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The hypoglycemic activity of Thai traditional medicine "Yahom"

Lerluck Steinrut[1*], Arunporn Itarat[2**], Juntipa Purintrapiban[3]

[1] Student of Doctor of Philosophy, Faculty of Medicine, Thammasat University.
[2] Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University.
[3]Department of Biomedicine, Faculty of Medicine, Prince Song Khla University, Thailand.
*Present Author, ** Corresponding author

Abstract

Thai tradition medicines called Yahom, has been used for treatment in many chronic disease patients such as hypertension, heart disease, diabetes mellitus, hyperlipidemia. Ten plants are ingredient of this preparation. This study evaluated the ethanolic extract of Yahom preparation and each plant as ingredients of this preparation for promote the glucose transport. The uptake of radioactive 2-deoxyglucose in L6 myotube was used to test hypoglycemic activity assay. The antioxidant activity by DPPH assay was also tested. The extraction method is maceration in 95% ethanol. The result showed Yahom and *Syzgium aromaticum* exhibited good antioxidant activity, while *Syzgium aromaticum* and *Mesua ferrea* Linn. increase glucose uptake. The study conclude that ingredient in this preparation enhance the glucose transport.

Keywords: antioxidation activity, hypoglycemic activity, glucose uptake, L6 muscle cell, Diabetes mellitus

Introduction

Diabetes mellitus is chronic disease and there are going to be the large public health problem of Thailand and all developed country. Many Patients suffer from this disease. The prevalence and incidence of patients rapidly increase every year. Government budget on treatment and health serving of these patients have to increase follow to the number of patients to maintain quality of life of these population group.

Yahom, one of the most popular Thai tradition medicines, has been used for treatment of fainting, nausea, vomiting, flatulance and and unconcious. Containing of several herb in Yahom preparation make it's have many biological activity include anti-inflammation, antibiotic, antifungal, astringent, would healing, antioxidant, anti-glycemic and anti-hypertensive effect. The several pharmacology action indicated that yahom widely use in many chronic disease patients include hypertension, heart disease, diabetes mellitus, hyperlipidemia especially elderly which over 40 years persons. Thus, the aim of this study was focused to investigate the pharmacology activity of Yahom only on hypoglycemic effect by using cell based assay such as L6 muscle cells. The knowledges from this research could support using yahom of folk doctors to treatment Diabetic patients.

Methods

Plant materials and extraction method

Ten plants in Yahom preparation were collected from natural sources on all part of Thailand. All plants were dried in well ventilation open air and ground to give the powder. Each 100 gm. of the powder was macerated by 95 % ethanol for three days, filtrate and evaporate by evaporator and repeat for 3 times, filtrate was concentrated and dry by evaporator. All extracts were kept in -20 °C before reaction testing. The percentage of yield were showed in table 1

Glucose uptake assay [1-6]

L6 muscle cells is the most preferred cell lines to study insulin-stimulated glucose uptake .In this study , cell culture and glucose transport measurements by using monolayers of L6 muscle cell were grown at 37 ^{o}C . in 48-well culture dishes in dulbecco's modified Eagle's medium (DMEM, low glucose), containing 10% fetal bovine serum (FBS), penicillin (100 units ml.$^{-1}$) and streptomycin (100 μg/ml.$^{-1}$) in incubators equilibrated with 5% CO_2. At 95% confluence (day 0) and there after, differentiation was induced and maintained in the low serum (2% horse serum, HS) containing medium. Cells were used to investigate the effects of plant extracts on 2-DG transport between days 7 and 9 at which ~ 80-90% of myotubes were formed. The cells were stimulated in HEPES-buffered saline, pH 7.4 (HBS) solution with 2% HS, 15mM glucose with different concentrations of test agent for 1 h. Control cells were also performed in the absence of test sample for the same incubation periods. At the end of the incubation, the test media were washed out with HBS followed by incubation in HBS-2-dG solution (1 μCi ml^{-1},0.01 μM) for 10 min in the presence of absence of insulin. The radioactivity associated with the cells was determined by cell lysis in 0.05 N NaOH, and then subjected to liquid scintillation counting.

