Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTIES ON THREE MEDICINAL PLANTS FOR POTENT ANTI-SOFT ROT AGENTS

(Fitokimia dan Sifat Antibakteria Tiga Jenis Pokok Berubat sebagai Agen Anti Reput Buah)

Airiel Daniel Daud[1], Nur Suhada Azman[1], Nur Izni Ainina Alias[1], Norfatihah Syaifudin[1], Aiza Harun[1]*, Noorshilawati Abdul Aziz[2]

[1] Faculty of Applied Science,
[2] Faculty of Plantation and Agrotechnology
Universiti Teknologi MARA Pahang, Malaysia. 26400 Bandar Tun Razak Jengka, Pahang, Malaysia

**Corresponding author: aizaharun@.uitm.edu.my*

Received: 28 September 2020; Accepted: 13 December 2020; Published: xx February 2021

Abstract

Gynura procumbens, *Murraya koenigii*, and *Cosmos caudatus* are among the readily homegrown medicinal plants possessing ethnomedicinal applications. The lack of studies on the utilisation of these plants against plant pathogen has driven this study to investigate these plants as a potential for plant pathogen soft rot disease and proposed structures. Antibacterial activity of three types of extracts of each plant species was conducted against a gram-positive soft rot disease bacterium of *Erwinia chrysanthemi*. An agar disc diffusion method was used to evaluate antibacterial activity on the extracts against *E. chrysanthemi*. A thin layer chromatographic technique (TLC) was used to screen the presence of phytochemicals. The molecular structures of the anti-soft rot agents were based on the proton nuclear magnetic resonance (NMR), infrared (IR) spectroscopy and comparison with literature. The phytochemicals of alkaloid, terpenoid, and phenolics were detected in all plant species. All extracts exhibited antibacterial activity against *E. chrysanthemi*, and the activity was in a concentration-dependent manner. The highest inhibition zone was recorded as 12 mm from the methanol extract of *C. caudatus*. Infrared analysis recorded functional groups of hydroxyl (OH), carbon double bond (C=C), and carbonyl (C=O) in which these groups of molecules might be retained inside the active compounds. Using TLC, IR, NMR and comparison with the available literature, the anti-soft rot agents were proposed as a phenolic compound known as rutin **(1)**, triterpenoid saponin **(2)**, hydroxycinnamic acid ester **(3)**, caffeic acid **(4)**, quinic acid **(5),** and alkaloid derivatives **(6)**. These results could provide scientific baseline information for the development of natural pesticide to control anti-soft rot disease.

Keywords: antibacterial activity, anti-soft rot, phytochemicals, medicinal plants, *Erwinia chrysanthemi*

Abstrak

Gynura procumbens, *Murraya koenigii*, dan *Cosmos caudatus* merupakan pokok tumbuhan berubat yang sangat dikenali kerana penggunaannya dalam perubatan tradisional. Akibat kekurangan kajian ketiga-tiga pokok ini terhadap patogen tumbuhan, maka fokus kajian adalah untuk melihat potensi sifat antibakteria ketiga-tiga pokok ini terhadap patogen penyakit reput buah dan juga mencadangkan struktur agen anti reput buah. Aktiviti antimikrob ini telah dilakukan ke atas satu bakteria gram positif iaitu *Erwinia chrysanthemi*. Teknik penyerakan cakera agar telah digunakan untuk menilai keberkesanan aktiviti antibakteria untuk semua jenis ekstrak tumbuhan. Teknik pemisahan kromatografi lapisan nipis (KLN) telah digunakan untuk melihat kehadiran bahan fitokimia.

