Ministry Of Education
Department of Research and Innovation
Chemical Technology Research Centre

Investigation of Some Compounds and Antioxidant Activity of Myanmar Amber

Kay Thi Kyaw
Principal Scientist
Pharmaceutical Research Department

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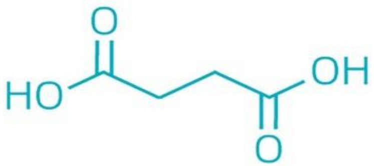
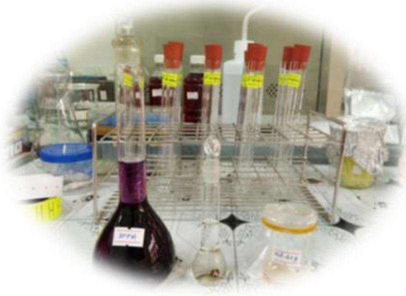
Objectives

- To observe some compounds from Myanmar Amber



Succinic acid

- To evaluate the antioxidant activity of amber



Outline

- Introduction
- Methodology
  - ✓ Preparation of amber ethanol extract
  - ✓ Separation of petroleum ether soluble fraction
  - ✓ Thin layer chromatographic determination of succinic acid
  - ✓ Determination of total phenolic content
  - ✓ Determination of antioxidant activity by DPPH free radical scavenging method
- Result and Conclusion

Introduction

Amber is precious stone resulting of the plant resin fossilization after millions of years. Found in Baltic Sea and is known as Baltic amber or succinate and also found in the Dominican Republic, Spain, Sicily, Lebanon, Myanmar, and Romania. The amber from Myanmar named Burmite. (John. A.,et al., 2004)

Amber is not a mineral, because it has an organic origin and amorphous structure. The main characteristic actives in amber are terpenes and succinic acid. (Georgiana I.T.et al., 2012)

Antioxidant compounds play a vital role as a health- protecting factor. Antioxidant compounds like phenolic acid , polyphenols and flavonoids scavenge the free radicals and thus inhibit the oxidative mechanisms that lead to degenerative disease. Antioxidant reduces risk for chronic diseases such as cancer and heart disease.

Methodology
Preparation of amber ethanol extract

Collect the amber, is floating on the saturated salt solution

↓

Cleaning, Drying and Grinding

↓

Reflux extraction at 80°C for 10 hour with 97% ethanol

↓

Filtered, extract is concentrated by rotary evaporation

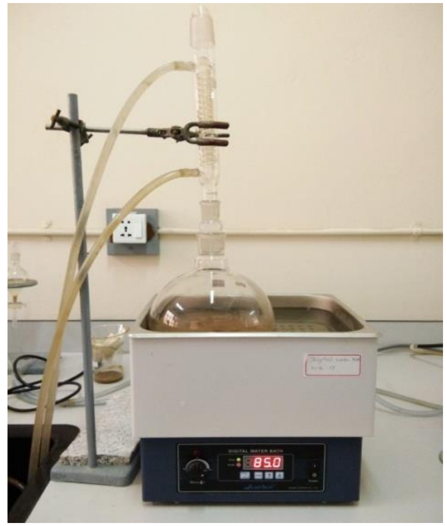
↓

amber extract

Collect amber floating on saturated salt solution

Air dry

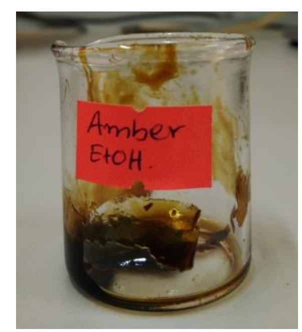
Grind by grinder



Reflux extraction at 80°C water bath

Filtration

Rotary Evaporation- < 50 ºC



Amber extract

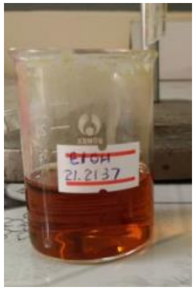
Separation of petroleum ether soluble & insoluble fraction

Ethanol extract (100 mg)

↓ **dissolved in 97%EtOH (9 ml)**

Extracted with pet ether by using separating funnel

Insoluble in pet ether



Soluble in pet ether

Evaporated and Dried under vacuum at 40°C

Thin layer chromatographic determination of succinic acid

TLC Plate	- Silica Gel
Sample	- ethanol extract (insoluble in pet ether) & Ref. std. succinic acid
Mobile phase	- MeOH : NH_4OH (4 : 1 v/v)
Spray Reagent	- bromocresol green

