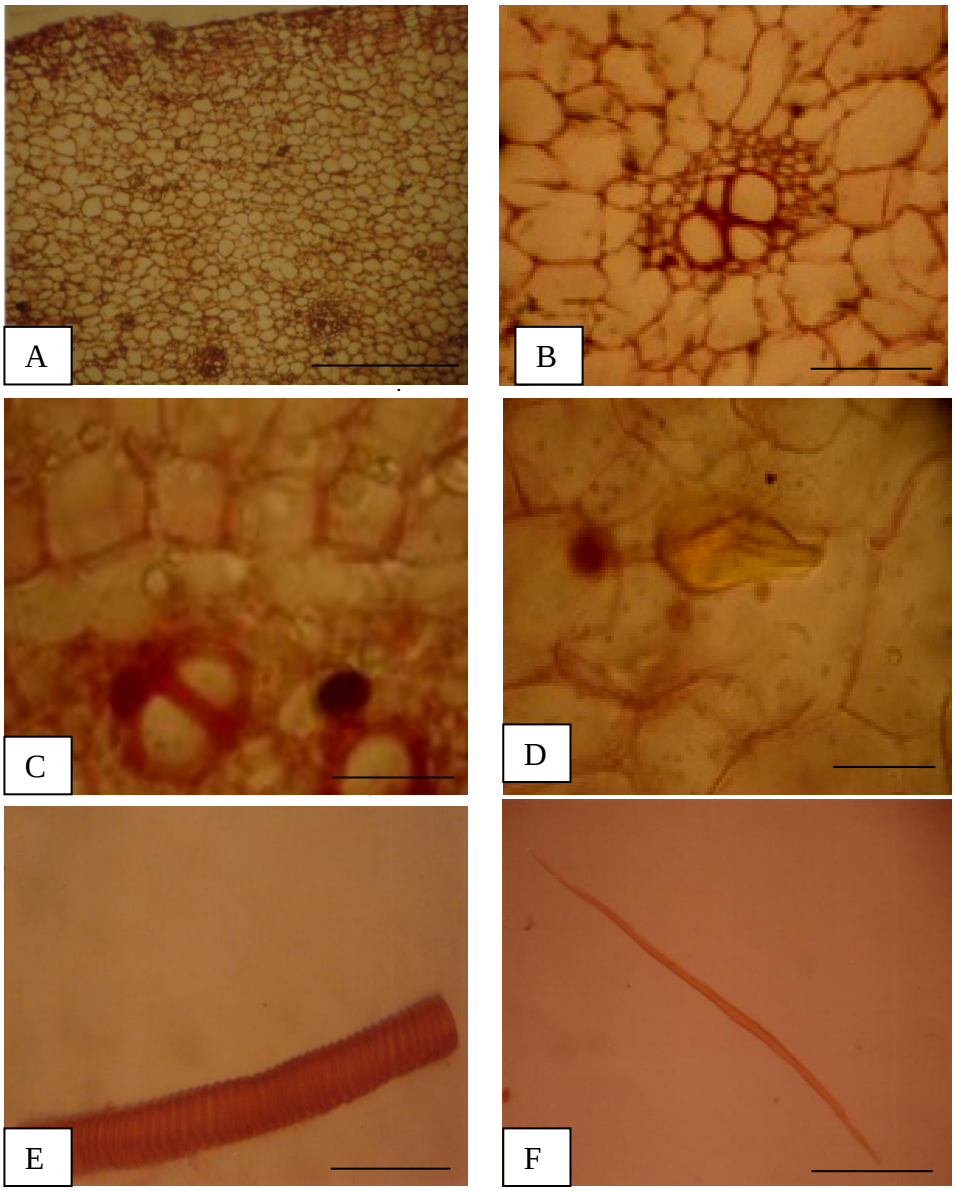

Fig:(2) Microscopical characters of *Zingiber officinale* Rosc.

