



Acute toxicity study and antioxidant activity of herbal tonic "ဇေဝ" EVA

Department of Medical Research

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Introduction

- Antioxidant from natural substances such as plants, fruits vegetables, spices and herbs that are consumed as food or ingredients have been widely investigated for several biochemical and pharmacological properties.

Plants sourced food antioxidants

- vitamin C, vitamin E, β - carotene, polyphenols, phenolic acids, flavonoids
- have been recognized as having the potential to reduced diseases risk and **inhibit the oxidative mechanism.**

- Antioxidants have antiageing property.
- Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress².

- Most of antioxidants compounds in a typical diet are derived from plants sources
- Some traditions recommend daily consumption of honey (ပျဉ်းရည်) mixed with warm water to strengthen the circulatory system and for asthma

Aim and Objectives

- to determine the phytochemical constituents
- to evaluate the free radical scavenging activity (antioxidant activity)
- to find out acute toxicity for herbal formulation, test drug

Materials

- The test sample was supported by "၈၀" traditional medicine clinic

(တိုင်းရင်းဆေးဝါးမှတ်ပုံတင်အမှတ် TMR 010126
(0216))

❖ 11 kinds of ingredients

Sr	Scientific name	Myanmar name	Amount in g/100g
1.	Honey	ဗျားရည်	24
2.	<i>Myristica fragrans</i> Houtt	(Za-deik-pho) ဇာတိပ္ပိုလ်သီး	4
3.	<i>Syzygium aromatum</i> (L.)	(Lay- hnyin) လေးညှင်း	4
4.	<i>Piper betle</i> L.	(Kun) ကွမ်းရွက်	10
5.	<i>Tinospora cordifolia</i> Miers.	(Sindone-ma-nwe) ဆင်တုံးမနွယ်	10
6.	<i>Santalum album</i> Linn.	(Natha-phyu) နံသာဖြူ	11
7.	<i>Glycyrrhiza glabra</i> L.	(New-gyo) နွယ်ချို	10
8.	<i>Pterocarpus santalinus</i> L.	(Natha-ni) နံသာနီ	10
9.	<i>Mesua ferrca</i> Linn.	(Gant kaw) ကံ့ကော်ဝတ်ဆံ	3
10.	<i>Carum copticum</i> Benth& Hook.f. <i>Lepidium sativum</i> Linn. <i>Peucedanum graveolens</i> Benth& Hook <i>Foeniculum vulgare</i> Gaetin. <i>Peucedanum graveolens</i>	စမုန်မျိုးငါးပါး (Samon-byu) စမုန်ဖြူ (Samon-ni) စမုန်နီ (Samon-ni) စမုန်ညို (Samon-saba) စမုန်စပါး (Samon- pwe) စမုန်ဖွဲ	10
11.	salt		4

- Oil of *Myristica fragrans* Houtt
(Za-deik-pho) (ဆာတိပျိုလ်သီး)

is used to conceal

the taste of various drugs and

as a local stimulant to the gastro-intestinal tract⁵.



- Essential Oil of
Syzygium aromaticum (L)
(Lay- hnyin) (av;nSif;) is
used as painkiller for dental
emergencies⁶.

- Betel leaves (Kun) (ကွမ်းရွက်) are used as an **antiseptic** and a breath-freshen



Tinospora cordifolia Miers.
(Sindone-ma-nwe)

used in general
debility, dyspepsia, fever
and urinary diseases in
Ayurvedic literature⁸.

- *Santalum album* Linn (Natha-phyu) (နံသာဖြူ)
wood is used in the treatment of
dysentery⁹.



- *Glycyrrhiza glabra* L. (Nwe-gyo) (နွယ်ချို) has also demonstrated **antiviral, antimicrobial, anti-inflammatory, hepatoprotective, and blood pressure-increasing** effects *in vitro* and *in vivo*¹⁰.



- *Pterocarpus santalinus* L.f. (Fabaceae) (Natha-ni) (နံသာနီ) is commonly called as Red Sander wood is used as, **tonic, as external application for wounds, cuts and inflammations¹¹.**



- InThe flowers of *Mesua ferrca* Linn (Gant Kaw) (ကုံကော်ဝတ်ဆံ) are useful in **bleeding piles**¹².





