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Full Length Research Paper

Antifungal activity of extracts and phenolic compounds from *Barringtonia racemosa* L. (Lecythidaceae)

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The antifungal activity of methanolic, ethanolic and boiling water extracts of *Barringtonia racemosa* leaves, sticks and barks were investigate against *Fusarium* sp., *Tricoderma koningii*, *Penicillium* sp., *Ganoderma tropicum, Ganoderma lucidum, Aspergillus* sp. and *Rhizopus* sp. at concentration of 50 mg/ml. Better antifungal activity was observed with the methanolic extracts in all aerial parts of *B. racemosa* that showed excellent inhibitory activity against all the fungi tested. The strongest inhibitory activity effect was observed with the methanolic extract of leaf against *Fusarium* sp. (53.45%), *G. lucidum* (34.57%), *Aspergillus* sp. (32.27%) and *T. koningii* (20.99%). Remarkable are also the specific effects of the boiling water extract of leaf against *Fusarium* sp. (51.72%) and with the ethanolic extract of bark against *Rhizopus* sp. (37.50%). None of the boiling water extracts of leaf, stick and bark showed inhibitory activity effect against *G. tropicum* and *T. koningii.* Among different fungi tested, *Fusarium* sp. was found to be more sensitive to *B. racemosa* extracts when compared to others. The increase in the production of phenolics in the extracts can be correlated with the induction of resistance in treated plant against phytopathogenic fungi. HPLC analysis of the extract of *B. racemosa* (leaves, sticks and barks) showed two different phenolic acids (gallic acid and ferrulic acid) and four different flavonoids (naringin, rutin, luteolin and kaempferol). The results of present study provide scientific basis for the use of the plant extract in the future development as antifungal, antibacterial, antioxidant and anti-inflammatory agent.

Key words: *Barringtonia racemosa*, antifungal, HPLC, phenolic acids, flavonoids.

INTRODUCTION

Medicinal plants have been associated with the prevention of degenerative diseases such as cancer and cardiovascular diseases. The presence of wide range of phytochemicals such as phenolics, thiols, carotenoids, anthocyanins and tocopherol have been suggested to exert chemopreventive and cardio protective effects as well as protecting the human body against oxidative damage by free radicals (Bakar et al., 2009). Natural phytochemicals derived from fruits, vegetables and herbs have been reported to possess a wide range of biological effects, including antioxidant, antimicrobial and anti-inflammatory actions (Brunet et al., 2009).

Among them, phenolic acids and flavonoids have been the object of a great number of studies of their antioxidative activity which is mainly because of their capacity to act as free radical scavengers and/or metal chelators (Anderson et al., 2001; Hameed, 2009). Both compounds have attracted considerable interest in the past few years due to their many potential health benefits. As polyphenols, phenolic acids and flavonoids are powerful antioxidants and have been reported to demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasiodilatory actions (Galeotti et al., 2008; Mattila and Hellstrom, 2007).

Barringtonia racemosa is a tropical higher plant and is a member of the Lecythidaceae family. Locally known as 'Putat Kampung', *B. racemosa* is an evergreen tree found in East Africa, South East Asia (including Malaysia) and Pacific Islands. This plant is a small tree capable of

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reaching 20 m or more with leaves tufted at the ends of stout twigs and it is common in the moist low country, especially near the shores of back waters, lakes, rivers and the banks of paddy fields (Deraniyagala et al., 2003). Ethnomedical survey has shown that the seeds of *B. racemosa* are traditionally used in certain remote villages of Kerala (India) to treat ulcer and cancer (Thomas et al., 2002). The roots of *B. racemosa* showed antibacterial activity against several Gram positive and Gram negative bacteria (Khan et al., 2001) and are a rich source of phytomedicine. The barks and leaves are used for antidote to snake-bites, rat-poisoning and on boils.

Seeds along with other ingredients are employed in the preparations for the treatment of itch, piles and typhoid fever while the bark is claimed to be specific for treatment of gastric ulcers (Deraniyagala et al., 2003). Secondary metabolites such as diterpenes, triterpenoids, steroids and saponins were previously isolated constituent from *B. racemosa* (Deraniyagala et al., 2003; Khan et al., 2001).

