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Hemorrhoid Therapy with Medicinal Plants: Astringency and Inhibition of Lipid Peroxidation as Key Factors

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Abstract: Free radicals are generated in ano-rectal diseases and stress process results in pain, inflammation, swelling, itching and tenderness. The present study investigates the benefit of astringent herbs in hemorrhoid therapy. Astringent herbs used locally in the treatment of hemorrhoids [*Achyranthes aspera* Linn. (Amarantheceae), *Adansonia digitata* Linn. (Bombacaceae), *Dialium guineense* Willd (Leguminosae), *Harungana madagascariensis*, *Kigelia africana* (Lam.) Benth. (Bignoniaceae), *Newbouldia leavis* Seem. (Bignoniaceae) and *Spondias mombin* Linn. (Anacardiaceae)] were subjected to assay. Astringency was measured as the amount of tannin precipitated by a standard protein Bovine Serum Albumin (BSA) using ferric chloride blue chromophore at an absorbance maximum at 510 nm. The effects of these plant extracts on *Scomber japonicum* Houttuyn (Scombridae) lipid peroxidation was accessed by thiobarbituric acid reactivity method measured at UV 532 nm and expressed as MDA equivalent/mg of tissue. Total phenol and flavonoid contents were determined as gallic acid and rutin equivalents, respectively. Astringency of extracts was in the order of Spondias leaves>Dialium seeds>Dialium leaves>Newboldia leaves>Kigelia fruit>Spondias fruit>Kigelia bark>Harungana bark>Newboldia bark>Harungana leaves >Adansonia leaves>Achyranthes leaves. Astringency correlated positively with total phenols ($R^2 = 0.7944$), inhibition of lipid peroxidation ($R^2 = 0.6596$ with raw homogenate and 0.9220 with cooked homogenate), low correlation with flavonoid ($R^2 = 0.059$) and no correlation between total phenol and flavonoid content ($r^2 = -0.0387$). It is proposed that these astringent herbs accomplish haemorrhoid therapy by inhibiting lipid peroxidation and plugging up minute leaks and holes in the veins and capillaries thereby promoting vein elasticity and acting as vasoconstrictors in the perianal area.

Key words: Hemorrhoid, medicinal plants, astringency, total phenol, lipid peroxidation, vasoconstriction

INTRODUCTION

Over three-quarters of individuals in the world have hemorrhoids at some point in their lives and hemorrhoids are vascular cushions, consisting of thick sub mucosa containing both venous and arterial blood vessels, smooth muscle and elastic connective tissue (MacKay, 2001). While, everyone has this tissue, it is the enlargement, bleeding and protrusion due to venous insufficiency that create pathology and its manifestations are an extremely common medical problem that every physician should be prepared to treat. Treatment includes over-the-counter topical medications, surgery and herbal therapy.

Medical treatment of hemorrhoids is aimed initially at relieving symptoms using ointments, creams, gels, suppositories, foams and pads. Analgesics relieve pain, itching and burning by depressing receptors on pain nerves. Corticosteroids reduce inflammation and can relieve itching, but their chronic

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use can cause permanent damage to the skin. Application of vasoconstrictors to the anus, make the blood vessels become smaller, which may reduce swelling. They also may also reduce pain and itching due to their mild anesthetic effect (Thomson, 1975; Left, 1987; Dennison *et al*., 1989; Liebach and Cerda, 1991).

A major component of a safe and effective therapy for hemorrhoids, often overlooked, is the use of plant extracts. Several plant extracts have been shown to improve microcirculation, capillary flow and vascular tone and strengthen connective tissue of the peri vascular amorphous substrate. The low compliance associated with treatments and lifestyle changes renders plant products an attractive option. This is possibly the missing link to an effective conservative approach to the management of this disease. Early intervention with conservative therapies may prevent time-consuming and expensive complications of hemorrhoids.

