Phytochemical Analysis and Anti-Inflammatory Effect of Kenaf and Roselle Seeds

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ABSTRACT

Introduction: Both kenaf (Hibiscus cannabinus) and roselle (Hibiscus sabdariffa) belong to the Malvaceae family. Methods: In this study, the phytochemical analysis and anti-inflammatory activity of kenaf seed oil (KSO), kenaf seed extract (KSE), roselle seed oil (RSO) and roselle seed extract (RSE) were investigated. Results: The flavonoids content present in the roselle seed oil (RSO), roselle seed extract (RSE), kenaf seed oil (KSO) and kenaf seed extract (KSE) ranged from 52.94±7.31 mg catechin/100g of sample (KSE) to 290.05±12.04 mg catechin/100 g of (RSE); phenolic content ranged from 108.46±6.40mg GAE/ 100g of sample (RSO) to 229.65±7.91 mg GAE/ 100g of sample (RSE); saponin content ranged from 68.14±3.46 mg saponin/ 100 g of sample (KSO) to 98.50±2.44 mg saponin/ 100g of sample (RSE); terpenoid content ranged from 148.76±9.69 mg linalool/100g of sample (KSO) to 294.74±16.14 mg linalool/100g of sample (RSE); and alkaloid content ranged from 17.40±1.346%/g (KSO) to 46.95±1.792%/g (RSE). The results showed that KSE, RSO and RSE significantly inhibited (p<0.05) inflammation compared to the control. Conclusion: The present study demonstrates that KSE, RSO and RSE exhibit potent anti-inflammatory property and offer potential for use as a therapeutic regiment in managing inflammatory conditions.

Key words: Alkaloids, arachidonic acid (AA) induced paw edema, carrageenaninduced paw edema, flavonoids, histamine induced paw edema, phenolics, saponins, terpenoids

INTRODUCTION

Kenaf (Hibiscus cannabinus L.) is a fibre plant native to East-central Africa where it has been grown for several thousand years for food and fibre. The plant's active components include tannins, saponins, polyphenolics, alkaloids, essential oils, and steroids which have long been prescribed in traditional folk medicine in Africa and India (Kobaisy et al., 2001). Kenaf seed

contains 9.6% moisture, 6.4% ash, 20.4% oil, 21.4% nitrogenous matter and 12.9% crude fibre. Kenaf seeds yield vegetable oil that is edible for human consumption (Nyam *et al.*, 2009). Kenaf seed oil contains vitamin E that is high in antioxidants, β -sitosterol and alpha-linolenic acid (ALA). The essential omega-3 fatty acid has been proven to have anti-inflammatory and antithrombotic activity (Ruiz *et al.*, 2002)

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and is known to be a chemopreventive agent. The chemopreventive effect of the extract is attributed to the action of various bioactive compounds such as vitamin E, phytosterol, alpha-linolenic acid and other antioxidants that are present in kenaf seed SFE extract (Nyam *et al.*, 2009).

Roselle is often used for medicinal purposes, especially in alternative medicine. Recent scientific work has established the anti-inflammatory effect of the dried flower extract of Hibiscus sabdariffa (Dafallah & Al-Mustafa, 1996) and the anti-mutagenic effect of the dried calyx extract (Chewonarin et al., 1999). Roselle seed oil belongs to the linoleic/ oleic category with its most abundant fatty acids being C18:2 (40.1%), C18:1 (28%), C16:0 (20%), C18:0 (5.3%), and C19:1 (1.7%). The major sterols present include beta-sitosterol (71.9%),campesterol (13.6%), delta-5-avenasterol (5.9%) and cholesterol (1.35%). Total tocopherols were detected at an average concentration of 2000 mg/kg, including alpha-tocopherol (25%), gamma-tocopherol (74.5%), and delta-tocopherol (0.5%) (Nyam et al., 2009). According to Meraiyebu et al. (2013), the methanolic extract of Hibiscus sabdariffa also exhibits therapeutic properties that are significant at high treatment does (500mg/ kg). The therapeutic effect of this extract has encouraged its use in the treatment of inflammation.

