STUDY ON SOME DERMATOLOGICAL PROPERTIES AND CHEMICAL CONSTITUENTS OF MYANMA NATURAL COSMETIC *Feronia limonia* (L.) SWINGLE (THEE) BARK

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Abstract

This research is concerned with the chemical investigation and some dermatological activity of *Feronia limonia* (L.) Swingle (Thee) bark. *Invivo* antistaphylococcal activities of pet-ether, ethanol and water crude extracts were evaluated by investigating the healing potential on *Staphylococcus aureus* induced excision type cutaneous wounds. All extracts took 6 days to heal wound. No significant difference on wound healing effect between different doses of 2 - 6 mg/day was observed. Control group needed 8 days to heal wound. Skin whitening activity was determined by using a modification of Imokawa Method. In this study, guinea pig was chosen as the experimental animal because its skin histologically and biochemically is similar to the human skin. Pet-ether andethanol extracts, and kojic acid (control) were topically applied on sun rays induced hyperpigmented skin of guinea pigs for three successive weeks. After 3 weeks, pet-ether and ethanol extracts treated portion were found to be whiter than that of untreated hyperpigmented skin. The whitening effect of pet-ether and ethanol extracts were found to be similar, however, lower than that of kojic acid. In chemical investigation, two compounds were isolated from chloroform extract of *Feronia limonia* (Thee) bark. These were: Compound I (Bergapten) (0.0008% yield, mp.188-189ºC) and Compound II (marmesin) (0.0022% yield, mp.160-163ºC).These compounds have been identified by spectroscopic measurement (UV, FTIR,^{1}H NMR and ^{13}C NMR).

Keywords: *Feronia limonia* (L.) Swingle, bergapten, marmesin, skin whitening, antistaphylococcal.

Introduction

Myanmar people frequently use the fragrant liquid powder of the bark of *Feronia limonia* (L.) Swingle (Thee) as a substitute for Tha-nat-khar (a famous natural cosmetic bark). It is recognized to be of great help to bear

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the heat of sun and thus an ideal cosmetic for those who have to work under direct sunshine. Women who work in paddy fields always wear thick layers of Tha-nat-khar to help themselves tolerate the intense heat of the sun. "Thee" paste also has the same properties as Tha-nat-khar making the skin cool and smooth, having a refreshing and cool fragrance, beautifying the users. It also cures pimples and acne. *Feronia limonia* (L.) Swingle (Figure1) commonly known as Wood apple chosen for present investigation is a tropical fruit plant native to Myanmar, India, Malaysia and Sri Lanka.

Botany of *Feronia limonia* (L.) Swingle

Taxonomical classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Sapindales
Family	:	Rutaceae
Genus	:	Limonia

Figure 1: Photograph of *Feronia limonia* (L.) Swingle (Thee) bark in Myanmar.

Material and Methods

Plant Material

Stem bark of *Feronia limonia* (L.) Swingle was collected from Pyay Township, Bago Region. It was cut into small pieces and air-dried at room temperature. Then it was ground into powder and stored in air-tight container to prevent moisture changes and contamination. Authentication of plant was done by the authorized botanist of Department of Botany, Yangon University.

Preparation of Extracts

Four crude extracts of *Feronia limonia* (L.) Swingle bark were prepared by successive extraction of plant material with pet- ether, followed

by chloroform, ethyl acetate finally with ethanol in Soxhlet extractor for 12 h. Water extract was prepared by refluxing the bark with distilled water. These extracts were individually evaporated in rotatory evaporator at 50ºC and subjected to column chromatography and biological activity tests.

Isolation and Purification of Some Chemical Constituents from Chloroform Extract of *Feronia limonia* (L.) SwingleBark

The chloroform extract (5.0 g) was dissolved in minimum volume of pet-ether and thoroughly adsorbed on silica gel (2 g). The adsorbed material after being dried was transferred to a silica gel column. The column was eluted consecutively with pet ether-ethyl acetate solvent system. A quantity of 10 mL was collected for each fraction and checked by TLC using 5% H_2SO_4 solution as spraying reagent. The fractions that showed similar TLC patterns were combined together and concentrated. These fractions were further purified by crystallization and provide the compounds. Fraction F_4 (10 mg) was further chromatographed over a silica gel column (10 g, column 1 cm in diameter) by eluting with danger : acetone (35 : 1) to afford five sub-fractions, F_a to F_e. Fraction F_d was further purified by crystallization with acetone-methanol provided yellow needles (4 mg, 0.0008 % yield; mp.=188-190ºC; R_f=0.56 in benzene: acetone 9:1)which was denoted as compound1. Fraction F_7 provided compound 2 (11 mg; 0.0022% yield; mp (160-163) ºC; R_f = 0.22 in benzene : acetone, 9 : 1) after being crystallized in benzene-acetone.

