

Original Article

Polyphenol-enriched extract of oil palm fronds (*Elaeis guineensis*) promotes vascular relaxation via endothelium-dependent mechanisms

Mahinda Abeywardena¹ PhD, Irine Runnie² BSc, Mohd Nizar BSc,² Suhaila Momamed² and Richard Head¹

¹CSIRO, Health Sciences and Nutrition, Adelaide, South Australia, Australia

²Department of Food Science, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

Plant-based polyphenolic compounds have been reported to possess cardiovascular health benefits. Several dietary sources, including herbs and spices, fruits and vegetables, and tea and wine, contain an array of biologically active compounds that have been shown to be effective in retarding oxidation of low-density lipoproteins (LDL) and promoting vascular relaxation. In the present study four different plant sources, both edible and non-edible, were evaluated for potential activity. Organic extracts enriched in polyphenols were prepared from palm fronds (*Elaeis guineensis*); lemongrass (*Cymbopogon citrates*); papaya shoots (*Carica papaya*) and green chilli (*Capsicum frutescenes*) and tested for their ability to prevent *in vitro* oxidation of LDL, and for potential vascular relaxation actions. Rings of rat thoracic aorta and isolated perfused mesenteric vascular beds were mounted in organ baths, contracted using a half-maximal dose of noradrenaline and exposed to cumulative additions of test extracts. Palm frond extract resulted in considerable relaxation (>75%) in both preparations and was found to be endothelium-dependent as removal of endothelium or inhibition of endogenous nitric oxide (NO) led to a total loss in relaxant activity. Lemongrass extract caused a greater relaxation action in the mesenteric preparation compared to aortic rings, and appears to be mediated via NO-independent and non-prostanoid mechanisms. Of the extracts tested, palm fronds also demonstrated the highest antioxidant capacity, as determined by the ferric reducing activity/potential assay, and resulted in a significant delay ($P < 0.05$) in the oxidation of LDL. Collectively, these preliminary findings lend further support to the potential cardiovascular actions of plant polyphenols and also identify oil palm fronds as containing constituents that promote vascular relaxation via endothelium-dependent mechanisms.

Key words: Endothelium, oil palm, polyphenols, rat, vasorelaxation.

Introduction

The vascular endothelium is a monolayer of cells that lies between circulating blood and the smooth muscles of blood vessels and plays an important regulatory role. Accordingly, a healthy endothelium not only acts as a selective barrier against infiltration of various molecules into the underlying tissue, but also modulates vascular tone, controls inflammatory processes and cell growth, and maintains cardiovascular homeostasis through the production of an array of both relaxant and constrictor compounds.^{1,2} Endothelial dysfunction has been recognised as central to the development of many cardio- and cerebrovascular diseases over a long period of time, as well as triggering more acute vascular syndromes such as vessel spasm and thrombosis leading to ischaemia.^{2,3} Impaired vascular relaxation is commonly observed in several disease states with increased cardiovascular risk, such as hypertension, diabetes and atherosclerosis. In such conditions, in addition to a selective reduction of endothelium-derived vasorelaxant compounds (e.g., nitric oxide (NO) and prostacyclin (PGI₂)), increased

production of vasoconstrictor mediators (e.g., thromboxane (TxA₂) and free radicals) has been observed.^{2,3} Therefore, agents that restore impaired vascular relaxation can be regarded as beneficial.

While several pharmacological agents exist to preserve vascular function and promote vascular relaxation either directly or indirectly,^{2,4} recent reports also suggest that several naturally existing compounds, including specific polyunsaturated fatty acids and phytochemicals, may also share these actions.^{5,6} For example, the benefits of a Mediterranean diet rich in these dietary constituents as well as the protective actions of wine polyphenols (the 'French paradox') against the development of coronary heart disease (CHD) are now well recognised.^{8,9} In this regard it has been

Correspondence address: Dr Mahinda Abeywardena, CSIRO Health Sciences and Nutrition, PO Box 10041, Adelaide BC, SA 5000, Australia.