Cadangan struktur kimia agen anti-reput buah telah dilakukan menggunakan spektroskopi nukleus hidrogen teraruh, spektroskopi infra merah dan perbandingan dengan kajian lepas. Fitokimia jenis alkaloid, terpen dan fenolik telah dikesan di dalam ekstrak ketiga-tiga jenis pokok. Kesemua ekstrak menunjukkan aktiviti antimikrob dan aktiviti ini berkadar terus dengan kepekatan ekstrak. Zon perencatan yang tertinggi diperolehi daripada ekstrak methanol *C.caudatus* iaitu sepanjang 12 mm. Analisa infra merah ekstrak ketiga-tiga pokok telah merekodkan kehadiran kumpulan berfungsi jenis hidroksil (OH), karbon ikatan ganda dua (C=C) dan kumpulan karbonil (C=O) di mana ia berkemungkinan berada di dalam struktur aktif ekstrak pokok. Selepas analisa KLN, infra merah dan spektroskopi nukleus magnetik resonan dan perbandingan dengan kajian lepas dijalankan terhadap ekstrak tumbuhan, struktur agen anti reput buah telah dicadangkan sebagai sebatian fenolik jenis rutin **(1)**, terpen saponin **(2)**, ester asid hidroksisinamik **(3),** asid kafeik **(4)**, asid kuinik **(5)**, dan terbitan alkaloid **(6)**. Hasil kajian diharap dapat menyediakan maklumat saintifik asas dalam pembuatan racun makhluk perosak semulajadi untuk mengawal penyakit anti reput buah.

Kata kunci: aktiviti antibakteria, anti reput buah, fitokimia, tumbuhan berubat, *Erwinia chrysanthemi*

Introduction

Plants are able to produce secondary metabolites such as flavonoids, alkaloids, phenols, and saponins that are critical for their survival [1]. These metabolites enable plants to shield themselves from pathogens, herbivory effects, competition with other plants, and to preserve them from unfavourable physical conditions such as harmful UV radiation, water deprivation, and low temperatures. The antimicrobial activity is dependent on the types of pathogen and the presence of the active compounds due to the variation on the results, which could be due to the restriction of the active components inside the extract to cause antimicrobial action [2]. *Cosmos caudatus* was reported to exhibit antimicrobial action against five microbial strains which are *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* with the MIC concentration of the extract ranging from 6.25 mg/mL to 12.5 mg/mL. *M. koenigii* has an antimicrobial effect on *Candida albicans, Aspergillus niger*, and *Trichophyton rubrum.* The antimicrobial effect of could be due to the presence of monoterpenoids and sesquiterpernoids in the *M. koenigii's* leaves [3].

An antibacterial activity using hexane, methanol, and chloroform from *M. koenigii* against *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli* as well as *Salmonella typhi* was also conducted [4]. The flavonoid compounds in *M. koenigii* were found to be responsible for the remarkable antibacterial activities and the antioxidant activity [5]. *G. procumbens* showed remarkable antimicrobial activity against gram-positive and gram-negative bacteria such as *Bacillus cereus*, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, and *Salmonella typhi* as well as antifungal activity against fungi such as *Candida albicans* and *Aspergillus niger*. These antimicrobial effects established the traditional use of *G. procumbens* to treat infections by herpes simplex virus and malaria parasites [6]. However, the limitation of applying these plants species to combat plant pathogen of soft rot disease *in vitro* study is the reason to further this investigation. Therefore, this study aims to evaluate the potency of plant extracts of *C. caudatus*, *M. koenigii*, and *G. procumbens* against *E. chrysanthemi*.

Materials and Methods

Preparation of leave extracts

The plant samples were collected at Herbs Nursery UiTM Pahang. The samples were washed and placed in an oven at a temperature of 40 ˚C until fully dried. The dried samples were then ground into a fine powder using an electric blender. The soaking process of a measured mass of the powdered sample was performed consecutively using three types of solvents with increasing polarities namely hexane, ethyl acetate (EA), and methanol (MeOH). The soaking process was held for three days and repeated three times for each solvent until no colour was detected. Then, the solvent mixture was filtered, evaporated to dryness using a rotary evaporator, and was kept until further use.

