African Journal of Pharmacology and Therapeutics Vol. 6 No. 4 Pages 178-182, 2017
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Research Article

Antioxidant and anti-inflammatory activities of selected medicinal plants from western Kenya

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Background: Globally, the increase in the burden of diseases related to oxidative damage and inflammation, coupled with the high cost of medication and the side effects of these therapies necessitates a need for more effective, affordable and safer remedies. Thus there still exists a demand for new antioxidant and anti-inflammatory agents.

Objectives: To screen selected medicinal plants from Kakamega County for their antioxidant and anti-inflammatory activities.

Methodology: Seven medicinal plants used to treat ailments related to oxidative damage and inflammation were selected and extraction was carried out using methanol. Antioxidant activity was screened using 2, 2-diphenyl-1-picrylhydrazyl assay while carrageenan induced rat paw edema assay was used to screen for their anti-inflammatory activity.

Results: The methanolic leaf extracts of *Rhus vulgaris* and *Phyllanthus fischeri* displayed good antioxidant activity with percentage inhibition of 71.4% and 66.7 % respectively. Furthermore, the methanolic leaf extract of *Rhus vulgaris* displayed significant anti-inflammatory activity while *Phyllanthus fischeri* had mild activity. Results were considered to be statistically significant when *(P*<0.05).

Conclusion: These results support the use of *Rhus vulgaris* and *Phyllanthus fischeri* in traditional medicine to remedy oxidative damage and inflammatory related diseases. These two plants are potential sources of natural antioxidant and anti-inflammatory agents.

Key words: Medicinal plants, Kakamega County, Antioxidant, Anti-inflammatory

Received: September, 2017
Published: December, 2017

1. Introduction

Nature, through plants has been a source of dietary antioxidants for years. Since the discovery and isolation of ascorbic acid, a natural antioxidant, there has being great interest in exogenous antioxidants (Kasote et al, 2015). Plants have the ability to biosynthesize a wide range of non-enzymatic antioxidants which can counteract the Reactive Oxygen Species (ROS) – induced oxidative damage. Krishnaiah et al, 2011 reported that almost two-thirds of the world's plant species have medicinal value and most of these plants possess favorable antioxidant potential.

Wilcox et al, 2004 noted that in plants, some of the antioxidants are naturally occurring or are formed in response to abiotic and biotic stress conditions. Common natural antioxidants include vitamins such as vitamin E and vitamin C. Similarly, a considerably large number of plants have been scientifically validated to exhibit anti-inflammatory activity and are sources of natural anti-inflammatory agents such as colchicine,

curcumin and capsaicin from *Colchicum autumnale*, *Curcuma longa* and *Capsicum* species respectively (Fürst and Zündorf, 2014).

The selection of plants based on their ethnomedicinal use has been proven to be an effective approach in drug discovery from higher plants (Kloucek et al, 2005). Newman and Cragg (2012) revealed that between 1981 and 2010, about 1073 new chemical entities belonging to the group of small molecules were approved and that only 36% were purely synthetic, while more than the half were derived from natural sources. Various phytochemicals present in plants such as alkaloids, volatile essential oils, glycosides, resins, tannins, terpenes and phenols are responsible for their medicinal properties (Aneesh, 2010)

For this study, plants selected based on their ethnomedicinal use were: *Kalanchoe densiflora* used to maintain general well-being, *Phyllanthus fischeri* is used to treat skin diseases, *Justicia betonica* is used to treatInflammation and *Senna diymobotrya* is used to treat back aches. Plants selected based on literature review were: *Rhus vulgaris* since other plants belonging to this genus such as *Rhus coriara*, have displayed good antioxidant activity. They contain alkaloids, phenols (Gabr et al, 2014). Similarly for *Solanum dasyphylum,* other related species such as *Solanum nigrum* and *Solanum torvum* have shown antioxidant activity (Loganayaki, 2010). For *Warburgia ugandensis,* traditional use reported in literature was the treatment of joints (Kokwaro, 1993). Phytochemical studies showed the plant contains flavonoids (Were et al, 2015).