Tel: +61 8 8303 8889; Fax: +61 8 8303 8899

Email: mahinda.abeywardena@csiro.au

reported that several flavonoid and non-flavonoid antioxidants of plant origin, including tocopherols and tocotrienols, β -carotene, tea catechins, grape and wine polyphenols, prevent the oxidation of low-density lipoproteins (LDL) and may retard the development of CHD.¹⁰ In addition, most of these compounds have been shown to exert benefits on the vasculature by promoting relaxation and by modifying specific events in the inflammatory process.^{6,7,11}

In addition to the dietary sources that have been widely reported (including fruits, vegetables, wine and tea)^{7,12–14} there exists the possibility that plant-based polyphenols with potential health benefits may also be present in alternative crops grown for commercial purposes. In the present study, we evaluated the effects of polyphenol-enriched extracts of oil palm fronds (*Elaeis guineensis*) and several other plant materials for their ability to prevent *in vitro* oxidation of LDL, and for possible vascular-protective properties using isolated vascular tissue preparations.

Material and methods

Plant extracts

Four types of plant material were used in the present study: palm (*Elaeis guineensis*) fronds, lemongrass (*Cymbopogon citratus*) trunks, papaya (*Carica papaya*) shoots and green chilli (*Capsicum frutescens*) fruits. Oil palm fronds were collected from the plantation at the Universiti Putra Malaysia. Other plant materials were purchased from the local market (Serdang, Malaysia). The fresh materials were weighed, washed and cut into small pieces and oven dried at 40°C overnight. The dried material was ground using a blender and extracted three times with methanol (1:10 v/v). The pooled extracts were dried under vacuum using a rotary evaporator and the resultant waxy residue collected, freeze dried, flushed with nitrogen and stored at –20°C. For vascular function studies, a stock solution (100 mg/mL) was prepared using a 1:1 v/v mixture of methanol: saline and serially diluted. Aliquots (25–50 μ L) were added directly in a cumulative fashion to the bath (aortic rings) or injected intraluminally (mesenteric vascular bed). Vehicle controls were performed and found to be without effect.

Antioxidant capacity

The antioxidant capacity of various extracts was determined *in vitro* using the ferric reducing activity/potential (FRAP) assay, as described by Benzie and Strain.¹⁵ This assay is based on the reducing ability of an antioxidant sample to reduce Fe³⁺ to Fe²⁺ at low pH. Plant extracts were added to the FRAP reagents, incubated and the change in absorbance determined. The FRAP value for each extract was calculated using this information.

In vitro LDL oxidation

Low-density lipoprotein was isolated from human plasma by ultracentrifugation and subjected to oxidation in the presence of copper sulphate at 37°C for up to 3 h.¹⁶ Oxidation of LDL was determined as the production of conjugated dienes and

measured at 234 nm. The lag phase, a measure of the time during which LDL is protected from oxidation by antioxidants present in the LDL and in the incubation mix, was determined.

Aortic ring preparation

Four-month-old male rats (Wistar–Kyoto; Animal Resource Centre, Canning Vale, Australia) were housed in stainless steel wire cages and maintained on standard rat pellets. Rats were killed and the descending thoracic aorta isolated, cleared of adhering tissue and cut into eight rings, approximately 3 mm in length. The rings were mounted in stainless steel stirrups and placed in an organ bath chamber, as described previously.^{14,17} The tissues were bathed in Krebs-Henseleit buffer solution comprised of the following: 113 mmol/L NaCl, 4.8 mmol/L KCl, 1.2 mmol/L KH₂PO₄, 1.2 mmol/L MgSO₄, 25 mmol/L NaHCO₃, 2.5 mmol/L CaCl₂, 11.2 mmol/L glucose and 0.57 mmol/L ascorbic acid, in de-ionised water. The buffer solution was continuously bubbled with an O₂/CO₂ mixture (95%/5%) and maintained at 37°C. The aortic rings were allowed to equilibrate for 60 min under 4 g of resting tension before contracting with KCl (20 mmol/L) to test the tissue viability. Concentration response curves to noradrenaline (NA; 10^{–10}–10^{–5} mol/L) were constructed by cumulative additions to the bath. Vascular relaxation to acetylcholine (ACh) was studied in tissues precontracted with the half-maximal dose (EC₅₀) of NA. In brief, after repeated washing and re-equilibration for 1 h, the rings were precontracted with NA EC₅₀ before concentration response curves to ACh (10^{–10}–10^{–5} mol/L) were constructed. Relaxation to various test compounds were studied using tissues precontracted with NA (EC₅₀). The changes in tension were recorded on a Graphtec Lineacorder (FW33701; Graphtec Corporation, Yokohama, Japan) or using a computer-based data acquisition system (Biopak MP100A-CE; Biopac Systems Inc. Santa Barbara, CA, USA) via an amplifier connected to a Grass FT03 force transducer.