A. Transverse section of rhizome (200 µm)
B. Close up view of vascular bundle (50µm)
C. Close up view of endodermis and starch grains (30µm)
D. Close up view of secretory cell (15µm)
E. Vessel element (50µm) F. Fiber (50µm)

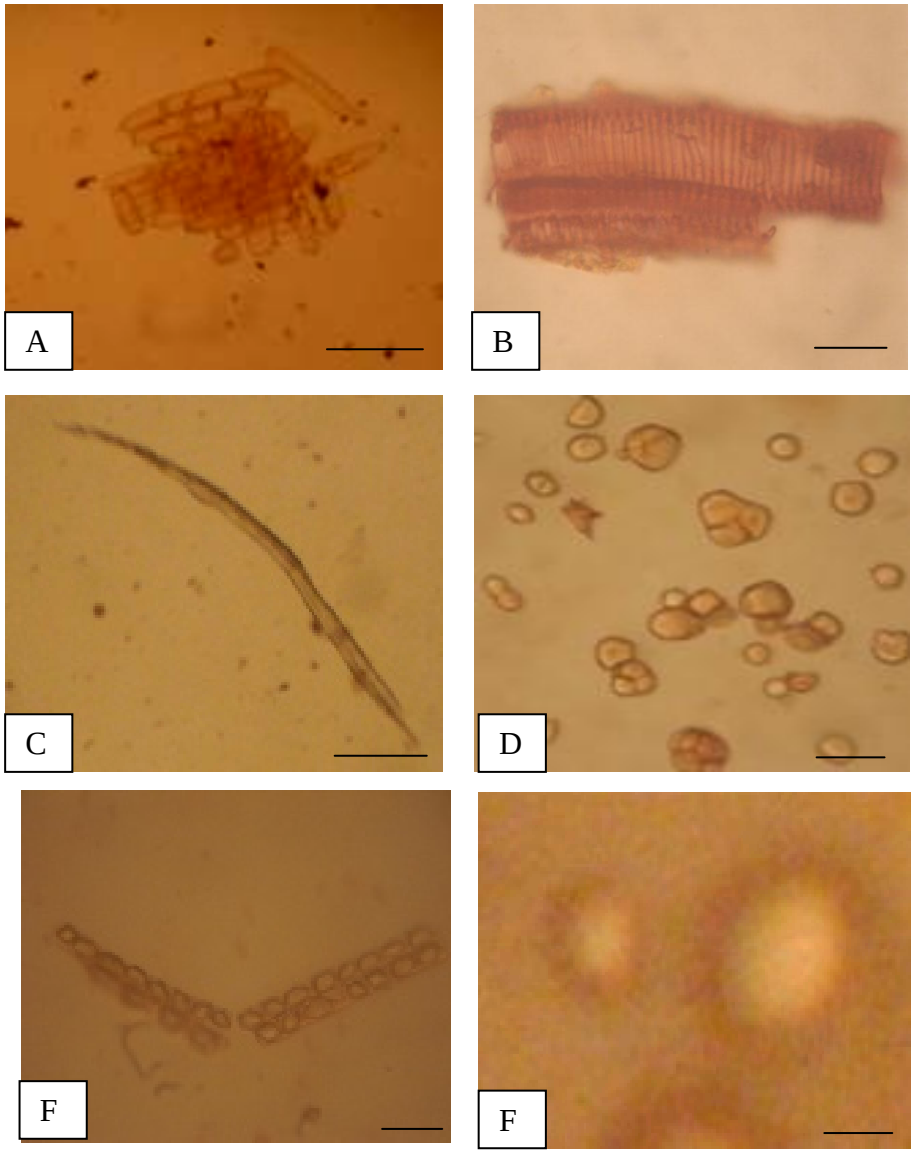

Fig: (3) Powder microscopical characters of *Zingiber officinale* Rosc.

A. Parenchyma cells (50 µm)
B. Vessel elements (30 µm)
C. Fiber (50µm)
D. Srarch grains (10 µm)
E. Two rows of crystals (5µm)
F. Oil drops (10µm)

Phytochemical analysis

Table 2. Phytochemical Tests of *Zingiber officinale* Rosc.

