Study on the Antimicrobial Activity, Nutritional Compositions and Extracted Essential Oil from the Fruits and Seeds of *Illicium verum* Hook. f.

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Abstract

In this research work, *Illicium verum* Hook. f. English name (Star Anise) was selected for chemical analysis. The phytochemical screening of the Star Anise was tested by using standard methods. The mineral contents in the powder of sample were determined by EDXRF method. The antimicrobial activities of the crude extract in three solvent systems were tested by agar well diffusion method on six selected organisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonus aeruginosa*, *Bacillus pumilus*, *Candida albican* and *Escherichia coli*. The ethanol extract of the sample showed high activities on all types of organisms. The n-hexane and ethyl acetate extract of sample observed medium activities on all types of organisms. The protein content was determined by using Kjeldahl's method. The fat content of selected sample was studied by using Soxhlet extraction method. The reducing sugar content was also studied by iodometric titration. The nutritional compositions of the sample were found to be (4.725%) of protein, (7.610%) of fat and (1.350%) of reducing sugar. The essential oils in this sample were also extracted by steam distillation and hydro distillation. Essential oil (hydro distillation) in this sample was analyzed by Gas Chromatography-Mass Spectrometry. The essential oils in this sample are D-Limonene, Linalool, 4-Terpinenol, α -Terpinenol, Chavicol, Anisaldehyde, Anethole, *p*-Acetonylanisole and Chavicol acetate.

Key words; Star Anise, Kjeldahl, Soxhlet, Iodometric, Essential oils

Introduction

Star anise (*Illicium verum* Hook.f.) is a spice that closely resembles anise in flavour obtained from the star-shaped pericarp. It is native to southern China and northern Vietnam and is grown almost exclusively in Southern China, Indochina and Japan. The ripe, strongly anise-smelling fruits open up in a star. They are used as a spice and for the production of star anise oil by steam distillation. Star anise oil is a colorless to pale yellow liquid which solidifies on cooling. (Beissels, 2002) Its fruit is an important traditional Chinese medicine as well as a commonly used spice. Spices are the alternate sources of antioxidant, which used in time immemorial. It is widely used as herbal medicine and cosmetics. The fruits are commonly used as a spice and pharmaceutical treatment for flatulence and spasmodic pain (Lobo, 2010). Polysaccharides of plant sources have drawn the attention of biochemical and nutritional researchers in recent years due to their various biological activities. Polysaccharides offer health benefits such as anti-cancer effects, immuno modulation, anti-bacterial and anti-cardiovascular disease effects. Except for its usage in food, star anise is still a natural nutrient and traditional Chinese medicine which may be especially useful for combating colic and rheumatism. It also has carminative, stomachic, stimulant and diuretic properties. (Bendahous, 2007).

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Botanical Description

Family - Schisandraceae
Scientific name - *Illicium verum* Hook.f.
English name - Star anise
Myanmar name - Nar nat pwint
Part Used - Fruits and Seeds
Medicinal uses - Stimulates Pancreas, Antiflu , Heart Health and Stomachic
[Loi and Thu (1970) (Wong,1999)

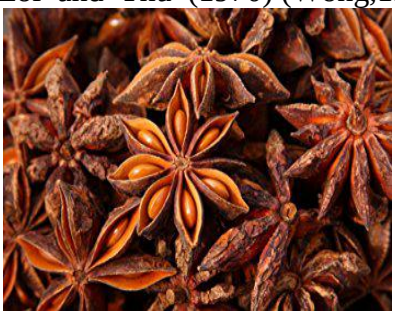


Figure (1) Fruits and Seeds of Star Anise

Material and Methods

Sampling

The fruits and seeds of star anise were collected from Zay Cho Market, Chanayetharsan Township, Mandalay Region, Myanmar. The sample was chopped into small pieces and dry in the well ventilated shade. These pieces of sample were ground to powder. It was stored in a well-stoppered bottle and used throughout the experiment.

Preliminary Phytochemical Tests of the fruits and seeds of star anise

The phytochemical tests were carried out to detect the presence or absence of organic constituents in the fruits and seeds of star anise. (Harborne J B, 1973)

Mineral Contents of the fruits and seeds of star anise

The mineral contents of the fruits and seeds of star anise were examined by the Energy Dispersive X-ray Fluorescence (EDXRF) spectrophotometer at Department of Physics, University of Mandalay. (SPECTRO XEPOS EDXRF Spectrometer, Germany)

Antimicrobial Activities of Crude Extracts of the fruits and seeds of star anise

The antimicrobial activities of crude extract sample of the fruits and seeds of star anise were examined by using Agar well diffusion method at Central Research and Development Centre (CRDC), Insein, Yangon. (Magaldi , 2004) (Valgas, 2007)

Determination of nitrogen and protein content by Kjeldahl's method

The protein content of the fruits and seeds of star anise was determined by using Kjeldahl's method. (AOAC, 2000)

Determination of fat content by Soxhlet Extraction method

The fat content of the fruits and seeds of star anise was determined by using the Soxhlet extraction method. (AOAC, 2000)

Determination of Reducing Sugar Contents of the sample

The reducing sugar content of the fruits and seeds of star anise was determined by iodimetric titration using sodium hydroxide.

