ASIAN JOURNAL OF PHARMACOGNOSY Research Article
Asian J. Pharmacogn 3(1):23-31

eISSN-0128-1119

Skin Care Active Ingredients from *Senna alata* (L.) Roxb Extracts

Saidatul Husni Saidin[a*], Nor Azah Mohamad Ali[a], Nurhazwani Mohd Hirmizi[a], Norulaiman Yusoff[a], Zunoliza Abdullah[b], Shalini Markandan[c], Mary Khoo[c], Mazura Pisar[c], Mailina Jamil[a], Tan Ai Lee[d], Nuziah Hashim[b] and Safiatunnuur Mohamed Salleh Caadir[a]

[a]*Herbal Product Development Programme,* [b]*Phytochemistry Programme,* [c] *Bioactivity Programme,* [d]*Bioresources Programme*
Natural Products Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor.

Received January 15, 2018; accepted March 6, 2019; published online March 25, 2019

[*]For correspondence: Saidatul Husni Saidin: saidatul@frim.gov.my

Abstract: *Senna alata* (L.) Roxb. (*S. alata*) locally known as "gelenggang daun kecil" in Malay language has been used traditionally to treat skin ailments such as rashes and ringworm. In this study, the leaf extracts of *S. alata* were prepared by (i) undergoing proper species authentication, (ii) quality control of the raw materials, (iii) extraction, (iii) chemical profiling, and (iv) bioactivity evaluation. Antioxidant and anti-inflammatory properties of the extracts were determined by the DPPH radical scavenging assay, total phenolic content assay, tyrosinase inhibitory assay, and lipoxygenase assay. The safety of the extract was determined by conducting cytotoxic test on normal kidney and hepatic cell lines. Based on the bioactivity properties, an active ingredient for skin care formulations was developed and incorporated in facial serum formulation as a prototype product. The prototype product was tested for stability, pH value, physical, and skin irritation. *S. alata* has the potential to be developed as skin care active ingredient however further study is required in addressing the mechanism of action of the extract in skin care protection.

Keywords: *Senna alata,* Medicinal plant, Skin care active ingredient

ASIAN JOURNAL OF PHARMACOGNOSY Research Article
Asian J. Pharmacogn 3(1): 21-29

eISSN-0128-1119

INTRODUCTION

Senna alata (L.) Roxb is a medicinal plant from the family Fabaceae. Previously it was known as *Cassia alata*. This plant is native to Southeast Asia, Northern Australia, Africa and Latin America. It also known as gelenggang, solok, ketepeng badak, seven golden candlesticks, Christmas senna and daun kurap or gelenggang gajah. In Malay traditional medicine, this plant is used as treatment of various skin diseases such as 'kudis', 'kurap', 'kayap' and 'panau' (Mastura, 2016). Other uses are to treat hemorrhoids, constipation, inguinal hernia, intestinal parasites, syphilis, diabetes, convulsion, heart failure, abdominal pain and oedema (Gritsanapan & Mangmeesri, 2009). Pharmacological investigations performed so far on *S. alata* have shown that this herb has several biological activities, such as antimicrobial, antifungal, anti-inflammatory, analgesic, antitumor and hypoglycaemic activities (Somchit et al. 2003, Moriyama et al. 2003). This species is regarded as one of the important species in genus senna which is rich in flavonoids, anthraquinones and polysaccharides. Some of chemical constituents reported from *S. alata* are rhein, emodin, aloe-emodin, crysophanol, glycoside rhein iso-crysophanol, sitosterol, kaempferol 3-O-gentibioside, sennoside A, B and C (Gritsanapan & Mangmeesri, 2009).Despite of many studies were carried out on *S. alata*, it is an interesting idea to developed a herbal product from this species, especially a product that is supported with scientifically fact and evidences. Moreover, *S. alata* was listed among 18 species under Agriculture National Key Economic Area (NKEA), 10th Malaysian Plan. In this study, *S. alata* was investigated for its potential to be develop into active ingredient for skin care product through chemical and biological analyses, formulation study and safety aspect.

