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Antimicrobial Activity and Isolation of Some Organic Compounds on the Seed of *Hygrophila phlomoides* Nees (Migyaung-Kun-Bat)

By

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ABSTRACT

The selected medicinal plant, *Hygrophila phlomoides* Nees (migyaung-kun-bat) seed was chosen for the investigation of the physicochemical properties and some pharmacological actions and the isolation of some organic compounds. Preliminary phytochemical investigations on dried powder of *H. phlomoides* Nees seed indicated the presence of α-amino acids, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, steroids, terpenoids and tannins were present in sample. The elemental analysis of *H. phlomoides* Nees sample was carried out by EDXRF method. By EDXRF method, it was found that calcium was the most abundant element and no heavy toxic elements were detected. In the *H. phlomoides* Nees seed, Ca (0.67 %), K (0.48 %), P (0.34%), Si (0.27 %), S (0.26%) and Fe (0.033 %) were found to be present. Nutritional values such as moisture content (10.00%), ash content (5.00%), protein content (30.63%), fiber content (32.30%), fat content (7.10%), carbohydrate content (14.98 %) and energy value (246.30(kcal / 100g)) were also determined on the *H. phlomoides* Nees seed.

In antimicrobial activity of the different crude extracts were screened by using agar well diffusion method. Ethyl acetate extracts show more significant antimicrobial activity (zone of inhibition ranged 40 mm) than that of other crude extracts. The PE extract exhibited the inhibition zone ranging between 18 to 22 mm testing with all species of microorganism. MeOH crude extract from *H. phlomoides* Nees seed was investigated by using rapid screening of antioxidant activity by dot-blot and DPPH staining method. MeOH extracts of *H. phlomoides* Nees seed showed potent activity at dry matter amount (3.125 µg to 400µg dry matter/mL). Two isolated compounds, MKB-1 (terpenoid) and MKB-2 (lupeol) were isolated from ethanol extract of *H. phlomoides* Nees seed. Isolated compounds were identified by TLC, UV and FTIR. Above the scientific finding, *H.phlomoides* seed can be used for antimicrobial and antioxidant agents in traditional medicine.

***Keywords*: *Hygrophila phlomoides* Nees, Antimicrobial activity, Antioxidant activity**

Aim

To investigate the pharmacological activity and to isolate and classify the active constituents present in *Hygrophila phlomoides* (migyaung-kun-bat) seed

Objectives

- To collect and identify the selected sample
- To isolate some chemical constituents from active crude extracts
- To investigate the antimicrobial activities of various crude extracts of sample
- To evaluate the antioxidant activity of the crude extracts by DPPH method
- To classify the isolated compound by physicochemical properties such as TLC, UV and FT IR spectroscopy.

INTRODUCTION

Scientific Classification & Medicinal Uses

Kingdom	-	**Plantae**
Family	-	**Acanthaceae**
Botanical name	-	***Hygrophila phlomoides* Nees**
Genus Name	-	***Hygrophila***
English name	-	**Burma lin-seed**
Myanmar name	-	**migyaung- kun-bat, meegyaung-kun-hpat**
Parts used	-	**Seed**

Medicinal uses - Antioxidant, anti bacteria, skin infections

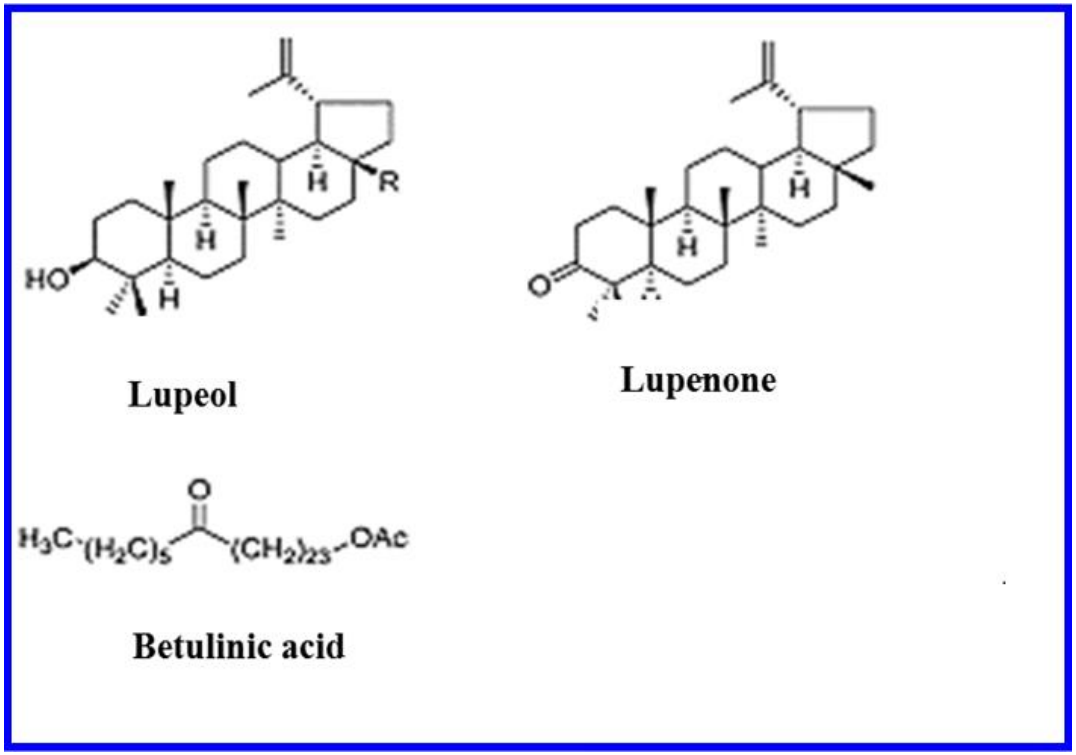


Figure 1. Structure of some chemical constituents from migyaung-kun-bat seed

Terpenoids

- widely distributed in nature, and occurs in nearly all living plants
- .They are generally regarded as derivatives of isoprene (CH_2= $C(CH_3)$–CH = CH_2) or iso-pentane ($(CH_3)_2CHCH_2CH_3$) units where in the isoprene units are arranged in a head to tail fashion
- Terpene hydrocarbons are classified as monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), triterpenes ($C_{30}H_{48}$), tetraterpene ($C_{40}H_{64}$) and polyterpenes $(C_5H_8)_n$.
- Terpenes are widely used in the food, pharmaceutical and perfume sectors, as well as in a wide range of pharmacological applications

