ANTIBACTERIAL ACTIVITIES OF LEAF EXTRACTS FROM *CODARIOCALYX MOTORIUS* (HOUTT.) H.OHASHI

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Abstract

Codariocalyx motorius (Houtt.) H.Ohashi belonging to family Fabaceae was collected from Pyin Sar village, Pyin Oo Lwin Township during the flowering and fruiting periods from August to November, 2017. The botanical identification, preliminary phytochemical screening, physicochemical evaluation, elemental analysis and antibacterial activities of leaf extracts were carried out. According to preliminary photochemical examinations, the flavonoids, glycosides, phenolic compounds, polyphenols, saponins, amino acids, carbohydrates, reducing sugars and tannins were present while alkaloids, phytosterols and cyanogenic substances were absent. Physicochemical characterization showed that water soluble ash was found more than acid insoluble ash. The studied species showed more amounts of water extractable matter than ethanol, ethyl acetate and pet ether extractable matter. The elemental analysis showed that potassium, calcium, sulphur, iron, manganese, zinc and copper were present. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were detected according to microdilution method. The antibacterial activities were determined with four extracts, namely pet ether, ethyl acetate, ethanol and aqueous against on gram-positive bacteria; *Enterococcus faecalis*, *Staphylococcus aureus* and gram-negative bacteria; *Eschericha coli*, *Pseudomonas aeruginosa*. The MIC values were between 3.9 mg ml^{-1} to 125 mg ml^{-1} and MBC were 31.25 mg ml^{-1} to >250 mg ml^{-1}. This plant showed more sensitive effects on gram-positive bacteria than gram-negative bacteria. These results showed that *C. motorius* (Houtt.) H.Ohashi may be useful to treat diseases caused by *Enterococcus faecalis* and *Staphylococcus aureus* infections.

Keywords: *Codariocalyx motorius* (Houtt.) H.Ohashi, Morphology, Phytochemical, Physicochemical, Elemental, Antibacterial.

Introduction

Medicinal plants are nature's gift to human beings for disease free healthy life. The use of herbs as medicine is the oldest form of health care known to humanity and has been used in cultures throughout history (Barnes *et al*. 2007).

Herbal medicines usually contain in a range of pharmacologically active compounds. Bioactive compounds are normally accumulated as secondary metabolites in all plant cells. Leaf is one of the highest accumulated plant part of such compounds and people are generally preferred it for therapeutic purposes. Some of the active compounds inhibit the growth of disease causing microbes either singly or in combination (Selvamohan *et al*. 2012).

Increasing development of drug resistance in human pathogens as well as the appearance of side effect of synthetic drugs needs to develop new antimicrobial drugs from natural sources (Mondel *et al.* 2004). This situation has forced to search for new antimicrobial sources like medicinal plants (Doshi *et al*. 2011). Prevention of bacterial infections, using plant extracts, is highly desirable due to low cost, environmental friendliness, and effectiveness against certain bacteria, compared to antibiotics which might be harmful to the environment (Cheng *et al.* 2014).

Codariocalyx motorius (Houtt.) H.Ohashi, commonly known as Dancing plant or Se ka myin or Shik kho pin and are widely distributed in Myanmar (Kress *et al*. 2003), is famous for its rapid movement of lateral leaflets. This plant is popularly used in Indian traditional and folk medicine since its leaves have diuretic, febrifugal and tonic properties and roots are used as a remedy for asthma, coughs, as antidysenteric and as emollient. It possess a remarkable wound

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healing effect (Vipin *et al.* 2015). It has traditionally been used in Chinese Medicine to treat various ailments such as rheumatism, cough, malaria, pyrexia, dysentery, hepatitis, haemoptysis (Ma *et al.* 2011). *C. motorius* (Houtt.) H.Ohashi has been demonstrated to possess wound healing activity (Gopalakrishnan *et al*. 2015, Gopalakrishnan & Rajameena. 2012), antimicrobial properties (Kalirajan *et al*. 2012), antithrombotic and anticoagulant activities (Vipin *et al*. 2015) and anti-oxidant activity (Chidambaram *et al*. 2013, Gopalakrishnan & Rajameena 2014). This plant is used in Myanmar traditional medicine, such as, vitamin B deficiency diseases, abscesses and wound healing activities.

The description of morphological characters and phytochemical constituents, physicochemical properties, elemental analysis and antibacterial activity of leaf extracts of *C. motorius* (Houtt.) H.Ohashi were carried out in present study.