Results and discussion

The percentage of yield from Yahom extract and its ingradients showed in table 1. The highest percentage of yield is *Syzgium aromaticum* (Kaan Phluu), fallowed by *Mammea siamensis* Kosterm.(Saaraphee) and *Mesua ferrea* Linn.(Bunnaak) (33 %, 32% and 30 % respectively).

Table 1 The percentage of yield of the ethanolic extract Yahom preparation and its plant ingredients.

Scientific name	Thai name	% yield	EC_{50} (μg/ml)
Aquilaria crassna	Kritsanaa	4.3	29.36
Syzgium aromaticum	Kaan Phluu	32.55	5.57
Amomum krervanh Pierre.	Krawaan	2.96	82.79
Myristica fragrans Houtt.	Jaan Thet	10.25	23.65
Albizia myriophylla Benth.	Cha Em Thai	22.38	15.64
Jasminum sambac(Linn.) .Ait.	Mali Laa	13.36	100
Mammea siamensis Kosterm.	Saaraphee	31.99	9.12
Nelumbo nucifera Gaertn.	Bua Lung	10.88	100
Mimussops elengi Linn.	Pikun	19.86	74.68
Mesua ferrea Linn.	Bunnaak	30.27	29.01
Yahom		12.65	11.92
BHT			12.23

Yahom extract showed antioxidant activity evaluate by DPPH radical scavenging assay (EC_{50} = 12 μg/ml). The ingredients which exhibited the three highest antioxidant activity are *Syzgium aromaticum* (Kaan Phluu), *Mammea siamensis* Kosterm.(Saaraphee) *and Albizia myriophylla* Benth.(Cha Em Thai) by 5.57, 9.12 and 15.64 μg/ml respectively.

The results of glucose uptake in L6 myotubes evaluated to be fold increase of basal were found that *Mesua ferrea* Linn. (Bunnaak) extract at concentration 100 μg/ml showed the highest potent glucose uptake in cell as 1.5 , *Syzgium aromaticum* (Kaan Phluu) at 100 μg/ml is 1.3 , *Myristica fragrans* Houtt.(Jaan Thet) at 50 μg/ml is 1.3 and *Mimussops elengi* Linn.(Pikun) at 200 μg/ml. is 1.3.