Screening of *In Vivo* Antistaphylococcal Activity of Different Crude Extracts of *Feronia limonia* (L.) Swingle Bark

PE, EtOH and water extracts of *Feronia limonia* (Thee) bark were used for this activity**.** Twelve rats were randomly divided into four groups; each group contained 3 rats. Surgical wound (2 cm in length and 0.5 cm in depth) were made on dorsal under septic condition. *Staphylococcus aureus* suspension (0.1 ml per wound) was introduced into all rats. Inflammation of infected wounds were observed. Wound inflammation was found to be maximal after 24 h. After 24 h inoculation of *S. aureus*, each extracts (PE, EtOH and H_2O extracts) in three different concentrations (0.2%, 0.4% and 0.6%) were applied topically into inflamed wounds (1 mL/day on a daily basis). For control, 1 mL of ethanol was used.

Evaluation of the Skin Whitening Effect of *Feronia limonia* (L.) Swingle Bark

Skin whitening activity of *Feronia limonia* bark was evaluated via inhibitory effect on sun light-induced hyperpigmentation using guinea pigs (Shigeta *et al.,* 2004).The dorsal skin of the three guinea pigs was washed and about (2 cm x 2 cm) area was cleanly shaved. These guinea pigs were exposed sunlight daily; starting from 11 am to12 am (1h /day) for two consecutive weeks. After two weeks, the hyperpigmentation (tanning) on skin was clearly noticed by naked eye.

Individual guinea pigs (**1**, **2** and **3**) were then treated respectively with PE extract, EtOH extract and kojic acid (control). The hyperpigmented area of each animal was imaginarily divided vertically into two portions (1 cm x 2 cm). The area of left portion on guinea pig **1** was topically applied evenly with the solution of PE extract (0.1mL , i.e., 25 μg extract per cm^2 skin area) and the right portion of it was treated with pet-ether (0.1 mL). Similarly, left portion of guinea pig **2** was treated with EtOH extract (0.1mL , 25 μg/cm^2) and the right side was treated with ethanol (0.1mL). Guinea pig **3** received kojic acid (0.1mL , 10 μg/cm^2) on left portion and ethanol on right portion.

Treatments were continued for three successive weeks one time per day. Finally, the blanching effect of individual samples on hyperpigmented skin was evaluated by viewing with naked eye while comparing with control area.

Results and Discussion

Spectroscopic Identification of Isolated Compounds

Identification of Isolated Compound 1

Study on UV Spectrum

The ultraviolet spectrum of isolated compound 1 in methanol is shown in Figure 2. Isolated compound **1** displayed wavelength of maximum absorption (λ_{max}) at 222, 239, 248, 267 and 310 nm (Table1) characteristic of a coumarin nucleus. A UV maximum near 310 nm suggested a bergaptol or isobergaptol derivatives. The reported λ_{max} for bergapten was 223, 243, 249, 259, 268 and 311 nm (Steck and Bailey, 1969).

Study on FTIR Spectrum

The FTIR spectrum of isolated compound 1 is shown in Figure 3 and the band assignment is reported in Table 2.

FTIR absorption peaks also displayed characteristic of a coumarin nucleus. Peaks in weak intensity at 3110 and 3010 were attributed to the C-H stretching vibration of coumarin. In addition, aliphatic C-H stretching vibration appeared at 2947 cm^{-1}. A strong band at 1720 cm^{-1} that represented C=O stretching vibration revealed the presence of lactone ring in this compound. In addition, C=C stretching vibration appeared at 1604 and 1473 cm^{-1}. The bending vibration of aliphatic C-H groups appeared at 1350 cm^{-1}. Bands at 1141 and 1072 cm^{-1} can be interpreted as C-O stretching vibration. In addition, =C-H out of plane bending vibration of aromatic system was found at 825 cm^{-1}.

Muller *et al.* (2004) reported characteristic absorption peaks of bergapten at $\bar{\nu}$ 3100, 3070, 3050, 1726, 1620, 1602, 1575, 1540 and 885 cm^{-1} and those of xanthotoxin at $\bar{\nu}$ 3110, 3080, 3040, 1705, 1626, 1580, 1540 and 875 cm^{-1}.

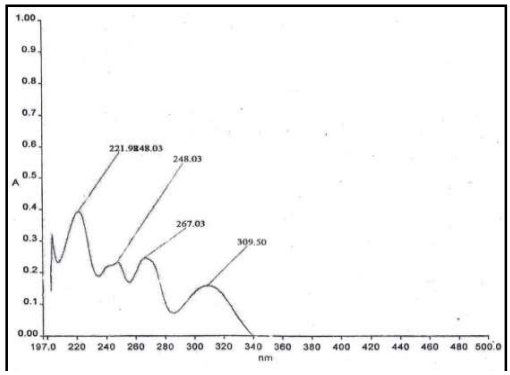


Figure 2: UVspectrum of isolated compound 1 (in MeOH)