Honey & salt
ဗျားရည် နှင့် ဆား



Peucedanum graveolens Benth & Hook
စမုန်ညို



Carum copticum Benth & Hook.f.
စမုန်ဖြူ



Lepidium sativum Linn.
စမုန်နီ

အသုံးပြုပုံ (အရှင်နာဂသိန် ပုံပြဆေးအဘိဓာန်/အရှင်နာဂသိန် လက်ဆွဲဆေးဆေးအဘိဓာန်)

- ❖ ဇာတိပွိုလ်သီး
 - ဝမ်းမီးနံ၊ နှလုံးအားနည်း၊ ဝမ်းသက်၊
ကာလဝမ်း
- ❖ လေးညှင်း
 - စမြင်ခံခြင်း၊ အဖျားရောဂါ၊ ကိုယ်ဝန်တည်
စပျို့အန်ခြင်း၊ အဆစ်အဆက်ကိုက်ခြင်း၊
ခေါင်းကိုက်ခြင်း၊ သွားနာသွားကိုက်၊
ပန်းနာရင်ကြပ်၊ နှာရည်ယို
- ❖ ကွမ်းရွက်
 - ချောင်းဆိုး၊ ပန်းနာ၊ မျက်စိပျောက်ဆေး၊
အဆစ်ယောင်၊ ခေါင်းကိုက်

❖ ဆင်တုံးမနွယ်

- လေ၊ သည်းခြေ၊ သလိပ်၊ အစာမကြေ၊
လေပြည့်၊ အဆစ်ယောင်

❖ နံ့သာဖြူ

- သလိပ်၊ သည်းခြေကိုနိုင်သည့် အဆိပ်၊
ရေငတ်၊ သွေးအန်၊ သွေးကျ

❖ နွယ်ချို

- ခံတွင်းအပူဖု၊ ကြို့ထိုးခြင်း၊ ဝက်ရှူးနာ၊
နှလုံးရောဂါ

❖ နံသာနီ

- အော့အန်၊ ရေငတ်၊ အနာပေါက်၊ မျက်စိ
ရောဂါ၊ စိတ်ဖောက်ပြန်ရှူးသွပ်သောရောဂါ

❖ ကံကော်ဝတ်ဆံ

- အလွန်ပြင်းသောအဖျား၊ ဦးခေါင်းသို့သွေး
ဝင်သောအနာ၊ မီးယပ်သွေးသန့်

❖ စမုန်ဖြူ

- ဝမ်းဗိုက်နာ၊ ဝမ်းကိုက်၊ ဝမ်းမီးနှိုးရောဂါ
ပျောက်

❖ စမုန်နီ

- အစာအိမ်ထိုးကျင့်၊ နာကျင်ခြင်း၊ ရောင်ရမ်း
နာ၊ ချောင်းဆိုး၊ သွေးမြင်းသရိုက်

❖ စမုန်ညို

- ရင်ဘတ်ထိုးအောင့်၊ နာကျင်သောရောဂါ၊
ဝမ်းမီးနှိုးခြင်း၊ အစာမကြေခြင်းပျောက်ကင်း

❖ စမုန်စပါး

- အပူဖျား၊ အစာမကြေ၊ ဆီးချုပ်၊ မျက်စိ
ကြည်ဆေး

Chemicals and Reagents

- 1, 1-diphenyl-2-picryl-hydrazyl (DPPH),
- Ascorbic acid,
- Ethanol and Distilled water (DW)

Method

Preparation of extracts

- ❑ One hundred and eighty gram of a mixture of dried coarse powder was extracted with 1000 ml of 50 % ethanol in a boiling water bath for 6 hours at 60°C.
- ❑ Then the extract was evaporated at 50° C until solid residue was obtained¹³.

Experiment on Acute toxicity

- According to OECD guideline 423 (*Organization for Economic Co-operation and Development*)
- 24 female mice ddy strain mice (Dutch Denken Yoken) ,Body weight 25-30gm were randomly selected and divided into 4 groups



Experiment on Acute toxicity

- ❑ kept in their cage *for at least 5 days prior to the experiment to allow for acclimatization* for Laboratory condition.
- ❑ before the experiment, *the animals were kept fasting over night for 18 hours* but were allowed with free access to water and weighed

- *Experimental animals* : ICR mice, 4 groups (n=6)
- *Control group* : only distilled water was given orally
- *Dose* : 300 mg/kg (0.46 ml)body weight
2000mg/kg (3.08 ml)
5000 mg/kg (7.7 ml)



- *after dosing the first 30 minutes, the observation for toxic signs* with 24 hours were done as follows:
- changes in skin and fur, eyes, mucous membranes, respiratory, autonomic, central nervous systems and behavioral pattern up to 14 days

Phytochemical test for types of compounds

- Preliminary phytochemical constituents of the test drug was done by according to the Harborne methods (Harborne, J.B, 1984)¹⁴

*Measurement of DPPH Radical
Scavenging Activity by
Spectrophotometric method at 517nm*