The literature survey revealed that there are no scientific studies carried out regarding antifungal activity of the leaves, sticks and barks of *B. racemosa*. Hence, the present study is focused to evaluate the antifungal potentials including determining and quantifying the major phenolic acid and flavonoid compounds present in *B. racemosa* different aerial parts (leaves, sticks and barks) by using high performance liquid chromatography (HPLC) method.

MATERIALS AND METHODS

Plant materials

Plant materials used in this study include fresh *B. racemosa* leaf, stick and bark that were obtained from Kampung Kuak Luar, Pengkalan Hulu, Perak Darul Ridzuan, Malaysia. The plant sample was identified by Mrs. Latifah Zainal Abidin from the Department of Herbarium, Faculty of Forestry, University Putra Malaysia. The voucher number is NMH01/09. The samples were washed with running tap water and separated before being chopped into pieces. They were freeze-dried for 24 h between -50 to -54°C and ground into powder.

Extraction of *Barringtonia racemosa* aerial parts

Aliquots of 0.5 g of the freeze-dried leaves, sticks and barks were extracted with 40 ml absolute methanol and ethanol. Then, 10 ml 6 M hydrochloric acid (HCl) was added to each extract. The mixture solution of the extract was hydrolysed by refluxing at 90°C for 2 h. The hydrolysed extracts were then filtered separately through a layer of Whatman No.1 filter paper (Whatman, England). The filtered extract solution was vacuum-dried in a rotary evaporator at 40°C until all the solvent evaporated. For boiling water extraction, 10 g of freeze-dried samples (leaves, sticks and barks) were extracted with 400 ml boiling water and incubated at 100°C for 15 min while being stirred. The extracts were then filtered separately through a layer of Whatman No. 1 filter paper (Whatman, England).

The filtrate was vacuum-dried in a rotary evaporator at 40°C. To proceed for further analyses, the crude extracts from the three solvents were re-dissolved in 5 ml of absolute methanol to be used in the antifungal activity test.

Antifungal activity test

Seven fungi (*Fusarium* sp., *Tricoderma koningii*, *Penicillium* sp., *Ganoderma tropicum, Ganoderma lucidum, Aspergillus* sp. and *Rhizopus* sp.) were used as test organisms and were provided by the Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, University Putra Malaysia. Cultures of each of the fungi were maintained on potato dextrose agar (PDA) and were stored at 22 ± 2°C.

Antifungal activity was determined as previously described by Kotan et al. (2008) with slightly modification. Briefly, PDA plates were prepared using 9 cm Petri dishes. The methanolic, ethanolic and boiling water extracts were dissolved in methanol (concentration of 50 mg/ml) and 200 µl amount (10 mg/Petri dish) of the extract solutions were added to each of the Petri dishes containing 25 ml PDA. A diameter disc 0.5 cm of the fungal species was cut from 1 week-old cultures on PDA plates and then mycelial surface of the disc was placed upside down on the centre of dish.

Therefore, fungal species was contacting to growth medium on dish. Then, the plates were incubated in the dark at 22 ± 2°C. The extension diameter (cm) of hypae from centre to the sides of dishes was measured at 24 h intervals for 6 days. Mean growth measurements were calculated from three triplicates of each of the fungal species. PDA plates containing methanol (200 µl/Petri dish), without extract solutions, were used as negative control. In addition, PDA plates treated with benomyl (10 mg/Petri dish) were used as positive control.

Analysis of phenolic compounds by high performance liquid chromatography (HPLC)

The content of phenolic acids and flavonoid glycosides and aglycones of *B. racemosa* were quantitatively measured in different parts (leaf, stick and bark) by reverse phase HPLC technique based on the previous method as described by Crozier et al. (1997) with some modifications. Aliquots of 0.5 g of freeze-dried leaf, stick including bark of *B. racemosa* were separately extracted with 40 ml of 60% (v/v) aqueous methanol containing 20 mM butylated hydroxyl toluene (BHT) as an antioxidant. 10 ml of 6 M HCl was then added to the rest of each extract and was then hydrolyzed by refluxing at 90°C for 2 h. The *B. racemosa* containing phenolic acids and flavonoid glycosides and the resulting aglycon, was then filtered through a 0.45 µm Milipore membrane filter (Schleicher and Schuell, Germany) and an aliquot of 20 µl of the filtrate was taken for the HPLC analysis. The elution was performed at a flow rate of 1.0 ml/min and detection was done at 280 nm for phenolic acids and 365 nm for flavonoids.

