

INVESTIGATION AND PHYTOCHEMICAL CONSTITUENTS OF GINGER (*Zingiber officinale*)

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Abstract: The aim of study is to identify the antimicrobial property of ginger. It was collected from Magway Township, Magway Region. This sample was dried in air for one week. The phytochemical screening of rhizome of Ginger extracts gave rise to some constituents such as alkaloid, flavonoid, steroid, glycoside, reducing sugar, phenolic compound and tannin, respectively. Moreover, the antimicrobial activities of four Solvent (n-Hexane, CHCl₃, EtOAc, EtOH) extracts were detected by using agar well diffusion methods on six selected Microorganism, such as *Bacillus Subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albicans* and *E-coil*. Among them, ethyl acetate extract of Ginger responds high activity on all tested microorganisms. This study shows that ethyl acetate extract of Ginger rhizome can be used as a potential source of antimicrobial agents.

Key Words: ginger, Magway Township, ethanolic, *Zingiber officinale*.

1. INTRODUCTION:

The rhizome of ginger has been widely distributed in the tropical and semi-tropic regions of the world. It belongs to the family Zingiberaceae. Ginger is a rain forest monocot about a meter high, with long, narrow leaves and spicate flowers. It has been grown in China since Antiquity. Seeds have never been found, ginger propagates through buddings from its knotty rhizome. The fresh ginger rhizome is a versatile ingredient of the far eastern cuisine, and is now commonly used in most of the world. Its flavour is lemony-balsamic and its taste is medium hot.

The pungent principles are the most medicinally potent because they inhibit prostaglandin and leukotriene formations. They also give ginger its pungent aroma. Aromatic, pungent rhizome is used as a spice, flavouring, food and medicine. Ginger is now recognized for helping to treat stomach upset and prevent symptoms of motion sickness. It has been studied for its antibacterial, antifungal, pain-relieving, anti-ulcer, antitumor, and used to treat nausea related to both motion sickness and morning sickness. Ginger's anti-inflammatory properties help relieve pain and reduce inflammation associated with arthritis, rheumatism and muscle spasms. Ginger's therapeutic properties effectively stimulate circulation of the blood, removing toxins from the body, cleansing the bowels and kidneys, and nourishing the skin. Other uses for rhizome of ginger include the treatment of asthma, bronchitis and other respiratory problems by loosening and expelling phlegm from the lungs. Rhizome of ginger may also be used to help break fevers by warming the body and increasing perspiration.

Ginger rhizome can be employed in the form of fresh paste, ginger tea (flavoring), dried powder and preserved slices (EL-Ghorabet al.,2010) Ginger can be available in different commercial products like cookies, candy, teas, tinctures, sodas, jam, beer, capsule and syrup (Mexwell,2008).

Zingiber officinale rhizome oil is commonly known as ginger oil, it is an essential oil derived from rhizome of ginger, it stimulates detoxification and micro-circulation, purifies blood. This study is thus aimed to investigate scientifically the presence of active phytochemical constituents and antimicrobial activities of the rhizome of ginger.

2. MATERIALS & METHODS

In this research work, commercial grade reagents from BDH, MERK were used except ethanol, benzene and ethyl acetate, which were distilled for two times before they were applied. In analytical procedure of experimental runs, recommended standard methods and techniques were applied.



Figure 1: Ginger

Sample Collection and Preparation

The Ginger (*Zingiber Officinale*) was collected from Magway Township, Magway Region. The collected sample was cut into small pieces. Since certain compounds get denatured in sunlight, it was dried under shade to avoid decomposition. The dried sample was then pulverized well and about 20g of powdered sample was soaked in 100ml ethanol. It was left for 24 hours to dissolve alkaloid, terpene and other constituents, if they were present. The ethanolic extract was filtered using filter paper. It could be dried by using sodium sulphate in order to remove the traces of moisture.

Preliminary Phytochemical Screening

The preliminary phytochemical tests (Harbonne; J.B. 1993) Were done on the plant extract to determine the presence of various phytochemical components.

Test for Alkaloid

The sample about (2 g) was boiled with 1% hydrochloric acid (10 ml) for 10mins, allowed to cool and filtered. The filtrate was divided into two portions in two test tubes. One portion was tested with Dragendorff's reagent and other with Wagner's reagent.

The formation of deep brown precipitate and reddish brown color solution indicate the presence alkaloid.