➤ *Preparation of different concentrations*

100 mg/10ml

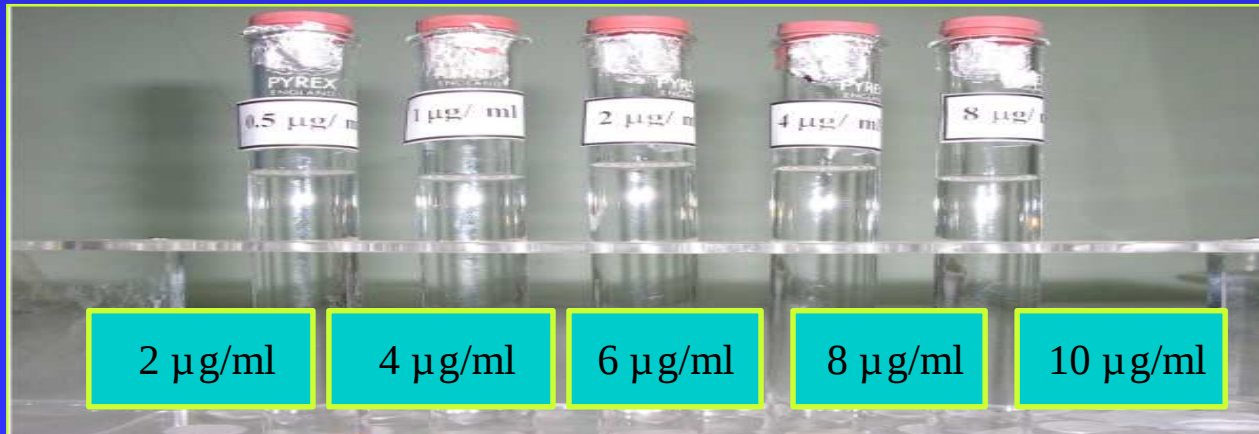


50% ethanol

100 μ g/ml



Serial
dilution



2 μ g/ml

4 μ g/ml

6 μ g/ml

8 μ g/ml

10 μ g/ml

Measurement of radical scavenging activity

Different concentration of tested solution (2 ml)

60 μ M DPPH solution (2 ml)



30 mins



Results and Discussion

Results of phytochemical test

No	Phytochemical test	Results
1	Alkaloids	absent
2	Flavonoids	present
3	Glycosides	present
4	Resins	present
5	Polyphenols	present
6	Carbohydrate	present
7	Amino acid	present
8	Saponins	present
9	Tannins	present
10	Steroids /Triterpene	present
11	Reducing sugar	present
12	Cyanogenic glycoside	absent

Acute toxicity study of test drug on albino mice

- no toxic signs and lethality were observed up to the maximum dose of 5000 mg/kg, for 14 days
- gross finding of the internal organs were observed to be normal

- at the end of 14 days, no significant body weight changes of test group when compared with the control group
- Therefore, *the test drug (herbal tonic "EO") showed free from toxic effect up to the dose level of 5000 mg/kg*

DPPH Radical Scavenging Activity of Eva

- ❑ Percent inhibition of 50% ethanolic extract of the test drug showed **51.4 %** at the concentration of **10 µg/ml**
- ❑ antioxidant activity, with **IC₅₀ values of 12.39 µg/ml**

CONCLUSION

It could be concluded that the herbal tonic possess antioxidant activity in the experimental study. Therefore, this herbal medicine is experimentally proven as a safe and a good source of traditional medicine for use as tonic.

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THANK YOU





Myristica fragrans Houtt.

ဇာတိပျိုလ်သီး



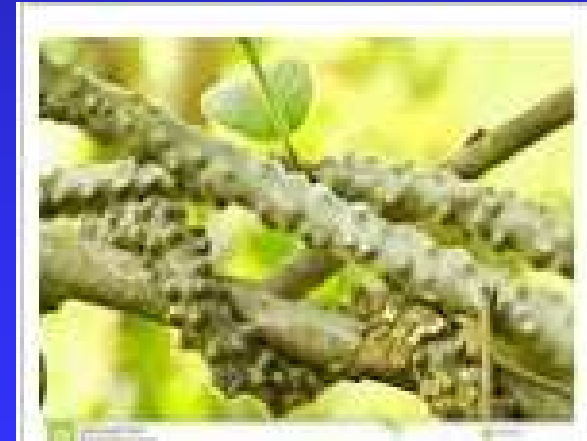
Syzygium aromaticum L.

လေးညှင်း



Betel leaves

ကွမ်းရွက်



Tinospora cordifolia Miers.

ဆင်တုံးမနွယ်

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