Perfused mesenteric vascular bed

The superior mesenteric artery was catheterised at the junction of the aorta, followed by removal of the entire mesenteric bed (including the intestinal tract) and cleaning of the gut contents by flushing with warm (37°C) Krebs-Henseleit solution.¹⁷ The preparation was mounted in a 50-mL organ bath chamber and perfused at a constant flow rate of 4 mL/min with oxygenated Krebs-Henseleit buffer. The tissue was aerated continuously. Changes in perfusion pressure were monitored using Statham P23 AC pressure transducers coupled to a Graphtec Lineacorder (FW33701). Intraluminal injection established dose–response curves to various agonists (KCl, NA). To monitor vasorelaxant actions of pharmacological agents and test extracts, the EC₅₀ dose of NA was determined and included in the perfusate to attain a partial pressure development state. Drugs such as ACh and histamine, and test extracts, were injected intraluminally to establish the extent of reduction in pressure due to vasorelaxation.

Myograph methodology

Three to four mesenteric arcades from the intact vascular were dissected from normotensive WKY rats after they were killed by a single intraperitoneal injection of Nembutal (Sodium pentobarbitone, 60 mg/kg bodyweight; Lippard Veterinary Supplies, Thebarton, Australia). A small incision was made under the dissection microscope at the distal end of the artery using ocular scissors, allowing insertion of the mounting wires. Two 40- μ m mounting wires, 5 cm in length, were inserted into the vessel and fed through the lumen for a distance of approximately 4 mm. The artery was cut at the distal end, transferred to the chamber of the myograph (JP Trading, Aarhus, Denmark) and mounted as described previously.¹⁸ In brief, the vessel segments were mounted between the two jaws before being held in place with the four mounting screws. The wires were separated along their length by the gradual extension of the micrometer. The vessels were gassed in a closed chamber with carbogen at 37°C for 45 min and normalised as described.^{18,19}

Normalised vessels were allowed to stabilise for 15 min before viability testing to the compounds KCl (2 mol/L), NA and Ach. Only vessels that exhibited a contraction greater than 4 mN when exposed to NA and 25% relaxation when exposed to Ach (in the presence of NA EC₅₀) were used for further experimentation.

Statistical analysis

Contractile responses were calculated as percentage maximum contraction to KCl (20 mmol/L) for each ring. Relaxation to Ach is presented as percentage contraction of the half-maximal (EC₅₀) response to NA. Where appropriate, the results are presented as mean \pm SEM. The means were compared using the Student's *t*-test and *P*-values of <0.05 were considered statistically significant.

Chemicals

Acetylcholine chloride, noradrenaline bitartrate, N_ω-nitro-L-arginine (NOLA) and indomethacin were purchased from the Sigma Chemical Company (St Louis, MO, USA). All other chemicals and solvents were of analytical grade or the highest purity available.

Results

The antioxidant capacities of the extracts, as assessed by the *in vitro* FRAP assay, are shown in Table 1. Compared to the other three extracts, palm frond displayed a considerably greater value reflecting a higher antioxidant capacity.