No	Test	Extract	Test reagent	Observation	Result
1.	Alkaloids	1% HCl	Dragendroff's reagent Mayer's reagent Wagner's reagent	orange ppt. white ppt. reddish ppt.	+ + +
2.	Glycoside	Water extract	10% lead acetate solution	white ppt.	+
3.	Phenol	Water extract	1% $K_3Fe(CN)_6$ + 1%$FeCl_3$ solution	dark blue ppt.	+
4.	Tannin	Water extract	1% Gelatin solution + 1%$FeCl_3$ solution	white ppt.	+
5.	Saponin	Water extract	$NaHCO_3$ solution (or) DW	frothing	+
6.	Reducing sugar	Water extract	Benedict's solution	no ppt.	+
7.	Carbohydrate	Water extract	Benedict's solution + Conc: H_2SO_4	not colour change	+
8.	Lipophilic	Water extract	KOH solution	not colour change	+
9.	Flavonoid	EtOH extract	Conc: HCl + Mg	not colour change	+
10.	Polyphenol	EtOH extract	10% $FeCl_3$ solution	blue colour	+

(+) = present, (–) = absent

Phytochemical analysis indicated the presence of alkaloids, glycoside, phenol, tannin, saponin, reducing sugar, carbohydrate, lipophilic, flavonoid and polyphenol in both water and ethanol extract were shown in Table 2.

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RAW MATERIAL ANALYSIS REPORT

Scientific Name - *Zingiber officinale* Rosc.

Souce of Sample - Pyin-oo-lwin Township, Mandalay Division

Register No. - 2455

Machine - FTIR (FTIR-8400S) Date 24.3.08

Analysis method - KBr Method

Analysis date - 19-03-2008

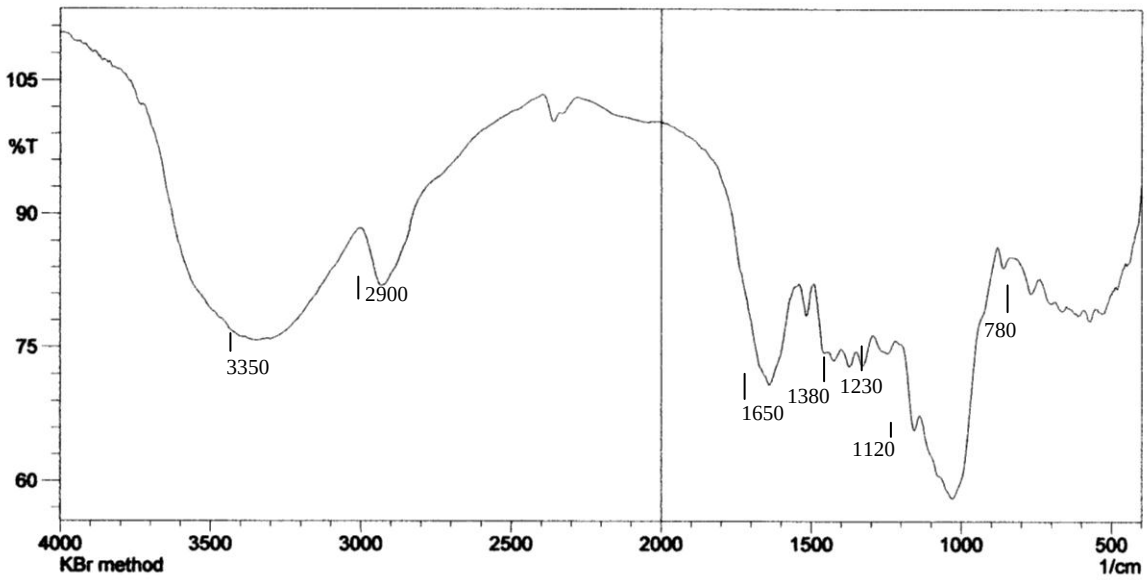

Figure 4. FTIR spectrum of *Zingiber officinale* Rosc.