The present study was conducted to quantify the phytochemicals content, such as phenolic, flavonoid, saponin, terpenoid and alkaloid in kenaf and roselle seeds. Besides, the present study also evaluated the anti-inflammatory effect of the oral administration of the oil and extracts of kenaf and roselle seeds on male Sprague dawley rats.

METHODS

Materials

An amount equivalent to 20 kg of dried kenaf and roselle seeds were obtained from

the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia and Department of Agriculture Plantation, Rembau, Negeri Sembilan, Malaysia, respectively.

Kenaf (KSO) and Roselle Seed Oil (RSO) Extraction

The kenaf and roselle seed oils were extracted according to the methods by Nyam *et al.* (2009).

Kenaf (KSE) and Roselle Seed Extract (RSE) with Pulsed Ultrasound-Assisted Extraction (PUAE)

The kenaf (KSE) and roselle seed extracts (RSE) were obtained according to the methods of Wong *et al.* (2014).

Quantification of phytochemicals Total flavonoids content (TFC)

Aluminium trichloride colorimetric method (Ogbunugafor, Eneh & Ozumba, 2011) was applied to determine the total flavonoids content of KSO, KSE, RSO and RSE.

Total phenolic content (TPC)

Phenolic compounds in KSO and RSO were extracted using extraction method of Nyam *et al.* (2013). Total phenolic content was estimated using Folin-Ciocalteu assay, based on the method described by Wong *et al.* (2014).

Total saponin content

Total saponin content was determined based on the method of Abramovic & Abram (2006).

Total terpenoid content

Total terpenoids was determined based on the method described by Mboso *et al.* (2013).

Total alkaloids content

Total alkaloids content was determined based on the method described by Ghorai et al. (2012).

Experimental animals

Male Sprague dawley rats weighing 200-250g were obtained from the institutional animal house of Universiti Kebangsaan Malaysia (UKM), Bangi, Malaysia. All of the animals were in healthy condition, with 3 housed in each transparent plastic cage under standard laboratory conditions (temperature at 22 ± 2 °C, and humidity at 75 ± 5 %) with a 12 h dark-light cycle. The animals were fed with meat free rat and mouse diet (as normal standard diet) purchased from Speciality Feeds (Glen Forrest, Western Australia) and given water ad libitum. The animals were allowed to acclimatise for two weeks before the treatments started. Approval for experiment procedures was received from the Faculty's adhoc ethics committee of UCSI University, Cheras, Malaysia (Proj-FAS-EC-13-039). The experiments were carried out in strict, accordance with animal ethical committee guidelines for the care and use of laboratory animals.

Experimental design

The rats were divided into 6 experimental groups (4 sample groups, 1 control group and 1 indomethacin group), with 6 rats each (n=6), labeled and weighed. The weight of each rat was recorded.

Histamine induced paw edema

The diameter (cm) of the rat's right hind paw was measured using a vernier caliper. Each member of the group was fed with a different sample through a feeding tube as shown in Table 1. After 1 h, 0.05 ml of 1 % histamine was injected into the plantar side of the right hind paw of all the 6 experimental groups of rats. The paw diameter was measured by using a ruler; at 1-h intervals after the injection for 5 h and recorded. The results of the four sample groups were compared with the results from control and indomethacin group.

Carrageenan induced paw edema

The diameter (cm) of the rat's right hind paw was measures using a vernier caliper. Each member of the group was fed with a different sample as shown in Table 1 through tube feeding. After 1 h, 0.1 ml of 1% carrageenan was injected into the plantar side of the right hind paw of all the six experimental groups of rats. Paw thickness was measured just before the carrageenan injection, that is, at "0 h" and then at 1st, 2nd, 3rd, 4th, and 5th h after carrageenan injection. The results of the four sample groups were compared with the results from control and indomethacin group.