Research Plan

migyaung-
kun-bat seed

Crude Extracts
Preparation

Isolation of
Phytoconstituents

Antimicrobial Activity
Antioxidant Activity

Classification

Materials and Methods

Sample Collection

- Collection Place ⏩⏩ Danuphyu Township
- Botanical Identification ⏩⏩ Botany Department, Maubin University

Collected sample → 1. clean & air dried at RT → 2. powdered with machine → **Stored in air-tight container**

Preliminary Phytochemical Test

Dried Powder sample

- Alkaloids

- Glycosides
- α-amino acids
- Carbohydrates
- Starch
- Saponins
- Reducing sugars

- Steroids

- Flavonoids
- Tannins
- Phenolic compounds
- Terpenoids

Preparation of Crude Extracts

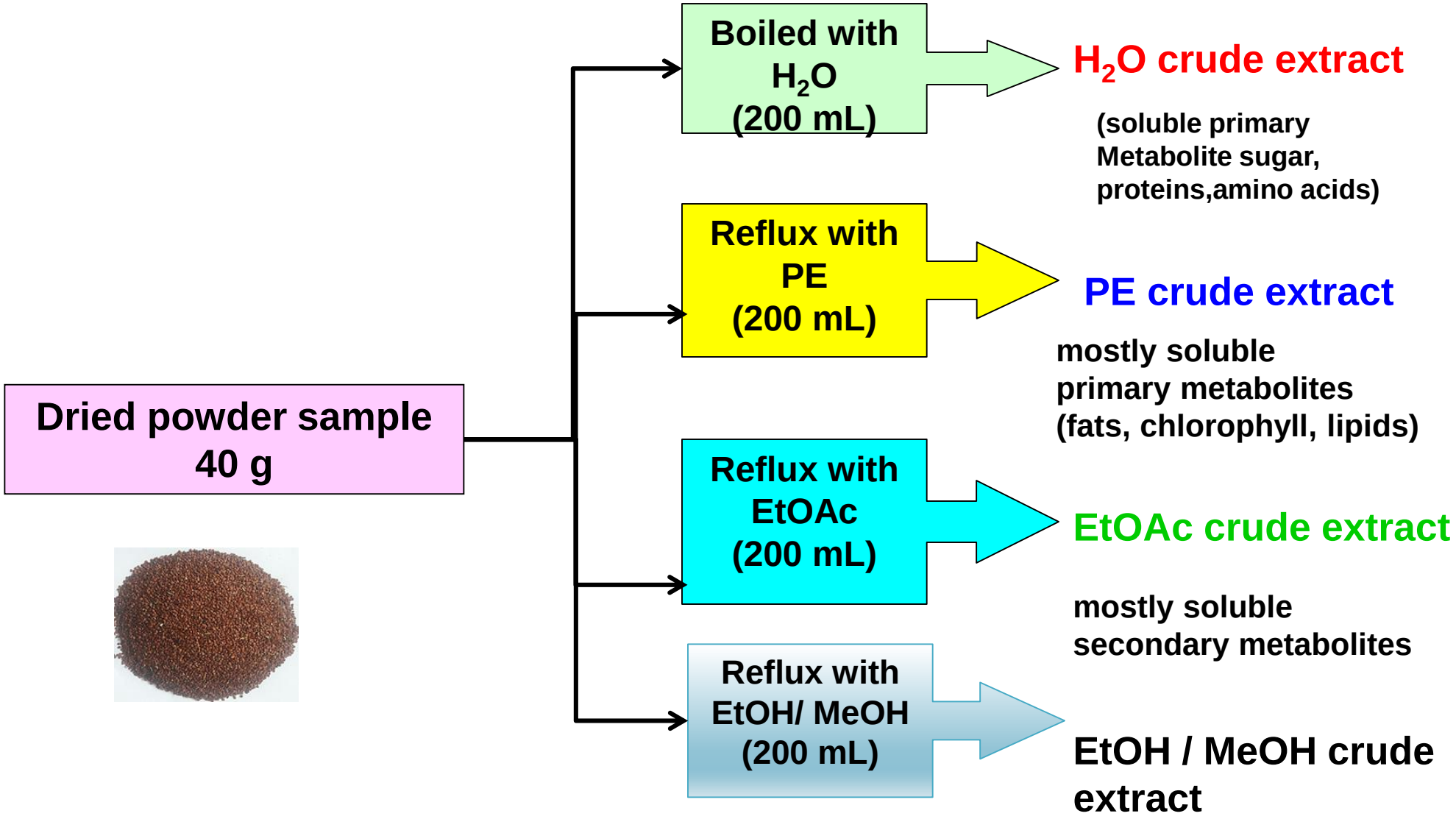


Figure 2. Flow diagram of preparation of crude extracts for bioactivity test

Screening of Antimicrobial Activity

- Test Sample : H_2O, EtOH, MeOH,EtOAc, PE extracts of sample
- Microorganism Tested : 6- pathogenic strains
  - (1) *Bacillus subtilis*
  - (2) *Staphylococcus aureus*
  - (3) *Pseudomonas aeruginosa*
  - (4) *Bacillus pumilus*
  - (5) *Candida albicans*
  - (6) *Escherichia coli*
- Method Used : Agar well diffusion method