MATERIALS AND METHODS

Plant materials: This study is focusing on leaf of *S. alata*. 5kg of dried and ground leaves were bought from Herbagus Sdn. Bhd., Bertam, Penang. Authors manage to get fresh material from the plantation for authentication purposes and a voucher specimen was prepared for references. The dried samples were tested for microbial contamination analysis and heavy metal test to determine the safety.

Microbial contamination analysis: Total aerobic microbial count (TAMC) and total yeasts and moulds count (TYMC) in plant samples were determined according Norulaiman et al, 2011. Acceptance criteria of TAMC and TYMC were 5 x 10^7 CFU/g and 5 x 10^5 CFU/g, respectively (Drug Registration Guidance Document (DRGD), 2013)

Heavy metal test: The concentrations of heavy metals in plant sample were determined according to Nurhazwani et al, 2015, by using Perkin Elmer Model Analyst 600 Atomic Absorption Spectrometry (AAS). Graphite Furnace Atomic Absorption Spectrometry (GFAAS) was used to measure lead and cadmium metals, while Flow Injection for Atomic Spectroscopy System (FIAS 100) was used to determine arsenic and mercury metals. Heavy metal concentration in plant sample must comply and did not exceed maximum limit as regulated by National Pharmaceutical Regulatory Agency (NPRA) in DRGD. The maximum limits for heavy metals are as follows (Table 1).

ASIAN JOURNAL OF PHARMACOGNOSY Research Article
Asian J. Pharmacogn 3(1): 21-29

eISSN-0128-1119

Table 1. The maximum limit for heavy metals in DRGD, First Edition, January 2013, revised July 2015

Heavy metal elements	Maximum limit (mg/kg or mg/litre or ppm)
Lead	NMT 10.0
Cadmium	NMT 0.3
Mercury	NMT 0.5
Arsenic	NMT 5.0

Plant extraction: Water extract was prepared through refluxed process and freeze dried method. Meanwhile, ethanolic extracts were prepared by soaking in ethanol at room temperature. After three days, the sample was filtered. Then the filtrate was concentrated using rotary evaporator. These extracts were prepared for bioactivity testing and HPLC analysis.

Phytochemical screening: Dried samples were used to screen the presence of alkaloids, saponin, flavonoids, tannin and polyphenol compounds, triterpenes and steroids. The test methods were carried out according Saidatul Husni et al, 2015.

HPLC analysis: HPLC analysis was carried out using the filtered ethanolic extract and kaempferol 3-*O*-gentibioside was used as standard. The HPLC parameter as follow (Table 2):

Table 2. HPLC parameter to analyse *S. alata* ethanolic and water extract

	Information
Reference	Kaempferol 3-*O*-gentibioside
Instrument	Waters Delta 600 with photodiode array detector
Column	C18 (5 um, 4.6 mm I.d.x 250 mm)
Mobile phase	0.1% aqueous formic acid & Acetonitrile
Flow rate	1 ml/min
Detection	PDA 280 nm & 340 nm

Bioactivity analyses: In order to develop a skincare active ingredient, *S. alata* extracts were tested for selected biological activities that relate to skin properties especially involving antioxidant and anti-inflammatory. The safety of the extracts were determined by conducting cytotoxicity test.

DPPH radical scavenging activity: 4 mL of the plant extracts was added to 1 mL of 1mM 1,2-diphenyl-2-picrylhydrazyl (DPPH). The mixture was shaken and left a room temperature for 10 minutes. The absorbance of resulting solution was measured spectrophotometrically at 520nm.

Total phenolic content (TPC) assay: Determination of TPC was carried out using Folin-Ciocaalteu method (Velioglu, 1998). The absorbance was recorded at 752nm using 80% (v/v) methanol as a blank. Total phenolic content was expressed as milligram of gallic acid equivalents (GAE) per 100 gram of samples.