Arachidonic acid induced paw edema

The diameter (mm) of the rat's right hind paw was measured using a vernier caliper. Each member of the group was fed with a different sample as shown in Table 1 through tube feeding. After 1 h, 0.1 ml of 0.5% arachidonic acid in acetone was injected into the plantar side of the right hind paw of all the six experimental groups of rats. The paw diameter was measured by using a vernier caliper at 1-h intervals after the injection and recorded. The results of the four sample groups were

Table 1. Experimental treatment of respective groups

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Groups	Treatment (per oral)
Group 1 (Control group)	Distilled water (500mg/kg)
Group 2 (KSO)	Kenaf seed oil (500mg/kg)
Group 3 (KSE)	Kenaf seed extract (500mg/kg)
Group 4 (RSO)	Roselle seed oil (500mg/kg)
Group 5 (RSE)	Roselle seed extract (500mg/kg)
Group 6 (Indomethacin)	Indomethacin (5mg/kg)

compared with the results from control and indomethacin group.

Statistical Analysis

Analysis of variance (ANOVA) was carried out and the average values were compared with Fisher's Multiple Comparison Test. Differences were considered statistically significant at P<0.05. All statistical analyses were performed using Minitab 15 for Windows.

RESULTS AND DISCUSSION

Total phenolic content

Results showed that the samples obtained by using different solvents and extraction methods had different concentrations of total phenolic contents (Table 2). The differences observed in yields could be related to the polarity of the particular solvent used in the extraction. Nyam et al. (2012) reported that phenolic compounds from seeds are better extracted with ethanol than with hexane because most of the phenolic compounds are hydrophilic. Using polar solvent to extract phenolic compounds from the seeds was found to be more efficient. In KSE and RSE samples, both of which were extracted using ultrasonic extraction, high phenolic content was obtained. However, in the case of KSO and RSO, polyphenol reduction occurred as a result of high temperature in the soxhlet extraction leading to lower total phenols in oil. Further, ultrasound can disrupt cell walls, enhancing the mass transfer of cell contents, and resulting in a higher yield of phenolic content within a shorter period of time compared to the soxhlet method. Furthermore, for the extraction of KSE and RSE, a bipolar solvent was used, i.e. 80% ethanol; the presence of water in the extraction facilitates the release of phenolic compounds, which are hydrophilic (Wong et al., 2014).

Studies have shown that gene expression for pro-inflammatory factors can be suppressed by phenolics (Whent *et al.*,

2013). Polyphenols have been reported to exhibit significant inhibitory activities on nitric oxide implicated in physiological and pathological processes as chronic inflammation (Joseph *et al.*, 2009; Cheol-Hyun *et al.*, 2014).

Total flavonoid content

KSE and RSE contained higher total flavonoid content compared to KSO and RSO. KSE and RSE were extracted by using a binary solvent system whereas KSO and RSO were extracted by using a mono solvent system. Previous findings reported that the binary solvent system yields a higher flavonoids content compared to the mono solvent system (Wang et al., 2008). When 80% ethanol was used as solvent, the water content in the solvent helped to dissolve the flavonoids (Bohm, 1999). Several studies have reported that a variety of flavonoid molecules exhibit antiinflammatory activity both, in vitro and in various animal models of inflammation (Wang et al., 2008).

Total saponin content in Kenaf and Roselle seeds

The saponin content in KSE and RSE was higher compared to the saponin content in KSO and RSO. Ultrasonic energy has a higher rate of extraction of triterpenoid saponins. This is due to its higher diffusion rates by virtue of eliminating mass transfer resistances. During ultrasonic extraction, the physical separation is further enhanced by the restricted stirring occurring as a consequence of cavitation. The combination of this stirring effect and the repeated washing of the triterpenoid saponins with solvents are far superior to the simple washing procedure in soxhlet extraction (Jadhav et al., 2009). Saponin derivatives are potent inhibitors of acute inflammation, especially in the second phase of the carrageenan-induced rat paw edema (Gephiremen et al., 2005)