Antibacterial activity

The antibacterial work of all extracts of the plant species against *E. chrysanthemi* was conducted based on the previously published study [7]. The strain of the bacteria

was obtained from Biology Laboratory UiTM Pahang. All extracts were two-fold serially diluted from 400 mg/mL until 50 mg/mL and were used to prepare 6 mm impregnated disc. The effectiveness of the extracts was determined by the size of the inhibition zone (in mm) through the disc diffusion method.

Thin layer chromatographic analysis

The TLC analysis of extracts was performed according to the previously published study [8]. The developed TLC was sprayed with chemical reagents such as vanillin/H_2SO_4 reagent, Dragendorff's reagent, or ferric chloride reagent to ascertain any occurrence of phytochemicals such as terpenoid, alkaloid, and phenolic compounds.

Fourier transform infrared analysis

The FTIR analysis of extracts was performed using the same technique as previously described [9]. The information of functional groups recorded from infrared spectrum provided a basic structural formula of phytochemicals.

Proton nuclear magnetic resonance analysis

The proton NMR analysis was conducted to determine the types of proton presents. It was recorded on JEOL FTNMR 400 MHz transformed spectrometer in $CDCl_3$ or acetone-D6 using tetramethylsilane (TMS) as the internal standard at 400 MHz. The data from the proton NMR spectrum provide the type of proton.

Results and Discussion

Table 1 depicted the *in vitro* antibacterial activity for each extract of *C. caudatus*, *M. koeginii*, and *G. procumbens*. The size of the inhibition zone appeared from the antibacterial action reveals the effectiveness of the extracts. The greater the inhibition zone, the more effective the extracts with higher antibacterial activity.

Generally, all types of extracts for all plant species exhibit antibacterial activity in a concentration-dependent manner. As the concentration of the extract increases, the size of the inhibition zone also increases enhancing the antibacterial activity. The accumulation of active compounds in the extracts are sufficient to affect the growth of *E. chrysanthemi* by preventing cell wall synthesis or cell content and inhibit DNA replication, which will then stop the growth of *E. chrysanthemi* [10]. As the polarity of the extracts increases from hexane to methanol, the inhibition zone also increases except for methanol extract of *G. procumbens* stem. Methanol extract from *C. caudatus* leaves was found as the most active extract since it inhibited the growth of *E. chrysanthemi* prominently with an inhibition zone of 12 mm at 400 mg/ml compared to other species and among other extracts from *C. caudatus* itself. However, this extract inhibited the growth of *E. chrysanthemi* in a moderate action in accordance with its inhibition zone at 400 mg/ml. The higher antibacterial activity was methanol extract of *C. caudatus* compared to ethyl acetate and hexane extract, and this is aligned with previous studies [10, 11] that showed antibacterial activity of a plant if methanol extract was used.

Meanwhile, another previous antibacterial study using an ethanol extract of *C. caudatus* recorded promising antibacterial activity against selected human bacteria [2]. According to previous reports, phenolic compounds such as flavonoid was found to take part in suppressing bacterial growth [1, 12, 13] and it was isolated and identified from the polar extract. Therefore, the existence of phenolic compound in *C. caudatus* leaves as tabulated in Table 2 is consistent with previous studies and its presence might be the reason for the antibacterial action revealed by the extracts against *E. chrysanthemi*. However, the trend of antibacterial activity for *G. procumbens* stem does not follow the polarity of the extracts as in other tested species. The extract from nonpolar extract hexane with an inhibition zone of 8.5 mm at 400 mg/ml was recognised as an active extract. These different trends of antibacterial activity may be in part caused by the presence of alkaloid and terpenoid that would be found in the hexane extracts of *G. procumbens* stem as illustrated in Table 2.

Phytochemical investigation of anti-soft rot agents

An active compound such as rutin, which possessed antibacterial properties [14, 15] is one of the phenolic compounds isolated from the leaves extract of *C. caudatus*. Since the phenolic compounds were also found in all extracts of *C. caudatus* leaves (Table 2) and are also an antibacterial agent, it is hence suggested that

rutin is one of the phytochemicals that are responsible for inhibitory action against *E. chrysanthemi*. Based on the structure of rutin (**1**), the common groups or functional groups are phenol rings, which contain C=C, OH groups, C-O-C linkage as well as CH_3 and CH groups (Figure 1).