(at Pharmaceutical Research Department)

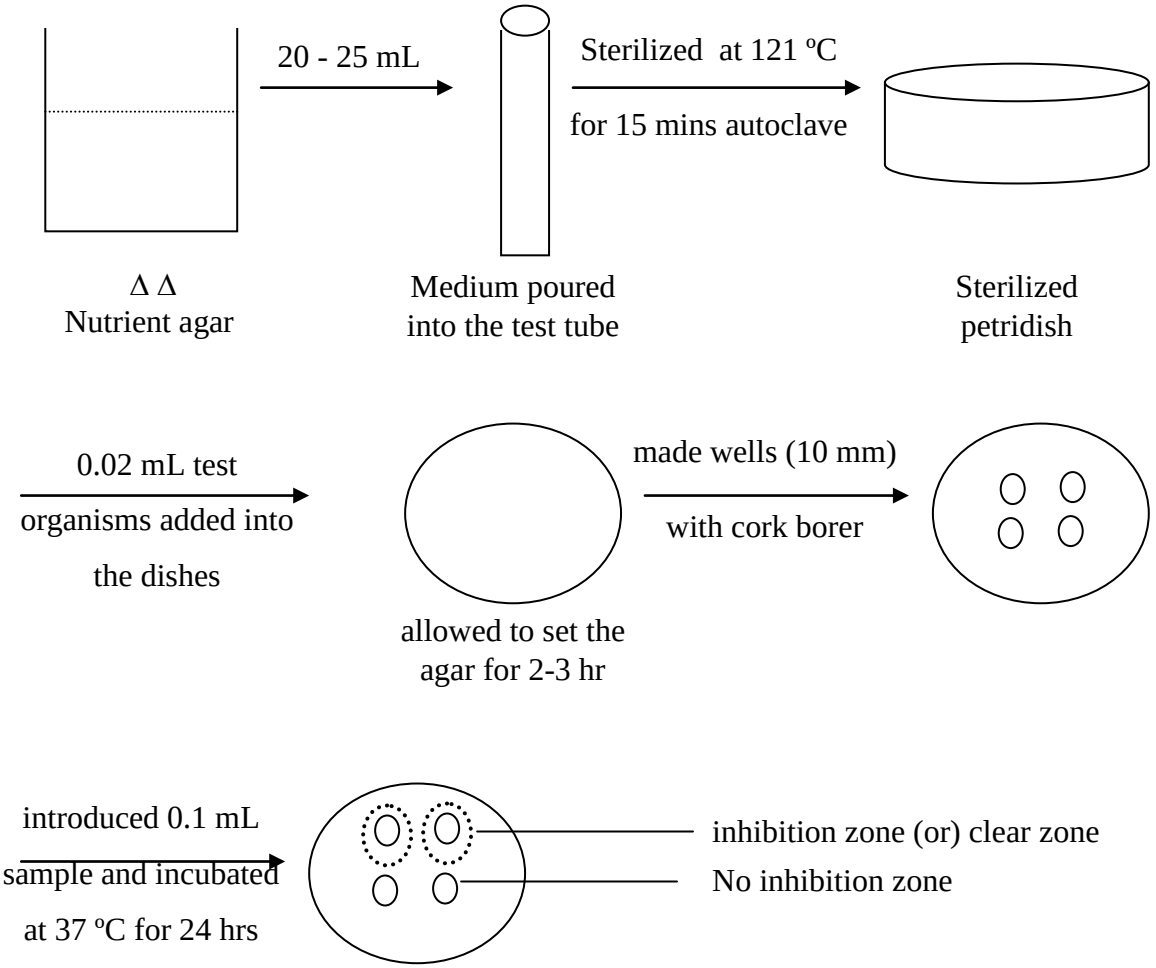


Figure3 Flow diagram for the procedure of agar well diffusion method

Rapid screening of antioxidant by dot-blot and DPPH staining

-Each MeOH extracts of (MKB)was carefully loaded onto a 6cm × 6 cm TLC layer and allowed to dry (3 min)

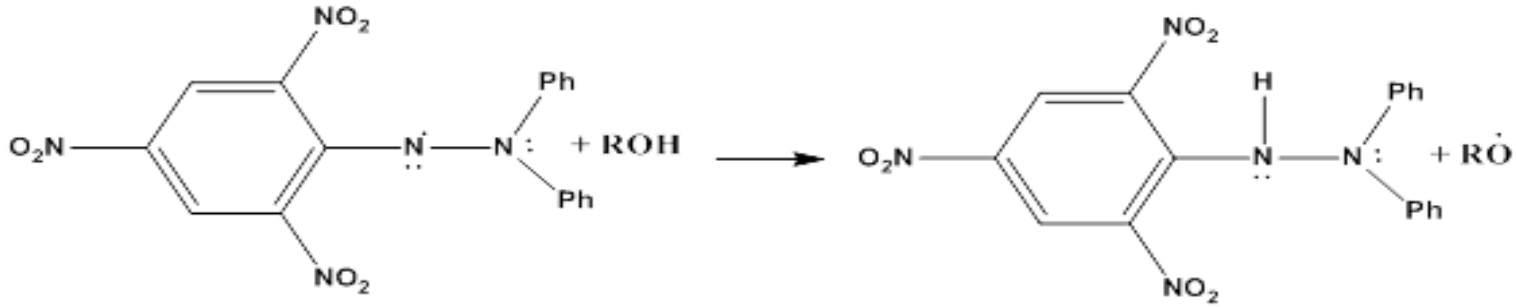
-Drops of each sample were loaded, in order of decreasing concentration (400 to 3.125 µg/mL), along the row.