Tyrosinase inhibitory activity: A pre-incubation mixture consisting of 1.8 ml of 0.1 M phosphate buffer (pH 6.5).),0.6 ml of H_20_2, 0.1ml of the sample solution and 0.1 ml of the aqueous solution of the mushroom tyrosinase (9600 U/ml) was pre-incubated at 250C for 5 minutes. Then, 0.4 ml of 6.3 mM L-Dopa was added and the reaction was monitored at 475 nm for 1000 seconds.

ASIAN JOURNAL OF PHARMACOGNOSY Research Article
Asian J. Pharmacogn 3(1): 21-29

eISSN-0128-1119

L-cystein (8 mg/ml) was used as the positive control. The negative control has ethanol instead of the sample.

Lipoxygenase inhibitory assay: Lipoxygenase inhibition activity was determined using spectophotometric method. Stock solutions of test samples and nordihydroguaiaretic acid (NDGA) at a concentration of 20 mg ml-1 were prepared by dissolving the extracts in DMSO. Sodium phosphate buffer of 160 µl (0.05 M, pH 7.5), 10 µl of test solution and 20 µl of soybean lipoxygenase solution were mixed and incubated for 10 min at 25 °C. The reaction was then initiated by the addition of 10 µl of the substrate in the form of sodium linoleic acid solution. The enzymatic conversion of sodium linoleic acid to (9Z, 11E)- (13S)-13-hydroperoxyoctadeca-9,11-dienoate was measured by monitoring the change of absorbance at 295 nm over a period of 6 min using a spectrophotometer. Another reaction mixture (control) was prepared by replacing 10 ml of test solution with 10 ml of DMSO in order to obtain maximum uric acid formation. NDGA was used as the positive control in this assay. All tests were performed in triplicates in 96- well UV microplate.

Cytotoxicity Evaluation: WRL-68 (human liver) and Vero (African green monkey kidney) cell lines were used in this study. Both cell lines were obtained from ATCC. They were cultured in Dulbecco's Modified Eagle Medium supplemented with 5% foetal bovine serum and 1% penicillin-streptomycin. Cells were maintained in an incubator containing 5% CO_2/ 95% air at 37 ºC. Cells at exponential growth phase were seeded in a 96-well plate (6,000 cells/well; 100 ul/well complete medium). After an overnight recovery period, cells were exposed to the plant extracts at various concentrations (highest concentration = 1000 µg/ml) for 72 h. After 72 h of incubation, cells viability were measured using MTT assay, Paclitaxel was used for comparison. Results were expressed as percentage of cell survival and the median inhibition concentration (IC_{50}) was determined using non-linear regression from the corresponding dose-response curve.

Product formulations: Formulation of skin care active ingredients from *S. alata* was developed by modifying method by Saidatul Husni et al, 2013. The active ingredient then was formulated into facial serum as skin care prototype product. The ingredients for facial serum were deionised water, carbomer, glycerine, propylene glycol, xanthan gum, polyglyceryl-6 distearate (and) jojoba esters (and) polyglyceryl-3 beeswax (and) cetyl alcohol, ethyl methicone. preservative, pH modifier and fragrance. In a beaker A, carbopol was sprinkled over deionised water and leave until hydrated. Glycerine, propylene glycol and xanthan gum were added into the hydrated carbopol and the mixture were heated at 75°C. In a separate beaker, beaker B, Polyglyceryl-6 distearate (and) jojoba esters (and) polyglyceryl-3 beeswax (and) cetyl alcohol were heated at 75°C until melted completely and was added into beaker A under rapid mixing by rotor stator at 2000 rpm for 5 minutes. Then, ethyl methicone and pH modifier were added into mixtures. When mixture was cooled down until 35°C and *S. alata* active ingredients and fragrance were added.