Extract of *Ruscus aculeatus* is effective in increasing venous tone because of its anti-inflammatory and astringent properties. The active biochemical constituent is proposed to be the saponin glycoside ruscogenin (MacKay, 2001). *Hamamelis virginiana* (Witch Hazel) extract, high in tannins and volatile oils, has a long therapeutic tradition and is used primarily for its astringent, anti-inflammatory and local haemostatic effects. In folk medicine it has been used for venous conditions, including hemorrhoids. Witch hazel decoctions are easily found on the shelf of most pharmacies, yet the literature available regarding its efficacy and mechanisms of action is limited (Bouskela *et al*., 1994).

Studies have investigated the mechanism of action responsible for the astringent and antiphlogistic properties of Witch hazel. Witch hazel extract has been shown *in vivo* to inhibit alpha-glucosidase as well as human leukocyte elastase, enzymes which contribute to the degradation of connective tissue (Erdelmeier *et al*., 1996). Rigorous clinical investigation of *Centella asiatica* has been conducted on chronic venous insufficiency and hemorrhoids. Centella has the potential to enhance connective tissue integrity and improve capillary permeability (Brinkhaus *et al*., 2000).

Extract of the Japanese pagoda tree (Sophora japonica) has also been shown to be effective in the strengthening of vein walls, normalization of the permeability of veins and capillaries and maintenance of good vein health. Cells subjected to oxidative stress may severely affect cellular function and cause damage to membrane lipids, to proteins, to cytoskeletal structures and to DNA.

The muco-cutaneous repair processes are common to inflammation, infections and surgical repairs. Ano-rectal and cutaneous tissues exposed to injury react so that water molecules contained within cells are altered and lipids of membranes or extracelluar tissues are also injured resulting in the formation of a number of noxious free radicals that affect ano-rectal diseases. Acute damage to these tissues results in muco-cutaneous inflammatory response. Clinical symptoms include discomfort, pain, tenderness, itching. Inflammation associated with itching results in scratching, which further traumatizes these tissues, particularly in pruritus ani. This trauma causes bleeding into the affected tissues, such that haemoglobin is released from the red blood cells. When the haemoglobin is exposed to the hydrogen peroxide generated from neutrophils and xanthine oxidase in inflamed tissues, there is haemoglobin degradation and consequent release of catalytic iron ions and toxic free haem that are capable of initiating or aggravating lipid peroxidation. These events in damaged tissues increase the inflammatory response while the consequent excoriations from the pruritus make these affected surfaces more likely to become infected by secondary bacterial contamination (Gough *et al*., 2000).

Despite the impressive pharmacologic activity directed to reducing pain and promoting healing of ano-rectal conditions, the benefits that antioxidants can play in hemorrhoid therapy are not highlighted. Since, natural body antioxidants cannot cope with the stress process involved in hemorrhoids these could be supplemented by using herbs rich in antioxidants both internally and locally to prevent lipid peroxidation.

The purpose of this study is to investigate the use of astringent herbs in reducing the free radical and inflammatory cell response due to the putative anorectal condition(s) and provide a natural

composition to neutralize and scavenge free radical species generated in ano-rectal tissues. As part of our ongoing investigation on astringent herbs, some medicinal plant extracts used locally in the treatment of hemorrhoids [*Achyranthes aspera* Linn. (Amarantheceae), *Adansonia digitata* Linn. (Bombacaceae), *Dialium guineense* Willd (Leguminosae), *Harungana madagascariensis*, *Kigelia africana* (Lam.) Benth. (Bignoniaceae), *Newbouldia leavis* Seem. (Bignoniaceae), *Spondias mombin* Linn. (Anacardiaceae)], were subjected to astringency and lipid peroxidation assays as possible key factors in hemorrhoid therapy.

MATERIALS AND METHODS

The study was carried out (May 2007 to January 2009) in our laboratory at the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria.