-The sheet bearing the dry spots was placed upside down for 10 s in a 60µM DPPH solution

(yellow spots with strong intensity appeared quickly)

- DPPH ➡ 1,1- Diphenyl - 2 Picryl-Hydrazyl)

($C_{18}H_{12}N_5O_6$)



DPPH (purple) **DPPH-H (yellow)** **Radical**

Dried powder MKB seed (200 g)

extracted with 95% EtOH, RT Conc.

EtOH extract

successive partitioned with PE

EtOH extract (defatted)

Column chromatography, silica gel, PE:EtOAc gradient elution (9:1 → EtOAc), EtOAc: MeOH (1:1) and MeOH only

Only PE → **F-1**

F-2 ← PE:EtOAc (9:1-7:1)

→ **MKB-1**
Colourless amorphous
R_f = 0.85
(PE:EtOA, 9:1)
0.71 %

PE:EtOAc (7:1-5:1) → **F-3**

→ **MKB--2**
white crystal
R_f = 0.45
(PE:EtOA, 9:1)
0.21 %

PE:EtOAc (5:1 – 1:1) → **F-4**

washed with MeOH →

MKB-3
pale yellow amorphous
R_f = 0.35 (PE:EtOAc, 5:1)
0.17%

Figure 4. Flow diagram for isolation of some constituents from the migyaung-kun-bat seed

Results and Discussion

Table 1. Results of Phytochemical Investigation of *Hygrophila phlomoides* Nees Seed

No	Types of compounds	Extract	Reagent used	Observation	Remark
1	Alkaloids	1% HCl	Mayer's reagent	White ppt	+
			Dragendoff's reagent	Orange ppt	+
2	α - amino acids	H_2O	Ninhydrin reagent	Violet spot	+
3	Carbohydrates	H_2O	10% α-naphthol , conc: H_2SO_4	Red ring	+
4	Flavonoids	EtOH	Mg ribbon, conc: HCl	Pink colour	+
5	Glycosides	H_2O	10% lead acetate solution	White ppt	+
6	Phenolic compounds	EtOH	5%$FeCl_3$ sol:, $K_3Fe(CN)_6$ sol:	Deep blue colouration	+
7	Reducing sugars	H_2SO_4	Benedict's solution	no ppt	+
8	Saponins	H_2O	Distilled water	Frothing	+
9	Starch	H_2O	I_2 solution	No blue colour	-
10	Tannins	EtOH	5% $FeCl_3$ solution	Green colour	+
11	Steroid/ Terpenoide	PE/ $CHCl_3$	Acetic anhydride &conc: H_2SO_4	Green colour/ pink colour	+

(+) = presence (-) = absence (ppt) = precipitate

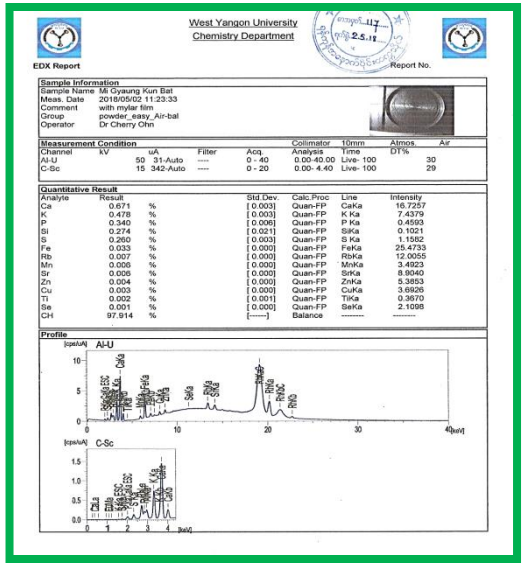


Figure 5 EDXRF spectrum of *H. phlomoides* Nees seed

Among them, calcium peak was the most predominant in *H. phlomoides* Nees seed.

