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Aqueous Co-extract Mixture of *Combretum molle* (stembark) and *Xylopia aethiopica* (fruit) show Phytochemical Synergy in its Anti-fungal and Antioxidant Bioactivities

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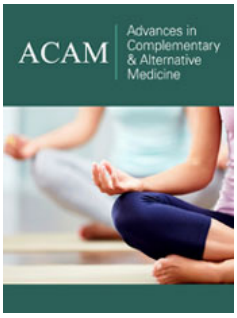
ISSN: 2637-7802



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Submission: July 10, 2020

Published: July 24, 2020

Volume 6 - Issue 2

How to cite this article: John Kenneth Mensah, Mustapha Abubakari, Yakubu Jibira. Aqueous Co-extract Mixture of *Combretum molle* (stembark) and *Xylopia aethiopica* (fruit) show Phytochemical Synergy in its Anti-fungal and Antioxidant Bioactivities. Adv Complement Alt Med. 6(2). ACAM.000634.2020.
DOI: 10.31031/ACAM.2020.06.000634

Abstract

Background: Ethnomedicinal practices typically employ botanical medicines as complex mixtures of two or more plants whose net bioactivity is the culmination of its phytochemical additivity or phytochemical synergy or phytochemical antagonism among discreet phytochemical entities. The aqueous co-extract mixture of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) is an example of this practice where the two botanicals are popularly co-used ethnomedicinally for the promotion of healthy wound healing.

Aim: To evaluate the potential phytochemical synergy of the combined methanol extracts of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) and to examine the effect of this co-extract on the relative anti-inflammation efficacy *in vivo*, on the relative attenuation of microbial growth in vitro and on the relative mitigation of oxidative stress *in vitro*.

Methods and Materials: The antioxidant effect of the combination was assessed through the DPPH radical scavenging activity *in vitro*. Broth dilution evaluations of anti-microbial susceptibility was taken as the *in vitro* anti-microbial response. Oral administration of co-extract to suppress the carrageenan-induced foot oedema in 7-day chicks was chosen for the *in vivo* assessment of the anti-inflammatory response.

Results: Although the phenolic content of *Combretum molle* (stem bark) was 1.6-fold higher than that of the *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) co-extract, its DPPH radical scavenging activity was 38.2-fold lower leading to the conclusion that the co-extract yielded robust synergistic attenuation of oxidative stress through DPPH radical scavenging activities *in vitro*. Co-extracts produced a synergistic inhibition of fungal cell growth (2-fold higher inhibition over individual extracts) in the two fungal species (*Candida albicans* and *Taenia corporis*) *in vitro*. Not only did the co-extract efficacy outperformed the efficacy of individual extracts at the dose-response endpoints of the carrageenan-induced inflammation in 7-day old chicks but it also matched the efficacy of the control drug Diclofenac.

Conclusion: Clinically relevant phytochemical combinations from ethnomedicinal sources acting synergistically can exert net potent biological effects of medicinal importance. The *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) co-extract ethnomedicinal wound care have implications for healthy wound healing and can be brought forward into the clinical and public health realms for translational impact.

Keywords: *Combretum molle, Xylopia aethiopica*, anti-microbial, antioxidant, anti-inflammation, phytochemical synergy

Introduction

Ethnomedicinal practices typically employ botanical medicines as complex mixtures of two or more plants whose net bioactivity is mechanistically linked to its phytochemical additivity or its phytochemical synergy or its phytochemical antagonism evoked by discreet phytochemical entities at multiple target sites. The aqueous co-extract mixture of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) is an example of this practice where the mixture

of the two botanical extracts is popularly used ethnomedicinally to increase its efficacy for skin, epithelia and mucosal wounds as well as to manage wound-related diseases including the management of diabetic wounds.

Individual extracts from each plants have been shown *in vitro* and/or in small animals to be anti-cancer [1], anti-malarial [2], anti-inflammatory [3-5], antihelmintic [6], neuroprotective [7], anti-oxidant [8], anti-microbial [9-11], anti-diabetic [8,12,13] and analgesic [5,14]. While the co-use of extracts of *Combretum molle* and *Xylopia aethiopica* have a long history of traditional use in wound healing, the underlining mechanism for its therapeutic efficacy has not been previously described in the scientific literature. Since the co-extract of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) continues to be popular alternatives to conventional pharmacological treatment of wounds, it is urgent that its purported ethnomedicinal efficacy be assessed through scientific investigation and the breadth and scope of its underlying mechanism of action on wound healing be clarified.

Healthy wound healing encompasses a broad spectrum of features, most notably suppression of microbial proliferation and repression of microbial virulence, mitigation of concurrent cellular oxidative stress and inhibition of downstream inflammatory events. But the clinical management of chronic wounds has become a vexing problem for healthcare professionals, particularly because antimicrobial resistance to existing cures has developed in wound healing where co-infections among multiple microbial species, inflammation of tissues and cells and the evocation of cellular oxidative stress have combined to cause both diagnostic and therapeutic complications.

The study hypothesized that treatment of wounds with the combined extracts of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) offers a therapeutic advantage over that of individual plant extracts through the robust mechanistic evocation of phytochemical synergy in mechanisms that are absent when either plant is used alone. This study utilized a two-fold approach to test this hypothesis. First, it investigated the effects of *Combretum molle* (stem bark) alone and that of *Xylopia aethiopica* (fruit) alone on anti-microbial and antioxidant effects *in vitro* and on *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) on anti-inflammation effects *in vivo*. Second, it investigated the effects of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) in combination on anti-microbial, antioxidant effects *in vitro* and on anti-inflammation effects *in vivo*.