Extraction of Essential Oil by Steam Distillation and Hydro Distillation Methods

The essential oils in the fruits and seeds of star anises sample were also extracted by steam distillation and hydro distillation.

Determination of Chemical Compositions by GC-MS

The extracted essential oils (Hydro distillation) in the fruits and seeds of star anise were measured by GCMS at the Department of Chemistry, University Research Center (URC) ,Mandalay. (GC-MS- QP2010, SHIMADZU),(David O Sparkman; 2011), (Canbay, H.S, 2011)

.Results And Discussion

Preliminary Phytochemical Screening of the star anise

The fruits and seeds of star anise were tested by phytochemical screening and these results are shown in Table (1).

Table (1)Phytochemical Tests of the sample

No.	Constituents	Reagents used	Observation	Result
1.	Alkaloid	Wagner's reagent	Reddish brown ppt	+
2.	Flavonoid	Conc: HCl, Mg pieces, Δ	Pink color solution	+
3.	Glycoside	10% Lead acetate	White ppt	+
4.	Phenolic	10% $FeCl_3$	Brown color solution	+
5.	Polyphenol	1% $FeCl_3$ + 1% $K_3[Fe(CN)_6]$	Greenish blue color solution	+
6.	Reducing sugar	Benedict's solution, Δ	Brick-red ppt	+
7.	Saponin	$NaHCO_3$	Frothing	+
8.	Steroid	Acetic anhydride, $CHCl_3$, Conc: H_2SO_4	Nogreen color solution	–
9.	Tannin	1% $FeCl_3$, H_2SO_4	Brown ppt	+
10.	Terpene	Acetic anhydride, conc: H_2SO_4, $CHCl_3$	Pink color color	+

(+) Presence (—) Absence (ppt) Precipitate

According to this table, the fruits and seeds of star anise consist of alkaloid, flavonoid, glycoside, and phenolic, polyphenol, reducing sugar, saponin, tannin and terpene.

Determination of the Mineral Contents of the star anise

The mineral contents of the fruits and seeds of star anise were shown in Table (2).

Table (2) The Mineral Contents of the sample

No.	Elements	Symbols	Relative Abundance (%)
1.	Potassium	K	61.541
2.	Calcium	Ca	17.775
3.	Sulphur	S	7.993
4.	Manganese	Mn	3.434
5.	Iron	Fe	3.110
6.	Phosphorus	P	3.067
7.	Silicon	Si	1.544
8.	Zinc	Zn	0.565
9.	Copper	Cu	0.331
10.	Rubidium	Rb	0.328
11.	Nickel	Ni	0.314

According to this table, potassium is the highest amount in the sample. Potassium is a very important mineral for the proper function of all cells, tissue and organs in the human body.

Determination of Antimicrobial Activities of the star anise

The results of antimicrobial activities of the fruits and seeds of star anise were shown in Table (3).

Table (3) Antimicrobial Activity of the Crude Extract of sample

Samples	**Solvents**	**Inhibition zone**					
		I	II	III	IV	V	VI
Star anise	*n-hexane*	18 mm (++)	15 mm (++)	16 mm (++)	15 mm (++)	15 mm (++)	17 mm (++)
	EtOAc	19mm (++)	18 mm (++)	16mm (++)	17mm (++)	18mm (++)	19 mm (++)
	EtOH	21mm (+++)	21 mm (+++)	21mm (+++)	20mm (+++)	20mm (+++)	21 mm (+++)

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

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

According to experimental data, the ethanol extract showed high activities on all types of organisms. The n-hexane and ethyl acetate extract of sample observed medium activities on all types of organisms.

Determination of Nutritional Compositions of the Sample

The nutritional values such as protein, fat and reducing sugar contents were determined according to the appropriate reported methods. The results are shown in Table (4).

Table (4) Results of Nutritional Compositions of the sample

No	**Experiments**	**Percent (%)**
1	Protein content	4.725
2	Fat content	7.610
3	Reducing sugar content	1.350

According to this table, the fruits and seeds of star anise, protein content is 4.725 %, fat content is 7.610 %, and reducing sugar content is 1.350 %.