The aim of the present research was to study the antibacterial activities of various leaf extracts of *C. motorius* (Houtt.) H.Ohashi. The objectives were to study the morphological characters, to investigate the qualitative and quantitative analysis and to detect the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of leaf extracts of *C. motorius* (Houtt.) H.Ohashi.

Materials and Methods

Sample collection and identification

The plant specimens were collected from Pyin Sar village, Pyin Oo Lwin township, Mandalay region during their flowering and fruiting period from August to November 2017. The collected specimens were identified with the help of literatures: Hooker (1879), Nian-he (2009) and Xu Langran (2010).

Phytochemical investigation

The preliminary phytochemical tests of the powdered leaf were carried out at Research Division, University of Traditional Medicine, Mandalay according to Harbone (1998) and Raaman (2006) methods.

Physicochemical properties

Physicochemical properties were determined for the quality control parameter of medicinal purposes (WHO, 2011) at Research Division, University of Traditional Medicine, Mandalay.

Elemental analysis

Elemental concentration were analyzed by using Energy Dispersive X-ray Fluorescence Spectrophotometer (EDXRF) at Chemistry Department, Western Yangon University and Atomic Absorption Spectrophotometer (AAS) at Amtt Laboratory Department, Yangon.

Antibacterial activities

Antibacterial activity of pet ether, ethyl acetate, ethanol and aqueous extracts of *C. motorius* (Houtt.) H.Ohashi were tested by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using microdilution method with resazurin (Sarker *et al.* 2007). Twelve concentrations (0.12 to 250 mg ml^{-1}) of various extracts were tested in *vitro* antibacterial activity against four pathogenic bacterial strains. The ciprofloxacin was used as positive control. Test organisms used in this study were supplied from Upper Myanmar Public Health Laboratory, Mandalay and Biotechnology Research Department, Kyaukse. The test organisms were *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923. The bacteria

concentration was (5×10^5 $CFUml^{-1}$). The antibacterial activity test was done at Medical Laboratory Technology Department, University of Medical Technology, Mandalay.

Results

***Codariocalyx motorius* (Houtt.) H.Ohashi** J. Jap. Bot. 40: 367. 1965

Desmodium motorium (Houtt.) Merr., J. Arnold Arbar. 19(4): 345–346.1938.

Hedysarum motorium Houtt., Nat. Hist. 2(10):246.1779.

Desmodium gyrans (L.f.) DC. Prodr. 2: 326. 1825.

Family : Fabaceae

Myanmar name : Say ka myin or Ship kho pin

English name : Nra lap or Dancing plant

Flowering time : August to January

Fruiting time : October to January

Annual, erect herbs, 0.5 – 1.0 m high; stems and branches slender, glabrous. Leaves pinnately trifoliolate compound, alternate; stipules lanceolate, 3.0 - 4.0 mm long; petioles terete, 0.8 – 1.8 cm long; stipels 2.0 - 3.0 mm long; leaflets oblong-lanceolate to lanceolate, the terminal one larger than the two lateral leaflets, 1.5 - 7.5 cm by 0.2 - 2.0 cm, rounded or obtuse at the base, entire along the margin, acute at the apex, glabrous above and densely pubescent beneath. Inflorescences terminal racemes, 2- to 5-flowered, peduncles 4.5 - 10 cm long, sparsely pubescent. Flowers bisexual, zygomorphic, pentamerous, hypogynous, purple, 1.0 - 1.3 cm in diameter; pedicels 2.0 - 3.0 mm long, pubescent; bracts ovate, 5.0 - 7.0 mm long; bracteoles ovate-linear, about 2.0 mm long. Calyx campanulate, 2-lipped; tube about 2.0 mm long; upper lip 2-lobed, orbicular, about 5.0 mm long; lower lip 3-lobed, lobes lanceolate, 2.0 - 5.0 mm long, densely pubescent. Corolla papilionaceous; standard obovate, 5.0 - 7.0 mm by 4.0 - 6.0 mm; wings linear-oblong, 4.0 – 5.0 mm; keels obtuse, 5.0 – 6.0 mm long. Stamens 10, diadelphous, (9)+1; staminal tubes about 7.0 mm long, glabrous; anthers dithecous, dorsifixed, oblong, dehiscing longitudinally. Carpel 1; ovary superior, oblongoid, 6.0 - 7.0 mm long, unilocular with few ovules in the locule on the marginal placentae, pubescent; styles filiform, 3.0 - 4.0 mm long, glabrous; stigma penicillate. Pods oblongoid, 4 - 10 jointed, compressed, 2.0 - 3.5 cm long, densely pubescent. Seeds oblongoid, green, glabrous.