Table 2. Phytochemical analysis of kenaf and roselle seeds

	Alkaloid (%)	17.40±1.35 ^d	27.80±1.83b	17.40±1.35°	46.95±12.74ª
	Linalool (mg linalool/ 100 g of sample)	148.76±9.69°	294.74 ± 16.14^{b}	$188.95\pm13.30^{\circ}$	381.35±12.74ª
	Saponin (mg saponin/ 100 g of sample)	68.14±3.46°	89.89±3.43b	54.10 ± 2.80^{d}	98.50±2.44ª
	TFC (mg catechin/ 100 g of sample)	52.94±7.31 ^d	165.05±11.53 ^b	95.16±8.75°	290.05±8.75ª
,	TPC (mg GAE/ 100g of sample)	108.46±6.40 ^d	170.72 ± 16.63^{b}	$136.71\pm3.53^{\circ}$	229.65±7.91ª
	Sample	KSO	KSE	RSO	RSE

Mean \pm standard deviation (n=4) with different superscript letters abcd indicate significant differences (p<0.05) between the same column. KSO- kenaf seed oil; KSE- kenaf seed extract; RSO- roselle seed oil; RSE- roselle seed extract

Table 3. Percentage increase in paw diameter for different treatment groups in histamine induced edema model and percentage of inhibition.

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Treatment	Dose		% of incre	% of increase in paw diameter (% inhibition)	inhibition)	
		1st h	2nd h	3 rd h	4 th h	5 ^н Л
Saline (Control)	500mg/kg	25.757±1.311	45.707± 4.171	32,930±12.730	40.488± 6.643	34.930± 10.819
Indomethacin 5mg/kg	5mg/kg	11.150±3.902* (56.71%)	23.080 ±0.000* (49.50%)	20.510 ±4.450* (37.72%)	17.950± 8.885* (55.67%)	15.383±7.695 (55.96%)
KSO	500mg/kg	19.393±1.051 (24.71%)	29.090±1.576* (36.36%)	48.790±11.870* (-48.16%)	32.423± 6.702* (19.92%)	29.393±10.923 (15.85%)
RSO	500mg/kg	7.507±7.697* (70.85%)	15.017±13.643* (67.15%)	15.020±15.400* (54.39%)	9.890± 8.582* (75.57%)	4.943± 4.290* (85.85%)
KSE	500mg/kg	0.000± 0.000* (100%)	9.047±10.135* (80.21%)	14.130 ±7.380* (57.09%)	13.970± 0.554* (65.5%)	9.367± 4.270* (73.18%)
RSE	500mg/kg	11.113±7.696* (56.85%)	13.333± 6.665* (70.84%)	15.550± 3.850* (52.78%)	11.110± 3.845* (72.56%)	9.367± 4.270* (73.18%)

Mean \pm SD (n=6). *p < 0.05 as compared to the control; % of inhibition is given in parentheses.

Total terpenoid content

Generally, samples the extracted contained more terpenoid compared to the oil samples. This is consistent with our results; KSE and RSE, which were extracted by using ultrasonic extraction, higher terpenoid content contained compared to KSO and RSO. In ultrasonic extraction, the cavitation effect caused by ultrasonic radiation facilitates penetration of solvent into the cells, resulting in the organic compounds passing easily into the extrahent.

Total alkaloid content

From our results, it is found that the samples extracted by hexane, that is, KSO and RSO, had lower alkaloid content compared to KSE and RSE, which were extracted by 80% ethanol. This indicates the alkaloids present in the samples were mostly polar, and that more alkaloids were being extracted by using 80% ethanol, a polar solvent.

The lengthy process involved in Soxhlet extraction, can cause alkaloid degradation. Besides, it has been verified that the presence of water increases the extractive power of the solvents and speeds up the extraction process (Wang *et al.*, 2006). KSE and RSE contained higher alkaloid content due to the water that was present in the 80% ethanol solvent.