Five standard phenolic acids and six standard flavonoids were used in determining individual polyphenol compound in *B. racemosa* which includes gallic acid, caffeic acid, syringic acid, ferrulic acid, salicylic acid, rutin, quercetin, myricetin, luteolin, kaempferol and naringin (SIGMA, USA). All standards were diluted in methanol with concentration of 1 mg/ml. The standards, glycosides and the resulting aglycon from *B. racemosa* were quantified by Agilent 1100 series HPLC (Germany) on C_{18} symmetry Nova Pack Waters column.

A solution of 15-35 % (v/v) acetonitrile (HPLC grade) in water adjusted to pH 2.5 with trifluoroacetic acid (TFA) was used as a mobile phase solvent for gradient solution (20 min). Quantification of polyphenol compounds in *B. racemosa* extract samples was based on peak area as compared to the peak area of the standards. To identify the peaks, the spectra patterns and retention time of the samples were compared with standards.

Statistical analysis

Each *in vitro* experiment was performed in triplicate and repeated

three times. Experimental results were expressed as means ± standard deviation (SD) of three parallel measurements with one way ANOVA, Dunnett's Test. Statistical analysis was performed by using Software Graph Pad, Version 5.0. Probability $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Antifungal activity

The results of the antifungal activity of methanolic, ethanolic and boiling water extracts of *B. racemosa* against seven fungi species are summarized in Table 1.

Comparing the antifungal activity of the extracts prepared by different solvents, methanolic extracts from leaf, stick and bark of *B. racemosa* exhibited the most interesting results among the seven fungi tested. The strongest inhibitory activity effect was observed with the methanolic extract of leaf against *Fusarium* sp. (53.45%) followed by *G. lucidum* (34.57%), *Aspergillus* (32.27%) and *T. koningii* (20.99%). Remarkable are also the specific effects of the boiling water extract of leaf against *Fusarium* sp. (51.72%) and with the ethanolic extract of bark against *Rhizopus* sp. (37.50%). None of the boiling water extracts of leaf, stick and bark showed inhibitory activity effect against *G. tropicum* and *T. koningii.* Among different fungi tested, *Fusarium* sp. was found to be more sensitive to all the extracts when compared to others.

Various extract of medicinal plants have shown inhibitory effects against phytopathogenic fungi *in vitro* (Shalini and Srivastava, 2009). The present results of the antifungal activity were support by the previous study that showed the crude methanolic extract of *Barringtonia asiatica* (leaves, fruits, seeds stem and root barks), same species with *B. racemosa*, exhibited a very good level of broad spectrum antifungal activity (Khan and Omoloso, 2002). The methanolic extract of *B. asiatica* flower had also exhibited the growth of *Microsporum canis* and *Trichophyton rubrum* at 1000 µg/ml while *Epidermophyton floccosum* at 125 µg/ml (Locher et al., 1995).

HPLC analysis

Resent researchers indicate that polyphenols being secondary metabolites, are present in rich amount in several plants. Many of them posses antioxidant, anti-inflammatory, antimicrobial and several others therapeutic properties (Falleh et al., 2008; Shalini and Srivastava, 2009). As for the chromatograms obtained from the HPLC study (Figures 1 and 2), it is clearly shown that crude extracts from different parts (leaf, stick and bark) of *B. racemosa* exhibited variable patterns of phenolic acid and flavonoid compounds (Table 2). From the study, it was found that gallic acid, ferrulic acid, naringin, rutin, luteolin and kaempferol are present as the major phenolic acid and flavonoid compounds in the extracts of *B. racemosa* leaf, with values of 171.81, 65.80, 62.94, 59.10, 10.29 and 5.75 µg/g freeze-dried weight tissue, respectively. The results also revealed that traces amounts of gallic acid, naringin and luteolin (103.53, 51.17 and 5.22 µg/g freeze-dried weight tissue respectively) could be detected from the extracts of *B. racemosa* stick. Further HPLC analysis on the extracts of *B. racemosa* bark revealed that gallic acid, ferrulic acid and naringin were present as the major phenolic compounds with values of 56.92, 25.67 and 13.76 µg/g freeze-dried weight tissue respectively.