FTIR,QC,MPF(sgg)(24-03-2008)

~ 3350	OH stretching vibration
~2900	CH stretching vibration of saturated hydrocarbons
~1650	C = O stretching vibration
~1380	CH in plane bending vibration
~1230	C – O – C stretching vibration
~1120	OH deformation bands in alcohol group
~780	CH out of plane banding vibration

As shown in Figure 4, the peak at 3350 cm $^{-1}$ implied OH stretching vibration. The peak at 2900 cm $^{-1}$ indicated CH stretching vibration of saturated hydrocarbons. The peak at 1650 cm $^{-1}$ assigned C = O stretching vibration. The peak at 1380 cm $^{-1}$ showed CH bending vibration. The peak at 1230 cm $^{-1}$ showed C - O - C stretching vibration. The peak at 1120 cm $^{-1}$ implied OH deformation bends in alcohol group. The peak at 780 cm $^{-1}$ assigned CH out of plane bending vibration. According to this FTIR spectrum, the crude drug powder of *Zingiber officinale* could be contained OH, CH, C = O, C - O – C, CH, OH, CH respectively functional group as shown in Table 3.

Discussion and Conclusion

In morphological study, *Zingiber officinale* Roscoe., is an upright rhizomatous herb. The stem is green, erect, usually unbranch. Bright green leaves are narrow, lanceolate with acute at the apex. Inflorescence is terminal spikes and flowers are bisexual, zygomorphic with yellowish green or white. The calyx is split down on one side, white and corolla infundibuliform. Stamen as long as the lip; filament filiform and anther dithecous,The ovaries are inferior, globoid, trilocular with 2 rows of ovules in each locule on the axile placentae; style filiform; stigma subgloboid. These characters were agreed with those mentioned by Chevalliers (1996), Kress (2003) and Akhani (2004).

Characteristics of the rhizomes of *Zingiber officinale* were observed by microscope. In transverse section of the rhizome was circular, oval or broadly elliptical in outline. In rhizome, the epidermal cells were one layered and made up of parenchymatous cells and the ground tissues consisted of outer collenchymatous layers with several layers of parenchymatous cells. The various sizes of vascular bundles were scattered in the ground tissue. The cortical regions were divided into outer and inner parts by a layer of thin-walled endodermis and the bundles were scattered throughout the cortex and more numerously just beneath the endodermis. The vascular bundles composed of phloem and xylem, consisted of 2 to 6 vessels with spiral or scalariform thickenings. Moreover the fragments of parenchymatous cells containing yellowing matters of oil droplets, annular or spiral thickenings vessel, fibers, single or compound starch grains were found in the rhizome powder of *Zingiber officinale*. Powder microscopy showed vessels, tracheidal fibres with bordered pits and horizontal perforations, starch grains oval to rounded, mostly simple but sometimes as compound grains. These characters were agreement with According to Metcalfe and Chalk (1950), Pandy (1993) and Trease & Evans (2002).

In the present study of phytochemical screening showed alkaloid, glycoside, phenol, tannin, saponin, reducing sugar, carbohydrate, lipophilic compound, flavonoid and polyphenol. According to Sharma *et.al* 2005 and penna *etal* 2003 stated that the presence of alkaloids, glycosides, tannins, saponins, reducing sugars, carbohydrates, lipophilic compounds, flavonoids and polyphenols absence phenol. According to present FTIR spectral data, the peaks of the curves of *Zingiber officinale* showed that the plants roughly contained OH stretching of alcohol or phenol groups, CH stretching vibration of aliphatic hydrocarbon groups, C = O stretching of the unsaturated carbonyl groups, C - O - C stretching vibration of ethers and esters respectively functional groups. Sharma *et al.* (2013) stated that the FTIR spectrum of the *Zingiber officinale* have showed the seven functional group (OH), (C-H),(C=C), (C=O),(C-O-C), (CH_3) stretching functional group that are agreed with the presence of OH, C=O, C-O-C compounds by phytochemical studies in this research.

In conclusion, *Zingiber officinale*., is a common condiment for food beverages and sources of significant medicines. Moreover, several *Zingiber* species produced a spice or essential oil used as adulterants or substitutes for others plant parts. The morphological and microscopical characters of rhizome were also studied which can be used for determine the identification and standardization of medicine. The basic chemical constituents of plants have been found to be medicinally useful and interesting to be secondary metabolites. It is hoped that in this study will provide medicinal information for other researcher and traditional medicine.

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