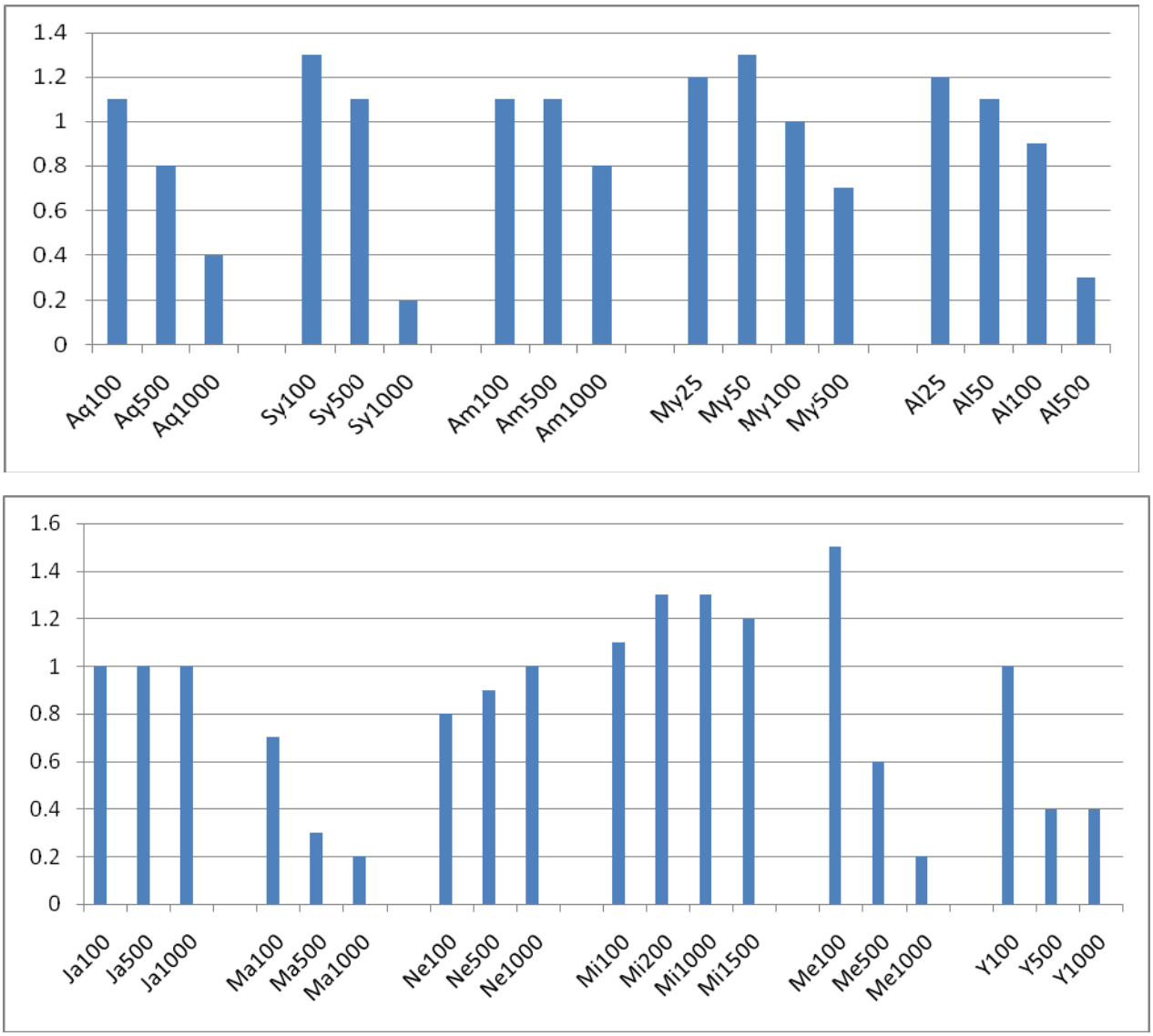

Figure 1, 2 Glucose uptake measure by fold of basal increasing by vary concentration in Yahom and single plants. *(Aq* = Kritsanaa *, Sy* = Kaan Phluu *, Am* = Krawaan *, My* = Jaan Thet , *Al* = Cha Em Thai , *Ja* = Mali Laa *, Ma*=Saaraphee , *Ne* = Bua Lung , *Mi* =Pikun , *Me* = Bunnaak, Y= Yahom)

From these investigation, they showed that *Syzgium aromaticum* (Kaan Phluu) have the hightest antioxidant activity by EC_{50} at 5.57 µg/ml and it also exhibit hypoglycemic effect at concentration 100 µg/ml. as 1.3 fold of basal. This results support theory of antioxidant can reduce glucose uptake or reduce blood sugar. However, the ethanolic extract of *Mesua ferrea* Linn.(Bunnaak) showed the highest hypoglycemic activity at dosage of 100 µg/ml by 1.5 fold of basal but it has low antioxidant activity (EC_{50} = 29 µg/ml.). However, Yahom , showed less active hypoglycemic activity because of this method is not appropriate for yahom which its ingredients have principle component as volatile oil . It should be change in study as adipose cells instead myotube or muscle cells., The result showed that low dose of *Myristica fragrans* extract exhibited more effective than high dose so it is benefit for find plant extract showed high activity by low dose using to treatment disease .

Conclusion

From the study ,*Syzgium aromaticum* (Kaan Phluu) extract enhance glucose transport and highest activity of antioxidant activity, while *Mesua ferrea* Linn.(Bunnaak) showed the highest hypoglycemic activity but low antioxidant activity.

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