Animal inflammatory model Histamine induced paw edema

The paw diameter of each experimental rat was measured and recorded at 0 hour, 1st hour, 2nd hour, 3rd hour, 4th hour and 5th hour. The percentage increase in paw diameter and inhibition percentage were calculated and are summarised in Table 3. RSO, KSE, RSE and indomethacin (a prototype of a non-steroidal antiinflammatory drug) showed significant inhibitions (p<0.05) at both early and late phases compared with the control group. The maximum inhibition of RSO was at the 4th and 5th hour (75.57 and 85.85%). As for KSE, the maximum inhibition was at 1st and 2nd hour (100.00 and 80.27%). In RSE, the maximum inhibition was at the 4th and 5th hour (72.56 and 73.18%). Besides, RSO, KSE and RSE also showed higher % of inhibition when compared with indomethacin treatment. The inhibition

Table 3. Percentage increase in paw diameter for different treatment groups in histamine induced edema model and percentage of inhibition

Treatment	nt Dose % of Increase in Paw Diameter (% inhibition)					
		$1^{st}h$	2 nd h	$3^{nl}h$	4 th h	5 th h
Saline (Control)	500mg/kg	25.757±1.311	45.707± 4.171	32.930± 12.730	40.488± 6.643	34.930± 10.819
Indomethacin	5mg/kg	11.150±3.902* (56.71%)	23.080 ±0.000* (49.50%)	20.510 ±4.450* (37.72%)	17.950± 8.885* (55.67%)	15.383± 7.695 (55.96%)
KSO	500mg/kg	19.393±1.051 (24.71%)	29.090± 1.576* (36.36%)	48.790± 11.870* (-48.16%)	32.423±6.702* (19.92%)	29.393±10.923 (15.85%)
RSO	500mg/kg	7.507± 7.697* (70.85%)	15.017± 13.643* (67.15%)	*15.020± 15.400* (54.39%)	9.890± 8.582* (75.57%)	4.943± 4.290* (85.85%)
KSE	500mg/kg	0.000± 0.000* (100%)	9.047± 10.135* (80.21%)	14.130 ±7.380* (57.09%)	13.970± 0.554* (65.5%)	9.367± 4.270* (73.18%)
RSE	500mg/kg	11.113± 7.696* (56.85%)	13.333± 6.665* (70.84%)	15.550± 3.850* (52.78%)	11.110± 3.845* (72.56%)	9.367± 4.270* (73.18%)

Mean \pm SD (n=6). *p < 0.05 as compared to the control; % of inhibition is given in parentheses.

of the edema produced by histamine may indicate that the extract exhibited its antiinflammatory action by means of either inhibiting the synthesis, release or action of inflammatory mediators of histamine, or antagonise the activity of the mediators after their release (Ganesh, Vasudevan & Kamalakannan, 2008). KSO did not show significant inhibition when compared with the control group. Therefore, KSO pretreatment was not effective in histamine induce paw edema model.

The activity noticed in RSO, KSE and RSE may be due to the presence of phytochemicals. Previous findings report that RSO, KSE and RSE generally contain higher concentrations of phytochemicals such as flavonoids, phenols and saponin, alkaloids and terpenoids compared to KSO. The additional presence of phytochemicals, especially phenolic and terpenoid content may contribute somewhat major antiedematous activity.

Carrageenan induced paw edema

Injection of carrageenan into the hind paw induced a progressive edema reaching its maximum at the 3rd h. For the control

group, the edema reached a maximum (48.74% increment in paw diameter) at 3rd h and remained elevated until the last measurement at the 5th h (Table 4). As for the other treatment groups like indomethacin, RSO, KSE and RSE, the treatment showed a bell shaped effect of down-regulating carrageenan-induced paw swelling. The edema reached a maximum at the 3rd h but did not remain elevated as the % of increment in paw diameter decreased at 4th and 5th h.