Interestingly, among the phenolic acids detected, gallic acid was found significantly ($p < 0.05$) highest in the leaf and stick extracts (171.81 and 103.53 µg/g freeze-dried weight tissue respectively). These values are higher than the gallic acid value in fresh Mauritian black tea leaves (0.006 µg/g dry weight) (Ramma et al., 2005). Besides gallic acid, the naringin was found significantly ($p < 0.05$) highest flavonoid content in *B. racemosa* leaf, stick and bark (62.94, 51.17 and 13.76 µg/g freeze dry weight tissue respectively).

The results of the antifungal activity of the various crude extracts were in agreement with the uses of the extract of *B. racemosa* in traditional medicine (Thomas et al., 2002) and also with those reported by Galeotti et al. (2008) on the antifungal activity of flavonoid compounds against the fungus *Verticillium alba-atrum*. Flavonoids are abundant in *D. caryophyllus* and widespread in many other plants. Although the mechanism of action of such compounds against fungi is still unknown, their efficacy, availability at low cost, and low toxicity to humans give the phenolic acids and flavonoids potential as natural fungicides (Mattila and Hellstrom, 2007). Flavonoids have been proven for use against fungal pathogens of man since they have the ability to inhibit spore germination of plant phatogens (Cushnie and Lamb, 2005).

According to Crozier et al. (1997) and Hertog et al. (1992), HPLC analysis is the best way for chemical characterization and therefore this study also established HPLC analysis for the active phenolic compounds from *B. racemosa* that can act as antifungal. Gallic acid, ferrulic acid with others phenolic acids has been found to posses antifungal, antibacterial, anti-inflammation, antioxidant, antipyretic and externally used as antiseptic for various skin conditions (Shalini and Srivastava, 2009). The degradation of phenolic compounds in the extracts samples of *B. racemosa* might due to inappropriate acid conditions and reaction time used during the hydrolysis period. The presence of some unknown compounds in the extraction medium accelerating the degradation of phenolics also should be considered as suggested by Hertog et al. (1992).

Conclusion

In conclusion, this study provides new scientific informa-

Table 1. Inhibition of mycelial growth of fungal species by the crude extracts of *B. racemosa*.