Using well-characterized arrays of robust bioassays to evaluate *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extracts for antioxidative activity, anti-inflammatory activity and microbial growth-inhibiting properties, this study reports that *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) combination treatments specifically relies on synergistic activity of their co-extracts' phytochemical pool in its anti-fungal and antioxidant activities during wound healing. The study results agree with the ample extant literature support for the scientific consensus that has emerged concerning the proposition that the net biological effect of crude botanical extracts is the result of the combined bioactivities of multiple constituents that are either additive, synergistic or antagonistic in functional consequences and in bioactive outcome.

Materials and Methods

Experimental goal

The study evaluated the relative ability of the co-extract of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) to synergistically: inhibit growth properties of pathogenic microbial cell lines *in vitro*, evoke DPPH anti-oxidative response *in vitro* and, mitigate inflammatory effect *in vivo* using well-established battery of biochemical assays. A two-fold approach was utilized to test the hypothesis that the co-extracts of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) offers a therapeutic advantage over that of individual *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) plant extracts through its mechanistic evocation of a robust phytochemical synergy that is unavailable when either *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) plant is used alone. The experimental effort, therefore, concentrated on comparison of the individual effects of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) alone with the combined effect of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) on microbial growth proliferation *in vitro*, on antioxidant efficacy *in vitro* and on anti-inflammation activity *in vivo*.

Co-extract mixture ratio and concentrations of extracts

The optimal co-extract mix, with an efficacious balance of potentially synergistic bioactivity was maintained at a ratio of 3:2:*Combretum molle* (stem bark):*Xylopia aethiopica* (fruit) (weight/weight) using the historical precedent set by ethnomedicine as its reference source. Stock solutions of 20mg/mL botanical *Combretum molle* (stem bark) and *Xylopia aethiopica* extracts and of *Combretum molle* (stem bark)-*Xylopia aethiopica* co-extracts, throughout this work, were prepared in 99% ethanol and kept frozen until the day of use. All bioassays included a solvent/vehicle control and the synergy assay contained *Combretum molle* (stem bark):*Xylopia aethiopica* (fruit) (3:2 ratio weight/weight).

Botanical acquisition and authentication

Botanically authenticated *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) plant materials for extraction were acquired and samples kept in the herbarium archive. Voucher plant specimens were retained at the School of Pharmacy, KNUST. The generation of phytochemical enriched plants extracts for study was done with cold maceration using methanol as solvent in a protocol previously described in the published literature [15]. Extraction solvent was evaporated, and dried extracts stored at -20 °C until needed.

Thin layer chromatography

In-house prepared thin-layer chromatographic plates with silica gel as adsorbent was utilized for the assessment of the number of individual compounds present in each extract. The TLC plate size, the thickness of the silica gel adsorbent on the plate, the solvent composition of the mobile phase, iodine vapor visualization and Rf calculations of resolved bands are described elsewhere [16].

Microbial cell lines

The genotypic and the phenotypic characteristics of the pathogenic microbial cell lines utilized for the assessment of microbicidal activities of extracts have been described elsewhere [15]. Similarly, the culture, storage and maintenance of the microbial cell lines have been reported earlier [15]. The 6 pathogenic microbial panel of cell lines that were used as microbial targets included 2 gram-positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes*), 2-gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) and 2 fungi (*Candida albicans* and *Taenia corporis*). Individual *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extracts and *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extracts were sequentially tested for both antibacterial and antifungal activities. A 10^6CFU/mL of each cell line was used as McFarland standard for each microbial working culture [17].

Microbiological activity

To characterize the microbiocidal activities of the *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extract and of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract, including determining their spectrum of activity against a panel of 4 pathogenic bacteria *(Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa*) and 2 clinically relevant fungi (*Candida albicans and Taenia corporis*), the broth dilution method was used. Microbial cell lines were assayed for growth inhibition in 96 well plates as previously described. Growth suppression was quantitatively estimated as the minimal inhibitory concentration (MIC) of individual *Combretum molle* (stem bark) and *Xylopia aethiopica* extracts and of *Combretum molle* (stem bark)-*Xylopia aethiopica* co-extracts as described in an earlier report [15]. Vehicle and pharmacological drugs (ciprofloxacin, Ketoconazole) were included as the respective negative and positive controls as earlier noted [15]. Cultured microbes were treated with *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extracts and with *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extracts [(*Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit):3:2] at concentrations specified in the results (Tables 1-3). Potential synergy-mediated suppression of microbial growth by *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract was evaluated using estimated MICs in both bacterial and fungal cell lines.