Specimen examined: Mandalay Region, Pyin Oo Lwin Township, Pyin sar Village, 22°02'N, 96°28' E, elevation 1000 m; 17 September 2017; Aye Aye Myat, collection no. 1, 2, 3

Habit

Inflorescenc

Flower as

L.S of flower

Stamens Carpel Fruits Seeds

Figure 1 Morphological characters of *Codariocalyx motorius* (Houtt.) H.Ohashi

Phytochemical constituents

Phytochemical constituents showed that flavonoids, glycosides, phenolic compounds, polyphenols, saponins, amino acids, carbohydrates, reducing sugars and tannins were present while alkaloids, phytosterols and cyanogenetic substances were absent in studied species.

Physicochemical properties

Quantitative evaluation revealed that physicochemical parameter are pH 6.3%, ash content 6.45%, acid insoluble ash 3.38%, water soluble ash 93.55%, water extractable matter 22.64%, ethanol extractable matter 14.81%, ethyl acetate extractable matter 3.05% and petroleum ether extractable 1.61%.

Elemental analysis

According to the EDXRF, the macro elements consists of potassium 1.145%, calcium 0.805% and sulphur 0.208%. The microelements of iron 0.023%, manganese 0.009%, zinc 0.002% and copper 0.002% were found. According to AAS, the contents of toxic elements, lead and cadmium were not detected.

Antibacterial activity

The results of antibacterial activity of pet ether, ethyl acetate ethanolic and aqueous extracts of *C. motorius* (Houtt.) H.Ohashi was presented in Table 1. The positive control was used in ciprofloxacin.

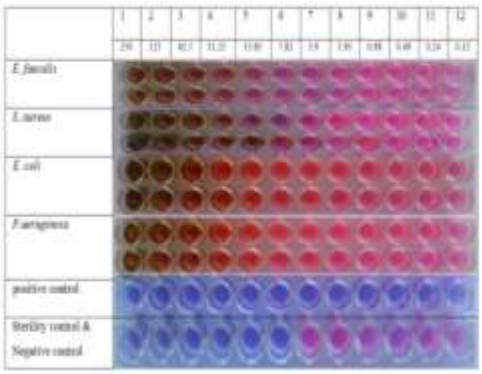


Figure 2MIC of pet ether extracts from leaf of *C. motorius* (Houtt.) H. Ohashi against bacteria

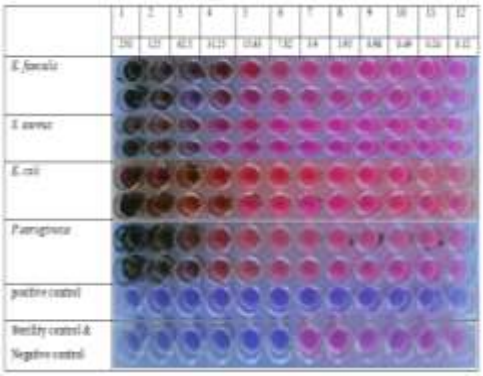


Figure 3 MIC of ethyl acetate extracts from leaf of *C. motorius* (Houtt.) H. Ohashi against

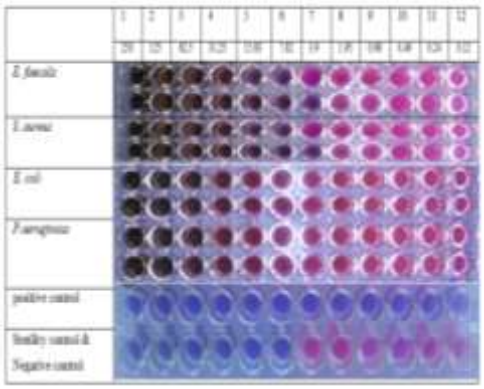


Figure 4 MIC of ethanolic extracts from leaf of *C. motorius* (Houtt.) H. Ohashi against bacteria