Extraction of polyphenols

Grinded amber + Hot 70% MeOH

↓ Mix

Extraction in the water bath at 70ºC

↓

Centrifuged at 3500 rpm/10 min

↓

Decant the supernatant

↓

Made up to 10 ml with 70% MeOH

↓

Stored at 4ºC/24 hr

ISO:14502-1: 2005(E)

Determination of total phenolic content

Sample extract / Std. Gallic acid solution

↓

10% Folin-Ciocalteu phenol reagent

↓

7.5% Sodium carbonate solution

↓

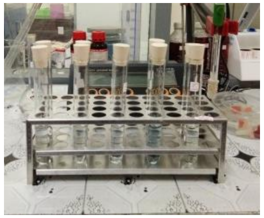
Stand at R/T, 1hr

↓

Spectrophotometric measurement at 765 nm

↓

Calculation



UV 1800, Shimadzu

ISO:14502-1: 2005(E)

$$TPC(\%) = \frac{(Dsampl_e - Dinter_{cept}) \times Vsample \times d \times 100}{S_{std} \times msampl_e \times 10000 \times WDM_{,\ sample}}$$

D_{sample} = the optical density obtained for the sample test solution

$D_{intercept}$ = the optical density at the point the best-fit linear calibration line intercepts the y axis

V_{sample} = the sample extraction volume, in milliliters

d = the dilution factor used prior to the colorimetric determination

S_{std} = the slope obtained from the best-fit linear calibration

m_{sample} = the mass, in grams, of the sample test portion

$W_{DM,sample}$ = the dry matter content.

Determination of antioxidant activity by DPPH free radical scavenging method

Preparation of extract and DPPH solution with 95% methanol

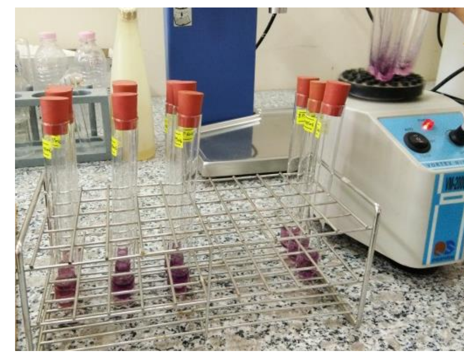
Serial dilution of extract solution (100,80,60,40,20 μg/ml)

Adding of DPPH solution

Complete mixing with vortex mixer

Left in the dark room for 30 minutes

Spectrophotometric measurement at 517nm



Calculation

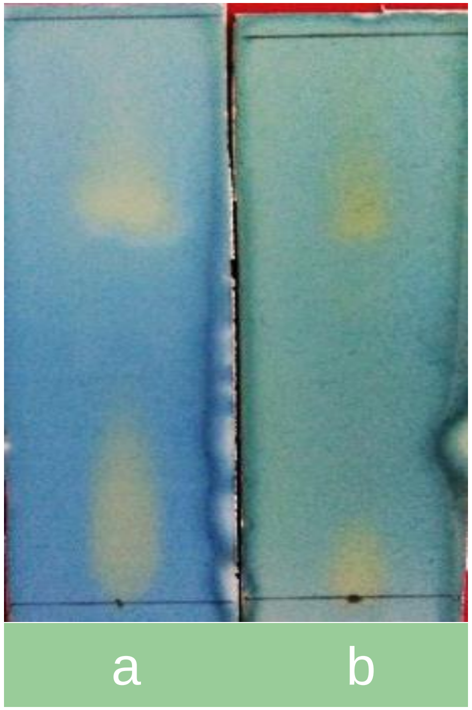
$$DPPH scavenging\ activity(\%) = [\frac{Abs_{control} - Abs_{Sample}}{Abs_{control}}] \times 100$$

Where,

Abs_{sample} = Absorbance of extract and methanol

$Abs_{control}$ = Absorbance of DPPH and methanol

Results
Succinic acid of amber ethanol extract



Mobile : MeOH : NH_4OH (4 : 1)