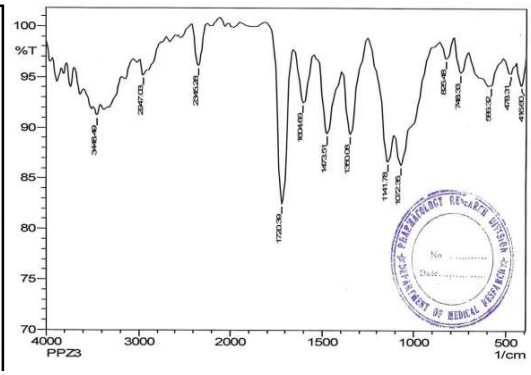


Figure 3: FT IR spectrum of isolated compound 1

Table 1: UV-Vis Spectral Data (λ_{max}) of Isolated Compound 1

Compound	**λ_{max} (nm)** (in MeOH)
Compound	222, 239, 248, 267, 310
Bergapten*	223, 243, 249, 259, 268, 311
Xanthotoxin*	223, 242, 248, 269, 273, 313

* (Muller *et al.*, 2004)

Table 2: FTIR Band Assignment of Isolated Compound 1

Wave number (cm^{-1})	**Band Assignments**
3110, 3010	C-H stretching vibration of coumarin
2947	Aliphatic C-H stretching vibration
1720	C=O stretching vibration of lactone
1604, 1473	C=C stretching vibration
1350	Aliphatic C-H bending vibration
1141, 1072825	C-O stretching vibration
	= CH out of plane bending vibration

Study on ^{1}H NMR Spectrum

The ^{1}H NMR spectrum of isolated compound 1 was measured in $CDCl_3$ and shown in Figure 4 and assignments are presented in Table 3.

The spectrum of compound 1 showed pyrone ring proton signals at δ 8.15 ppm (1H, *d*, *J*=10.0 Hz, H-4) and 6.31 ppm (1H, *d*, *J*=10.0 Hz, H-3), both doublets and coupling with each other with *ortho* coupling constant. H-4 signal at δ > 8.0 indicated substitution occurred at C-5 position. Accordingly, aromatic methoxyl group appeared at δ 4.19 ppm (3H, *s*) would be at C-5. In addition, a benzene ring proton signal at δ 7.26 ppm (1H, *s*, H-8) was observed. The protons on furano ring appeared as two doublets at δ 7.02 ppm (1H, *d*, *J*=2.4 Hz, H-3′) and 7.65 ppm (1H, *d*, *J*=2.4 Hz, H-2′); both protons mutually coupled. Therefore, compound 1 should have the following molecular structure.

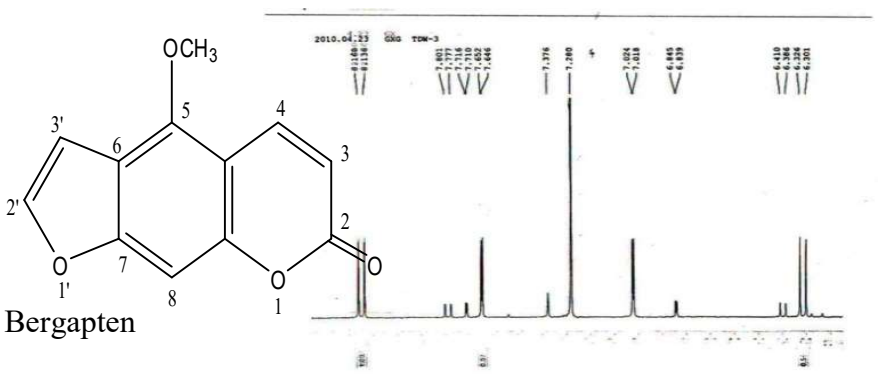


Figure 4: ^{1}H NMR spectrum of isolated compound 1 ($CDCl_3$, 400 MHz)

Table 3: ^{1}H NMR Spectral Data and Peak Assignment of Compound (in $CDCl_3$)

Chemical shift (ppm)	
Observed (Compound 1)	**Literature* (Bergapten)**
8.15 (1H, *d*, *J*=10.0Hz, H-4)	8.16 (*d*, 9.8 Hz)
7.65 (1H, *d*, *J*=2.4Hz, H-2′)	7.60 (*d*, 2.5 Hz)
7.26 (1H, *s*, H-8)	7.16 (*s*)
7.02 (1H, *d*, *J*=2.4Hz, H-3′)	7.01 (*d*, 2.5 Hz)
6.31 (1H, *d*, *J*=10.0Hz, H-3)	6.26 (*d*, 9.8 Hz)
4.19 (3H, *s*, 5-OMe)	4.26 (*s*)

Identification of Isolated Compound 2

Study on UV-Vis spectrum

Figure 5 represents the UV spectrum of compound 2 in methanol. It provided the wavelength of maximum absorption (λ_{max}) at 209, 234, 262, 275, 320 and 360 nm. It could be characterized as a furanocoumarin since absorption at two regions, 290-310 and 240-270 nm appeared (Muller *et al.*, 2004).