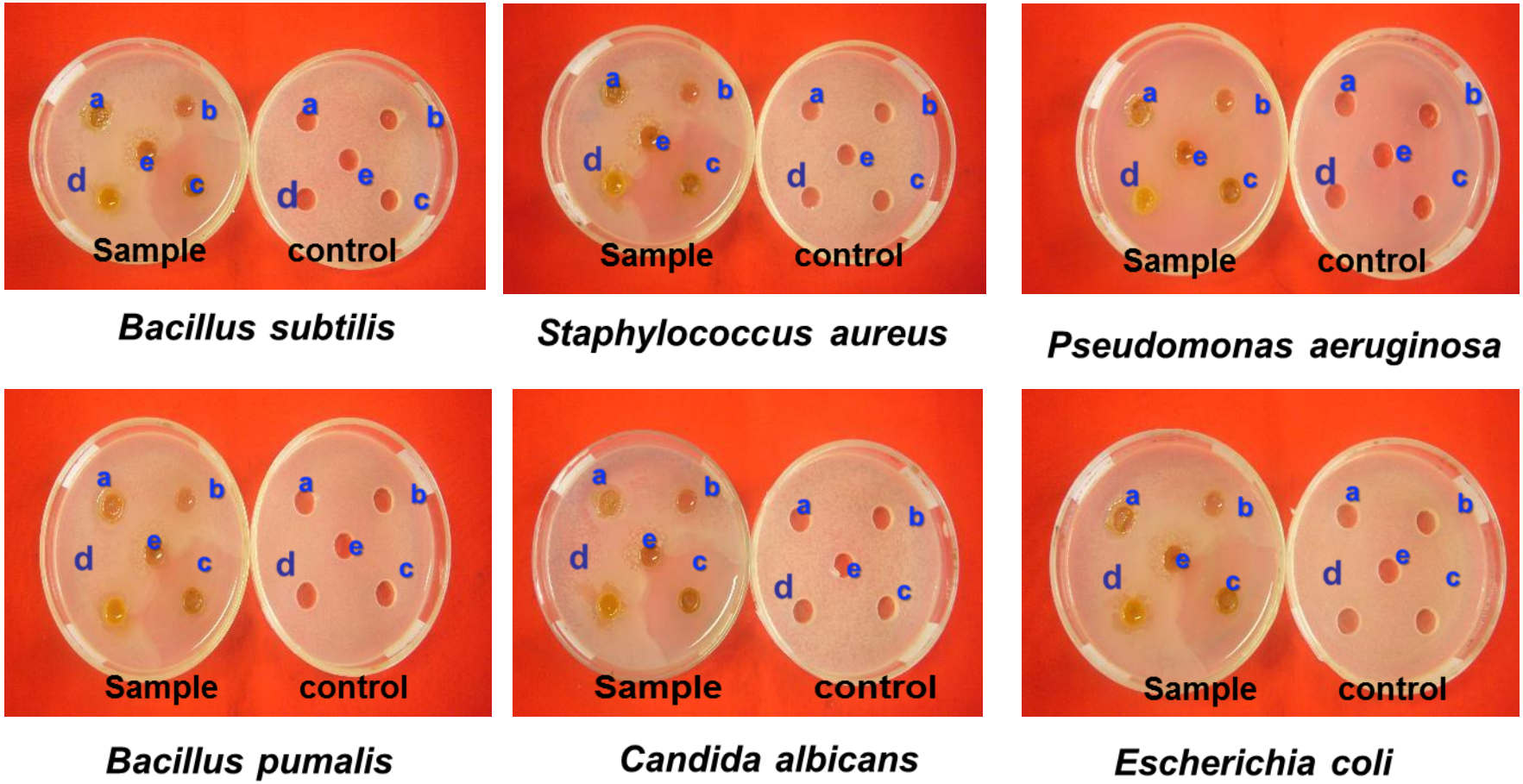
Table 3. Results of Elemental Analysis *H. phlomoides* Nees seed

Element	Relative abundances (%)
Ca	0.671
K	0.478
P	0.304
Si	0.274
S	0.260
Fe	0.033
Rb	0.007
Mn	0.006
Sr	0.006
Zn	0.004
Cu	0.003
Ti	0.002
COH	97.914

Table 1 **Nutritional Values (%) in *Hygrophila phlomoides* Nees seed**

No.	Parameters	Observed value
1	Moisture **(%)**	10.00
2	Ash **(%)**	5.00
3	Crude Fibre **(%)**	32.30
4	CrudeFat **(%)**	7.10
5	Protein **(%)**	30.63
6	Carbohydrate **(%)**	14.98
7	Energy Value (kcal / 100g)	246.30

As a result, it was found that fibres were present as major nutrient in samples.



Clockwise

a - PE extract b - EtOH extract c – EtOAc extract d - MeOH extract e- H_2O extract

Figure 6. Antimicrobial screening of crude extracts of migyaung- kun-bat seed

Table 1. Inhibition Zone Diameters of Crude Extracts from migyaung-kun-bat seed

Organisms used	Diameter of inhibition zone (mm) for migyaung-kun-bat seed				
	PE extract	MeOH extract	EtOH extract	EtOAC extract	H_2O extract
(1) *B. subtilis*	22 (+++)	16 (++)	15 (++)	40 (+++)	–
(2) *S. aureus*	18 (++)	16 (++)	13 (+)	40 (+++)	-
(3) *P. aeruginosa*	20 (+++)	15 (++)	15 (++)	40 (+++)	–
(4) *B.pumilus*	20 (+++)	18 (++)	15 (++)	40 (+++)	-
(5) C.albicans	18 (++)	17 (++)	14 (+)	40 (+++)	-
(6) E. coli	20 (++)	17 (++)	14 (+)	40 (+++)	-

Agar Well – 10 mm

10 mm ~ 14 mm	(+)	- lower activity
15 mm ~ 19 mm	(++)	- higher activity
20 mm ~ above	(+++)	- highest activity

EtOAc extract on the organisms are considerably high (zone of inhibition ranged 40 mm). The PE extract exhibited the inhibition zone ranging between 18 to 22 mm testing with all species of microorganism. The EtOH and MeOH extracts also showed antimicrobial activity against six species of microorganisms. (zone of inhibition ranged from 13 to 18 mm).