Fungal species	Crude extracts (10 mg/Petri dish)			Benomyl (10 mg/Petri dish)		Control
		Growth (cm)[a]	Inhibition (%)	Growth (cm)	Inhibition (%)	Growth(cm)[a]
***Aspergillus* sp.**	MeOH-Leaf	6.07 ± 0.06	31.27***	4.80 ± 0.10	46.91***	8.60 ± 0.00
	MeOH-Stick	6.23 ± 0.12	29.22***	4.80 ± 0.10	46.91***	8.60 ± 0.00
	MeOH-Bark	5.90 ± 0.00	3.33***	4.80 ± 0.10	46.91***	8.60 ± 0.00
	EtOH-Leaf	6.33 ± 0.06	27.99***	4.80 ± 0.10	46.91***	8.60 ± 0.00
	EtOH-Stick	6.70 ± 0.00	23.46***	4.80 ± 0.10	46.91***	8.60 ± 0.00
	EtOH-Bark	5.87 ± 0.12	33.75***	4.80 ± 0.10	46.91***	8.60 ± 0.00
	BW-Leaf	6.27 ± 0.06	28.81***	4.80 ± 0.10	46.91***	8.60 ± 0.00
	BW-Stick	6.97 ± 0.06	20.16***	4.80 ± 0.10	46.91***	8.60 ± 0.00
	BW-Bark	6.90 ± 0.00	20.99***	4.80 ± 0.10	46.91***	8.60 ± 0.00
***Fusarium* sp.**	MeOH-Leaf	3.20 ± 0.17	53.45***	2.57 ± 0.23	64.37**	6.30 ± 0.00
	MeOH-Stick	4.47 ± 0.06	31.61***	2.57 ± 0.23	64.37***	6.30 ± 0.00
	MeOH-Bark	3.90 ± 0.10	41.38***	2.57 ± 0.23	64.37***	6.30 ± 0.00
	EtOH-Leaf	3.70 ± 0.17	44.83***	2.57 ± 0.23	64.37***	6.30 ± 0.00
	EtOH-Stick	3.70 ± 0.10	44.83***	2.57 ± 0.23	64.37***	6.30 ± 0.00
	EtOH-Bark	3.60 ± 0.17	46.55***	2.57 ± 0.23	64.37***	6.30 ± 0.00
	BW-Leaf	3.30 ± 0.10	51.72***	2.57 ± 0.23	64.37***	6.30 ± 0.00
	BW-Stick	4.23 ± 0.12	35.63***	2.57 ± 0.23	64.37***	6.30 ± 0.00
	BW-Bark	3.87 ± 0.21	41.95***	2.57 ± 0.23	64.37***	6.30 ± 0.00
Ganoderma lucidum	MeOH-Leaf	5.80 ± 0.00	34.57***	2.87 ± 0.06	70.78***	8.60 ± 0.00
	MeOH-Stick	6.67 ± 0.12	23.87***	2.87 ± 0.06	70.78***	8.60 ± 0.00
	MeOH-Bark	6.90 ± 0.00	20.99***	2.87 ± 0.06	70.78***	8.60 ± 0.00
	EtOH-Leaf	7.47 ± 0.06	13.99***	2.87 ± 0.06	70.78***	8.60 ± 0.00
	EtOH-Stick	6.57 ± 0.06	25.14***	2.87 ± 0.06	70.78***	8.60 ± 0.00
	EtOH-Bark	7.40 ± 0.00	14.81***	2.87 ± 0.06	70.78***	8.60 ± 0.00
	BW-Leaf	6.43 ± 0.12	26.75***	2.87 ± 0.06	70.78***	8.60 ± 0.00
	BW-Stick	7.73 ± 0.06	10.70***	2.87 ± 0.06	70.78***	8.60 ± 0.00
	BW-Bark	7.40 ± 0.10	14.81***	2.87 ± 0.06	70.78***	8.60 ± 0.00
Ganoderma tropicum	MeOH-Leaf	7.13 ± 0.12	18.10***	2.93 ± 0.06	69.96***	8.60 ± 0.00
	MeOH-Stick	8.00 ± 0.00	7.41***	2.93 ± 0.06	69.96***	8.60 ± 0.00
	MeOH-Bark	8.60 ± 0.00	0.00	2.93 ± 0.06	69.96***	8.60 ± 0.00
	EtOH-Leaf	7.67 ± 0.16	11.52***	2.93 ± 0.06	69.96***	8.60 ± 0.00
	EtOH-Stick	8.60 ± 0.00	0.00	2.93 ± 0.06	69.96***	8.60 ± 0.00
	EtOH-Bark	8.60 ± 0.00	0.00	2.93 ± 0.06	69.96***	8.60 ± 0.00
	BW-Leaf	6.93 ± 0.06	20.57***	2.93 ± 0.06	69.96***	8.60 ± 0.00
	BW-Stick	8.60 ± 0.00	0.00	2.93 ± 0.06	69.96***	8.60 ± 0.00
	BW-Bark	8.60 ± 0.00	0.00	2.93 ± 0.06	69.96***	8.60 ± 0.00
***Penicillium* sp.**	MeOH-Leaf	6.37 ± 0.12	27.58***	3.33 ± 0.12	65.02***	8.60 ± 0.00
	MeOH-Stick	6.34 ± 0.06	27.57***	3.33 ± 0.12	65.02***	8.60 ± 0.00
	MeOH-Bark	8.60 ± 0.00	0.00	3.33 ± 0.12	65.02***	8.60 ± 0.00
	EtOH-Leaf	6.90 ± 0.10	20.99***	3.33 ± 0.12	65.02***	8.60 ± 0.00
	EtOH-Stick	6.27 ± 0.15	28.81***	3.33 ± 0.12	65.02***	8.60 ± 0.00
	EtOH-Bark	6.83 ± 0.15	21.81***	3.33 ± 0.12	65.02***	8.60 ± 0.00
	BW-Leaf	6.77 ± 0.06	22.63***	3.33 ± 0.12	65.02***	8.60 ± 0.00
	BW-Stick	6.83 ± 0.29	21.81***	3.33 ± 0.12	65.02***	8.60 ± 0.00
	BW-Bark	7.90 ± 0.00	8.64***	3.33 ± 0.12	65.02***	8.60 ± 0.00

Table 1. Contd.