Study on FTIR Spectrum

The FTIR spectrum of compound 2 (in KBr) is shown in Figure 6. The band assignments are presented in Table 5. Strong band centered at 3413 cm^{-1} indicated the presence of OH group in this compound. The aromatic C-H stretching vibration was observed at 3100 cm^{-1}. Bands at 2985 and 2880 cm^{-1} were attributed to aliphatic C-H stretching vibrations indicating the presence of methyl groups in this compound. The band at 1680 cm^{-1} was attributed to the C=O stretching vibration of linear furanocoumarin. The bending vibration of aliphatic OH was found at 1407 cm^{-1}. The C-O group was observed as a small band at 1249 cm^{-1}. The out of plane =C-H bending vibration of aromatic system was found at 988 cm^{-1}.

The position of IR bands for marmesin was reported at: 3479 (OH), 2977, 2929, 1703 (α-pyrone ring), 1630, 1572, 1485 (aromatic C=C), 1444, 1404 (CH), 1268, 1132 (C-O), and 819 cm^{-1}.

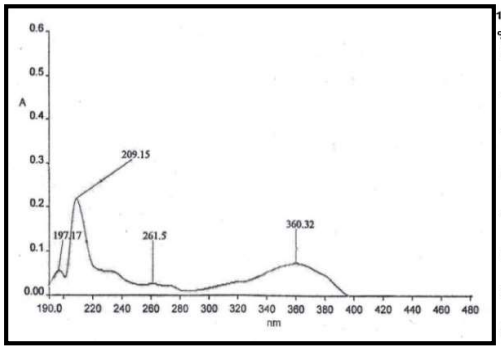


Figure 5: UV spectrum of isolated compound 2

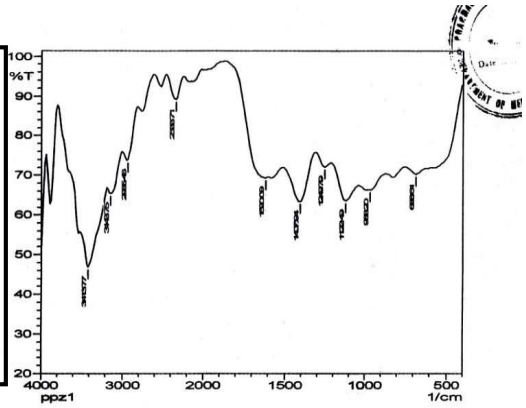


Figure 6: FTIR spectrum of isolated compound 2(inMeOH)

Table 4: UV-Vis Spectral Data (λ_{max}) of Isolated Compound 2

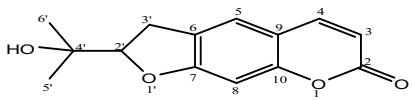
Solvent	Observed λ_{max} (nm)
MeOH	197, 209, 234, 261, 360

Table 5: FT IR Band Assignment of Isolated Compound 2

Wave number (cm^{-1})	**Band Assignments**
3413	O-H stretching vibration
3143	Aromatic C-H stretching vibration
2985, 2880	Aliphatic C-H stretching vibration
1680	C=O stretching vibration
1407	Aliphatic C-H bending vibration
1249, 1122	C-O stretching vibration
988	=CH out of plane bending vibration

Study on ^{1}H NMR Spectrum

The ^{1}H NMR spectrum of isolated compound 2was measured in $CDCl_3$ and shown in Figure7. Band assignment are presented in Table 6. In this spectrum, four aromatic protons were observed. Two doublets appeared at 7.58 (1H, *d*) and 6.19 ppm (1H, *d*), corresponding to H-4 and H-3 protons, were characteristic peaks of α–pyrone ring. Two singlet protons at 7.20 (1H, *s*) and 6.72 ppm (1H, *s*) were assigned to benzene protons that should be situated at *para* position. The former peak corresponded to H-5 and the later peak could be assigned to H-8. Instead, a triplet (1H) and a multiplet (2H) were observed at relatively higher field. Therefore, can be logically thought that furan ring in compound 2 was dihydrogenated. A proton triplet appeared relatively down field at 4.7 ppm was assigned to the proton of C-2′ (i.e. H-2′) that would be adjacent to electronegative oxygen atom. In addition, the splitting pattern revealed H-2′ had two neighbouring protons (two protons on C-3′, i.e. H_2-3′). Accordingly, H_2-3′ protons appeared as multiplet centered at 3.20 ppm (2H). Moreover, no proton should be present at C-4′. Remaining peaks that had not been identified yet were two methyl singlets at 1.21 and 1.38 ppm; and one broad peak at 1.82 ppm. Consequently, these three groups would be at C-4′. Finally compound 2 was assigned as marmesin, linear dihydrofuranocoumarin.