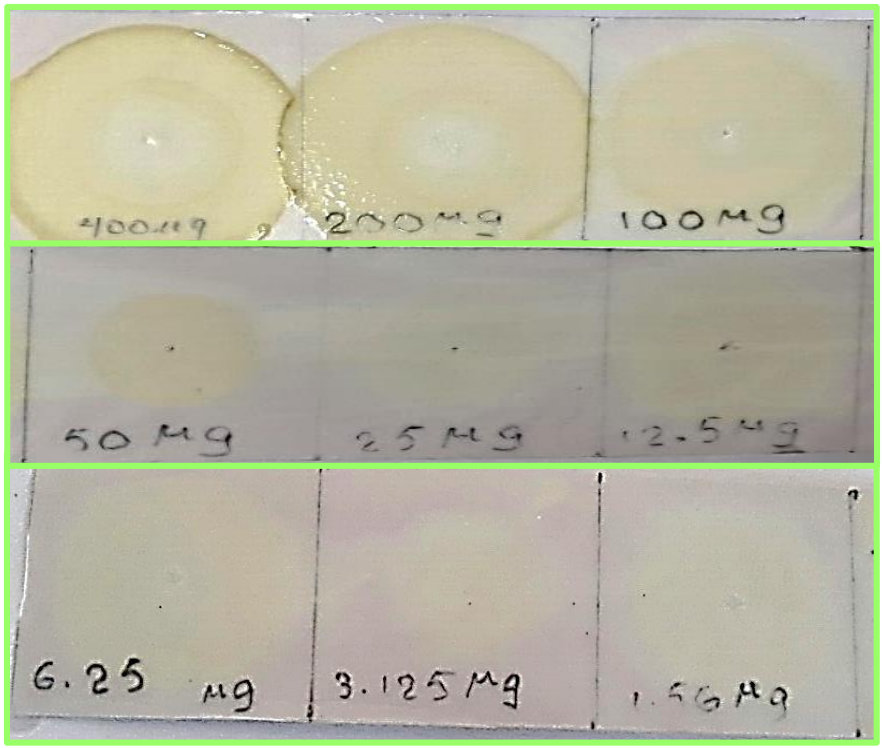


Figure 7. Screening of antioxidant activity of migyaung-kun-bat seed extracts(MeOH) by dot- blot and DPPH staining

Table 2. Some Physical Properties of Isolated Compounds from the migyaung-kun-bat seed

Isolated Compound	Physical State	Yield%	R_f value
MKB-1	Colourless amorphous	0.71%	0.85 PE:EA (9:1)
MKB-2	Colourless crystal	0.21 %	0.45 PE:EA (9:1)

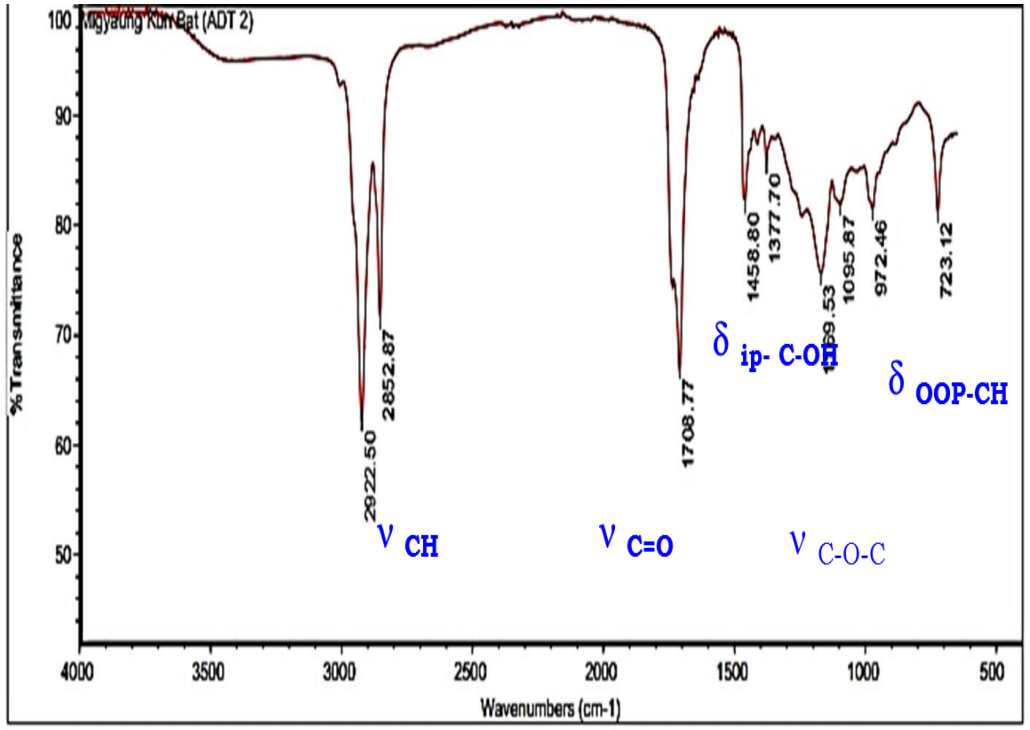
Characterization and Classification of Isolated Compound

Table 3. Results of Colour Test on TLC with Visualizing Agents

Colour on TLC					
Isolated compound	Libermann Burchard	10% H_2SO_4	5% $FeCl_3$	UV	Remark
MKB-1	purple	brown	ND	Inactive	Terpenoid
MKB-2	purple	brown	ND	Inactive	Terpenoid