In this model of inflammation, RSE had very consistent anti-inflammatory activity and showed significant inhibitions (p<0.05) at both early and late phases compared with the control group. RSE treatment reached a maximum inhibition of 90.18 and 87.97% at 4th and 5th h. Since RSE had shown significant inhibition at both early and late phases, RSE inhibited the synthesis and release or antagonised the action of inflammatory mediators like histamine and bradykinins (Ganesh et al., 2008). It can also be said to be an inhibitor of the cycloxygenase enzyme and lipoxygenase. Cycloxygenase is a key enzyme required for the conversion of arachidonic acid to

Table 4. Percentage increase in paw diameter in different treatment groups in carrageenan induced edema model and percentage of inhibition

Treatment	Dose	% increase in paw diameter (% inhibition)					
		1 st h	2 nd h	3 rd h	4 th h	5 ^н h	
Saline (Control)	500mg/kg	11.580±5.716	28.787±6.559	48.740± 6.532	48.483±2.627	48.483± 2.627	
Indomethacin	5mg/kg	14.643±10.935 (26.45%)	23.233±11.368 (19.29%)	33.673±2.327 (30.91%)	25.757±1.311* (46.87%)	19.950±4.438* (49.57%)	
KSO	500mg/kg	13.770±7.332 (18.91%)	30.200±3.035 (4.08%)	36.933±7.237 (24.22%)	30.570±12.550 (36.95%)	16.800±6.226* (57.54%)	
RSO	500mg/kg	9.707±3.979 (16.17%)	17.033±3.846 (40.83%)	12.453±9.203* (74.45%)	4.943±4.290* (89.80%)	2.563±4.440* (93.52%)	
KSE	500mg/kg	10.000±10.000 (13.64%)	16.667±5.774 (42.10%)	23.333±5.774* (52.13%)	20.000±10.000* (58.75%)	10.000±0.000* (74.72%)	
RSE	500mg/kg	9.523±4.128 (17.76%)	9.523±4.128* (66.92%)	14.287±7.145* (70.69%)	4.760±4.122* (90.18%)	4.760±4.122* (87.97%)	

Mean \pm SD (n=6). *p < 0.05 as compared to the control; % of inhibition is given in parentheses.

prostaglandins during acute inflammation (Williams, Mann & DuBois, 1999).

Indomethacin showed signification inhibition at the late phase (4th and 5th h) compared with the control group. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-anti-inflammatory agent drugs like hydrocortisone, phenylbutazone and indomethacin. RSO and KSE showed similar significant inhibition patterns as indomethacin with a significant inhibition of RSO and KSE being shown at 3rd, 4th and 5th hours. Indomethacin is a cycloxygenase inhibitor. Hence, RSO and KSE can be said to inhibit the cycloxygenase enzyme but lipoxygenase inhibitors also possess significant anti-inflammatory activity against carrageenan induced paw edema. Therefore, inhibition of carrageenan induced paw edema by crude extract could also be due to its inhibitory activity on the lipoxygenase enzyme (Kumari et al., 2012). Besides, RSO, KSE and RSE also showed a higher per centage of inhibition compared with indomethacin treatment. KSO did not show significant inhibition compared with the control group, which means that KSO pre-treatment was not effective on the rats in this carrageenan induce paw edema model. This might be related to the lower phytochemical content in KSO as the effectiveness of the anti-inflammatory property might be affected by the phytochemical concentration.

Arachidonic acid induced paw edema

In the control group and KSO group, edema reached a maximum increment in paw diameter (45.957%) and (33.787%) at the 4th h and remained elevated until the last measurement at 5th h(Table 5). As for RSO group, maximum edema was reached at the 3rd h. In the indomethacin group, edema reached maximum percentage of increase in paw diameter at the 2nd hour, reaching (30.3%) and showed a bell shaped effect of down-regulating arachidonic acid induced paw swelling.

In this inflammation model, KSE and RSE showed very consistent antiinflammatory activity with significant inhibition (p<0.05) throughout the 5 h compared with the control group. Indomethacin and RSO showed significant inhibition at a later stage, 4^{th} and 5^{th} h.