Sample Collection

The plant materials were earlier identified courtesy of Mr. Wale Ekundayo by comparing with herbarium specimens at FRIN (Forest Research Institute of Nigeria herbarium). Collection was through purchase from a local market in Lagos Nigeria, courtesy of Mr. Isaac Adeleke of the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos. The plant materials were dried in the oven at 50°C for 5 days, powdered, wrapped in newspaper and stored at room temperature until needed for the tests.

Extraction Procedure

A modified method of Muanya and Odukoya (2008) was used. Fifty grams of the powdered sample was weighed, put in a separating funnel and 300 mL of ethanol added. This was left to percolate for a week, the ethanol extract was filtered and the marc re extracted with 1×200 mL ethanol.

The ethanol extract was bulked together and filtered. The filtrate was concentrated by drying on a water bath by gentle heating. The dry extract was weighed, put in a sample container, properly labeled and kept in the refrigerator at 4°C until needed.

Source of Bovine Serum Albumin

Fatty acid free BSA (Sigma A-6003) was used.

Preparation of Buffer Solution

9.8 g of NaCl was dissolved in 800 mL of water, 11.5 mL glacial acetic acid was added, the solution was adjusted to pH 4.9 with a solution of NaOH and final volume made up to 1 L.

Qualitative DPPH Radical Scavenging Activity

Radical scavenging activity of plant extracts was determined by slightly modified method of Miliauskas *et al*. (2004) as used by Sofidiya *et al*. (2006). One milligram per milliliter ethanolic stock solution of extract was prepared and to this was added 0.5 mL ethanol solution of DPPH (1 mM). The mixture was shaken and then left to stand at room temperature for 10 min and colour reaction was noted. α-Tocopherol (Sigma) was used as standard antioxidant.

Determination of Astringency

A modified method of Hagerman and Butler (1978) was used. This involved the formation of a protein tannin complex. One milliliter extract was added to 2 mL solution of BSA (Bovine Serum Albumin 1 mg mL^{-1} in buffer solution prepared above). This was mixed together and incubated for 24 h at 4°C in the refrigerator. The mixture was centrifuged at 1500 r for 12 min and the supernatant poured off. The precipitate was dissolved in 5 mL mixture of [1% Sodium Dodecyl

Sulphate (SDS) and 5% v/v Triethanolamine (TEA) in distilled water]. One milliliter of acidic $FeCl_3$ was added, vortexed immediately and allowed to stand on the bench at room temperature for 10 min.

The tannin-BSA complexes present in the precipitate was measured spectrophotometrically (UV Spectrophotometer, Thermo Spectronic Genesys 20 model) using ferric chloride blue chromophore at an absorbance maximum of 510 nm and compared to a standard curve prepared with tannic acid. Astringency was reported as mg L^{-1} tannic acid equivalents.

Determination of Total Phenol Content

Total phenol contents in the extract were determined by the modified Folin-Ciocalteu method of Wolfe *et al*. (2003) as used by Sofidiya *et al*. (2008). An aliquot of the extract was mixed with 5 mL Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 mL (75 g L^{-1}) of sodium carbonate.

The mixture was allowed to stand for 30 min at room temperature and the absorbance was measured at 760 nm spectrophotometrically. The final results were expressed as Gallic Acid Equivalents (GAE).

Determination of Total Flavonoid

Total flavonoids were estimated using the method of Ordonez *et al*. (2006). To 0.5 mL of sample, 0.5 mL of 2% $AlCl_3$ ethanol solution was added. The absorption was measured at 420 nm after 1 h 10 min, at room temperature. Total flavonoid contents were calculated as rutin from a calibration curve.

Lipid Peroxidation

Antioxidant efficacy of the extracts towards lipid peroxidation in raw and cooked fish homogenate of *S. japonicum* was measured by thiobarbituric acid reactivity method. Thiobarbituric acid (TBA) reactivity in the homogenate was determined by following a modified method of Luotola and Luotola (1985) as used by Muanya and Odukoya (2008) and expressed as MDA equivalent/mg of tissue. The TBA chromogen (intensity of the pink coloured complex) was measured at 532 nm against blanks using UV Spectrophotometer, Thermo spectronic (Genesys 20 model). Water-soluble vitamin E was used as reference standard. A graph of absorbance against concentration was plotted using the data obtained for the pure vitamin E. The TBARS of the extracts were evaluated from the standard curve (absorption against concentration of tetraethoxypropane) and expressed as MDA equivalent/mg of tissue.