Mermasin(2)

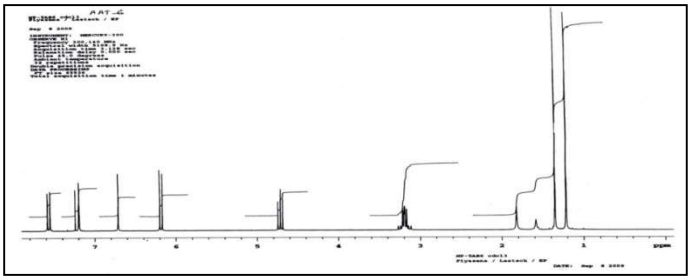


Figure 7: ^{1}H NMR spectrum of isolated compound 2 ($CDCl_3$, 300MHz)

Table 6: ^{1}H NMR Spectral Data and Peak Assignment of Compound (in $CDCl_3$)

Proton No.	Observed data, present work	Marmesin*
H-3	6.19 (1H, *d*)	6.21 (1H, *d*, *J*=9.5 Hz)
H-4	7.58 (1H, *d*)	7.59 (1H, *d*, *J*=9.5 Hz)
H-5	7.20 (1H, *s*)	7.22 (1H, *s*)
H-6	-	-
H-8	6.72 (1H, *s*)	6.74 (1H, *s*)
H-1′	3.20 (2H, *m*)	3.23 (2H, *br d*, *J*= 8.8 Hz)
H-2′	4.72 (1H, *t*)	4.74 (1H, *t*, *J*=8.8 Hz)
H-4′	1.38 (3H, *s*)	1.37 (>Cme_2)
H-5′	1.21 (3H, *s*)	1.23
3′-OH	1.82 (1H, *br*)	1.85 (1H, *br*)

Study on ^{13}C NMR spectrum

The^{13}C NMR spectrum of isolated compound 2 is shown in Figure 8 and peak assignment is reported in Table 7.The ^{13}C NMR spectrum revealed 14 carbon atoms that consistent with the molecular structure explored from ^{1}H NMR spectrum. These carbon peaks were classified as one carbonyl carbon; four quaternary aromatic carbons two of which are oxygenated; one

oxygenated quaternary aliphatic carbon; four aromatic 475 ethane carbons; one oxygenated aliphatic 475 ethane carbon; one methylene carbon; and two methyl carbons.

The down field peak at δ 163.0 ppm was assigned to carbonyl carbon of pyrone ring. Two peaks at 161.9 and 155.5 were corresponded to oxygenated quaternary carbons, C-7 and C-10, respectively. Remaining two aromatic quaternary carbon peaks were observed at 124.9 (C-6) and 112.6 (C-9). In addition, aliphatic quaternary carbon (C-4′) appeared at relatively low field δ 71.6 was due to the presence of –OH group. Four peaks correspond to aromatic CH at 143.5, 123.3, 112.1 and 97.8 ppm were respectively assigned to C-4, C-5, C-9 and C-8. Peak at 91.0 ppm was attributed to C-2′ that attached to electronegative oxygen atom. Peak at 29.5 ppm corresponded to methylene carbon (C-3′). Remaining peaks at 26.1 and 24.3 ppm were assigned to two methyl carbon. The chemical shift of marmesin (Lee *et al.*, 2002) from published data were also presented in Table7. It can be seen from the table that the chemical shift of compound 2 was consistent with that of marmesin {2-(2-hydroxypropan-2-yl)-2H-furo[3,2-g]chromen-7(3H)-one}.

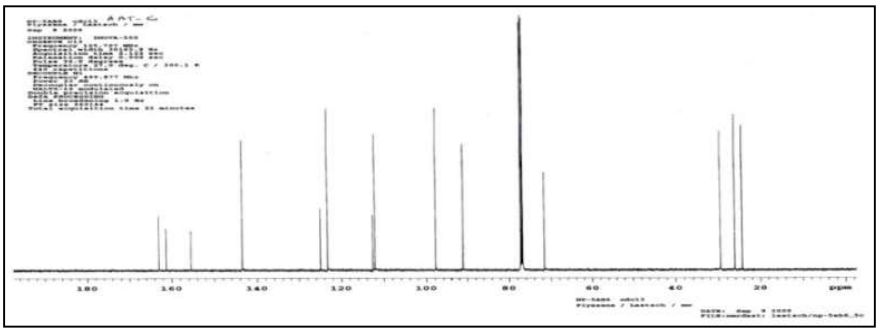


Figure 8: ^{13}CNMR spectrum of isolated compound ($CDCl_3$, 125 MHz)