Table 5. Percentage increase in paw diameter in different treatment groups in AA induced edema model and percentage of inhibition

Treatment	Dose	% increase in paw diameter (% inhibition)					
		1 st h	2 nd h	3 rd h	4 th h	5 ^н h	
Saline (Control)	500mg/kg	21.210±13.885	31.563±5.882	31.563±5.882	45.957±11.168	45.957±11.168	
Indomethacin	5mg/kg	11.363±4.611 (46.43%)	30.300±5.248 (4.00%)	27.270±9.090 (13.60%)	21.210±5.248* (53.85%)	21.210±5.248* (53.85%)	
KSO	500mg/kg	15.503±6.279 (26.91%)	21.313±5.421 (32.47%)	30.453±5.694 (3.52%)	33.787±7.824 (26.48%)	33.787±7.824 (26.48%)	
RSO	500mg/kg	16.670±0.000 (78.60%)	18.890±1.923 (40.15%)	20.000±0.000 (36.63%)	20.000±0.000* (56.48%)	20.000±0.000* (56.48%)	
KSE	500mg/kg	7.690±0.000 (63.74%)	7.690±0.000* (75.64%)	7.690±0.000* (75.64%)	7.690±0.000* (83.33%)	7.690±0.000* (83.33%)	
RSE	500mg/kg	0.000±0.000* (100%)	4.763±8.250* (84.91%)	11.750±4.399* (62.77%)	13.970±0.554* (69.60%)	13.970±0.554* (69.60%)	

Mean \pm SD (n=6). *p < 0.05 as compared to the control; % of inhibition is given in parentheses

As for KSO, no significant inhibition was shown.

AA-induced edema is correlated with the early phase of inflammatory pathology and involves the action of vasoactive amines, such as histamine, serotonin and kinins on vascular permeability. AA is a precursor of inflammatory eicosanoids, such as prostaglandin E2 and leukotrienes (produced via COX-1, COX-2 and 5-LOX enzymes). COX and LOX inhibitors classically cause significant reduction in AA-induced ear edema (Gabor, 2000). AA metabolites are also involved in mast cells degranulation, which is accompanied histamine release. Consequently, antihistamines are also able to reduce AA-induced edema. Hence, KSE and RSE are COX and LOX inhibitors and antihistamine. As for RSO and indomethacin, they are COX and LOX inhibitors but not anti-histamine as they only exhibit significant inhibition at a later stage.

CONCLUSION

The present results demonstrated that kenaf seed oil (KSO), roselle seed oil (RSO), kenaf seed extract (KSE) and roselle seed extract (RSE) contain significant levels of phytochemicals such as phenol, flavonoid, saponin, terpenoid and alkaloid. The present study also showed that RSO, KSE and RSE exhibited anti-inflammatory effects in edema induced rats. Hence, it is concluded that the phytochemical content may affect the anti-inflammatory activity of the samples studied.

REFERENCES

- Abramovic H & Abram V (2006). Effect of added rosemary extract on oxidative stability of Camelina sativa oil. *Acta Agric Slovenica* 87: 255-261.
- Bohm BA (1999). Introduction to flavonoids. Hardwood Academic Publisher, Italy.
- Cheol-Hyun K, Min-Kyung P, Sung-Kyu K & Young-Hee C (2014). Antioxidant capacity

- and anti-inflammatory activity of lycopene in watermelon. *Inter J Food Sci Technol* DOI: 10.1111/ijfs.12517.
- Chewonarin T, Kinouchi T, Kataoka K, Arimochi H, Kuwahara T, Vinitketkumnuen U & Ohnishim, Y (1999). Effects of roselle (Hibiscus sabdariffa Linn.), a Thai medicinal plant, on the mutagenicity of various known mutagens in Salmonella typhimurium and on formation of aberrant crypt foci induced by the colon carcinogens azoxymethane and 2-amino-1-methyl-6-phenylimidazol [4,5-b] pyridine in F344 rats. Food Chem Toxicol 37: 591-601.
- Dafallah AA & Al-Mustafa Z (1996). Investigation of the anti-inflammatory activity of Acacia nilotica and Hibiscus sabdariffa. *Am J Chin Med* 24: 263-269.
- Gábor M (2000). Mouse ear inflammation models and their pharmacological applications, pp. 407-409. Akadémiai Kiadó, Budapest.
- Ganesh M, Vasudevan M & Kamalakannan K (2008). Anti-inflammatory and analgesic effects of Pongamia glabra leaf gall extract. *Pharmacologyonline* 1:497-512.
- Gepdiremen A, Mshvildadze V, Süleyman H & Elias R (2005). Acute anti-inflammatory activity of four saponins isolated from ivy: alpha-hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F in carrageenan-induced rat paw edema. Inter J Phytother Phytopharmacol 12: 440-444.
- Ghorai N, Chakraborty S, Gucchait S, Saha SK & Biswas S (2012). Estimation of total terpenoids concentration in plant tissues using a monoterpene, linalool as standard reagent. *Protocol Exchange* doi:10.1038/protex.2012.055.
- Jadhav D, Rekha BN, Gogate PR & Rathod VK (2009). Extraction of vanillin from vanilla pods: A comparison study of conventional soxhlet and ultrasound assisted extraction. *J Food Eng* 93: 421-426.
- Joseph S, Sabulal BV, George TP & Smina Hanardhanan KK (2009). Antioxidative and anti-inflammatory activities of the chloroform extract of Ganoderma lucidum found in South India. Sci Pharm 77: 111-121.