Anti-inflammation activity

The anti-inflammation assay was performed with the *in vivo* carrageenan-induced foot swelling assay in 7-day old chick as previously described [15]. Briefly, induction of inflammation in the foot *via* subplantar injection into the right footpad with 1% suspension of carrageenan is followed by oral administration of serial dilutions of each *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extract to assess bioactivity and of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract to assess synergy [15]. Chicken were randomly segregated in groups of 5 and each group was exposed orally to one of five treatments: [(*Combretum molle* (stem bark) extract alone-30-300mg/kg, *Xylopia aethiopica* (fruit) extract alone-30-300mg/kg, *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract-30-300mg/kg, and two positive control drugs across different dose ranges (1-10mg/kg for Dexamethasone; 10-100mg/kg for Diclofenac)] in vehicle. Vehicle control animals received only vehicle (polyethylglycol 400 diluted 1:5 in 5% bovine serum albumin/H_2O). Oedema volumes were estimated as the difference between the foot volumes of each chick prior to carrageenan injection and the foot volume at hourly intervals (0-5h) post treatment. Anti-inflammatory responses of samples were taken as extract or control drug treatment-induced reduction in volume of swelling. Reductions in oedematous volumes estimated at hourly intervals was used to plot time-course depiction of sample-induced anti-inflammatory response. Net anti-inflammatory response after the time-course was portrayed in a dose-response curve. Computed sample ED50s relied on the use of linear regression as earlier described [15]. A one-way analysis of variance was used to analyze the raw data and the Bonferroni's modified t test used for the analysis of differences in groups of chicken. A p value pegged at 0.05 was considered significant.

Total phenolic content

Total Phenolic Concentrations of samples [*Combretum molle* (stem bark) alone, *Xylopia aethiopica* alone and *Combretum molle* (stem bark)-*Xylopia aethiopica* co-extracts] were determined spectrophotometrically using a modified Folin-Ciocalteu colorimetric method that relied on the use of Gallic Acid as standard [16]. Results were derived from interpolation from Gallic Acid standard curve and expressed in Gallic Acid Equivalents (GAE). Data are presented as Mean ± SD of three measurements.

Total antioxidant capacity

Individual *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extracts and *Combretum molle* (stem bark)-*Xylopia aethiopica* co-extract were assessed for Total Antioxidant Capacity *in vitro* using the Phosphomolybdenum method as detailed in a previously reported protocol [16]. A 5mL:5mL mixture of serially diluted standard Ascorbic Acid: Phosphomolybdenum reagent (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molyb-

date) were incubated at 95 ºC for 90mins. The absorbances of the solutions were subsequently taken at 695nm on a UV-VIS spectrophotometer. Serial dilutions of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extracts and of *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extracts (enabling assessment of synergy between *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) were similarly incubated with the Phosphomolybdenum reagent and the 695nm absorbance of the solution were likewise taken. Interpolation from a standard curve of absorbance versus concentration of Ascorbic acid provided the total antioxidant capacity of extracts and of co-extracts expressed in Ascorbic Acid Equivalents (AAE). The data is reported as the Mean ± SD of a triplicate experiments.

DPPH antioxidant activity

Assessment of the *in vitro* efficacy of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extracts and of *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extracts as antioxidant s was accomplished with the DPPH radical scavenging assay as described elsewhere [17]. Briefly, serial dilutions of the extracts and of the co-extract were treated with a standard solution of DPPH prior to the spectrophotometric-based estimation of the DPPH radical scavenging activity of the test samples as previously described [17]. Ascorbic acid was used as a positive control antioxidant. Phytochemical synergy was assessed via usage of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract mixture as antioxidant. Sample IC50s were estimated via linear regression analysis of respective dose-response curves of % DPPH inhibition versus sample concentration. Sample points are presented as Mean ± SD of three measurements.

Statistical Analyses

Experimental replicates were subjected to Mean and Standard Deviation calculation with the Microsoft Excel XP suite of programs.

Results

Phytochemical composition

Phytochemical compositions of natural product extracts are predictive of and indicative of bioactivity. To assess the phytochemical composition of extracts, standardized in-house diagnostic phytochemical screening was performed on the individual extracts as described in Materials and Methods.

As shown in Table 1, bioactive phytochemicals derive from different chemical and structural forms. *Combretum molle* (stem bark) extracts are enriched in phytochemical chemotypes that included Saponins, Tannins, Steroids, Flavonoids, Glycosides while *Xylopia aethiopica* (fruit) extracts contains Saponins, Steroids, Alkaloids, Terpenoids, and Anthraquinones (Table 1). Only Saponins and Steroids are common to both plants. Phytochemical-rich *Combretum molle* (stem bark) extract and phytochemical-rich *Xylopia aethiopica* (fruit) extracts are putatively considered to show multiple bioactivities. Comparison of phytochemical compositions of the individual plants suggests that the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract will possess a potential total pool of the most biologically active constituents than that of individual extracts alone.

Table 1: Total observable chemical entities as estimated by Thin layer chromatography (TLC) and the total phytochemical composition as screened by standard phytochemical analyses of the methanolic extracts of *Combretum molle* (stem bark) and of *Xylopia aethiopica* (fruit) plant samples.

TLC Results			
Sample	**Number of pots**	**Rf Values**	**Phytochemicals present**
C. molle	3	0.16, 0.42, 0.79	Saponins, Tanins, Steroids, Flavonoids, Glycosides
X. aethiopica	5	0.17, 0.57, 0.70, 0.83, 0.99	Saponins, Steroids, Alkaloids, Terpenoids, Anthraquinones

Thin layer chromatography

The three TLC bands for *Combretum molle* (stem bark) and the five TLC bands for *Xylopia aethiopica* (fruit) attest to the multi-constituent compositions of their respective polar extracts (Table 1). Rf values for all chromatographically separated bands showed sufficient band resolution with the chromatographic system (mobile and stationary phases) utilized for the assessment of the number of distinct chemical entities in the extracts. Additional confirmatory evidence supportive of the TLC is provided by the phytochemical composition that denoted a multiplicity of functional groups embedded, potentially, in different chemical entities.