***Rhizopus* sp.**	MeOH-Leaf	3.83 ± 0.06	16.67***	2.67 ± 0.06	45.83***	4.50 ± 0.00
	MeOH-Stick	3.60 ± 0.00	22.50***	2.67 ± 0.06	45.83***	4.50 ± 0.00
	MeOH-Bark	3.07 ± 0.12	35.83***	2.67 ± 0.06	45.83***	4.50 ± 0.00
	EtOH-Leaf	3.70 ± 0.00	20.00***	2.67 ± 0.06	45.83***	4.50 ± 0.00
	EtOH-Stick	3.20 ± 0.00	32.50***	2.67 ± 0.06	45.83***	4.50 ± 0.00
	EtOH-Bark	3.00 ± 0.00	37.50***	2.67 ± 0.06	45.83***	4.50 ± 0.00
	BW-Leaf	3.37 ± 0.06	28.334***	2.67 ± 0.06	45.83***	4.50 ± 0.00
	BW-Stick	3.32 ± 0.03	29.58***	2.67 ± 0.06	45.83***	4.50 ± 0.00
	BW-Bark	3.27 ± 0.06	30.83***	2.67 ± 0.06	45.83***	4.50 ± 0.00
Tricoderma koningii	MeOH-Leaf	6.90 ± 0.17	20.99***	4.33 ± 0.15	52.68***	8.60 ± 0.00
	MeOH-Stick	8.27 ± 0.06	4.14**	4.33 ± 0.15	52.68***	8.60 ± 0.00
	MeOH-Bark	8.07 ± 0.06	6.58***	4.33 ± 0.15	52.68***	8.60 ± 0.00
	EtOH-Leaf	8.17 ± 0.06	5.35***	4.33 ± 0.15	52.68***	8.60 ± 0.00
	EtOH-Stick	7.37 ± 0.12	15.23***	4.33 ± 0.15	52.68***	8.60 ± 0.00
	EtOH-Bark	8.00 ± 0.00	7.41***	4.33 ± 0.15	52.68***	8.60 ± 0.00
	BW-Leaf	8.60 ± 0.00	0.00	4.33 ± 0.15	52.68***	8.60 ± 0.00
	BW-Stick	8.60 ± 0.00	0.00	4.33 ± 0.15	52.68***	8.60 ± 0.00
	BW-Bark	8.60 ± 0.00	0.00	4.33 ± 0.15	52.68***	8.60 ± 0.00

*Significant at $p< 0.05$, **Significant at $p< 0.01$, ***Significant at $p< 0.001$ according to control.
[a]The growth of fungal species is given as mean ± standard deviation of three replicates.
MeOH: Methanol, EtOH: Ethanol and BW: Boiling water.

Table 2. Contents (µg/g freeze dry weight) of phenolic compounds in leaf, stick and bark extracts of *B. racemosa*.

Sample extract	Content (µg/g)										
	Phenolic acid								Flavonoid		
	GA	CA	SYA	FA	SA	N	M	Q	R	L	K
Leaf	171.81	-	-	65.80	-	62.94	-	-	59.1	10.29	5.75
Stick	103.53	-	-	-	-	51.17	-	-	-	5.22	-
Bark	56.92	-	-	25.67	-	56.92	-	-	-	-	-

- Not detectable.
GA: gallic acid, CA: caffeic acid, SYA: syringic acid, FA: ferrulic acid, SA: salicylic acid, N: naringin, M: myricetin, Q: quercetin, R: rutin, L: luteolin, and K: Kaempferol.

tion about *B. racemosa*, based on its antimicrobial potential and chemical profiling that has never been reported. The antifungal activity of *B. racemosa* may be attributed to the various phytochemical constituents present in the crude extract. The purified components may have even more potency with respect inhibition of microbes. Further work on the types of phytoconstituents and purification of individual groups of bioactive components can reveal the exact potential of the plant to inhibit several pathogenic microbes and encourage in developing a novel broad spectrum antimicrobial herbal formulation in future.