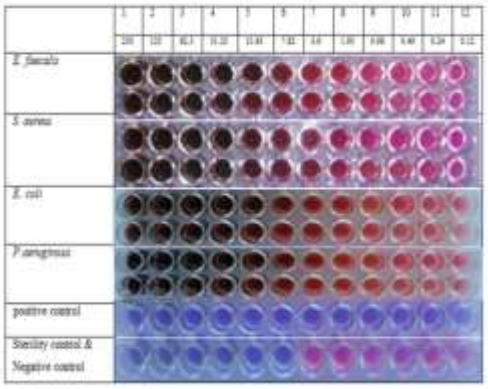
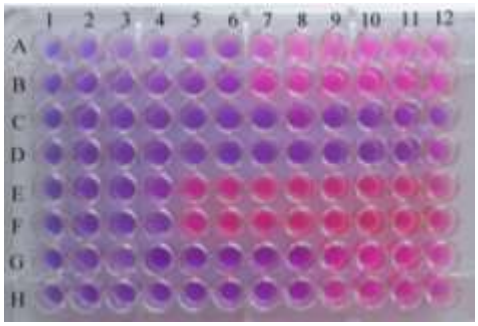


Figure 5 MIC of aqueous extracts from leaf of *C. motorius* (Houtt.) H. Ohashi against bacteria



A, B = *Enterococcus faecalis*

C, D = *Eschericha coli*

E, F = *Pseudomonas aeruginosa*

G, H = *Staphylococcus aureus*

* pink colour indicates growth and blue means inhibition of growth

Figure 6 MIC of Antibiotic (Ciprofloxacin) against on bacteria

E .faecalis *S. aureus* *E-coli* *P. aeruginosa*

Figure 7 MBC of pet ether extracts from leaf of *C. motorius* (Houtt.) H. Ohashi against bacteria

E .faecalis *S. aureus* *E-coli* *P. aeruginosa*

Figure 8. MBC of ethyl acetate extracts from leaf of *C. motorius* (Houtt.)H. Ohashi against bacteria

E .faecalis *S. aureus* *E-coli* *P. aeruginosa*

Figure 9 MBC of ethanolic extracts from leaf of *C. motorius* (Houtt.) H. Ohashi bacteria

Figure 10 MBC of aqueous extracts from leaf of *C. motorius* (Houtt.) H. Ohashi against

Figure 11 MBC of Antibiotic (Ciprofloxacin) against on bacteria

Table 1 Antibacterial activity of MIC and MBC values for various leaf extracts from *Codariocalyx motorius* (Houtt.) H.Ohashi

Tested Microorganisms	pet ether extract (mg ml^{-1})		ethyl acetate extract (mg ml^{-1})		ethanolic extract (mg ml^{-1})		aqueous extract (mg ml^{-1})		Ciprofloxacin (mg ml^{-1})	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. faecalis ATCC 29212	3.9	62.5	31.25	62.5	15.62	125	31.25	31.25	$1x10^{-3}$	$1x10^{-3}$
S. aureus ATCC 25923	7.81	125	62.5	125	7.81	125	15.62	62.5	$2.5x10^{-4}$	$5x10^{-4}$
E. coli ATCC 25922	125	125	62.5	125	62.5	250	15.62	>250	$5x10^{-4}$	$1x10^{-3}$
P. aeruginosa ATCC 27853	125	125	62.5	62.5	62.5	125	31.25	>250	$4x10^{-3}$	$8x10^{-3}$

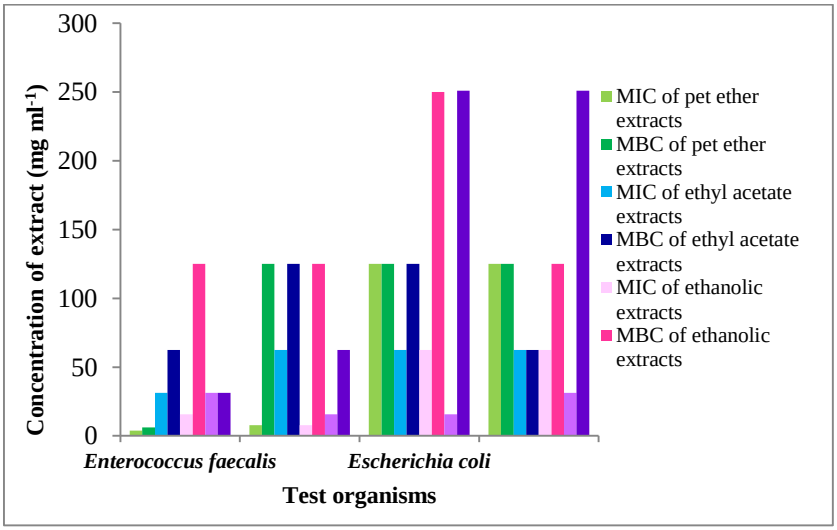


Figure 12 Antibacterial activity of MIC and MBC values for various leaf extracts from *Codariocalyx motorius* (Houtt.) H.Ohashi