Broth dilution assay

While microbial infections of wounds often cause diagnostic and therapeutic complications, anti-microbial effects of compounds on wounds is frequently associated with the promotion of healthy wound healing. To examine the anti-microbial effects of extracts and co-extracts, the standard broth dilution method was used and the estimated minimum inhibitory concentration (MIC) was utilized for the quantitative assessment of relative microbiocidal efficacies as described in Materials and Methods.

Pathogenic bacteria cell lines

Individual *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extracts as well as *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract inhibited bacterial growth with a range of MICs (708-6250µg/mL in Table 2) that were higher (10-100-fold difference higher) than that of the conventional antibiotic (ciprofloxacin has a range of 3.12-12.5µg/mL) used as positive controls (Table 3). MIC data demonstrate that both the individual *Com-*

bretum molle (stem bark) and *Xylopia aethiopica* (fruit) extracts and the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract are non-inhibitory to bacteria cells with the concentration range of 1.0-700µg/mL (Table 2).

Combretum molle (stem bark)

Of the individual extracts tested against bacteria cultures, *Combretum molle* (stem bark) was the most potent bactericide as its higher relative bactericidal potency was manifested in a stronger growth inhibitory effect than *Xylopia aethiopica* (fruit) (at least, a 2-fold lower MIC in Tables 2 & 3).

The bactericidal effect of *Combretum molle* (stem bark) extract on gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pyogenes,* and on the gram-negative bacteria *Pseudomonas aeruginosa* were demonstrably stronger than the inhibitory effect on *Escherichia Coli* as shown by MIC values (Table 3). Against the gram-positive *Streptococcus pyogenes, Combretum molle* (stem bark) shows the largest growth inhibitory effect with an MIC of 708µg/mL, a value that represents an 8.8-fold better anti-bacteria effect than that of *Xylopia aethiopica* (fruit) and a 2.2-fold better anti-biotic than that of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract (Table 3). Although growth suppressive effects of *Combretum molle* (stem bark) on the gram-positive bacteria *Staphylococcus aureus* and on the gram-negative *Pseudomonas aeruginosa* were lower (MIC for both cases is 1560µg/mL), it still exhibited a 3.78-fold better bactericidal activity than *Xylopia aethiopica* (fruit) and a 2-fold better anti-bacteria activity than *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract (Table 3).

Table 2: Growth inhibitory MICs assessed by the Broth Dilution assay for *Combretum molle* (stem bark) extract and for *Xylopia aethiopica* (fruit) extract and for *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract mixture.

Extract	Test Organism	Concentration (mg/ml)							
		25	12.5	6.25	3.125	1.562	0.78	0.39	0.195
C.molle	*S.aureus*	-	-	-	-	-	+	+	+
	S.pyogenes	-	-	-	-	-	-	+	+
	E.coli	-	-	-	-	+	+	+	+
	P.aeruginosa	-	-	-	-	-	+	+	+
	C.albicans	-	-	-	-	+	+	+	+
	T.corporis	-	-	-	-	+	+	+	+
X.aethiopica	*S.aureus*	-	-	-	-	+	+	+	+
	S.pyogenes	-	-	-	+	+	+	+	+
	E.coli	-	-	-	+	+	+	+	+
	P.aeruginosa	-	-	-	+	+	+	+	+
	C.albicans	-	-	-	-	+	+	+	+
	T.corporis	-	-	-	-	+	+	+	+
CMXA	*S.aureus*	-	-	-	-	-	+	+	+
	S.pyogenes	-	-	-	-	-	+	+	+
	E.coli	-	-	-	-	+	+	+	+
	P.aeruginosa	-	-	-	-	+	+	+	+
	C.albicans	-	-	-	-	-	+	+	+
	T.corporis	-	-	-	-	-	+	+	+

+ indicates microbial growth; - indicates no microbial growth; CMXA- *Combretum mole*; (stem bark)-*Xylopia aethiopica* (fruit) co-extract.

Table 3: MICs of all samples (*Combretum molle* (stem bark) extract, *Xylopia aethiopica* (fruit) extract, *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract mixture and control drugs (Ciprofloxacin and Ketoconazole) in the Broth dilution assay summarized for comparison against all test organisms.

Test Organisms	MIC (µg/ml)				
	C.molle	*X.aethiopica*	CMXA	Ciprofloxacin	Ketoconazole
S.aureus	1560	3120	1560	3.12	N/A
S.pyogenes	708	6250	1560	6.25	N/A
E.coli	3120	6250	3120	12.5	N/A
P.aeruginosa	1560	6250	3120	12.5	N/A

C.albicans	3120	3120	1560	N/A	25
T.corporis	3120	3120	1560	N/A	25

N/A-Not Applicable; CMXA- *Combretum molle*; (stem bark)-*Xylopia aethiopica* (fruit) co-extract

Among the botanical extracts, *Combretum molle* (stem bark) exerted its weakest bactericidal effect on *Escherichia coli* with an MIC of 3120µg/mL that accounted for at least a 2-fold difference weaker effect compared to that evoked against the other bacteria panelists (Table 2 & 3). Nevertheless, the individual *Combretum molle* (stem bark) bactericidal effect against *Escherichia coli* was still a 2-fold difference stronger than that of the individual *Xylopia aethiopica* (fruit).