Figure 1 summarises the data for Cu²⁺-mediated oxidation of LDL. Compared to the vehicle control, papaya, chilli and palm frond extracts caused an increase in lag time (*P* < 0.05) indicating protection of LDL against oxidation. The longest delay occurred with palm frond extract (77.9 \pm 0.6 min compared with 55.2 \pm 0.5 min for the control).

The effects of the test extracts on the vascular relaxing ability of the aortic rings precontracted with NA (EC₅₀) are summarised in Figure 2. It is clear that in endothelium-intact rings, oil palm frond extract resulted in considerable relax-

ation (84%), comparable to that achieved with Ach (10⁻⁵ mol/L). In comparison, the other three preparations exerted moderate activity with percentage relaxation values ranging from 32% for lemongrass to 45% for papaya.

Palm frond extract failed to exert any relaxant effect in endothelium-denuded rings or following inhibition of endogenous NO in endothelium-intact rings with NOLA. A similar effect was observed for papaya extract. In contrast, the polyphenol-rich extract from chilli demonstrated varying levels of residual activity, depending on the experimental condition.

The extent of loss of relaxation following de-endothelialisation and inhibition of endothelial NO is shown in Table 2. Removal of the endothelium resulted in an 83% loss in activity compared to 58% with NO inhibition (chilli), while lemongrass extract showed comparable effects (approximately 80%) following either treatment. Similarly, de-endothelialisation or inhibition of NO caused a total loss in the activity of palm frond and papaya extracts.

Table 1. Antioxidant capacity of various plant extracts as determined by the FRAP assay

Extract	FRAP value (μ mol)
Palm frond (<i>Elaeis guineensis</i>)	203 \pm 4
Green chilli (<i>Capsicum frutescens</i>)	43 \pm 2
Papaya shoot (<i>Carica papaya</i>)	58 \pm 1
Lemongrass (<i>Cymbopogon citratus</i>)	22 \pm 1

Values are mean \pm SEM of four separate determinations. FRAP, ferric reducing activity/potential.

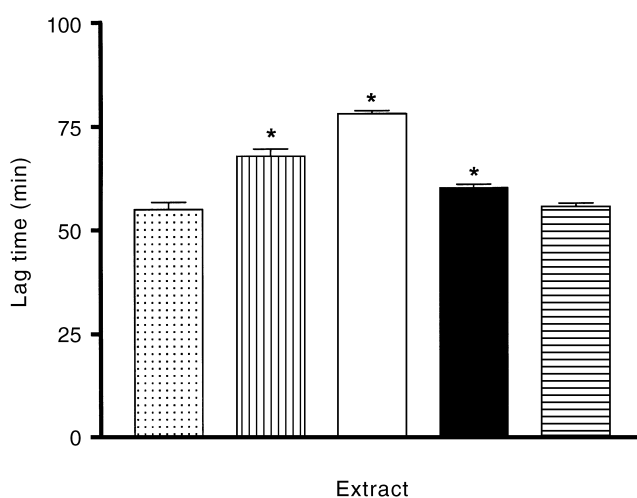


Figure 1. Effect of various plant extracts on Cu²⁺ mediated oxidation of low-density lipoprotein (LDL) *in vitro*. LDL was isolated from human plasma and subjected to oxidation. The extent of oxidation of LDL was measured as the production of conjugated dienes and quantified at 234 nm. Test extracts were incubated with LDL at a dose of 12.5 μ g/mL. Values shown are the mean \pm SEM for three separate triplicate determinations. Asterisk denotes significant difference from the control at the 5% level (*P* < 0.05). (□), Lemongrass; (▨), chilli; (▩), palm frond; (■), papaya; (□), control.

The relaxation actions of plant extracts in the mesenteric vascular bed preparation perfused with a half-maximal concentration (EC_{50}) of NA are summarised in Figure 3 and Table 3.