The skin care product prototype was tested for i) stability as described by Saidatul Husni et al, 2013, ii) physical properties; pH value, colour and odour and iii) skin irritation test; using *in vitro* skin model EpiDerm™ at SIRIM Berhad..

RESULTS

The plant samples, *S. alata* from Herbagus Sdn Bhd was found safe to be used as skin care active ingredient as fulfill the quality specification by DRGD (refer Table 3 and 4).

ASIAN JOURNAL OF PHARMACOGNOSY Research Article
Asian J. Pharmacogn 3(1): 21-29

eISSN-0128-1119

Table 3. Microbial contamination analysis on plant sample, dried and ground *S. alata* leaves.

Test	Result	Acceptance criteria
TAMC	8.2×10^4 CFU/g	5×10^7 CFU/g
TYMC	4.0×10^5 CFU/g	5×10^5 CFU/g

CFU: colony forming unit

Table 4. Heavy metal test on plant sample, dried and ground *S. alata* leaves.

Heavy metal elements	Result (mg/kg)
Lead	0.52
Cadmium	0.02
Mercury	0.01
Arsenic	1.29

NMT: not more than

From the phytochemical screening (Table 5), it was found that the plant sample contained saponin, flavonoids, condensed tannins, triterpenes and steroids. The presence of strong flavonoid in *S. alata*, 3+, may contribute to bioactivities such antioxidant, antimicrobial and anti-inflammatory. According to Sule et al, (2011), tannins has astringent property and can precipitating protein that leads to tissue improvement, while saponin has mild detergent property and has ability to permeate into the cell.

Table 5: Phytochemical screening on plant sample, dried and ground *S. alata* leaves.

Phytochemical constituents					
Flavonoid	Tannin	Alkaloid	Steroid	Saponin	Triterpene
3+	2+	-ve	3+	2+	3+

Note: -ve: not present, +1: low, +2: moderate, +3: high

Further chemical analysis was carried by using HPLC and kaempferol 3-*O*-gentibioside was detected in both water and ethanolic extract of *S. alata* (Figure 1 and 2). The presence of kaempferol 3-*O*-gentibioside (peak 4) indicated that the plant material is *S. alata* as this compounds was reported in other study (Moriyama et al.2003). However, kaempferol 3-*O*-gentiobioside was not confirmed yet as the bioactive compound. Based on the biological properties of *S. alata* water extract and ethanolic extract shown in Table 6 and Table 7, *S. alata* ethanolic extract show higher antioxidant and anti-inflammatory activities compared to *S. alata* water extract. However, the water extract's IC_{50} is higher than *S. alata* ethanolic extract and both extracts showed higher IC_{50} than Paclitaxel, negative control. This indicated that both extracts are non-toxic towards cell lines. In DPPH radical scavenging analysis, the reducing activity of the *S. alata* extracts was evaluated and determines its antioxidant potential. The antioxidant ability of *S. alata* extracts to inhibit the catalytic pathway of melanin pigment biosynthesis was evaluated in Tyrosinase inhibitory assay. Thus resulting in skin whitening properties. Amount polyphenols in the *S. alata* extracts was expressed equivalent to gallic acid were 100 mg/100g for *S. alata* ethanolic extract and 40mg/100g. Based on the biological activities findings, *S. alata* extract was found to have potential to be developed as active ingredient for skincare product. In order to determine whether the *S. alata* extracts as active ingredient is suitable to be incorporated into skin care product, a facial serum with *S. alata* extract was formulated as prototype product. This facial serum was assessed for its physical properties and stability. The results are stated in Table 8.