Analysis of Essential Oil Components (Hydro distillation) in the Fruits and Seeds of Star Anise by GCMS

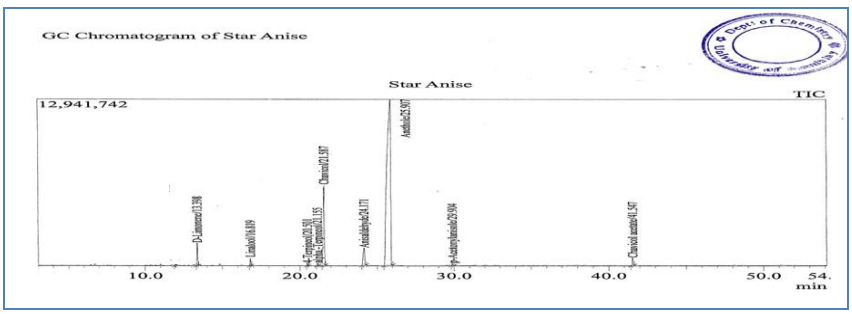
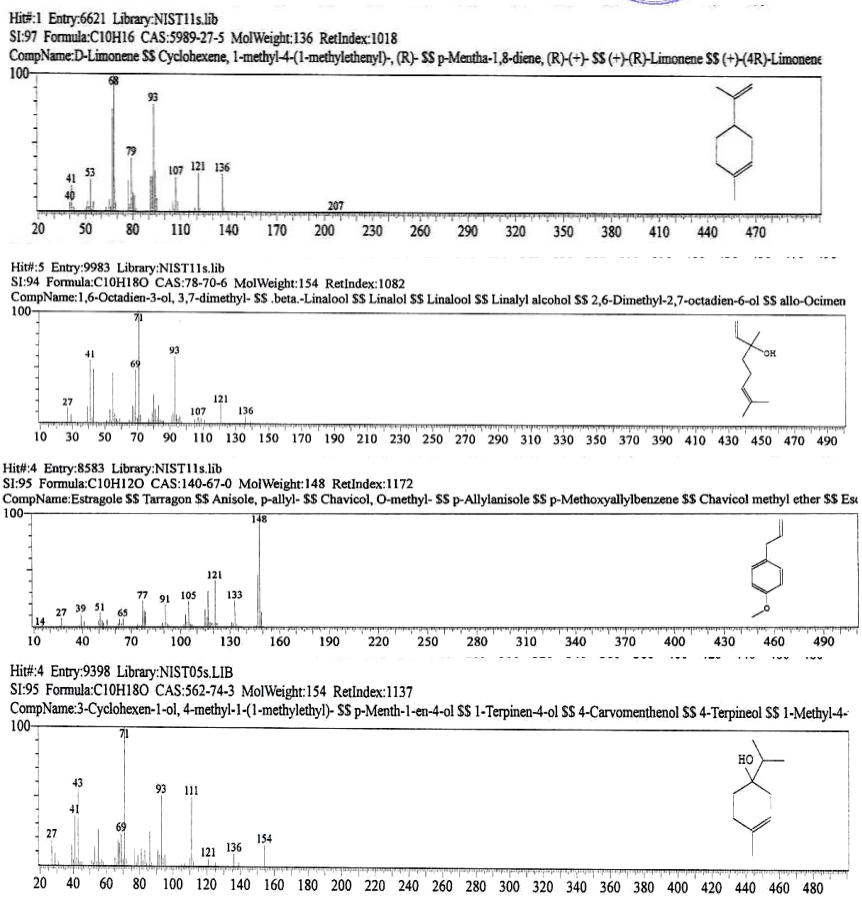