Test for Flavonoid

The sample about (2 g) was dissolved in 10 ml of 95% ethanol, boiled for 10mins and then filtered and cooled. Then 0.5 ml of conc: HCL and a few milligram of Mg turnings were in this filtrate. The occurrence of brown precipitate indicates the presence of flavonoid.

Test for Terpene

The sample nearly (2 g) was boiled with 10ml of 95% EtOH for 10 mins and then filtered. In this filtrate, 1ml of acetic anhydride, 1ml of chloroform and 2 ml of conc: H₂SO₄ were added and shaken.

The formation of pale yellow solution was observed, so it indicates the presence of terpene.

Test for Steroid

The sample about (2g) was heated with 10ml of ethanol for 10 mins and the mixture was filtered. Then 2-3 drops of conc: H₂SO₄ and acetic anhydride were added to this filtrate.

The formation of pale green color solution was observed indicating that steroid may be present.

Test for Glycoside

The sample (2g) was boiled with 10ml of distilled water about 10 mins allowed to cool and filtered. The filtrate was treated with 10% lead acetate solution.

The occurrence of white precipitates was observed and so glycoside may be present.

Test for Reducing Sugar

The sample about (2g) was boiled with 10ml of distilled water about 10mins and then filtered. The filtrate was boiled with Benedicts solution.

The formation of green precipitate was observed indicating that sugar may be present in ginger.

Test for Phenolic compound

The sample (2g) was heated with 10ml of distilled water. The mixture was filtered and filtrate was obtained. Then, 5-10 drops of 10% FeCl₃ solution was added to the filtrate where brown precipitate was observed.

Therefore, phenolic compound is present in ginger.

Test for Tannin

The sample nearly (2g) was boiled with 10ml of distilled water for 10mins and filtered. The filtrate was treated with 0.5ml of 2% NaCl and then the clear solution from filtrate was added with 0.5ml of 1% FeCl₃. The presence of brown precipitate showed the presence of tannin.

Antimicrobial Assay

The rhizome of ginger was extracted in four solvent systems (n-Hexane, CHCl₃, EtOAc, EtOH). The antimicrobial activities of crude extracts of this rhizome were determined by using agar well diffusion method at DCPT (Development Centre for Pharmaceutical Technology), Insein, Yangon.

Preparation of Antimicrobial Activity Test

The study of antimicrobial activities was performed by agar well diffusion method. Nutrient agar was prepared according to the method described by Cruick Shank.R.1986.

Nutrient agar was boiled and 20-25 ml of the medium was poured into the test tube and plugged with cotton wool and sterilized at 121°C for 15minutes in Autoclave. After autoclaving, the tubes were cooled down to 30-35°C and poured into sterilized petridishes and 0.1-0.2 ml of test organisms were also added into the dishes.

They were allowed to set the agar for 2-3 hours. After the agar was set 10mm petal agar wells were made by the help of the sterilized agar well culture. Then about 0.2ml of sample was introduced into the agar well and incubated at 37°C for 24-28 hours. The inhibition zone appeared around the agar well, indicating the presence of antimicrobial activity.

The extent of antimicrobial activities of crude extracts was measured from the diameter of zone inhibition.

3. RESUSTS AND DISCUSSION:

Phytochemical Screening of crude Sample

The preliminary phytochemical screening of rhizome of *Zingiber officinale* extract showed the presence of alkaloid, flavonoid, terpene, steroid, glycoside, reducing sugar, phenolic compound and tannin, respectively. The results are tabulated in table (1).

Table.1. Preliminary Photochemical Screening of Crude Extracts of Rhizome of *Zingiber officinale* (Gyin)

No	Tests	Solvent Extract	Test Reagents	Observation	Sample
1	Alkaloid	1% HCl	Dragendroff's Reagent	Deep brown	+
			Wagner's Reagent	Reddish brown	+
2	Flavonoid	95% EtOH	Mg turning, conc: HCl	Brown	+
3	Terpene	95% EtOH	Acetic anhydride, CHCl ₃ , conc: H ₂ SO ₄	Pale yellow	+
4	Steroid	95% EtOH	Acetic anhydride, cons: H ₂ SO ₄	Pale green	+
5	Glycoside	Distilled Water	10% lead acetate	White ppt	+
6	Reducing Sugar	Distilled Water	Benedict's solution	Green ppt	+
7	Phenolic	Distilled Water	10% FeCl ₃	Brown ppt	+
8	Tannin	Distilled Water	2% NaCl, 1% FeCl ₃	Brown ppt	+