Discussion and Conclusion

The antibacterial activities of pet ether, ethyl acetate ethanolic and aqueous extracts of leaf from *Codariocalyx motorius* (Houtt.) H.Ohashi was determined by microdilution method with resazurin. In addition, phytochemical constituents, physicochemical properties, elemental and heavy metal analysis of this plant was studied.

In the present study, the habit of *C. motorius* (Houtt.) H.Ohashi was annual herbs. Pinnately trifoliate compound leaves and the blades were oblong lanceolate. Inflorescences were terminal racemes with zygomorphic flowers. Papilionaceous corolla, stamens uniform and diadelphous (1+9), unilocular, few ovules with marginal placentation and sessile ovary were observed in studied species. The pods were curved and compressed which are agreed with those stated by Hooker (1885), Nian-he (2009) and Xu Langran (2010).

Phytochemical analysis is the best extraction method to indicate the presence of active constituents in herbal medicine. This species showed that the presence of flavonoids, glycosides, phenolic compounds, polyphenols, saponins, amino acids, carbohydrates, tannins, reducing sugar while alkaloids, phytosterol and harmful cyanogenic substance were absent. Due to the presence of effective bioactive compounds, this species possessed antibacterial and numerous pharmacological activities. Therefore, this plant plays a significant role in the treatment of various diseases.

In this study, the amount of water soluble ash was higher than acid insoluble ash. The low content of acid insoluble ash showed these plant extracts less amount of impurities. The extractable values were maximum in water extract followed by ethanol, ethyl acetate extract and least in pet ether extract which showed larger quantities polar phytoconstituents. These physicochemical data are pharmaceutically importance in herbal medicine.

Elemental analysis was done by Energy Dispersive X-Ray Fluorescence (EDXRF) Spectrophotometer. The macroelements; the content of potassium was mostly found. The second largest was calcium and the third was sulphur. Microelements; iron, manganese, zinc, copper were present. Moreover, the leaves powder of these plants were analysed by Atomic Absorption Spectroscopy (AAS) to know the present or absent of heavy metal. According to AAS, toxic elements; lead and cadmium were not present. Therefore this plant may be used in deficiency symptom of these elements and safely for long time.

In this study, antibacterial activities were determined by microdilution method using resazurin, as an indicator. This resazurin indicated the detection of bacterial growth. The results of present study showed that twelve different concentrations of leaf extracts were tested for their antibacterial activities. These bacteria were gram-positive bacteria; *Enterococcus faecalis*, *Staphylococcus aureus* and gram-negative bacteria; *Eschericha coli*, *Pseudomonas aeruginosa*.

The MIC results showed that all plant extracts (pet ether, ethyl acetate ethanolic and aqueous extracts) prevented the growth of all tested bacteria. The concentration for inhibition of growth of the test bacteria ranged from 3.9 mg ml^{-1} to 31.25mg ml^{-1} with the lowest MIC value against *Enterococcus faecalis* followed by *Staphylococcus aureus* (7.81 mg ml^{-1} to 62.5mg ml^{-1}) and *Eschericha coli* (15.62 mg ml^{-1} to 125mg ml^{-1}) while the highest *Pseudomonas aeruginosa* (31.25 mg ml^{-1} to 125 mg ml^{-1}). The MBC results showed that growth of the gram-positive bacteria was killed with a concentration ranged from 62.5 mg ml^{-1} to 125 mg ml^{-1} and gram-negative bacteria was from 62.5 mg ml^{-1} to >250 mg ml^{-1}. From these results, gram-negative bacteria are more resistant compared with gram-positive bacteria. Therefore, the plant extracts were more effective gram-positive bacteria than gram-negative bacteria. However, all of plant extracts possess antibacterial effects on both gram positive and gram negative bacteria. Kalirajan *et al*. (2012) reported that the methanol and aqueous extracts of *C. motorius* (Houtt.) H.Ohashi were tested for the antibacterial

activities by plate hole diffusion assay. They mentioned that methanol extract of this plant showed efficient antibacterial activity against *Eschericha coli* and *Staphylococcus aureus.* The aqueous extract was found to be effective against *Staphylococcus aureus*. Therefore, all of plant extracts have a deadly or killing effects on all test organism.