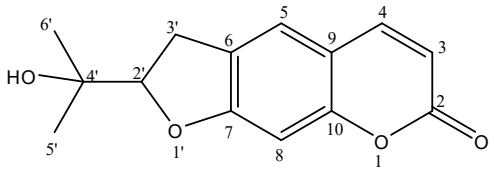
Table 7: ^{13}C NMR Spectral Data and Peak Assignment of Compound (in $CDCl_3$)

Chemical Shift (δ, ppm)	**Marmesin***
Observed value	**($CDCl_3$, 100 MHz)**
163.0	163.1 (C-2)
161.9	161.0 (C-7)
155.5	155.7 (C-10)
143.5	143.6 (C-4)
124.9	125.0 (C-6)
123.3	123.3 (C-5)
112.6	112.8 (C-9)
112.1	112.3 (C-3)
97.8	97.9 (C-8)
91.0	91.0 (C-2′)
71.6	71.6 (C-4′)
29.5	29.4 (C-3′)
26.1	26.1 (C-6′)
24.3	24.2 (C-5′)

Study on GC-MS spectrum

The GC-MS spectrum of compound 2 is shown in Figure 9. The molecular ion peak was observed at m/z 246 which was consistent with the molecular formula, $C_{14}H_{14}O_4$. The observed m/z values of compound **2** are 246 (M^+), 228, 213, 189, 187, 175, 160, 131, 103, 91, 77, 59 and 43. Reported EI MS data for marmesin m/z (%) 246(M^+,39), 213(20), 188(75), 187(100), 175(15), 160(30), 131(19), 59(66), and 43(7).

On the basis of the spectroscopic data, the isolated compound 2 was identified as marmesin ($C_{14}H_{14}O_4$, molecular mass = 246.26) with the following molecular structure.



Marmesin (2)

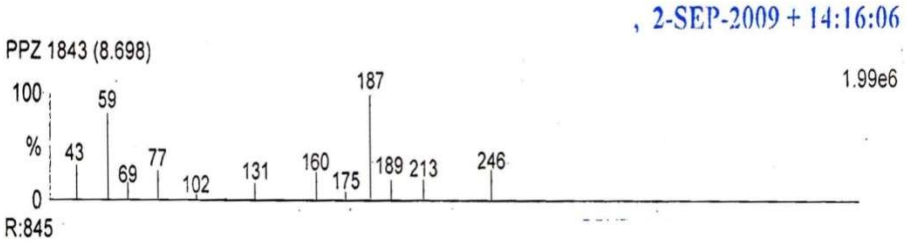
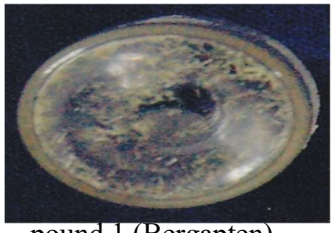


Figure 9: GC-MS spectrum of isolated compound 2

Table 8: Percent Yield and Melting Points of Isolated Compounds from *Feronia limonia* (L.) Swingle Bark

Isolated compounds	% Yield	Appearance	Melting point (°C)
1	0.0008	Yellow needles (Me_2CO-MeOH)	188-190 (sub.)
2	0.0022	White needles (benzene-Me_2CO)	160-163



pound 1 (Bergapten)

Compound 2 (Marmesin)

Figure 10: Photographs showing the Crystal form of isolated compounds

Study on *In Vivo* Antistaphylococal Activity of Crude Extracts of *Feronia limonia* (L.) Swingle Bark

In this study, *in vivo* antistaphylococal activities of three different extracts (PE, EtOH and H_2O extracts) of *F. limonia* bark were evaluated by investigating the healing potential on *Staphylococcus aureus* induced excision type cutaneous wounds.

Groups topically treated with any extracts needed 6 days to heal the wound. No significant difference on wound healing effect was observed between different doses of 2-6 mg/wound/day (topical application of 1 mL solution at 0.2-0.6% concentration of sample per day). Control groups (treated with ethanol only) needed 8 days to heal wound. The treated wounds exhibited remarkably dryness of wound margins with tissue regeneration and reduced wound area in comparison to controls.

The wound healing potency of different extracts are presented in Table 9. Figure11 represents the healing potency of *Feronia limonia* bark on staphylococcus induced excision type cutaneous wounds. Since *Feronia limonia* bark possess wound healing activity, it can be used as a traditional wound healer, and as an ingredient in skin cosmetic lotion.