ND= not detect

MKB-1



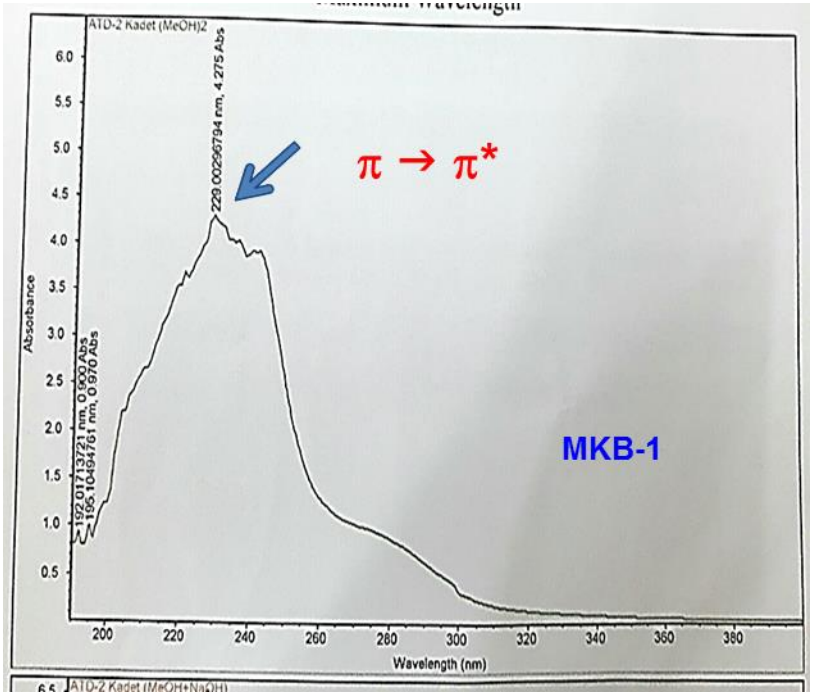


Figure 8. FT IR spectrum of isolated MKB-1 from the migyaung-kun-bat seed

Figure 9. UV spectrum of isolated MKB-1 from migyaung-kun-bat seed

Table 6. FT-IR Spectral Data of Isolated MKB-1 from the migyaung-kun-bat seed

Wave number (cm^{-1})	Band assignments
MKB-1	
2962,2924	C-H asymmetric and symmetric stretching vibration of CH_2 and CH_3 groups
1708	C=O stretching vibration of ketone group
1453,1377	in plane bending of CH_3 groups
1169	asymmetric C-O bond in -C-O-C group
1095	symmetric C-O bond in -C-O-C group
972	C-H out of plane bending

FT-IR

lupeol

MKB-2

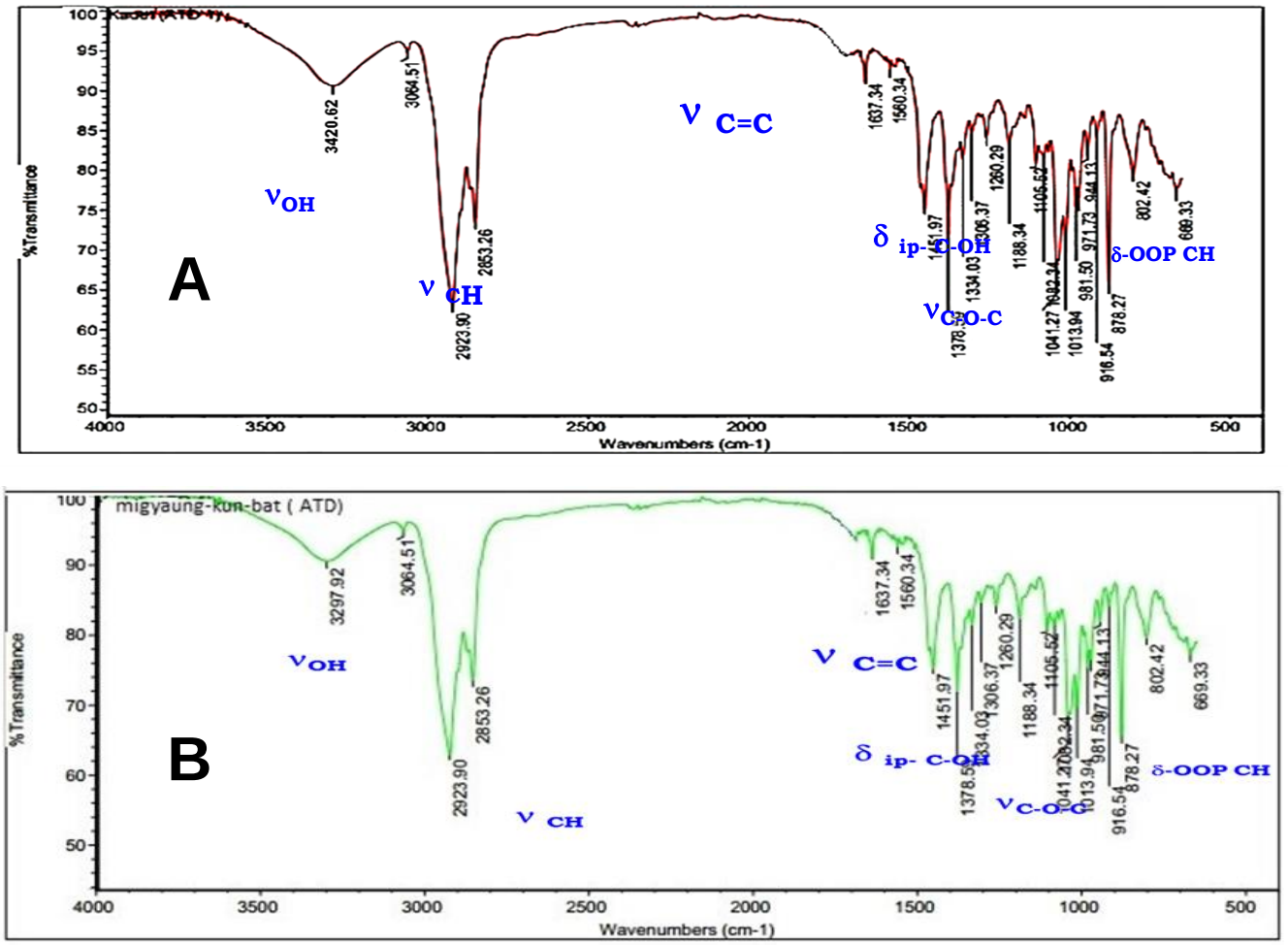


Figure 10. **(A) FT IR spectrum of authentic lupeol**
(B) FT IR spectrum of isolated MKB-2, (Lupeol) from the migyaung-kun-bat seed

Table 4. **FT-IR Spectral Data of Isolated Compound (MKB-2) Compare with Reported Lupeol**