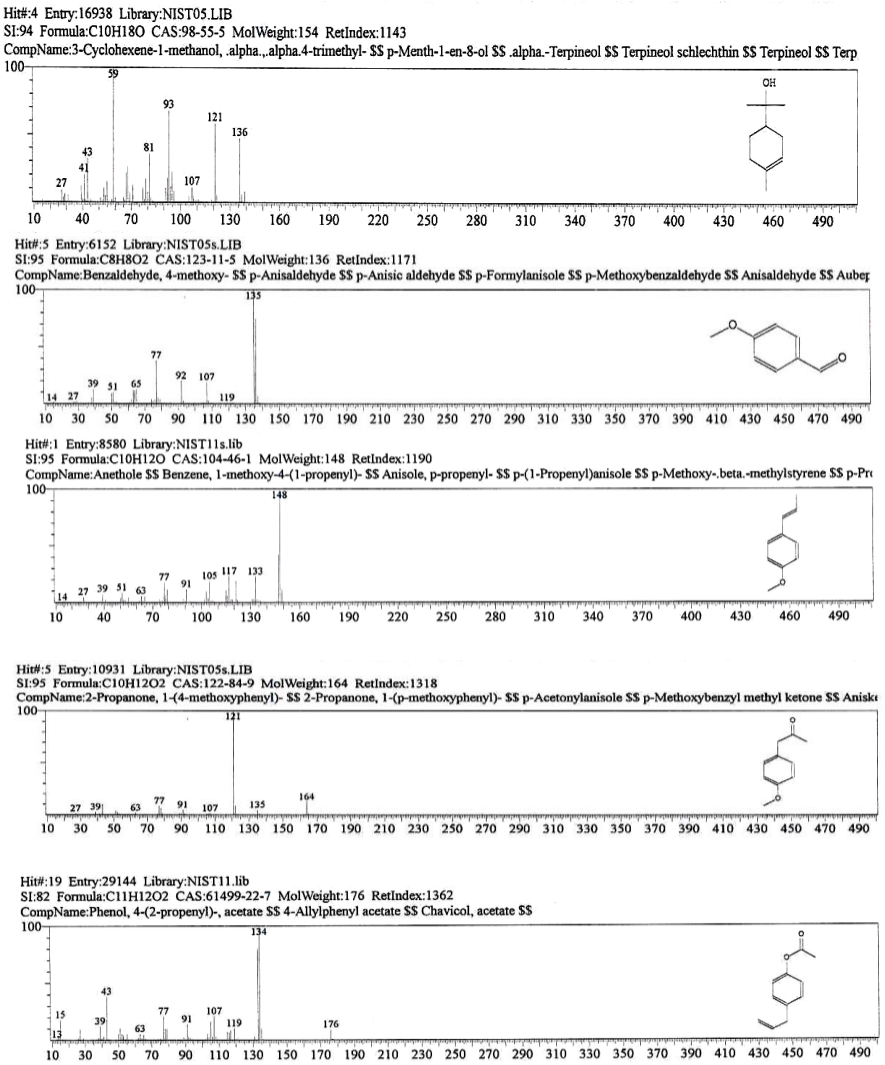
Figure (2) Analysis of Essential Oils in the fruits and seeds of Star Anise by GCMS

Table (5) The results of Essential Oils Components in the Fruits and Seeds of Star Anise

No	Name	Molecular Formula	Retention time (min)	Area (%)
1	D-Limonene	$C_{10}H_{16}$	13.398	2.20
2	Linalool	$C_{10}H_{18}O$	16.819	0.67
3	4-Terpineol	$C_{10}H_{18}O$	20.501	0.25
4	Alpha-Terpineol	$C_{10}H_{18}O$	21.155	0.26
5	Chavicol	$C_9H_{10}O$	21.587	11.61
6	Anisaldehyde	$C_8H_8O_2$	24.171	3.58
7	Anethole	$C_{10}H_{12}O$	25.907	79.21
8	p-Acetonylanisole	$C_9H_{10}O_2$	29.904	0.29
9	Chavicol acetate	$C_{11}H_{12}O_2$	41.547	0.93

Adam et al., 2008





Conclusion

In this study, star anise is used to investigate preliminary phytochemical screening, mineral contents, antimicrobial activity, and some nutritional values and essential oils components. From the result of phytochemicals tests, the sample contains alkaloids, flavonoid, glycoside, and phenolic, polyphenol, reducing sugars, saponin, tannin and terpene. As the results of EDXRF measurement, the amount of potassium (61.541 %) is the highest. Potassium is a very important mineral for the proper function of all cells, tissue and organs in the human body.The antimicrobial activities of various extract of sample were tested by Agarwell diffusion method on six selected organisms. It can be observed that n-hexane and ethyl acetate extract of sample gave medium activities on all organisms. The ethanol extract of sample gave high activities on all organisms. From the determination of nutritional compositions of the sample, the protein content is 4.725 %, the value of fat content is 7.610 %, and reducing sugar is 1.350 mg/g.

According to GC-MS spectrum, the essential oils present in this sample are D-Limonene, Linalool, 4-Terpineol, Alpha Terpineol, Chavicol, Anisaldehyde, Anethole, p-Acetonylanisole and Chavicol acetate. Among them, the percent of Anethole is the height level. Anethole is distinctly flavor and sweet , pleasant to the taste at higher concentration. The essential oils in this sample are monocyclic monoterpene except Linalool. Linalool is acyclic

monoterpene. Many monoterpenes have antimicrobial activities. From the experimental data, the fruits and seeds of star anise contain nutritional values and some mineral contents were responsible for health to humans.

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