ASIAN JOURNAL OF PHARMACOGNOSY Research Article
Asian J. Pharmacogn 3(1): 21-29

eISSN-0128-1119

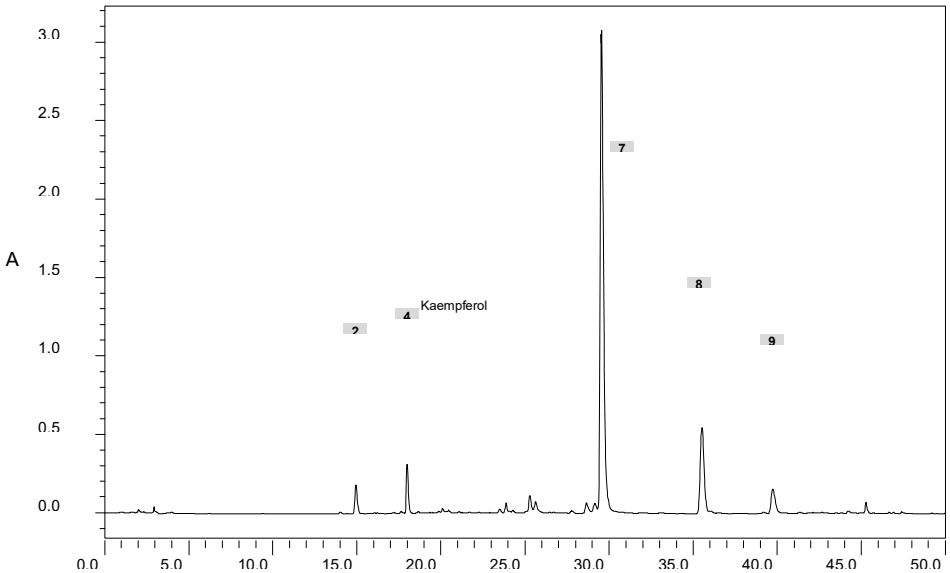

Figure 1: HPLC Chromatograph for *S. alata* ethanolic extract

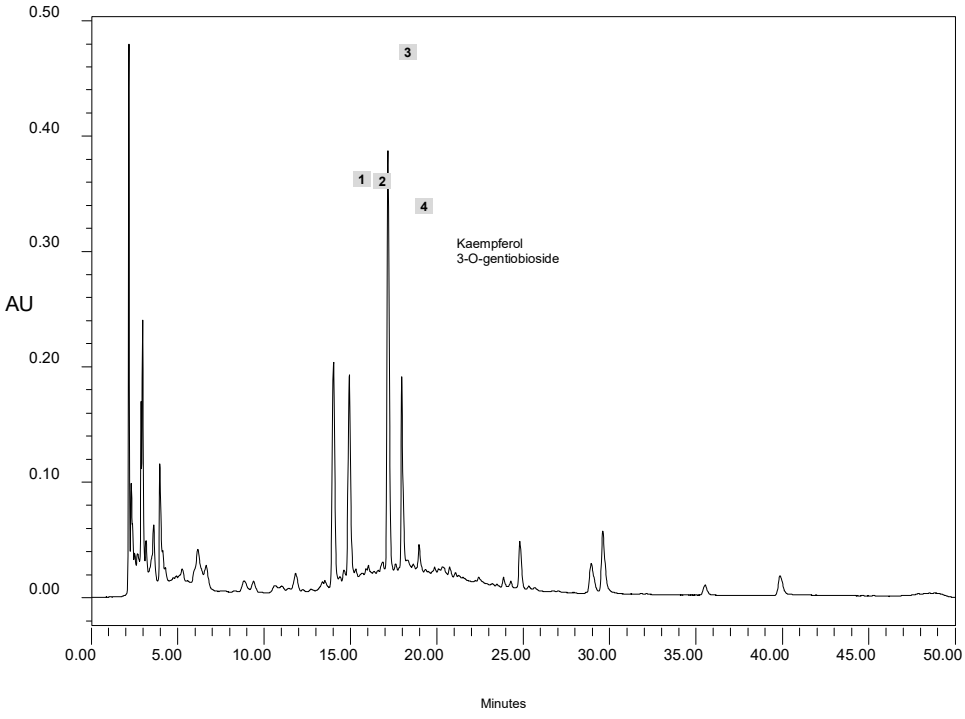

Figure 2: HPLC Chromatograph for *S. alata* water extract

ASIAN JOURNAL OF PHARMACOGNOSY Research Article
Asian J. Pharmacogn 3(1): 21-29

eISSN-0128-1119

Table 6. Antioxidant activity of *S. alata* ethanolic and water extract

	Antioxidant activity		
Plant extract	DPPH radical scavenging activity (%)	Tyrosinase inhibitory activity (%)	Total phenolic (TPC) mg/100g GAE
S. alata ethanolic extract	72.03±0.8	78.75±0.7	100.00
S. alata water extract	24.04±0.2	11.34±5.01	40.00

Table 7. Anti-inflammatory activity and cytotoxicity of *S. alata* ethanolic and water extract

	Anti-inflammatory	Cytotoxicity (IC_{50}(ug/ml))	
Plant extract	Lipoxygenase inhibitory (%)	Normal kidney cell lines (Vero)	Normal liver cell lines (WRL-68)
S. alata ethanolic extract	97.82±2.18	183.50±25.60	145.43±5.86
S. alata water extract	8.81±4.42	>1000	>1000
NDGA	99.72±0.28	-	-
Paclitaxel	-	0.0501±0.0103	0.0028±0.0004

Table 8. Assessment on physical properties (colour, odour, pH value) and stability of prototype skin care product, facila serum.

Test	Observation
pH	6.19
Colour	Off-white
Odour	Rose
Stability in 4°C after 3 months	Pass – no splitting phase
Stability in 40°C after 3 months	Pass – no splitting phase
Stability in 25-27 °C after 3 months	Pass – no splitting phase

pH value of 6.19 is considered as suitable for skin as the range of pH value suitable for healthy skin is 5-6.5. The colour of the prototype product is off-white due to the addition of *S. alata* extracts as active ingredient. The original colour of the extract is dark green. Prototype product has rose aroma due to the fragrance used in the formulation. Evaluation of skin irritation on the prototype product was carried out