The highest activity was observed with palm fronds (70%) while the chilli extract resulted in only 22% relaxation. Lemongrass exerted a greater relaxant effect ($P < 0.05$) in this vascular test system (55%) compared to its effects in the aortic rings (32%; Fig. 1). Inhibition of endogenous NO production (NOLA, 10^{-4} mol/L) and cyclooxygenase metabolism (indomethacin, 10^{-5} mol/L) led to varying levels of reduction in the vasorelaxant actions of the extracts (Table 3). For example, in the case of palm fronds, 80% ($P < 0.05$) of its relaxing effect was lost compared to 34% for lemongrass and 26% for chilli ($P > 0.05$; Fig. 2). The effect of sodium nitroprusside was also tested, particularly when low relaxation activity of extracts were observed,

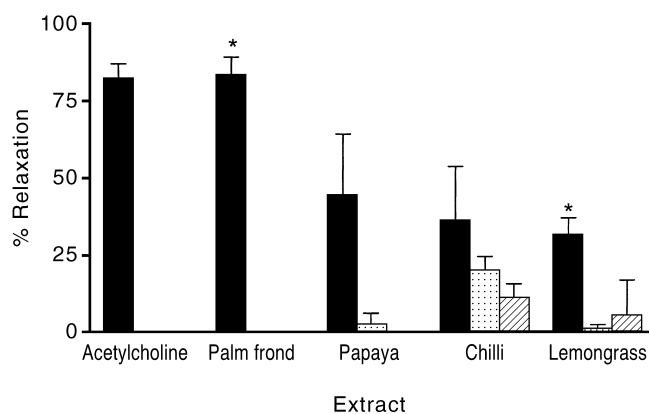


Figure 2. Relaxation response to various plant extracts in isolated aortic rings. The extracts were added in a cumulative fashion to rings precontracted (EC_{50}) with noradrenaline (NA). The endothelium was removed by careful rubbing of the intima with a cotton swab. For inhibition of nitric oxide endothelium intact rings were pre-incubated with N_{ω} -nitro-L-arginine (NOLA; 10^{-4} mol/L) for 30 min prior to contracting with NA. The data shows the extent of relaxation observed at the highest concentration tested ($37 \mu\text{g/mL}$). Results are the mean \pm SEM of four experiments. Asterisk indicates significant difference ($P < 0.05$). (■), +Endothelium; (▨), +NOLA; (▩), -endothelium.

Table 2. Loss of relaxing activity of plant extracts following de-endothelialisation and inhibition of nitric oxide in endothelium intact aortic rings

Extract	% Loss of activity	
	-Endothelium	+NOLA
Palm frond (<i>Elaeis guineensis</i>)	109 \pm 1	100 \pm 0.1
Green chilli (<i>Capsicum frutescens</i>)	83 \pm 1	58 \pm 1*
Papaya shoot (<i>Carica papaya</i>)	96 \pm 0.4	107 \pm 1
Lemongrass (<i>Cymbopogon citratus</i>)	82 \pm 2	87 \pm 1

The results presented were calculated using the maximum relaxation values observed in endothelium intact aortic rings. Values given are as mean \pm SEM ($n = 4$ rats per extract; duplicate rings). *Asterisk indicates significant difference ($P < 0.05$, Student's t -test) from the -endothelium preparation. NOLA, N_{ω} -nitro-L-arginine.

and found to cause complete relaxation of vascular preparations (data not shown).

Palm frond extract was further tested using the myograph technique, which utilises the smaller resistance vessels in the mesenteric vascular bed. The results confirmed that the relaxation properties observed with the larger conductance vessels (aortic ring preparation) and the perfused mesenteric vascular bed were also evident in microvessels of the mesenteric vasculature ($100\text{--}300 \mu\text{mol/L}$) (Fig. 4).