In accordance with the infrared result in Table 3, functional groups such as OH, benzene ring and C-O-C linkage are also detected in methanol extract of *C. caudatus* leaves. The presence of these functional groups is present in rutin as illustrated from the structure as well as the types of proton present (CH_3, CH_2 and CH) that is depicted in Table 4. Therefore, it is sugges ted that this structure would be one of the phenolic compounds retains in the methanol extract of *C. caudatus* and is responsible to enhance its antibacterial efficacy. Theoretically, the active groups such as OH groups in rutin weakens the cell wall of bacteria by making the lipid bilayer disconformated and disordered hence promoting vesicle leakage [16]. This phenomenon could be one of action mechanism of the phenolic compound from methanol extract of *C. caudatus* causing an inhibitory effect on the growth of *E. chrysanthemi*. Previous reports also claimed that other phenolic compounds such as apigenin, morin, and rhamnetin affect the thickness of lipid bilayer and fluidity level to decrease and increased membrane permeability, leading to the leaking of intracellular proteins in bacterial [17].

Ethyl acetate extract from *M. koenigii* and *G. procumbens* leaves is the active extracts against *E. chrysanthemi* with an inhibition zone of 11 mm and 7 mm, respectively. These inhibitory actions are considered as moderately active for *M. koenigii* leaves and weakly active for *G. procumbens* leaves. According to Table 2, since terpenoid and phenolic are detected in *M. koenigii* leaves and *G. procumbens* leaves as well as terpenoid and alkaloid are detected in *G. procumbens* stem, these might be the sources of inhibitory effect. Table 2 summarises the secondary metabolites or phytochemicals detected in all types of extracts of *C. caudatus, M. koenigii*, and *G. procumbens* through TLC separation. The presence of these compounds is unavoidable as these are the range of phytochemicals content contribute to the enhancement of antibacterial activity.

The antibacterial activity arises in ethyl acetate extract of *M. koenigii* and *G. procumbens* leaves extract might be due to the growth of *E. chrysanthemi* is inhibited by terpenoid and phenolic compound. Previous study [4] reported that the antibacterial activity increased with chloroform extract of *M. koenigii* leaves. Since the polarity index of ethyl acetate and chloroform is almost the same, which is 4.4 for ethyl acetate and 4.1 for chloroform, therefore, the finding in this study agrees with the previous studies. Another study reported that phenolic compounds were the major phytochemical in *M. koenigii* that caused antibacterial activity [5].

Previous study reported that inhibitory action against several human pathogens [18] is influenced by the presence of phenolic compounds in *G. procumbens* leaves. This is in line with the results in this study as tabulated in Table 2 in which the phenolic compound was also found in ethyl acetate and methanol extract of *G. procumbens* leaves. Several phenolic compounds known as flavanone, aurone, isoflavone, benzofuran, xanthone, and stilbene have been reported [19]. The proposed bioactive terpenoid such as terpenoid saponin **(2)** and phenolic compound such as hydroxycinnamic acid ester **(3)** from *M. koenigii* leaves agrees with the structures as previously reported [19]. These suggested that the structures fit with the infrared result of *M. koenigii* as in Table 3 whereby the functional groups of OH, C=O, C-O, and aromatic ring are present. The structures are comparable with the data on the types of the proton as in Table 4 whereby proton methyl ($R\text{-}CH_3$) methylene($R\text{-}CH_2$), methine(R-CH), vinyl proton(H-C=C-), proton hydroxyl (R-OH), and aryl proton (proton benzene) are consistent with the suggested structures in **(2)** and **(3)**.