Statistical Analysis

Three replicas were made for each experiment and all the data presented as the Mean±SEM. A regression analysis, using Excel for Windows Software, was established between astringency, lipid peroxidation, total phenols and flavonoid contents of different plant extracts.

RESULTS AND DISCUSSION

Astringency value, total phenol and flavonoid content of plant extracts are presented in (Table 1). Astringency of extracts was in the order of Spondias leaves>Dialium seeds>Dialium leaves> Newboldia leaves>Kigelia fruit>Spondias fruit>Kigelia bark>Harungana bark>Newboldia bark> Harungana leaves>Adansonia leaves>Achyranthes leaves. Astringency correlated positively with total phenols ($R^2 = 0.7944$), while correlation with flavonoid was low ($R^2 = 0.059$) this is due to the fact that the colour development in the Folin Ciocalteu procedure is due to the transfer of electrons at basic pH to reduce the phosphomolybdic/phosphotungstic acid complexes to form chromogenes thus measuring only phenols that will not bind to proteins. There was no correlation between total phenol and flavonoid content ($R^2 = -0.0387$).

Qualitative determination of free radical scavenging activity showed that all the of the extracts produced a positive colour change from purple to yellow.

Table 1: Astringency value, total phenol and flavonoid content of plant extracts

Plant material		Astringency value	Total phenol content	Flavonnoid content
Botanical name	Plant code used		(mg L^{-1})	
S. mombin leaves	SML	746.8551±0.6531	1.4680±0.0261	0.1363±0.0107
D. guineense seeds	DGS	549.7667±0.0556	0.4456±0.1152	0.4918±0.0749
D. guineense leaves	DGL	471.1736±0.6491	0.1424±0.0051	0.1563±0.1225
N. leavis leaves	NLL	258.3410±0.3891	0.3948±0.0447	0.6172±0.0299
K. africana fruit	KAF	197.9649±1.1864	0.2092±0.0009	0.4737±0.0185
S. mombin fruit	SMF	169.1974±1.0451	0.2746±0.0046	0.0646±0.0146
K. africana bark	KAB	140.0758±0.9327	0.1766±0.0542	0.2189±0.0461
H. madagascariensis bark	HMB	132.0892±0.0976	0.1758±0.0000	0.2795±0.0112
N. leavis bark	NLB	96.4752±0.0794	0.3004±0.1467	0.3211±0.0087
H. madagascariensis leaves	HML	92.6701±1.3277	0.1679±0.0108	0.6541±0.0233
A. digitata leaves	ADL	82.9841±0.4313	0.0623±0.0000	0.0318±0.0009
A. aspera leaves	ASL	36.0349±1.0466	0.0674±0.0003	0.0702±0.0056

Table 2: TBARS values (mean of 3 readings ± SEM) with raw fish homogenate in plant extracts