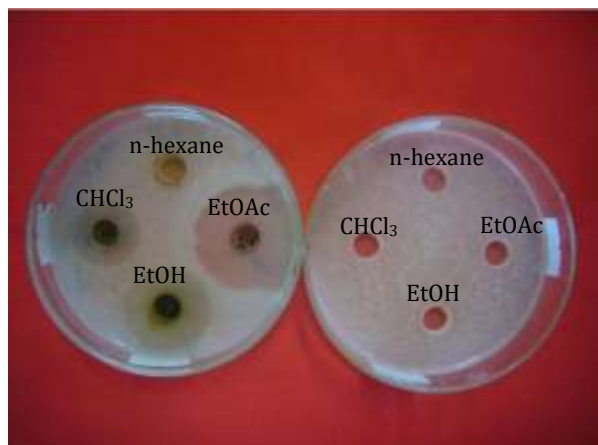
(+)= persence

(-)= absence

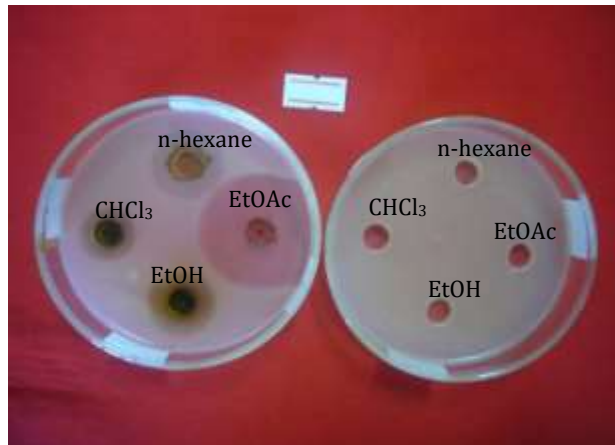
The presence of alkaloid and flavonoid in ginger may provide the medicinal benefits. The flavonoid provides health benefits against cancer and diseases and is used in several industries. Moreover, flavonoids have antioxidant activity, some of the activity attributed to flavonoids include anti-allergic, anti-cancer and anti-inflammatory. The presence of phenolic compound provide antioxidant properties and steroids has cardiotoxic activity.

Antimicrobial Activities of Various Solvent Extracts of Rhizome of *Zingiber officinale* (Gyin)

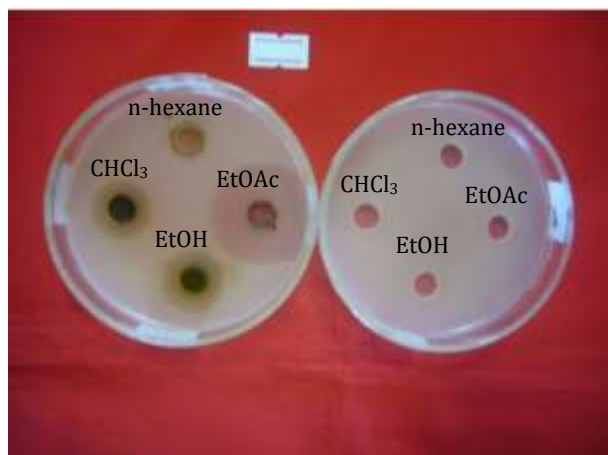
The potential sensitivities of the four solvent (n- Hexane, CHCl₃, EtOAc, EtOH) extracts were observed the six microorganisms and the zone of inhibitions were recorded in figure (2).