Polyphenol such as caffeic acid **(4)** and quinic acid **(5)** in Figure 3 was also reported as the bioactive constituents from *G. procumbens* leaves [20] and would possibly dwell in its ethyl acetate extract or methanol extract. The structure of **(4)** and **(5)** of *G. procumbens* might involve in inhibiting the growth of *E. chrysanthemi* although the inhibition zone is smaller

compared with *C. caudatus* and *M. koenigii*. The groups of OH, C=O, and C=C in the proposed structure of **(4)** are consistent with the functional groups from methanol extract of *G. procumbens* leaves. The proposed structure **(5)** contains common functional groups of OH, C=O, C=C, CH_2 groups and the finding of these functional groups in ethyl acetate extract of *G. procumbens* leaves from Table 3 and the types of the proton from Table 4 agrees with the proposed structures. Therefore, it is suggested that both structures could function as antibacterial agents to ensure the feasibility of inhibitory action.

Results in Table 2 illustrate that hexane extract of *G. procumbens* stem may contain an alkaloid, which may be in part responsible for its antibacterial effect against *E. chrysanthemi* although the activity is weaker than *C. caudatus* and *M. koenigii*. FTIR result of hexane extract of *G. procumbens* stem as depicted in Table 3 suggested the appearance of OH, C=O, C=C, and C-N functional groups, which likely consistent with alkaloid derivatives **(6)** [21]. The data on the types of proton such as methane proton, CH_2-N, methylene proton, and vinyl proton depicted in Table 4 are comparable with **(6)** structure. Therefore, the structure **(6)** is proposed as one of the active alkaloids from *G. procumbens* stem that may involve in antibacterial action.

The different profile of antibacterial activity between *C. caudatus*, *M. koenigii*, and *G. procumbens* is unique since they grow from different plant family with a wide range of phytochemicals. However, an assumption can be made in such a way that a powerful synergistic action of terpenoid and phenolic in *C. caudatus* might cause higher antibacterial activity against *E. chrysanthemi* compared to other tested plant extracts.

Table 1. The antibacterial activity on the crude extracts of *C. caudatus*, *M. koenigii*, and *G. procumbens* against *E. chrysanthemi*

Extract	**Concentration (mg/mL)**	***C. caudatus* Leaves**	***M. koenigii* Leaves**	***G. procumbens* Leaves**	***G. procumbens* Stem**
		Inhibition Zone (mm)	**Inhibition Zone(mm)**	**Inhibition Zone (mm)**	**Inhibition Zone (mm)**
Hexane	50	8.0	6.7	6.0	6.0
	100	8.0	6.7	6.0	6.5
	200	11.0	7.0	6.0	7.5
	400	8.0	8.7	6.0	8.5
Ethyl acetate	50	6.0	6.7	7.0	7.0
	100	6.0	6.7	7.0	7.0
	200	7.0	7.7	7.0	7.3
	400	9.0	11.0	7.0	7.7
Methanol	50	6.0	6.0	6.0	6.0
	100	6.3	8.0	6.0	6.0
	200	7.0	6.0	6.0	6.0
	400	12.0	10.0	7.0	6.0
Ampicillin	10 ug/ml	30	30	30	30

6 mm= not active (no inhibition zone); 7-10 mm = weakly inhibited; 11-14 mm = moderately inhibited; > 15 mm = strongly inhibited

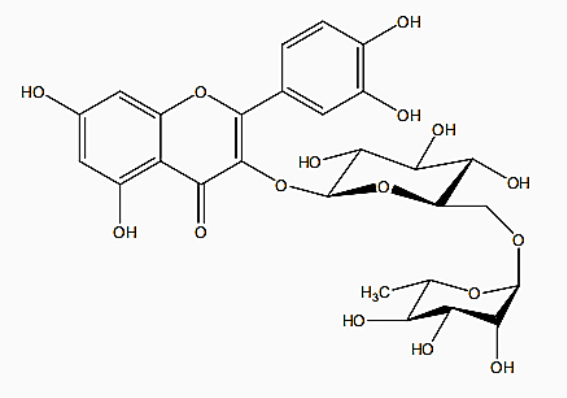


Figure 1. Based on the structure of rutin (**1**)