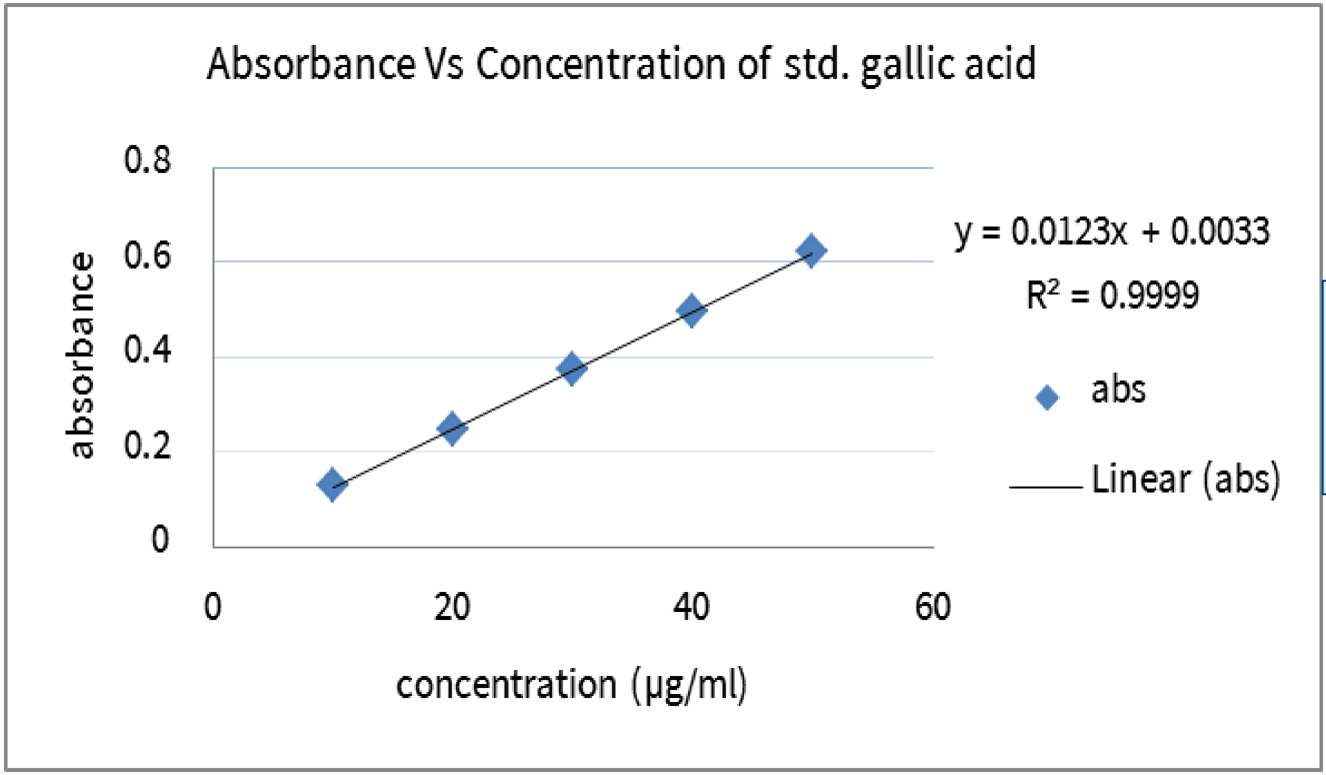
Spray Reagent: bromocresol green

Rf = 0.05, 0.71

a. Std. succinic acid

b. Amber ethanol extract (insoluble in pet ether)

Total phenolic content



Total Phenolic Content of amber = 2.3 %

Fig. Calibration Curve of Standard Gallic acid

DPPH scavenging activity of amber

	DPPH scavenging activity(%)					
	Concentration (µg/ml)	Amber extract	IC_{50}	Concentration (µg/ml)	Ascorbic acid	IC_{50}
1	20	3.5	279.75 µg/ml	2.5	17.56	7.5 µg/ml
2	40	7.7		5	34.31	
3	60	11.56		10	65.46	
4	80	15.41		20	91.92	
5	100	17.34		30	92.27	
6	-	-		40	92.39	

Conclusion and Suggestion

Amber had total phenolic compound

Amber possess succinic acid

Amber shows weak antioxidant activity

These results are potentially support to pharmaceutical products with respect to amber.

Further studies are required to isolate and quantitative measurements of the active constituents responsible for use in traditional medicine and cosmetics.

References

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- Qualitative and quantitative estimation of terpenoid contents in some important plants of Punjab, Pakistan. Pakistan Journal of Science (Vol.69, No. 2 June, 2017)
- Physical Properties of Amber. http://www.emporia.edu/ abersusa/ physic.htm.
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- **Neha, J**., Mishra R.N. 2011, “Antioxidant Activity of Trikatu mega Ext”, International Journal of Research in Pharmaceutical and Biomedical Science, www.ijrpbsonline, Vol. 2(2) pp. 624-628.
- **International Organization for Standardization:** Reference Number: ISO:14502-1: 2005(E): Determination of substances characteristic of green and black tea - Part 1: Content of total polyphenols in tea-Colorimetric method using Folin-Ciocalteu reagent.

Thank You