Wave number (cm^{-1})		Band assignments
lupeol	MKB-2	
3463	3420	OH stretching vibration of alcoholic group
3007	3064	$=CH_2$ stretching vibration of vinylidene group
2949	2923,2853	C-H asymmetric and symmetric stretching vibration of CH_2 and CH_3 groups
1539	1637,1580	C=C stretching vibration of alkenic group
1457	1451	in plane bending of CH_2 and CH_3 groups
1380	1378, 1334	in plane bending of gem dimethyl group
1189, 1076	1188, 1042	CHOH stretching vibration of cyclic alcohol
801	802	C-H out of plane bending

LUPEOL

UV

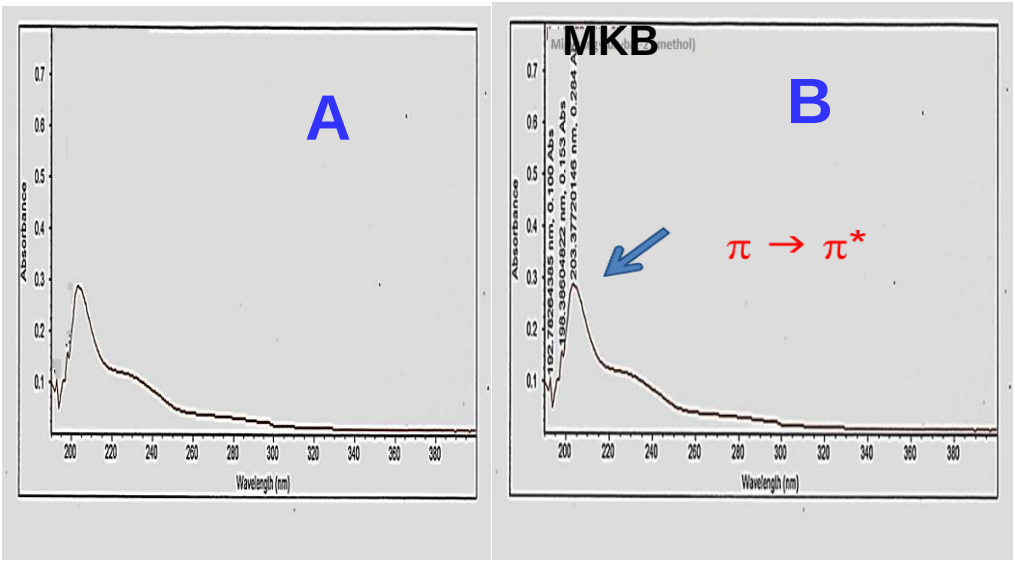
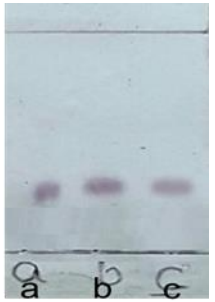


Table 5. UV Spectral Data of MKB-2 (Lupeol)

Observed λ_{max} (nm)	Assignment
203	$\pi \rightarrow \pi^*$

Figure 11. (A) UV spectrum of authentic Lupeol (B) UV spectrum of isolated MKB-2, (Lupeol) from the migyaung-kun-bat seed

Therefore, MKB-2 from the migyaung-kun-bat seed may be classified as lupeol by the arrangement of same R_f value with authentic sample on Co-TLC, its same colour reaction behaviors as lupeol and consistent UV and FT IR spectral data

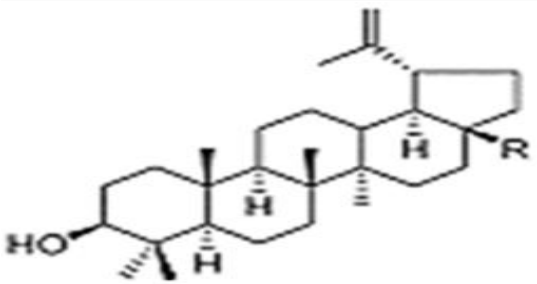


0.45

PE:EA (9:1)

a= standard lupeol
b= isolated compound
c=standard lupeol+ Isolated Comound

The most possible compound is



Lupeol

Conclusion

From this study of chemicals and bioactivity investigation of *H. phlomoides* Nees seed, the following inferences can be concluded. According to phytochemical investigation of selected plant was found to contained α-amino acid, alkaloid, carbohydrate, flavonoids, glycosides, phenolic compounds, reducing sugar, saponins, terpenoids, steroids and tannins. By EDXRF method, it was found that calcium was the most abundant element and no heavy toxic elements were detected. In the *H. phlomoides* Nees seed, Ca (0.67 %), K (0.48 %), P (0.34%), Si (0.27 %), S (0.26%) and Fe (0.033 %) were found to be present.

Nutritional values such as moisture content (10.00%), ash content (5.00%), protein content (30.63%), fiber content (32.30%), fat content (7.10%), carbohydrate content (14.98 %) and energy value (246.30(kcal / 100g)) were also determined on the *H. phlomoides* Nees seed. Antimicrobial activities of PE, MeOH, EtOH, EtOAc and H_2O extracts were screened by agar well diffusion method. All crude extracts of *H. phlomoides* Nees seed except H_2O extract showed the antimicrobial activity against all test organisms (zone of inhibition ranging from 13 mm to 40 mm).

MeOH crude extracts from *H. phlomoides* Nees seed were investigated by using rapid screening of antioxidant activity by dot-blot and DPPH staining method. In this method, MeOH extracts of *H. phlomoides* Nees seed showed potent activity at dry matter amount (3.125 µg to 400µg). The appearances of yellow colored spots have a potential value of antioxidant activity.

Isolated compounds, MKB-1 (terpenoid) and MKB-2 (lupeol) were isolated from ethanol extract of *H.phlomoides* Nees seed. Briefly, overall results of scientific finding, *H.phlomoides* seed can be used for antimicrobial and antioxidant agents in traditional medicine. Phytochemical constituents such as phenolic compounds terpenoid, and flavonoids present in this plant may be responsible for these activities.

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