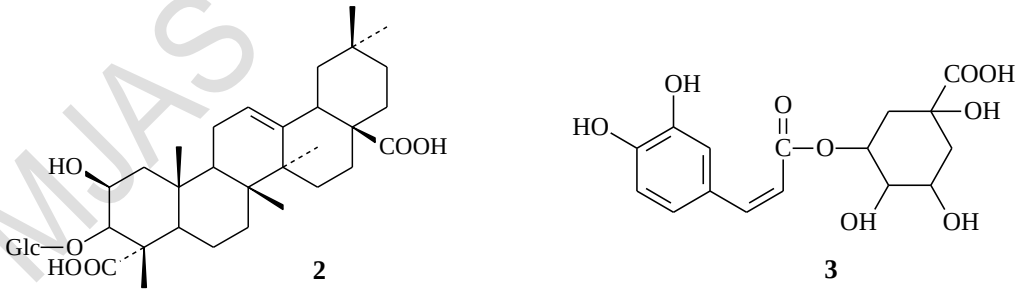
Table 2. TLC analysis of phytochemicals in *C. caudatus*, *M. koenigii*, and *G. procumbens*

Species	Terpenoid*			Alkaloid**			Phenolic***		
	Hex	EA	MeOH	Hex	EA	MeOH	Hex	EA	MeOH
C. caudatus	√	√	√	x	x	x	√	√	√
M. koenigii	√	√	√	x	x	x	√	√	√
G. procumbens leaves	x	x	x	√	x	x	x	√	√
G. procumbens stem	√	√	√	√	x	x	x	x	x

x= not exist; √ = exist

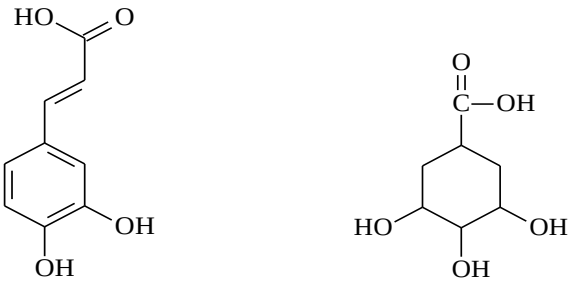
Hex=hexane extract; EA=ethyl acetate extract; MeOH= methanol extract

*using vanillin/H_2SO_4 spraying reagent; **using Dragendorff's spraying reagent; ***Ferric chloride spraying reagent.



2 **3**

Figure 2. The suggested structures for terpenoid saponin (**2**) and hydroxycinnamic acid ester (**3**)



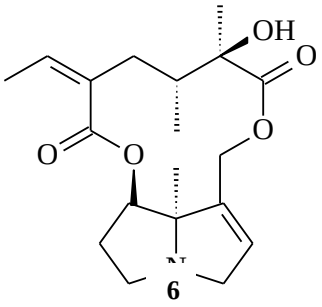
4 **5**

Figure 3. The suggested structures for caffeic acid **(4)** and quinic acid **(5)**

Table 3. FTIR spectroscopy of functional groups present in plant extracts of *C. caudatus*, *M. koenigii*, and *G. procumbens*

Extract	***C. caudatus*** **Leaves**		***M. koenigii*** **Leaves**		***G. procumbens*** **Leaves**		***G. procumbens*** **Stem**	
Hex	WN(cm^{-1})	FG	WN(cm^{-1})	FG	WN(cm^{-1})	FG	WN(cm^{-1})	FG
	3332.65	O-H	3374.79	O-H	3404.20	N-H	3418.62	O-H
	1651.45	C=O	2919.38	sp^3C-H	2850.09	sp^3 C-H	2915.19	sp^3C-H
	1603.23	aromatic	2850.83	sp^3C-H	2917.62	C-H	1740.98	C=O
	1200.69	C-O	1712.37	C=O	1735.12	C=O	1050.95	C-N
					1010.60	C-N		
EA	3334.60	O-H	1619.06	C=C	3383.74	O-H	3385.93	O-H
	2919.73	C-H	2918.89	sp^3C-H	2917.50	sp^3 C-H	2919.56	sp3C-H
	1611.08	aromatic	2850.81	sp^3C-H	2849.79	sp^3 C-H	1710.87	C=O
	1060.58	C-O	3352.25	O-H	1734.75	C=O		
MeOH	3259.13	O-H	3355.09	O-H	3362.10	O-H	3385.57	O-H
	1601.94	aromatic	2924.63	sp^3C-H	2919.14	sp^3 C-H	2919.21	sp^3C-H
	1057.32	C-O	2853.92	sp^3C-H	2851.04	sp^3 C-H	1600.25	C=C
					1632.08	C=C		