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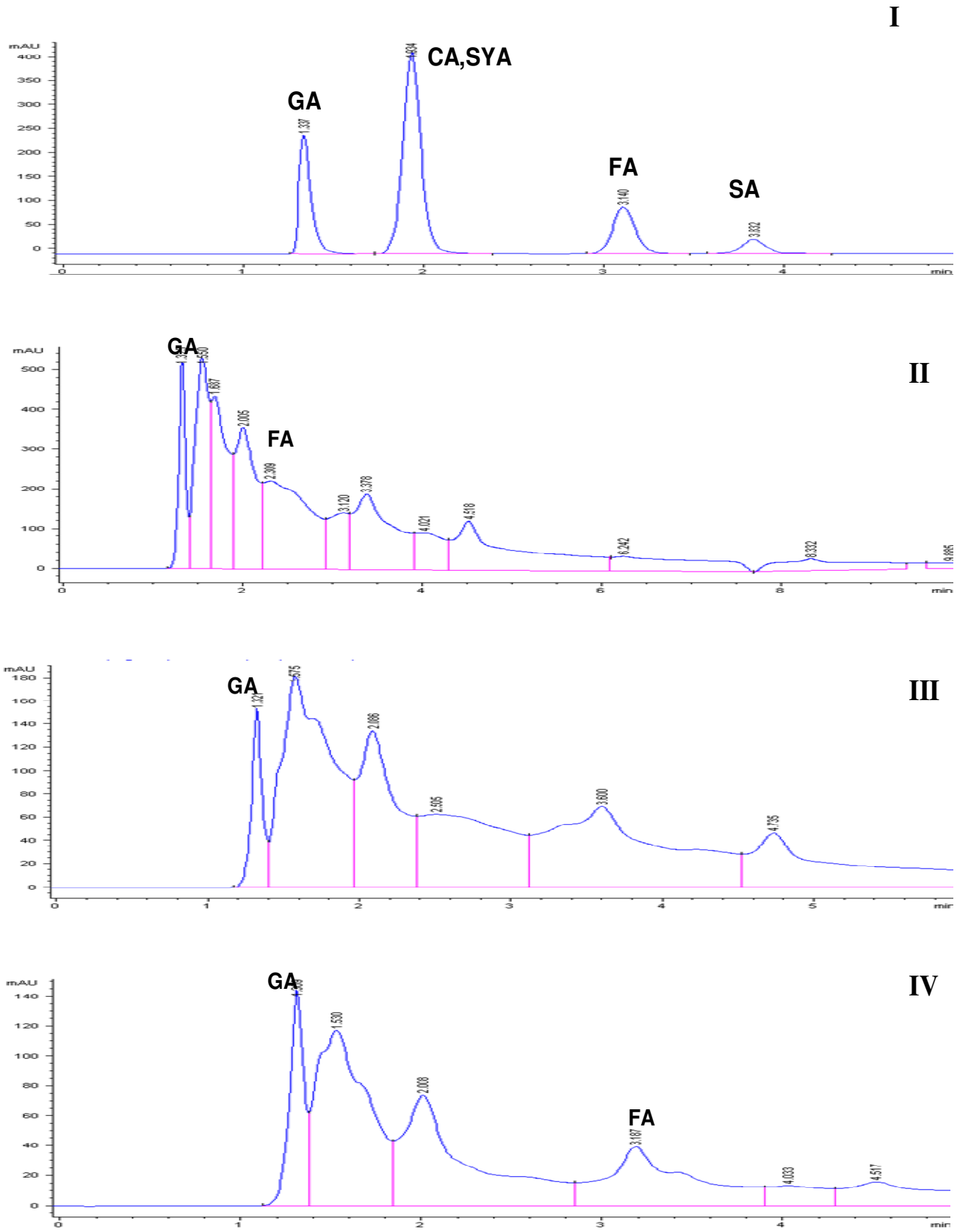

Figure 1. HPLC profiles of mixed standard phenolic acids (I), leaf (II), stick (III) and bark (IV) extracts of *Barringtonia racemosa* at 280 nm. GA: Gallic acid, CA: caffeic acid, SYA: syringic acid, FA: ferrulic acid, and SA: salicylic acid.