	Tbars (nmoles of malondialdehyde g^{-1} tissue)				
Plant code used	200.0	100.0	50.0	25.0	1.0
SML	6.0178±0.0672	21.4411±0.0572	33.9852±1.4281	39.4240±0.0801	51.1191±0.0732
DGS	5.9240±0.0052	13.0082±0.2117	14.6749±0.8954	27.4319±0.0568	44.9164±0.1824
DGL	6.1159±0.0471	19.4716±0.0956	26.0608±0.6533	31.4840±0.0771	38.2840±0.0741
NLL	6.0508±0.0212	10.0755±0.0076	10.7995±0.0035	9.3159±0.0035	ND
KAF	3.6607±0.0015	19.5728±0.0172	26.2801±0.0319	41.6236±0.0000	46.8984±0.0000
SMF	2.7401±0.1104	16.7820±0.0118	19.4366±0.2451	22.2075±0.1943	29.4451±0.0911
KAB	0.1992±0.0318	7.4341±0.0000	16.2115±0.0130	23.5505±0.1437	33.5742±0.0000
HMB	5.3502±0.0015	11.9025±0.0015	12.3695±0.0012	16.6827±0.0064	18.1992±0.0025
NLB	0.4973±0.0044	3.1113±0.0012	6.7376±0.0029	10.1635±0.0025	ND
HML	7.4931±0.0035	8.9821±0.0021	15.2774±0.0046	19.7143±0.0070	31.4863±0.0016
ADL	4.3219±0.0621	9.6755±0.0337	9.0681±0.0158	12.1197±0.0295	15.6801±0.8146
ASL	2.1145±0.0000	7.0531±0.0011	8.4459±0.0774	9.8763±0.0491	10.5227±0.0179

ND = Not Done

Table 3: TBARS values (mean of 3 readings ± SEM) with cooked fish homogenate in plant extracts

	Tbars (nmoles of malondialdehyde g^{-1} tissue)				
Plant code used	200.0	100.0	50.0	25.0	1.0
SML	2.9974±0.3275	2.7631±0.0726	11.6982±0.1106	19.4714±0.1191	23.6872±0.1146
DGS	3.5196±0.1149	5.9065±0.5431	16.2349±0.0498	17.7769±0.0672	20.9432±0.0387
DGL	2.4575±0.0764	5.8920±0.4456	10.4481±0.0927	9.9420±0.0947	13.4426±0.0772
NLL	1.4593±0.0026	1.8394±0.0006	2.3589 ±0.0040	2.9968±0.0050	ND
KAF	1.6655±0.0035	3.7632±0.0025	4.9535 ±0.0017	5.6405±0.0044	12.4238±0.0104
SMF	0.9786±0.2241	3.0067±0.0017	5.4421±0.5541	5.9865±0.0611	10.9865±0.0881
KAB	1.3018±0.0117	2.9835±0.0000	4.2387±0.0144	7.4684±0.0036	9.2682±0.0012
HMB	2.0393±0.0015	3.2010±0.0420	3.5269±0.0012	3.9474 ±0.1045	7.3763±0.0015
NLB	0.9425±0.0057	1.7992±0.0006	1.9907±0.0052	2.9104±0.0021	ND
HML	1.5356±0.0006	3.4280±0.0026	3.7608 ±0.0049	4.9824±0.0000	6.9170±0.0000
ADL	1.1462±0.0532	1.8974±0.0033	2.0329±0.6672	5.0076±0.0493	7.0453±0.0034
ASL	0.0327±0.5617	0.7654±0.0471	1.0684±0.4491	2.7753±0.0052	5.9749±0.0415

ND = Not Done

Absorbance decreased with increasing concentration of plant extract and thus a resultant increase in lipid peroxidation as shown in Table 2 and 3. Malondialdehyde (MDA) is formed during oxidative degeneration as a product of free oxygen radicals, which is accepted as an indicator of lipid peroxidation (Draper, 1999).

A rapid, simple and inexpensive method to measure antioxidant capacity involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity.

The qualititative analysis of the ethanolic extracts of the plants was carried out using the DPPH method. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turned from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H.

Astringency was measured as the amount of tannin precipitated by a standard protein Bovine Serum Albumin (BSA). Astringency is not a taste like sweetness, bitterness, sourness or saltiness but a feeling, of dryness as a result of increased friction caused by tannin, which reduces lubrication by precipitating proteins. The method of Hagerman and Butler (1978) used in this study involved formation of protein-tannin complex between the tannin containing extract and the protein, BSA.

Tannins have long been known to interact with proteins so assay used was devised to take advantage of this phenomenon. Ferric ion complexes with the tannins and forms a blue chromophore with an absorbance maximum at 510 nm. The blue product was determined spectrophotometrically.