The various studies on the effects of synthetic antioxidants on animal models have revealed toxicity such as liver toxicity and carcinogenesis related with use of synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate in high doses (Bauer et al, 2001; Gultekin and Doguc, 2013). Also despite inflammation being a defense mechanism by the body, the events and mediators involved initiate, maintain or potentiate other diseases such as rheumatoid arthritis, asthma, type 2 diabetes, neurodegenerative diseases, cancer and non-alcoholic fatty liver disease (NAFLD) (Sosa et al, 2002; Campbell, 2015). furthermore, current anti-inflammatory therapies mostly involve classes of drugs that produce serious side effects such as gastric intolerance, bone marrow depression and water and salt retention, resulting from prolonged use of these drugs (Das et al, 2014). These findings necessitate more research into natural antioxidants and anti-inflammatory agents and medicinal plants can act as a suitable source. Thus this study sought to identify medicinal plants from Kakamega County that have antioxidant and anti-inflammatory activities.

2. Materials and Methods

2.1 Chemicals, reagents and solvents

Methanol was used for extraction. Reagents were prepared as described in the established protocols. Carrageenan (Sigma chemical Co. USA, No. C-4014) was used in the anti-inflammatory assay with indomethacin (Dawa Ltd. Kenya) as a standard and sodium chloride (Sigma Aldrich GmbH Seelze, Germany) was used as the negative control. For the antioxidant assay, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich GmbH Seelze, Germany) was used and ascorbic acid (Sigma Aldrich GmbH Seelze, Germany) was the positive control.

2.2 Plant material and extraction

The seven plant species were collected from within Kakamega County and voucher specimens were prepared and submitted to the herbarium at the School of Biological Sciences, University of Nairobi, where authentication was done and the voucher specimens were deposited (**Table 1**).

Table 1: Selected medicinal plants for assay

Botanical name. Family	Voucher no.	Local name	Area collected from	Part used
Justicia betonica Linn.(Acanthaceae)	EAO2016/046	Shikuduli/ Amanyasi	Emakuche village, khwisero constiuency	Whole plant
Kalanchoe densiflora Rolfe (Crassulaceae)	EAO2016/047	Okwamatsi	Emakuche village, khwisero constiuency	Leaves
Phyllanthus fischeri Pax (Euphorbiaceae)	EAO2016/057	Olukhala	Eshtungu/Ekonyero village,	Aerial Parts
Rhus vulgaris Meikle(Anacardiaceae)	EAO2016/063	Omusangula	Mundatu village, Sabatia constituency	Leaves
Senna didimobotrya (Fresen.) Irwin and Barneby (Fabaceae)	EAO2016/070	Olubino	Eshikumu village, Ikolomani constituency	Leaves
Solanum dasyphylum Schumach. (Solanaceae)	EAO2016/077	Indula	Emanyasa village, Mumias constituency	Leaves
Warbugia ugandensis Sprague(Canellaceae)	EAO2016/093	Apaki	Eshtungu/ Ekonyero village, Butsotso west	Leaves

The dried plant materials were grounded into powder using a hammer mill (Muharatta mechanical grinder). 100gm of dry powdered plant material was subjected to cold maceration with 500 ml methanol in 1000 ml conical flask and left for about 24 h at room temperature with occasional shaking. Filtration was done using whatmann No: 1 filter paper and the filtrate concentrated in a vacuum at 65^0C using a rotary evaporator and stored at 4^o C for further use. The percentage yield was obtained as previously reported (Asha et al, 2015).

2.3 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay for antioxidant activity

Assay for antioxidant activity was carried out as described by Khalaf (2008) and Scio (2009).

The working solutions (50, 75, 100, 250, 500 and 1000 µg/ml) of the plant extracts were prepared from the stock solution using a suitable dilution and were added to 1 ml of 0.002% of DPPH prepared in methanol. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm. Ascorbic acid was used as a standard in concentrations ranging from 3.125 to 100 µg/ml. The assay was done in triplicates so as to ensure reproducibility (Khalaf et al, 2008).

The percentage inhibition of free radical formation was calculated as previously described (Kamkar et al, 2014). This percentage inhibition was determined at concentrations of 250 µg/ml, 500 µg/ml, and 1000 µg/ml of the various methanolic plant extracts.

The antioxidant activity of the samples was expressed as IC_{50} (inhibitory concentration), which is defined as the concentration (expressed in µg/ml) of sample required to inhibit the formation of DPPH radicals by 50%. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph that plotted inhibition percentage against extract concentration (Scio, 2009).