using *in vitro* reconstructed human epidermal model (EpiDerm™) skin irritation test. This test was out sourced to Institute Biotechnology Research Centre, SIRIM Berhad. The result of this test was the prototype was considered as non-irritant as the prototype product did not reduce the viability of EpiDerm™ tissue below 50% of the negative control.

CONCLUSION

Based on the findings in this study, *S. alata* is suitable to be developed as active ingredient for skincare product. The extracts were considered as safe based on the microbial contamination test, heavy metal analysis and cytotoxicity test. The extracts were recorded for good antioxidant and anti-inflammatory activity especially ethanolic extract. In the formulation study, the extract can be incorporated into facial serum and pass the stability test, has considerable pH value and acceptable odour and colour. The prototype product also was considered as non-irritant to skin. However

ASIAN JOURNAL OF PHARMACOGNOSY Research Article
Asian J. Pharmacogn 3(1): 21-29

eISSN-0128-1119

further study is required to address the mechanism of action of the extract in skin care protection and chemical constituent that contribute to the biological activity

ACKNOWLEDGMENT

The authors would like to acknowledge Majlis Amanah Rakyat (MARA) for the financial support under *Program Teknousahawan MARA-FRIM*. Our deepest appreciation goes to all Natural Product Division staffs that had helped us along the way.

DECLARATION OF CONFLICT OF INTEREST

No conflict of interest associated with this work

REFERENCES

Gritsanapan, W. and Mangmeesri, P. (2009) Standardized *Senna alata* Leaf Extract. Journal Health Research, 23 (2), 64-68.

Liu, M. and Yokomizo, T. (2015) The Role of Leukotrienes in Allergic Diseases. Allergology International, 64, 17-26.

Mastura, M. (2016) Herba Emas Negara, Mastura, M., Nik Zanariah, N.M. and Norini, H. (Ed.), pg 135-146, FRIM, Kuala Lumpur.