Discussion

The findings of the present study support earlier reports that demonstrated that many commonly consumed plant foods possess vascular relaxant properties. For example, in addition to the well-established actions of wine polyphenols and grape seed extracts,^{6,12} several common vegetables, nuts, fruits, herbs and spices have also been reported to possess vasorelaxant properties of varying potencies.^{7,13}

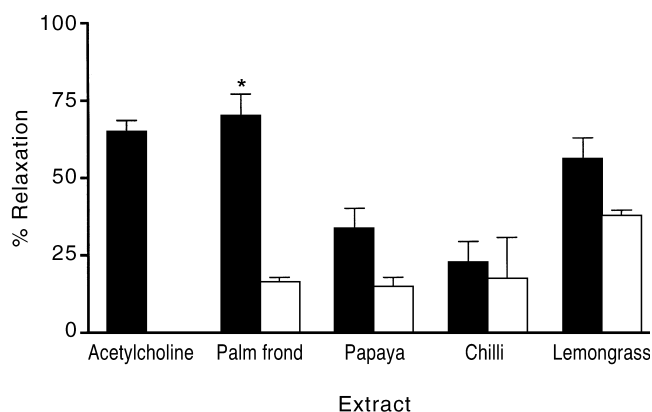


Figure 3. Vascular relaxation actions of test extracts on isolated perfused mesenteric vascular bed. The tissues were precontracted (EC_{50}) with noradrenaline (NA). Test compounds were injected intraluminally and the extent of relaxation calculated. The effect of pharmacological inhibitors of nitric oxide (NOLA; 10^{-4} mol/L) and cyclooxygenase (indomethacin; 10^{-5} mol/L) were studied by incorporating these agents into the perfusion buffer (30 min). Asterisk displays significant difference ($P < 0.05$; $n = 4$). (■), Basal, (□), +NOLA (10^{-4} mol/L) + INDO (10^{-5} mol/L). INDO, indomethacin.

Table 3. Loss of activity of various plant extracts following inhibition of nitric oxide (NOLA) and cyclooxygenase (indomethacin) in the perfused mesenteric vascular bed

Extract	% Loss of relaxation (NOLA + indomethacin)
Palm frond (<i>Elaeis guineensis</i>)	80 \pm 4*
Green chilli (<i>Capsicum frutescens</i>)	26 \pm 11
Papaya shoot (<i>Carica papaya</i>)	56 \pm 6*
Lemongrass (<i>Cymbopogon citratus</i>)	34 \pm 5

The percentage loss of activity was calculated using maximum relaxation values obtained in the absence of pharmacological inhibitors. Values given are mean \pm SEM for four separate determinations for all extracts, except chilli where $n = 3$. *Asterisk indicates significant difference ($P < 0.05$) from the respective control values observed in the absence of inhibitors. NOLA, N_{ω} -nitro-L-arginine.

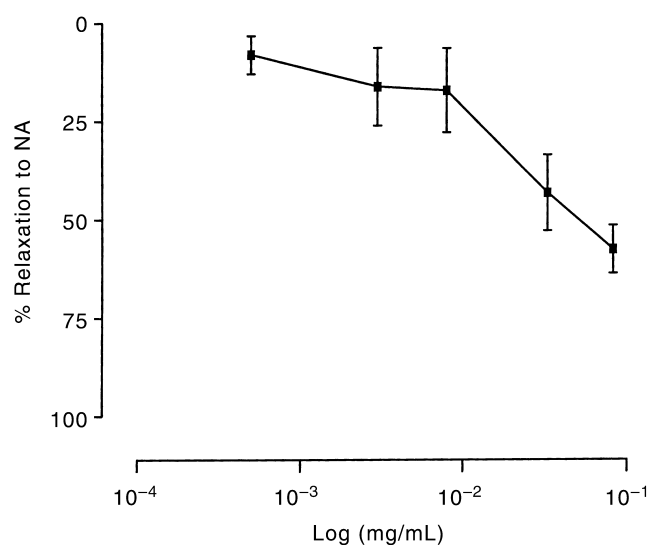


Figure 4. Dose-dependant relaxation response to palm frond extract-isolated mesenteric microvessels. The relaxation effects of the extract were tested on tissues precontracted with noradrenaline at approximate EC_{50} , as described in the text and in previous studies.¹⁸ Results are expressed as mean \pm SEM for duplicate determination from three rats.