2.4 Assay for anti-inflammatory activity

Carrageenan rat paw edema test was carried out as described in literature (Sawadogo et al, 2006; Igbe et al, 2012). Adult Wister rats were randomly divided into four groups. The test groups (A and B) were treated orally with 1000 mg/kg of the extract. The reference group (C) was administered with indomethacin (10 mg/kg) orally. The control group (D) received 10 mL/kg of distilled water. The animals were treated 1 hour before injection of 0.1 mL of 1% carrageenan into the sub-plantar tissue of the right hind paw (Igbe *etal.*, 2012). Changes in volume was measured using a phlerthysymograph at 0, 0.5, 1, 1.5, 2, 2.5 and 3 hours following carrageenan administration and increase in the volume of the right hind paws was taken as an indication of edema (Sawadogo et al, 2006). The percentage inhibition of the inflammation (hind paw edema) was calculated aspreviously described (Sawadogo et al, 2006).

Comparison between the treatment groups (controls and extact treated groups) was carried out using one way ANOVA. Results were considered significant when $P < 0.05$ (Igbe et al, 2012).

3. Results

Yield on extraction

Justicia betonica (whole plant), *Solanum dasyphylum* (leaves), *Senna didymobotrya* (leaves), *Rhus vulgaris* (leaves), *Warburgia ugandensis* (leaves), *Phyllanthus fischeri* (aerial parts) and *Kalanchoe densiflora* (leaves) were subjected to cold maceration with methanol. The yields are presented in **Table 2**.

Table 2: Yield on extraction of selected medicinal plants

Plant	Part used	% w/ w
Phyllanthus fischeri	Aerial parts	5.08
Kalanchoe densiflora	Leaves	5.15
Senna didymobotrya	Leaves	7.47
Solanum dasyphylum	Leaves	7.88
Justicia betonica	Whole plant	8.03
Warburgia ugandensis	Leaves	11.77
Rhus vulgaris	Leaves	13.76

1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay for antioxidant activity

The methanolic leaf extracts of *Rhus vulgaris* and *Phyllanthus fischeri* displayed good antioxidant activity with IC_{50} values of 163.63 µg/mL and 182.15 µg/mL respectively. The methanolic leaf extracts of *Senna didymobotrya*, *Justicia betonica, Warburgia ugandensis*, *Kalanchoe densiflora* and *Solanum dasyphylum* had weak antioxidant activity with IC_{50} ranging from 1029 to 4051 µg/ ml (**Table 3**). Ascorbic acid (standard) had an IC_{50} value of 49 µg/mL with percentage inhibition of 95.1%.

Table 3: IC_{50} µg/ ml and % inhibition of selected medicinal plants

Plant	IC_{50} µg/ ml	% inhibition
Rhus vulgaris	163	71.6[a]
Phyllanthus fischeri	182	66.7[a]
Warburgia ugandensis	4051	6.4[b]
Kalanchoe densiflora	1407	17.5[c]
Senna didymobotrya	1434	33.6[c]
Solanum dasyphylum	1029	48.4[c]
Justicia betonica	1671	31.1[c]
Ascorbic acid	49	95.1[d]

[a] - at 250µg/mL; [b] - at 500 µg/mL; [c] - at 1000 µg/mL; [d] - at 100 µg/mL

Anti-inflammatory activity

Based on their good antioxidant activity, *Rhus vulgaris* and *Phyllanthus fischeri* were selected for screening of their anti-inflammatory activity.

For normal saline, the paw size kept on increasing from baseline to a maximum size at 150 min (**Table 4**). Thereafter, the mean paw size declined slightly. For indomethacin (25 mg/kg body weight), the paw size

decreased continuously up to 90 min and there after increased. Similarly for the *Phyllanthus fischeri methanolic* leaf extract, the paw size decreased but after 30min, the paw size increased slightly.

The most effective agent was *Rhus vulgaris* methanolic leaf extract that caused a decrease for up to 90 minutes and there after the paw size increased slightly. At 1000 mg/kg, the dose seemed to be more efficacious than indomethacin (**Figure 1**).

For indomethacin and *Rhus vulgaris* extract, there was a statistically significant difference in the paw size when compared to the vehicle at all the different time points. On the other hand for *Phyllanthus fischeri,* the difference in paw size when compared to the vehicle was not statistically significant at all the different time points. P values less than 0.05 were considered to be statistically significant.