Hex = hexane; EA=ethyl acetate; MeOH= methanol; WN=wave number; FG=functional group



6

Figure 4. The suggested structures for alkaloid derivatives **(6)**

Table 4. General information on types of proton from ^{1}H-NMR spectrum

Species	**Range of Chemical Shift (ppm)**	**Types of Proton**
Compound from *C. caudatus*	0.9 - 1.5	R-CH_3
	1.2 - 1.4	R2-CH_2
	1.4 - 1.7	R3-CH
	0.5 - 5.0	R-OH
	4.5 - 5.0	sugar moiety/Ph-OH
Compound from *M. koenigii*	0.7 - 2.1	R-CH_3, R2-CH_2, R3-CH
	4.5 - 5.0	sugar moiety/R-OH
	4.5 - 6.5	C=C-H
	6.5 - 7.5	Benzene proton/ring
Compound from *G. procumbens* leaves	0.7 - 2.1	R-CH_3, R2-CH_2, R3-CH
	3.0 - 4.0	CH_2-O
	4.5 - 6.0	H-C=C-, OH
Compound from *G. procumbens* stem	0.7 - 2.1	R-CH_3, R2-CH_2, R3-CH
	3.0 - 3.5	CH_2-NR_2, CH_2-C=C-
	4.5 - 5.0	OH, CH_2-O

Conclusion

In conclusion, the methanol extract of *C. caudatus* leaves extract is regarded as the most active extract based on its higher inhibition zone against *E. chrysanthemi* compared to other plant extracts. Phenolic compound rutin **(1)** is suggested as the proposed anti-soft rot agent from methanol extract of *C. caudatus*. Other anti-soft rot agents are proposed as terpenoid saponin **(2),** hydroxycinnamic acid **(3),** ester caffeic acid **(4),** quinic acid **(5),** and alkaloid derivatives **(6)** from *M. koenigii* and *G. procumbens*. The establishment of antibacterial activity by evaluating the MIC as well as MBC values needed to be conducted. An extensive structure determination needs to be undertaken in detail such as 2D NMR analysis and LCMS to ascertain the anti-soft rot agent's structures.

Acknowledgement

The author would like to express the warmest gratitude to people who involve directly or indirectly for the completion of this research project.