Mohd Farhan, A.R., Muhammad Shahrul, M.N., Zamree, M.S., Pin, K.Y., Noor Rasyila, M.N., Nurhazwani, M.H., Mohad Kamal, N.H., Masitah, M.T., Firmansyah, J.S., Mohd Hapizul, U.L., Ameer Jasrie, J. and Rishwan Hakim, K. (2013) Preliminary Study on Antioxidant Activity and Total Phenolic Content of *Cynometra cauliflora* Leaves At Different Extraction Temperature in Translating Natural Products R,D &C Initiatives in Line with Economic Transformation Programme, Mastura M., Asiah, O., Adiana MA. & Hada Masayu ID (Ed.), Pg 192, FRIM, Kuala Lumpur.

Moriyama, H., Lizuka, T., Nagai, M. and Murata, Y. (2003) HPLC Quantification of Kaempferol 3-O-gentibioside in *Cassia alata*. Fitoterapia 74; 425-430.

Moriyama, H., Lizuka, T., Nagai, M., Miyataka, H. and Satoh, T. (2003) Antiinflammatory Activity of Heat-treated *Cassia alata* Leaf Extract and Its Flavonoid Glycoside. *Yakugaku Zasshi*, 123, 607-611.

Norulaiman, Y., Ong, B.K., Nor Azah, M.A. & Mohd Irman Shah, M. 2011. Microbial Load Enumeration of Various Form of Herbal Products for Oral Consumption in harnessing the tropical herbal heritage: recent advances in R&D and Commercialization. Proceedings of the Seminar on Medicinal and Aromatic Plants, Mastura, M., Adiana, M.A., Hada Masayu, I. & Pin, K.Y. (Ed.), pg 184, FRIM, Kuala Lumpur.

Nurhazwani, M.H., Saidatul Husni, S., Nor Azah, M.A., Azrina, A., Mailina, J., Norulaiman, Y., Wan Nurhafisya, M., Mohammad Faridz, Z.P. (2015) Quality Control of *Zingiber officinale* Oils in Product Formulations. Jurnal Teknologi (Sciences & Engineering), 77 (3), 39-42.

Saidatul Husni, S., Fauziah, A., Fadzureena, J., Siti Kamariah, H., Hani Idayu, H. & Nor Azah, M.A. (2013) Active Ingredient for Herbal Product from *Tetracera maingayi in* Proceedings of the 13th Seminar on Medicinal and Aromatic Plants. Translating Natural Products R,D &C Initiatives in Line with Economic Transformation Programme, Mastura M., Asiah, O., Adiana MA. & Hada Masayu ID (Ed.), Pg 142, FRIM, Kuala Lumpur.

Saidatul Husni, S., Norulaiman, Y., Fauziah, A., Mastura, M., Ong, B.K., Vimala, S., Nor Azah, M.A., Mary, K., Nuziah, H. and Mohd Irman Shah, M. (2015) Evaluation Biological Activities of Piper Sp Intended for Skin Formulation Preservative. Jurnal Teknologi (Sciences & Engineering), 72 (1), 1–6.

ASIAN JOURNAL OF PHARMACOGNOSY Research Article
Asian J. Pharmacogn 3(1): 21-29

eISSN-0128-1119

Somchit, M.N., Reezal, I., Nur Elysha, I. and Mutalib, A.R. (2003) In Vitro Antimicrobial Activity of Ethanol and Water Extracts of *Cassia alata.* Journal of Ethnopharmacology, 84, 1-4.

Velioglu, Y.S., Mazza, G., Gao, L. & Oomah, B.D. (1998) Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables and Grain Products. Journal of Agricultural and Food

Sule, W.F., Okonko, I.O., Omo-Ogun, S., Nwanze, J.C., Ojezele, M. O., Ojezele, O. J. & Olaonipekun, T. O. (2011) Phytochemical Properties and *in vitro* Antifungal Activity of *Senna alata* Linn. Crude Stem Bark Extract. Journal of Med Plants Research, *5*(2), 176-183.