Xylopia aethiopica (fruit)

In comparison, *Xylopia aethiopica* (fruit) extract was a relatively weak growth inhibitor of pathogenic bacteria exhibiting a higher range of MICs (3120-6250µg/mL) against bacteria panelists. MICs for *Xylopia aethiopica* (fruit) extracts was 3120µg/mL or higher (Table 3) and that compares unfavorably with MICs of the *Combretum molle* (stem bark) extract and with the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract. *Xylopia aethiopica's* (fruit) anti-bacteria effects resulted in decreased growth inhibition of gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* and in moderate growth inhibition of gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pyogenes*.

Combretum molle (stem bark)-*Xylopia aethiopica* (fruit) co-extract

The inhibitory effects of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract against pathogenic human bacteria was broad and largely of moderate species-specific efficacies. *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract showed higher relative potency against some bacteria species but lower effects against others when compared to the demonstrated bactericidal efficacies of individual *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extracts.

The *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract matched the potency of *Combretum molle* (stem bark) for the gram-positive *Staphylococcus aureus* and the gram-negative *Escherichia coli* yielding the same quantitative MIC value as that of *Combretum molle* (stem bark) alone (MIC against *Staphylococcus aureus* is1560µg/mL and against *Escherichia coli* is 3120µg/mL). However, the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract lagged behind *Combretum molle* (stem bark) in its inhibitory effect on gram-positive *Streptococcus pyogenes* and on gram-negative *Pseudomonas aeruginosa* showing in each case a 2-fold lower activity relative to that of *Combretum molle* (stem bark) (Table 3).

Compared to the drug control, the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract, shows minor anti-biotic activity against *Pseudomonas aeruginosa* and displays qualitatively insignificant anti-biotic activity against *Escherichia Coli* exhibiting the same MIC at 3120µg/ml (Table 3).

Control drugs

None of the growth inhibitory effects of the extract samples (*Combretum molle* (stem bark), *Xylopia aethiopica* (fruit) and *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract) outperformed that of the positive control drugs. Growth inhibition of all bacteria species by Ciprofloxacin occurred at relatively low concentrations with MIC values that are either 12.5µg/ml or better (Table 3).

Fungi

Against the two fungal species (*Candida albicans* and *Taenia corporis*), both *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) showed comparable anti-fungal activities exhibiting the same quantitative value of MICs at 3120µg/mL (Tables 2 & 3). Fungal susceptibility to *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract mixture's phytochemicals was moderately good as growth inhibition of both fungi occurred at relatively lower concentrations (co-extract evoked a 2-fold lower MIC for *Candida albicans* and *Taenia corporis* at 1560µg/mL) (Table 3). Although the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract outperformed the individual extracts, it failed to match the efficacy of the control drug Ketoconazole (Ketoconazole was an approximately 6-fold better anti-fungal agent than the co-extract) (Table 3).

Synergy in anti-fungal activity

The *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract screen revealed modest phyhtochemical synergy in reducing the proliferative growth of both fungal cell lines *(Candida albicans* and *Taenia corporis). Combretum molle* (stem bark) showed antifungal synergy with *Xylopia aethiopica* (fruit) *in vitro* inhibiting *Candida albicans* and *Taenia corporis* with relatively low MIC value (a 2.0-fold lower MIC in both *Candida albicans* and *Taenia corporis)* (Table 3). This quantitative level of growth inhibition evoked by the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract on the two fungal species is greater than the sum of the individual growth suppression achieved by distinct individual extracts.

Total phenolic content

Total phenolic contents of botanical extracts can be reliable predictors of antioxidant activities as a linear correlation of total phenolic content with antioxidant effects is a uniformly acknowledged relationship. To quantitatively estimate the total phenolic content, extracts and the co-extracts were subjected to the standard *in vitro* spectrophotometrical-based Folin-Ciocalteu colorimetric assay as detailed in Materials and Methods.

The data shows that *Combretum molle* (stem bark) is a polyphenol-rich plant whose total phenols exists in structurally distinct forms as Flavonoids and Tannins (Tables 1 & 4). Unlike *Combretum molle* (stem bark), *Xylopia aethiopica* (fruit) shows no detectable presence of either Tannins or Flavonoids (Tables 1 & 4) and consequently reported the lowest quantitative levels of total phenolic content (Table 4). The co-extract with its 3:2 weight/weight compositional mixture of *Combretum molle* (stem bark):*Xylopia aethiopica* (fruit) expectedly reported less polyphenol content than *Combretum molle* (stem bark) but demonstrated quantitatively more polyphenolic content than *Xylopia aethiopica* (fruit) (Table 4).

Table 4: Total Phenolic Content (gGAE/100g) and Total Antioxidant Capacity (gAAE/100g) of *Combretum molle* (stem bark) extract, *Xylopia aethiopica* (fruit) extract and *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract mixture.

Extract	Total Phenolic Content (gGAE/100g)	Total Antioxidant Capacity (gAAE/100g)
C.molle	61.65	14.65
X.aethiopica	4.6	12.4
CMXA	38.17	14.96

CMXA- *Combretum mole;* (stem bark)-*Xylopia aethiopica* (fruit) co-extract

Quantitative differences in the phenol levels of the extracts appear significant. In quantitative terms, *Combretum molle* (stem bark) reported a 13.4-fold higher total phenol content than *Xylopia aethiopica* (fruit) and a 1.6-fold higher total phenol content than the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract (Table 4). *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract shows an 8.3-fold higher total phenolic content relative to *Xylopia aethiopica* (fruit) (Table 4). The decreasing order of phenolic content as estimated by the Folin-Ciocalteu method is: *Combretum molle* (stem bark) >Co-extract of *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) >*Xylopia aethiopica* (fruit).