References

1. Salehan, N. M., Meon, S. and Ismail, I. S. (2013). Antifungal activity of *Cosmos caudatus* extracts against seven economically important plant pathogens. *International Journal of Agriculture and Biology*, 15(5): 864-870.
2. Rasdi, N. H. M., Samah, O. A., Sule, A. and Ahmed, Q. U. (2010). Antimicrobial studies of *Cosmos caudatus* kunth.(compositae). *Journal of Medicinal Plants Research*, 4(8): 669-673.
3. Handral, H. K., Pandith, A. and Shruthi, S. D. (2012). A review on *Murraya koenigii*: Multipotential medicinal plant. *Asian Journal of Pharmaceutical and Clinical Research*, 5: 5-14.
4. Subramanian, P. (2015). *Murraya koenigii* (Curry leave)- a review on its potential. *International Journal of PharmTech Research*, 7(4): 566-572.
5. Kumar, N. S., Simon, N. and Santhoshkumar, R. (2016). Invitro antimicrobial activity and phytochemical analysis of *Murraya koenigii* leaf extracts. *Global Journal of science Frontier Research*, 11(2): 17-20.
6. Tan, H. L., Chan, K. G., Pusparajah, P., Lee, L. H. and Goh, B. H. (2016). *Gynura procumbens*: An overview of the biological activities. *Frontiers in Pharmacology*, 7: 52.
7. Maha Yudden, N. K., Nordin, N. N., Harun, A., Mohd Rosli, N. H., Abdul Aziz, N. and Daud, S. (2019). Comparative study of antioxidant activity of stem and leaves of *Entada spiralis* and their antibacterial properties against *Erwinia chrysanthemi*. *Science Letters*, 13: 37-47.
8. Abdul Majid, N. and Harun, A. (2019). Isolation and tentative identification of antioxidative constituents from dichloromethane extract of *Muraya koenigii* leaves using chromatographic technique. *Gading Journal of Science and Technology*, 2: 31-37.
9. Mohd Padli, N. F. F., Sulaiman, S. N. A., Daud, S., Harith, S. S. and Abdul Aziz. N. (2019). Antioxidative constituents from twig and leaves of *Jasminum Sambac. Gading Journal of Science and Technology*, 2(2): 8-16.
10. Abdullah, E., Ahmad Raus, R. and Jamal, P. (2012). Extraction and evaluation of antibacterial activity from selected flowering plants. *American Medical Journal*, 3(1): 27-32.
11. Caunii, A., Pribac, G., Grozea, I., Gaitin, D. and Samfira, I. (2012). Design of optimal solvent for extraction of bio–active ingredients from six varieties of *Medicago sativa. Chemistry Central Journal*, 6(1): 123.
12. Zongo, F., Ribuot, C., Boumendjel, A. and Guissou, I. (2013). Botany, traditional uses, phytochemistry and pharmacology of *Waltheria indica L.*(syn. *Waltheria americana*): A review. *Journal of Ethnopharmacology*, 148(1):14-26.
13. Cushnie, T. T. and Lamb, A. J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents*, 38(2): 99-107.
14. Mediani, A., Abas, F., Khatib, A., Maulidiani, H., Shaari, K., Choi, Y. H. and Lajis, N. H. (2012). 1H-NMR-based metabolomics approach to understanding the drying effects on the phytochemicals in *Cosmos caudatus*. *Food Research International*, 49(2): 763-770.

15. Guardia, T., Rotelli, A. E., Juarez, A. O. and Pelzer, L. E. (2001). Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. *Il farmaco*, 56(9): 683-687.
16. Matijasevic, D., Pantic, M., Raskovic,B., Pavlovic,V., Duvnjak, D., Sknepnek, A, and Niksi, C. M. (2016). The antibacterial activity of *Coriolus versicolor* methanol extract and its efficient on ultrastructural changes of *Staphylococcus aureus* and *Salmonella Enteritidis*. *Frontier Microbiology*, 2016: 7-12.
17. Sanver, D., Murray, B. S., Sadeghpour, A., Rappolt, M. and Nelson, A. L. (2016). Experimental modelling of flavonoid–biomembrane interactions. *Langmuir*, 32: 13234-13243.
18. Zain, I. K. (2017). Effect of Cadmium and Copper Exposure on Growth, Secondary Metabolites and Antioxidant Activity in Cadmium (Cd). *Molecules*, 22(10):1623.
19. Dixon, R. A. (2001). Natural products and plant disease resistance. *Nature*, 411(6839): 843-847.
20. Murugesu, K., Mohammed Saghir, S. A., Sadikun, A., Khaw, K. Y. and Murugaiyah, V. (2020). Exploiting column chemistry for chromatographic separation and quantification of caffeoylquinic acids in *Gynura procumbens*. *Acta Chromatographica*, 2020: 1-9.
21. Schramm, S., Kohler, N. and Rozhon, W. (2019). Pyrrolizidine alkaloids: biosynthesis, biological activities and occurrence in crop plants. *Molecules*, 24: 1-44.