(a) *Bacillus subtilis*



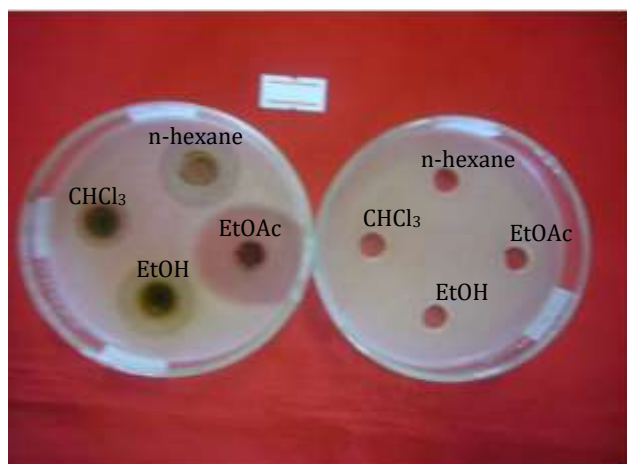
(b) *Staphylococcus aureus*



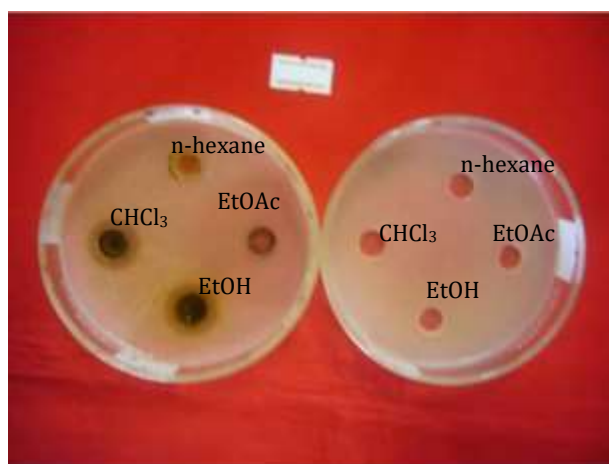
(c) *Pseudomonas aeruginosa*



(d) *Bacillus pumalis*



(e) *Candida albicans*



(f) *E. coli*

Figure 2: Zone of inhibitions on Nutrient agar

Table.2. Antimicrobial Activities of Various Solvent Extract of Rhizome of Zingiber officinale(Gyin)

Sample	Solvents	Organisms					
		1	2	3	4	5	6
Zingiber Officinale(Gyin)	n- hexane	-	(++)	-	(++)	(++)	-
	CHCl ₃	(+)	(+)	(+)	(+)	(+)	(+)
	EtOAc	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)
	EtOH	(++)	(++)	(+)	(++)	(++)	(+)
Agar well-10mm 10mm-14mm(+) 15mm-19mm(++) 20mm above(+++)	*Organisms* (1) <i>Bacillus subtilis</i> (2) <i>Staphylococcus aureus</i> (3) <i>Pseudomonas aeruginosa</i> (4) <i>Bacillus pumalis</i> (5) <i>Candida albicans</i> (6) <i>E-coil</i>						

The results show that ethyl acetate extract of rhizome of Zingiber officinale has high activities and chloroform extract shows low activity on all tested microorganisms. Staphylococcus aureus, bacillus pumalis and Candida albicans respond medium activity on n-hexane extract, Ethanol extract shows moderate activity on Bacillus subtilis, staphylococcus aureus, Bacillus pumalis, and Candida albicans. Moreover, it also gives low activity on Pseudomonas aeruginosa and E-coli.

4. CONCLUSION:

Zingiber officinale belongs to the family zingiberaceae. In this research ginger rhizome was selected for chemical analysis. The phytochemical and antimicrobial activity were investigated, scientifically.

The active phytochemical components such as alkaloid, flavonoid, steroid, glycoside, reducing sugar, phenolic compound and tannin could be observed. The presence of alkaloid, flavonoid, steroid, glycoside, reducing sugar, phenolic compound may provide pharmacological properties of ginger. Due to this properties, ginger can be widely used in medicine.

Furthermore, the four solvent (n-Hexane, CHCl₃, EtOAc, EtOH) extracts of rhizome of Zingier officinale were tested against six selected microorganisms. Among them, ethyl acetate extract of Zingiber officinale responds high activity and the chloroform extract shows low activity on all tested microorganisms. In addition, n-hexane extract gives moderately active on Staphylococcus aureus, Bacillus pumalis, Candida albicans but it was not effective on Bacillus subtilis, Pseudomonas aeruginosa and E-coli. Among the different microorganisms tested, moderate activity was found in Bacillus subtilis Staphylococcus aureus, Bacillus pumalis and Candida albicans on ethanol extract of ginger. The results of antimicrobial activities test also indicate that rhizome of ginger has anti- allergies, anti- inflammatory, antifungal and other medicinal properties.

Further investigation should be carried out its bioactive constituents in order to develop effective antimicrobial agents of ginger.

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