Total antioxidant capacity

Total antioxidant capacity encompasses the quantitative estimation of the sum of all antioxidant phytochemicals that are protective against oxidative stress in a sample. As a function of the combined action of all phytochemical entities (inclusive of polyphenols and any other anti-oxidative compounds) that have the capacity to neutralize several classes of reactive species, the quantitative value of Total Antioxidant Capacity correctly reflects antioxidant status and/or oxidative stress suppressive effects. To assess the total antioxidant capacity, extracts and the co-extracts were subjected to the standard *in vitro* Phosphomolybdenum assay as described in Materials and Methods.

High total polyphenolic content increases Total Antioxidant Capacities. However, neither the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract phenolic contents nor *Xylopia aethiopica* (fruit) phenolic contents correlated proportionally with their respective Total Antioxidant Capacities (Table 4). Total Antioxidant Capacities for the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract and for *Xylopia aethiopica* (fruit) were higher than would be expected based solely on their Total Phenolic contents (Table 4).

This observation suggests that other functionalities, including structurally distinct functional groups might be playing contributing roles in the elicitation of Total Antioxidant Capacities of the *Xylopia aethiopica* (fruit) extract and of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extracts. *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract's total Antioxidant Capacity was interestingly higher in quantitative terms than that of *Combretum molle* (stem bark) despite its comparatively lower (1.6-fold difference lower) total Phenolic content. Total Antioxidant Capacity of *Combretum molle* (stem bark) is only 1.18-fold higher than that of *Xylopia aethiopica* (fruit) but a meager 1.02-fold lower than that of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract (Table 4). *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract's Total Antioxidant Capacity is 1.2-fold higher than that of *Xylopia aethiopica* (fruit) (Table 4). The decreasing order of Total Antioxidant Capacities, as estimated by the Phosphomolybdenum assay, is: co-extract of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) >*Combretum molle* (stem bark) > *Xylopia aethiopica* (fruit).

DPPH radical scavenging antioxidant assay

Initiation and propagation of oxidative stress ultimately disrupts wound healing at skin and mucosal surfaces while antioxidant inactivation of concurrent cellular oxidative stress elicits cyto-protective and wound healing-enhancing effects. To assess the relative anti-oxidative response of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extracts and *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extracts, *in vitro* DPPH radical scavenging assay specified in Material and Methods section was used.

Combretum molle (stem bark)-*Xylopia aethiopica* (fruit) co-extract is a better antioxidant than Ascorbic acid showing a 3.6-fold lower IC50 than the Ascorbic acid control (Table 5). As noted, the highest phenolic content of *Combretum molle* (stem bark) did not translate into the highest proportionate DPPH antioxidant activity as the relatively lower phenolic content of *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract generated the highest DPPH scavenging. The DPPH radical scavenging activity of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract far outstripped that of the individual *Combretum molle* (stem bark) extract and of the individual *Xylopia aethiopica* (fruit) extract show-

ing a 38.2-fold and a 75.8-fold lower IC50s than the corresponding IC50s for the respective *Combretum molle* (stem bark) extract alone and for *Xylopia aethiopica* (fruit) alone (Table 5).

Table 5: Estimated DPPH antioxidant IC50s triggered by individual extracts, by the co-extract mixture and by the Ascorbic acid control.

Sample	IC50 (DPPH Scavenging)
C.molle	600.8
X.aethiopica	1191
CMXA	15.72
Ascorbic Acid	55.94
Gallic Acid	N/A

CMXA- *Combretum mole*; (stem bark)-*Xylopia aethiopica* (fruit) co-extract

For both *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extracts, DPPH antioxidant activity correlated with its Total Phenolic Content and with its Total Antioxidant Capacity (Tables 4-5). *Combretum molle* (stem bark) showed predictably higher DPPH antioxidant activities than *Xylopia aethiopica* (fruit), demonstrating a 1.98-fold higher efficacy (Table 5) that is expected based on its proportionate quantitative amount of Total Phenolic Content only. Although the rank of the DPPH antioxidant activity was observed to be a direct correlate of the rank of the Total Antioxidant Capacity of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract, the corresponding co-extract's DPPH antioxidant IC50 was disproportionately lower quantitatively (implying higher antioxidant activity) than it should have been if this activity were based solely on its relative phenolic content or on its relative Total Antioxidant Capacity (Tables 4 & 5). The decreasing order of antioxidant effects as estimated by the DPPH radical scavenging assay is: co-extract of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit)>Ascorbic Acid> *Combretum molle* (stem bark)>*Xylopia aethiopica* (fruit).

Synergy in antioxidant response

DPPH scavenging assay revealed, in quantitative terms, a strong synergy between the phytochemicals of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) of the co-extract in reducing oxidative stress. Compared to Ascorbic Acid, the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract phytochemicals were more efficient DPPH radical scavengers (*Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract showed a 3.6-fold better DPPH scavenging ability than individual plant extracts) (Table 5). The *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract's demonstration of higher levels of DPPH antioxidant activity than *Combretum molle* (stem bark) alone and/or than *Xylopia aethiopica* (fruit) alone is in line with the quantitative order of ranking of the Total Antioxidant Capacities of the extracts. But the unusually high quantitative differences in the DPPH value of the co-extract and of the individual extracts suggests other factors besides the Total Antioxidant Capacity values are in operation. This observation suggests that non-covalent co-extract phytochemical interactions can affect its biochemical constitution leading to the induction of phytochemical synergies and a higher than expected DPPH antioxidant effects.