Since, the BSA protein does not react with ferric chloride or interfere with the colour reaction, this analysis provided a convenient assay for phenols in the plant extracts as it only measured the phenols that could bind to BSA and precipitate along with the protein. The tannin phenol present is expressed relative to tannic acid.

The absence of correlation between total phenol and flavonoid content has also been found by (Meda *et al*., 2005; Moein *et al*., 2008) and could be explained by the presence of some other chemical groups other than proteins present in the plant extracts that can also react with the Folin Ciocalteu reagent.

Total flavonoid was calculated with reference to rutin. Though rutin helps those with capillary or venous fragility, easy bruising and a tendency of wounds not to heal well (Misra and Parshad, 2000). The low astringency correlation with flavonoid content indicated that the venous constriction in hemorrhoid therapy in this study might be as a result of astringency due to phenols that could bind to BSA and precipitate along with protein and not flavonoids. This result differs from previous studies as flavonoids are known to be effective for pain from injuries and bruises and possess the ability to protect, strengthen capillaries, aid circulation, lower cholesterol and reduce hemorrhoids (Misra and Parshad, 2000; Sandu and Singh, 2004).

Inflammation associated with anal itching results in scratching, which further traumatizes these tissues. This trauma causes bleeding into the affected tissues, such that hemoglobin is released from the red blood cells. When the hemoglobin is exposed to the hydrogen peroxide generated from neutrophils and xanthine oxidase in inflamed tissues, there is hemoglobin degradation and consequent release of catalytic iron ions and toxic free heme which are capable of initiating or aggravating lipid peroxidation (Gough *et al*., 2000). Astringency correlated positively with inhibition of lipid peroxidation ($R^2 = 0.6596$ with raw homogenate and 0.9220 with cooked homogenate). The results of our study indicate that there was protection of lipid peroxidation as evidenced by the low level of TBARS values with increasing concentration of the extracts suggesting a decrease in lipid peroxidation.

Fatty acids Omega-3 is an important polyunsaturated fatty acid found in fish oils and have been shown to decrease lipid peroxidation (Erdogan *et al*., 2004). Activity was more in cooked than raw fish homogenate. The present study confirms previous studies that cooking alters the physicochemical nature of the membranes thereby, the solubility and site of action is favoured by having more access to the radical and thus better antioxidant activity and effective inhibition of the free radical damage (Seeman, 1990; Muanya and Odukoya, 2008). Thus the inhibition of lipid peroxidation by these plant extracts is indicative of anti hemorrhoid activity.

Hemorrhoids refer to a condition in which the veins around the anus or lower rectum are swollen and inflamed.In hemorrhoid there is prolapsed of the veins in the anal area as a result of inflammation. Therefore the treatment of this condition requires herbs that will strengthen the veins and reduce inflammation and bleeding.

The herbal treatment in hemorrhoid therapy involves use of astringents. Astringency is not a taste like sweetness, bitterness, sourness or saltiness. It is a feeling of dryness or roughness that is sensed by the tongue as a result of increased friction between the tongue and the surfaces inside the mouth. This is caused by tannin, which reduces lubrication by precipitating proteins. Astringents will therefore cause coagulation of proteins in the cells of the perianal skin or the lining of the anal canal. This action promotes dryness of the skin, which in turn helps relieve burning, itching and pain. These extracts have an astringent effect which helps heal mucous membranes and exposed tissue and bring about contraction and firm up tissue.

It is proposed that these astringent herbs accomplish anti hemorrhoid activity by causing coagulation of proteins in the cells of the peri anal skin or the lining of the anal canal. These extracts have an astringent effect which helps heal mucous membranes and exposed tissues. They plug up minute leaks and holes in the veins and capillaries, promote vein elasticity and act as vasoconstrictors in the peri anal area. Thus promote dryness of the skin, which in turn helps relieve burning, itching and pain associated with hemorrhoids.

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