- Kobaisy M, Tellez MR, Webber CL, Dayan FE, Schrader KK & Wedge DE (2001). Phytotoxic and fungitoxic activities of the essential oil of kenaf (*Hibiscus cannabinus* L.) leaves and its composition. *J Agric Food Chem* 49: 3768-3771.
- Kumari STK, Lincy MP, Muthukumarasamy S & Mohan VR (2012). Anti-inflammatory activity of *Sarcostemma secamone* (L.) Bennet whole plant against carrageenan induced paw edema. *Biosci Discovery* 3: 288-291.
- Mboso OE, Eyong EU, Odey MO & Osakwe E (2013). Comparative phytochemical screening of *Ereromastax speciosa* and *Ereromastax polysperma*. J Nat Prod Plant Res 3: 37-41.
- Meraiyebu AB, Olaniyan OT, Eneze Anjorin YD & Dare JB (2013). Anti-inflammatory activity of methanolic extract of *Hibiscus sabdariffa* on carrageenan induced inflammation in wistar rat. *Inter J Pharm Sci Invent* 2: 9-11.
- Nyam KL, Tan CP, Lai OM, Long K & Che Man YB (2009). Physicochemical properties and bioactive compounds of selected seed oils. LWT- Food Sci Technol 42: 1396-1403.
- Nyam KL, Teh YN, Tan CP & Long K (2012). In vitro antioxidant activities of extract and oil from roselle (*Hibiscus sabdariffa* L.) seed against sunflower oil autoxidation. *Mal J Nutr* 18(2): 265-274.
- Ogbunugafor HA, Eneh FU & Ozumba AN (2011). Physico-chemical and antioxidant properties of Moringa oleifera seed oil. *Pakistan J Nutr* 10: 409-414.

- Ruiz ML, Castillo D, Dobson D, Brennan R & Gordon S (2002). Genotypic variation in fatty acid content of blackcurrant seed. *J Agric Food Chem* 50: 332-335
- Wang J, Sun B, Cao Y, Tian Y & Li X (2008). Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. *Food Chem* 106:804-810.
- Wang L, Tu YC, Lian TW, Hung JT, Yen JH & Wu MJ (2006). Distinctive antioxidant and anti-inflammatory effects of flavonols. *J Agric Food Chem* 54: 9798-9804.
- Whent M, Huang H, Xie Z, Lutterodt H & Yu L (2013). Phytochemical composition, anti-inflammatory, and antiproliferative activity of whole wheat flour. *J Agric Food Chem* 60: 2129-2135.
- Williams CS, Mann M & DuBois RN (1999). The role of cyclooxygenases in inflammation, cancer, and development. *Oncogene* 18: 7908-7916.
- Wong YH, Lau HW, Tan CP, Kamariah L & Nyam KL (2014). Binary solvent extraction system and extraction time effects on phenolic antioxidants from kenaf seeds (*Hibiscus cannabinus L.*) extracted by a pulsed ultrasonic-assisted extraction. The *Sci World J* Article ID 789346.