In the present study the most potent vasorelaxant activity was observed with a non-edible source of plant material – oil palm fronds. Similarly, the highest antioxidant capacity and the highest lag time for *in vitro* oxidation of LDL was observed with this preparation. (Table 1, Fig. 1). Because the extracts were treated the same and tested at the same dosage levels for biological activity, these findings suggest that either the profile of the polyphenols in palm frond extract is different from other preparations, or it is a more enriched source. Preliminary analysis indicates that palm fronds contain several flavonoid compounds, including quercetin, kaempferol, luteolin and myricetin (Runnie *et al.*, unpubl. obs, 2001).

Previous studies on structure–activity relationships have demonstrated vascular relaxation potencies of various bioflavonoids to be in the order of flavonoids > flavones > flavanols.^{20,21} Therefore, the differential potencies of various plant extracts observed in the present study are likely to be dependent not only on the total amount, but also on the composition of individual compounds. Although oil palm frond extract possessed significantly higher antioxidant capacity compared to the other three preparations (Table 1), it is unlikely that antioxidant activity per se is directly linked to the vascular relaxation actions.

Removal of the endothelium or inhibition of endothelial NO fully abolished (>82%) the relaxation actions of all extracts (except green chilli) in the aortic ring preparation. Therefore, as reported previously for several other plant sources,^{6,7,12} the present extracts also have the ability to promote production of endothelial NO. The chilli extract, however, led only to a modest loss of activity (58%) after inhibition of endothelial NO, while removal of the endo-

thelium resulted in a greater effect (83%; Table 2). These observations suggest a role for other vasodilatory mechanisms, in addition to NO, in mediating the vascular relaxant action of this preparation. It is also noteworthy that while flavones (e.g., apigenin, luteolin) exhibit endothelium-independent relaxation, the dose-dependent relaxant action of flavonols (e.g., quercetin) was considerably reduced compared to its effect on endothelium intact rings.²² Analytical studies on the composition of various extracts are needed to further the physiological findings of the present study, and are currently in progress.

As observed for larger conductance vessels (aortic rings), the resistance blood vessels (mesenteric vascular bed) and the isolated microvessels also showed significant relaxation ($P < 0.05$) in response to palm frond extract, while the papaya and chilli extracts were less effective. In contrast, lemongrass exerted a greater relaxant effect on resistance vessels (55%) compared to its effects on the aorta (32%; $P < 0.05$). Furthermore, while the inhibition of NO with NOLA totally abolished the relaxant effect of lemongrass in the aortic ring preparation, even the combined treatment with NOLA and indomethacin failed to cause a significant reduction in relaxation in resistance vessels. This observation tends to suggest the involvement of non-prostanoid and non-NO mechanisms in mediating relaxation in this preparation. Other relaxing mechanisms reported to be operative in the microvessels include endothelium-derived hyperpolarising factors, as well as several receptor-coupled ion channels, such as the recently described angiotensin II receptor-linked Ca^{2+} channel.²³ In addition, a direct effect on the vascular smooth muscle itself cannot be ruled out. Indeed, flavonoids have been reported to promote vascular smooth muscle relaxation via several different mechanisms, including changes in transmembrane calcium movement, inhibitory effects on cyclic AMP and cyclic GMP phosphodiesterases and on protein kinase C.^{19,24–26} Recent findings have also highlighted vascular bed heterogeneity with respect to NO and the nature of prostanoids produced in the aorta and mesenteric arteries,²⁷ which may in part explain the differential effects of lemongrass noted in the present study.

The potent vascular relaxation actions of oil palm frond extract observed in this preliminary study are worthy of further investigation. There is a need to further characterise the individual constituent or constituents responsible for the endothelium-dependant relaxation to better understand the precise mechanism(s) of action.

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