Anti-inflammation

Tissue and mucosal inflammations are early events in the pathogenesis of wounds and anti-inflammatory responses to wounds are similarly critical early predictors and indicators of healthy wound healing. To examine whether *Combretum molle* (stem bark) used alone or in combination with *Xylopia aethiopica* (fruit) slows the adverse etiological progression of wounds through inflammatory arrest, the carrageenan-induced chick assay in 7-day old chick as described in Materials and Methods was used. Test samples' anti-inflammatory responses were qualitatively and quantitatively presented as time-course outcomes (Figures 1 & 2) and displayed as net dose-response effects (Figures 3 & 4).

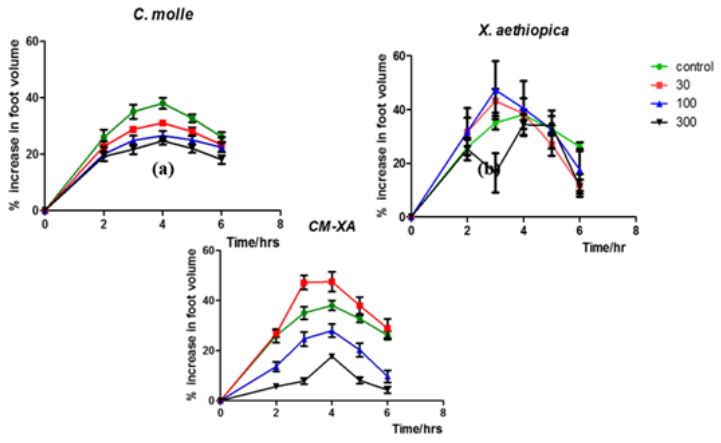

Figure 1: Time course of inhibitory effects of extracts on the carrageenan-induced inflammation of the 7-day old chick foot. Estimated oedema volume inhibitions (%) per time (h) of sample treatment (p.t.) are presented for: (a) *Combretum molle* (stem bark) extract (b) *Xylopia aethiopica* (fruit) extract and (c) *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract. Samples were administered in three different doses and each sample-induced inhibition in oedematous volume is depicted by a different color.

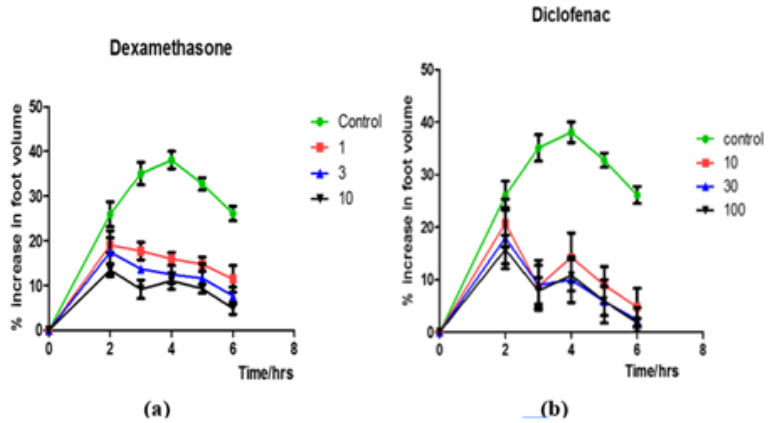

Figure 2: Time course of inhibitory effects of control drugs on oedema volumes in the carrageenan-induced chick foot swelling assay. Anti-inflammatory responses triggered by: (a) Dexamethasone and (b) Diclofenac treatments in three different doses are presented by the three different colored curves.

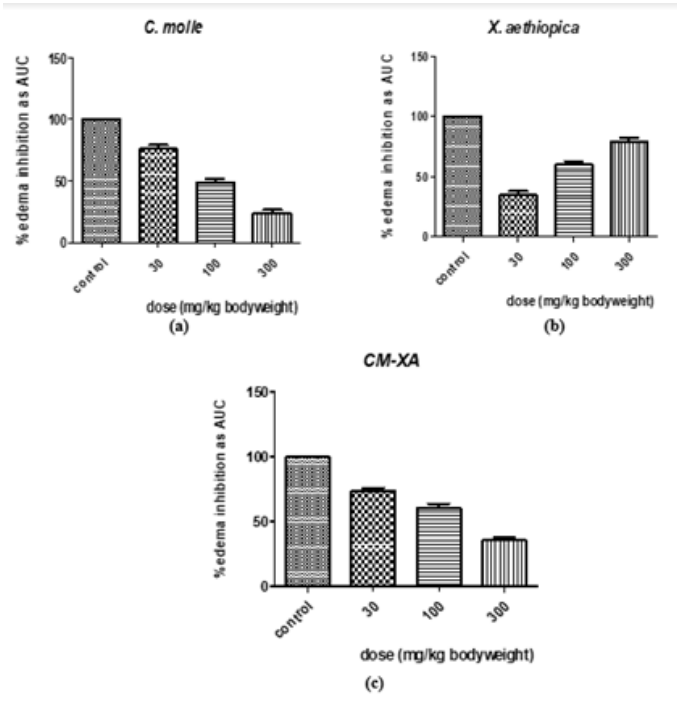

Figure 3: Measured inhibition in oedematous volume (%) in the carrageenan-induced inflammation of the 7-day chick foot after treatment with (a) *Combretum molle* (stem bark) extract (b) *Xylopia aethiopica* (fruit) extract and (c) *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract. Graded levels of anti-oedematous responses of extracts were estimated after treatment period. AUC refers to area under the curve.