Table 4: Effect of the various treatments on the paw volumes at different time points

	Mean and standard deviation of paw volumes (ml)			
	Saline (10mg/Kg) (n=6)	**Indomethacin (25mg/ Kg) (n=6)**	***Rhus vulgaris* (1000 mg/ Kg) (n=3)**	***Phyllanthus fischeri* (1000 mg/ Kg) (n=3)**
Before treatment	0.89±0.145	0.945±0.082	0.947±0.122	0.88±0.069
After treatment (180 min)	1.02±0.07	0.79±0.101 *	0.713±0.14*	0.927±0.122

* indicate significant ($p<0.05$) different when compared with the control group

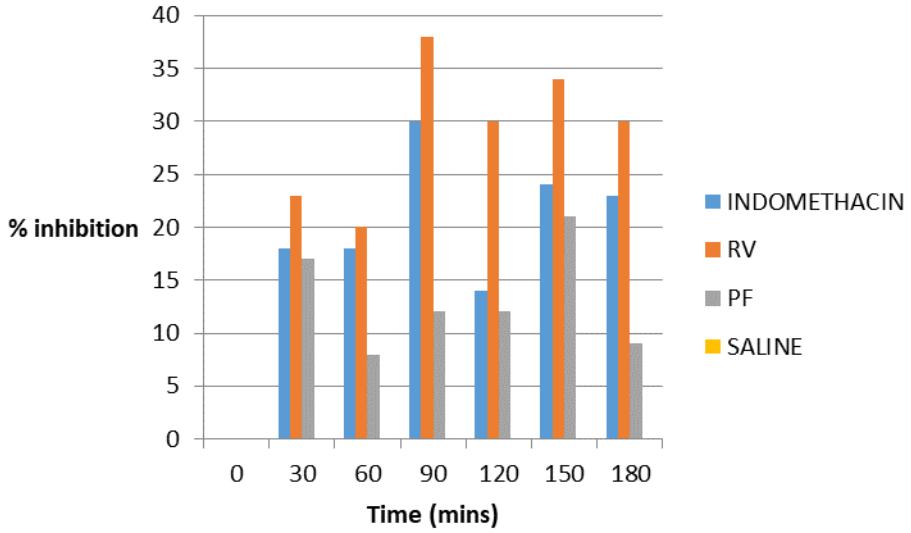

Figure 1: Comparison of % inhibition of inflammation for indomethacin, saline, *Rhus vulgaris* and *Phyllanthus fischeri*

4.0 Discussion

Maceration was preferred so as to reduce any possibility of thermal decomposition of any thermolabile compounds that may be present. This is the first report on the antioxidant activity of *Rhus vulgaris* and *Phyllanthus fischeri*. For *Rhus vulgaris,* other plants belonging to the Rhus genus such as *Rhus coriara*, have displayed good antioxidant activity. They contain alkaloids and phenols (Gabr et al, 2014). For natural antioxidants, phenolic compounds are mainly responsible for the antioxidant activity. Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins (Dai and Mumper, 2010). Notably, the extracts displayed a concentration dependent scavenging activity. *Rhus vulgaris* displayed the highest activity while *Warburgia ugandensis* had the lowest activity. The antioxidant activity of a plant extract is mainly determined by its phytoconstituents such as phenolic compounds (Rahman et al, 2013). The differences in the concentrations of these compounds in plants may explain the variations in IC_{50} values observed.

Based on their good antioxidant activity, *Rhus vulgaris* and *Phyllanthus fischeri* were selected for screening of their anti-inflammatory activity. Carageenan induced inflammation usually occurs in two phases (Barbosa, 2014). From the graph (**Figure 1**), *Rhus vulgaris* seemed to be effective only in the acute phase as opposed to indomethacin that had an effect in both the acute and delayed phases. The proposed mechanism of action of *Rhus vulgaris* in suppressing the first phase of inflammation may be through inhibition of the release

of early mediators, such histamine and serotonin that are responsible for inflammation.

5.0 Conclusion

The results of this study support the use of *Rhus vulgaris* and *Phyllanthus fischeri* in folk medicine as these two plant possess good antioxidant and anti-inflammatory activities. An extensive literature review revealed that this is the first report of the antioxidant and anti-inflammatory activities of these plants. Phytochemical studies should be undertaken to isolate compounds responsible for these activities.

Conflict of Interest declaration

The authors declare no conflict of interest.

Acknowledgements

The authors are grateful to Mr. Johnson Mwalukumbi and Mr. Raphael Ingwela for their help in carrying out the extractions, and Mr. William Njihia for assistance with the anti-inflammatory assay.

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