Table 9: *In Vivo* Antistaphylococal Activity (Wound Healing Property on *S. aureus* Induced Excision-Type Cutaneous Wound) of Different Crude Extracts of *Feronia limonia* (L.) Swingle Bark

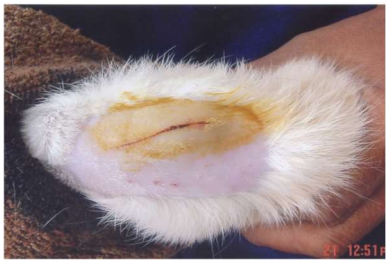
Concentration (%)	Dose (mg/day)	Time needed to heal wound (day)			
		Gp-1	Gp-2	Gp-3	Gp-4
0.2	2	6	6	6	8
0.4	4	6	6	6	8
0.6	6	6	6	6	8

Gp-1 Topically treated with pet-ether extract of *Feronia limonia* bark

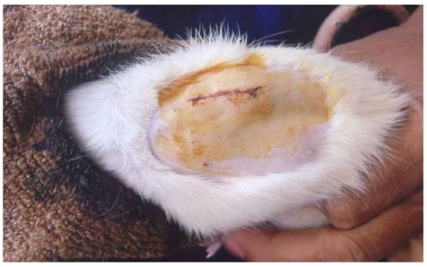
Gp-2 Topically treated with ethanol extract of *Feronia limonia* bark

Gp-3 Topically treated with water extract of *Feronia limonia* bark

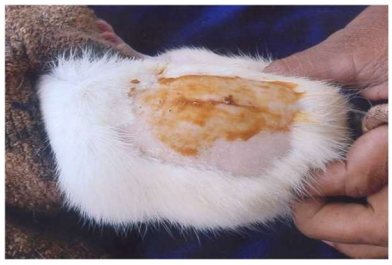
Gp-4 Topically treated with ethanol only



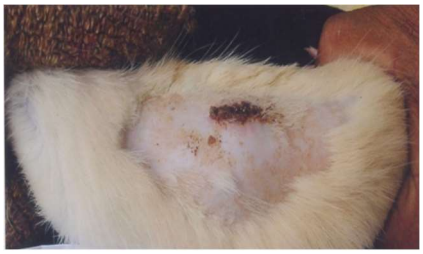
Group 1



Group 2



Group 3



Control

Figure11: *In vivo* antibacterial activity (wound healing property against *S. aureus* induced excision-type cutaneous wounds) of *Feronia limonia* (L.) Swingle bark

Group 1 Healing result after 6 days treatment with pet-ether extract

Group 2 Healing result after 6 days treatment with ethanol extract

Group 3 Healing result after 6 days treatment with water extract

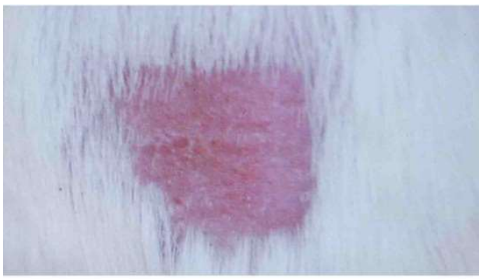
Study on the Skin Whitening Effect of *Feronia limonia* (L.) Swingle Bark

The back of guinea pigs was cleanly shaved and (2 cm x 2 cm) area on shaven skin was exposed to sunlight daily (11-12 AM, 1 h/day) for two consecutive weeks. When the hyperpigmentation on skin was clearly noticed, the area was imaginarily divided into two portions vertically (1 cm x 2 cm). The area of left portion was topically treated with sample solution and the right portion was treated with respective solvent for control purpose. Treatments were continued for three successive weeks one time per day.

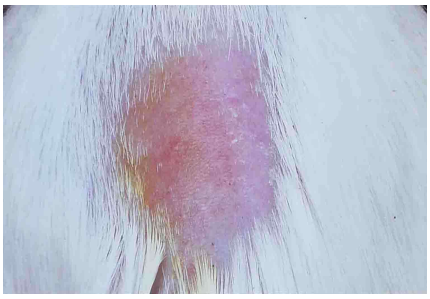
Finally, the blanching effect of individual samples on hyperpigmented skin was evaluated by viewing with naked eye while comparing with control area.

Figures 12,13 and 14 represent photographs showing whitening effect of samples (PE and EtOH extracts, and kojic acid) on sun light-induced hyperpigmentation after 21 days of topically application. It can be seen from the figures that the skin of guinea pigs receiving treatment was appeared to be whiter than that receiving treated with respective solvents. The extent of blanching effect of both extracts was seemed to be equal. The whitening level of control kojic acid was seemed to be higher than both extracts.

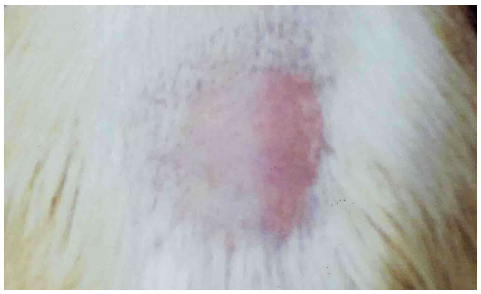
From this finding, it can be inferred that *F. limonia* bark possessed the ability to blanch hyperpigmentation of the skin and hoped to protect skin from photoaging. The level of UV radiation emitted by the sun is increasing due to the depletion of ozone layer. Skin is more exposed to UV radiation and often suffers from various harmful effects of UV. Melanin production in human skin is an important defense mechanism against UV and a major determinant of skin color. Therefore, this finding was envisaged to be beneficial for exploring plant-based cosmetics.