This result can be scientific evidence for the antibacterial activity of the leaf extracts of *C. motorius* (Houtt.) H.Ohash. Therefore, this plant may be used to treat many diseases caused by *Enterococcus faecalis* and *Staphylococcus aureus* infection.

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References

Branes, J., L. A. Anderson & J. D. Phillipson. (2007). Herbal medicine. 3rd Edition, Pharmaceutical Press, Londo.

Cheng, G., H. Hao, S. Xie, X. Wang, M. Dai, L. Huang & Z. Yuan. (2014). Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Frontiers in Microbiology, Vol. 5:217.

Chidambaram, U., V. Pachamuthu, S. Natarajan, B. Elango, Suriyanarayanan & K. M. Ramkumar. (2013). Invitro evaluation of free radical scavenging activity of *Codariocalyx motorius* root extract. Asian Pacific Journal of Tropical Medicine, Vol. 6 (3): p.188-194.

Doshi, G. M., S. S. Shidhaye, G. V. Aggarwal, P. P. Pillai, A. B. Bhalerao & S. K. Desai. (2011). Antibacterial potential of *Cassia auriculata* flowers. Journal of Microbiology and Biotechnology Research, Vol. 1(3): p. 15-19.

Gopalakrishnan, S. & R. Rajameena. (2014). Pharmacognostical and phytochemical studies on *Desmodium gyrans* DC. World Journal of Pharmacy and Pharmaceutical Sciences: Vol. 3 (8).

Gopalakrishnan, S. & R. Rajameena. (2012). Evaluation of ethanolic extract of *Desmodium gyrans* DC. leaves on wound healing activity in rats. Pharmaceutica Anal Acta, Vol. 3 (7).

Gopalakrishnan. S., R. Rajameena & R. Subramanian. (2015). In silico screening of phytochemical constituents from *Desmodium gyrans* DC. As glycogen synthase kinase- 3β (gsk-3β). International Journal of Pharma and Bio Sciences; 6 (2): p. 649-656.

Harbone, J. B. (1998). Phytochemical methods, third edition, Chapmen & Hall, London, UK.

Hooker, J. D. (1982). Flora of British India. Vol. II. L. Reeve & Co.5 Henrietta Street, Convert Garden, London.

Kalirajan, A., J. S. Michael & A. J. A. R.Singh. (2012). A preliminary screening of the medicinal plant *Desmodium gyrans* (Linn. f) DC for its antimicrobial, phytochemical and wound healing properties. International Journal of Pharmaceutical Sciences Research, Vol. 3 (6): p 1726-1730.

Kress, W. John, Robert A. De Filips, Ellen Farr and Yin Yin Kyi, (2003). A Checklist.

Ma, X., C. Zheng, C. Hu, K. Rahman & L. Qin . (2011.) The genus *Desmodium* (Fabaceae)-traditional uses in Chinese medicine, Journal of Ethnopharmacology, Vol.138(2): p. 314-332.

Mondal, S. & S. A. Kolhapure. (2004). Evaluation of the antimicrobial efficacy and safety of pure hands herbal hand sanitizer in hand hygene and on inanimate objects. The antiseptic, Vol. 101(2).

Nian-he. (2009). Flora of Hong Kong. Volume II. Agriculture, Fisheries and Conservation Department Government of the Hong Kong Special Administrative region. Hong Kong.

Raaman. N. (2006). Phytochemical techniques, New India Publishing Agency, Pitam Pura, New Delhi.

Sarker. S. D., L. Nahar & Y. Kumarasamy. (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Science direct. Methods Vol. 42(4): p.321-324.

Selvamohan T., V. Ramadas & S. S. S. Kishore. (2012). Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. Advance in applied science research, Vol 3(5): p. 3374-3381.

Vipin, P. S., N. M. Johannah, S. Menon, L. Lawrence & J. Padikkala. (2015). Antithrombotic and anticoagulant activities of *Desmodium gyrans* (DC). Journal of chemical and pharmaceutical research, Vol 7(5): p. 973-980.

World Health Organization (2011).. Quality control methods for herbal materials, WHO Press. Geneva Switzerland.

Xu Langran. (2010). Flora of China. Vol. 10. Science Press, Beijing.