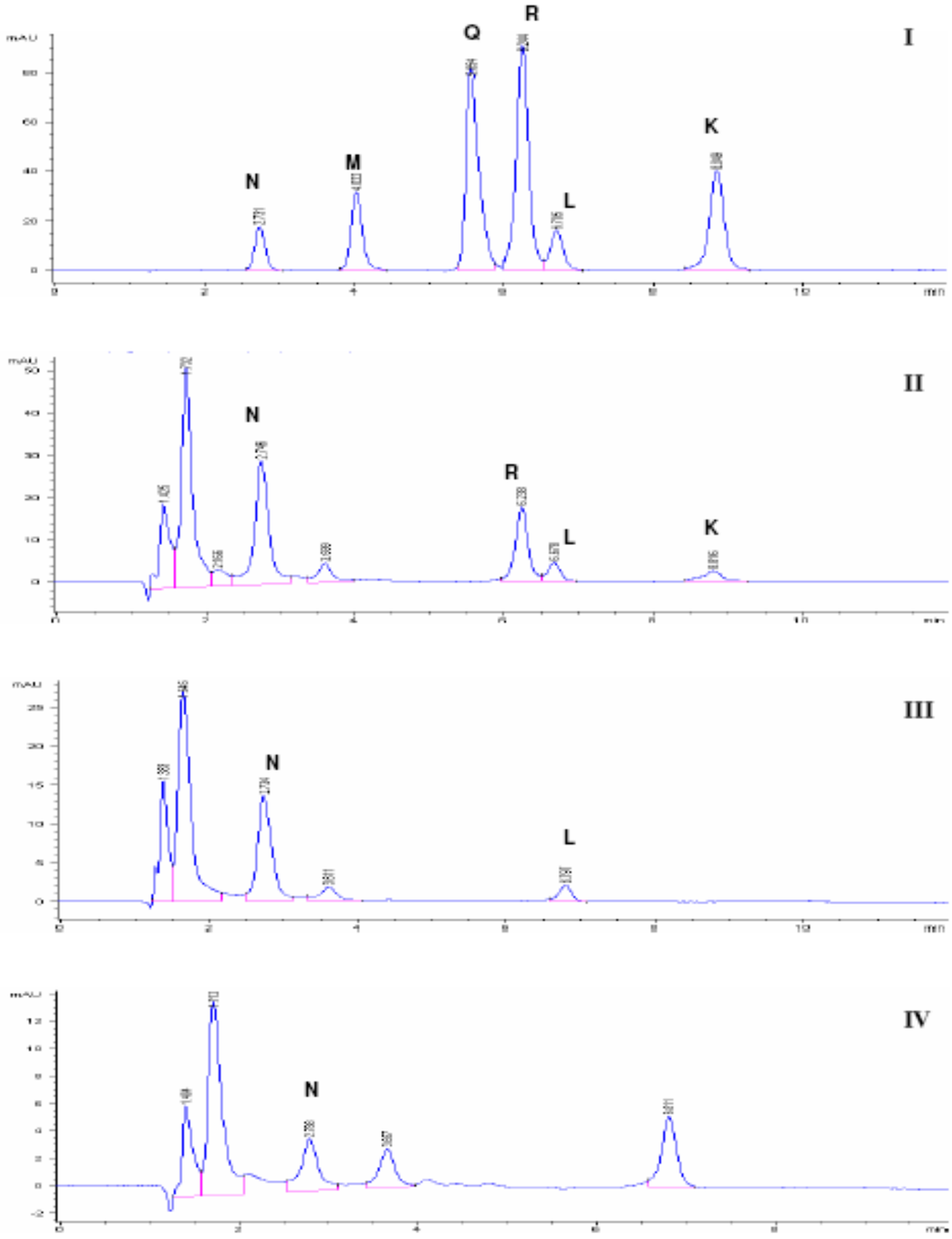

Figure 2. HPLC profiles of mixed standard flavonoids (I), leaf (II), stick (III) and bark (IV) extracts of *Barringtonia racemosa* at 365 nm. N: Naringin, M: myricetin, Q: quercetin, R: rutin, L: luteolin, and K: kaempferol.

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