Sun light-induced hyperpigmented skin of guinea pig 1

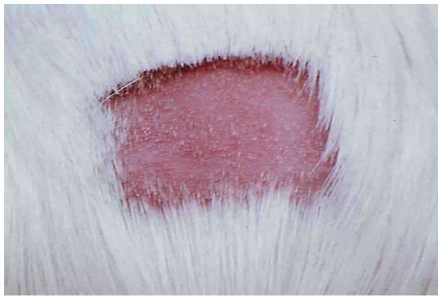


Providing topically treatment on half portion of hyper-pigmented skin

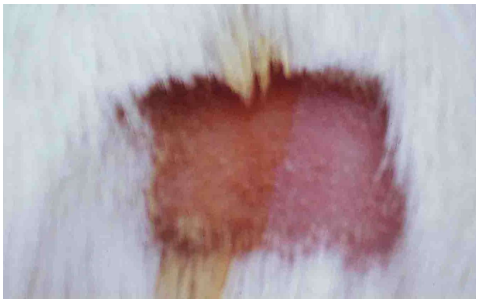


Whitening result after 3 weeks successive treatment

Figure 12 : Whitening effect of pet-ether extract of *Feronia limonia* (L.) Swingle bark on sun light-induced hyperpigmentation

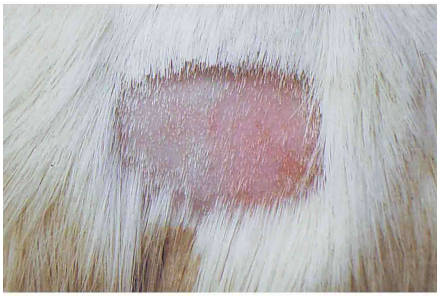


Sun light-induced hyperpigmented skin of guinea pig 2



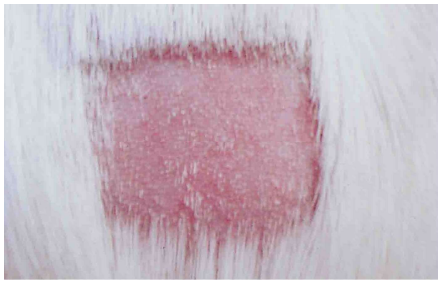
Providing topically treatment on half portion of hyper-pigmented skin

Figure 12 : Whitening effect of pet-ether extract of *Feronia limonia* (L.) Swingle bark on sun light-induced hyperpigmentation

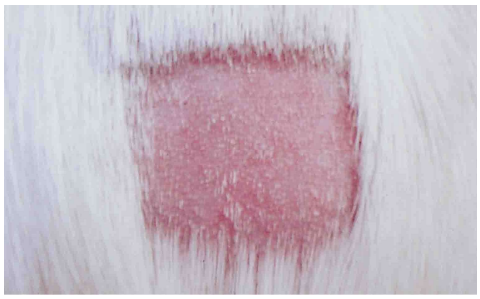


Whitening result after 3 weeks successive treatment

Figure 13: Whitening effect of ethanol extract of *Feronia limonia* (L.) Swingle bark on sun light-induced hyperpigmentation



Sun light-induced hyperpigmented skin of guinea pig 3



Providing topically treatment on half portion of hyper-pigmented skin

Whitening result after 3 weeks successive treatment

Figure 14: Whitening effect of kojic acid on sun light-induced hyperpigmentation

Conclusion

From the overall assessment concerning with chemical and biological activity investigation on stem bark of *Feromia limonia* (L.) Swingle (Thee in Myanmar), the following inferences may be deduced.

Compound **I** (bergapten) (0.0008% yield, mp 188-189ºC) and Compound **II** (marmesin) (0.0022% yield, mp160-163ºC).*S. aureus* induced excision-type cutaneous wounds when topically treated with PE, H_2O and EtOH extracts needed 6 days to completely cure the wound. No significant difference in wound healing effect between different doses (2-6 mg/day) was observed showing the bark possesses antistaphytococal effect. Control group *needed 8 days to cure wounds.* Skin whitening activity was observed by using sun light induced hyperpigmented guinea pig models. The hyperpigmented skin of guinea pig was topically treated with PE and EtOH extracts, and kojic acid (control) for 3 successive weeks. All extracts provided skin whitening effect but lower than that of kojic acid.

Skin whitening activity is related to inhibition of melanin formulation. Hence topical application of "*Feromia limonia*" bark is envisaged to protect individuals from sun burn, photo-aging and antiwrinkling; and to promote skin whitening by prevention of tanning. In addition, possessing the wound

healing activity of this bark further confirmed the use of this bark as a good candidate for natural cosmeceuticals.

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