Time course

The broad window of anti-inflammation effects characterized by its post treatment (p.t.) onset time, its magnitude of suppressive effect, and its duration of response is indicative of the strength of efficacy of the intervention. To assess whether or not critical sensitivity time points of anti-inflammatory responses are triggered by samples (individual *Combretum molle* (stem bark) and individual *Xylopia aethiopica* (fruit) extracts, combination extract of *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) and control drugs), time course studies were performed. Estimated hourly oedematous volumes were correlated with p.t. time at 2, 4 and 6h as described in Materials and Methods. Variations in the evoked % inhibition of oedema volume over the 6h time course by samples are depicted in Figures 1 & 2.

Combretum molle (stem bark)

Graded levels of the anti-oedematous responses evoked by *Combretum molle* (stem bark) extract over a 6h period (Figure 1) for all three doses suggests an anti-inflammatory response that was quick, effective and robust. Peak anti-inflammatory responses of 62% occurred at 2h for the 300mg/kg dose. All doses of *Combretum molle* (stem bark) extract lost the initial high inhibitory

effects attained on the 2 h mark and thereafter all high inhibitory levels decreased consistently to a maximum at the 4 h mark for all doses. All doses of extract recorded higher levels of inhibition at the final time-point of 6 h during which inflammation regresses to 50-60% (Figure 1).

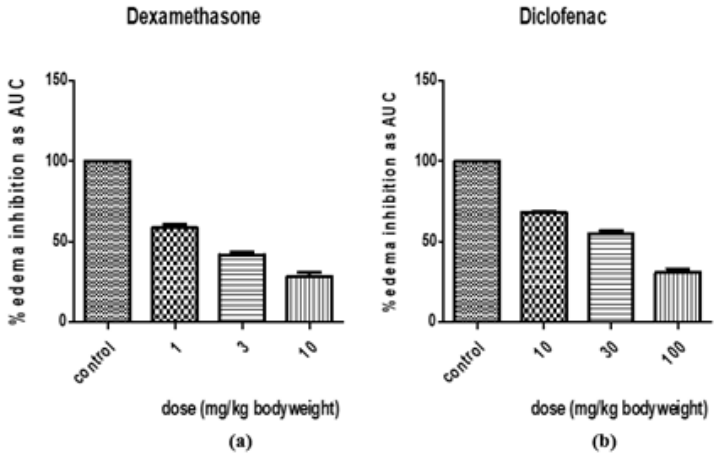

Figure 4: Estimated drug-induced inhibition of oedematous volume (%) in the carrageenan-induced inflammation of the 7-day chick foot. Inflammation suppression by (a) Dexamethasone and (b) Diclofenac treatments in three different doses are presented as bar graphs. AUC refers to area under the curve.

Xylopia aethiopica (fruit)

Quantitative variabilities in efficacy of *Xylopia aethiopica* (fruit) across the three dose ranges and within the 0-6h time course range shows uneven dose-specific anti-oedematous responses (Figure 1). Peak suppressive effects varied and occurred at different times for the different doses. The 300mg/kg dose showed a peak inhibition of 90% after 6h; the 100mg/kg resulted in a peak suppressive effect of 87% after 6h of ingestion; and 30mg/kg reached a peak anti-oedematous response of 86% after 6h) (Figure 1).

Table 6: Estimated anti-inflammatory ED50s triggered by individual extracts (*Combretum molle* (stem bark), *Xylopia aethiopica* (fruit)), by the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract mixture and by the standard control drugs (Dexamethasone, Diclofenac) on the carrageenan-induced oedema of 7-day chick foot.

Sample	ED50 (mg/kg)
C.molle Extract	59.77
X.aethiopica Extract	72.43
CMXA Extract	42.63
Dexamethasone	5.26
Diclofenac	166.3

CMXA- *Combretum mole*; (stem bark)-*Xylopia aethiopica* (fruit) co-extract

The 1-5h period was marked by declining inhibition as a function of time with the only exception of high anti-inflammation exhibited by the 300mg/kg dose to 85% after 3h of *Xylopia aethiopica* (fruit) extract ingestion. *Xylopia aethiopica* (fruit) was unable to maintain the decline in inflammation evoked by the 300mg/kg extract for 3-5h duration after ingestion.

Combretum molle (stem bark)-*Xylopia aethiopica* (fruit) co-extract

Anti-inflammatory response at 2h p.t. was greatest at the highest dose (300mg/kg) of *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract than at the lower doses (compare the three 2h time points of the 30mg/kg and 100mg/kg and 300mg/kg doses in Figure 1). For all administered doses of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract, inhibition declined at 4h before increasing at 5h on a route to the best inhibitory effect of 90% at 6 h. Decreases in oedema volumes evoked by 30mg/kg was larger than that evoked by 100mg/kg at 4h (compare the 4h time points of the two doses in Figure 1).

Time Course: Control Drugs Dexamethasone and Diclofenac

Dexamethasone

Anti-inflammatory responses were maximum at 6h post-treatment for all doses. The strongest anti-oedematous response occurred at the highest dose of 100mg/kg at 6h where the drug-induced decrease in inflammation was greatest (90%) (Figure 2). In all cases, inflammation inhibitory effects of the Dexamethasone after 4h increased with time and were still significant 5h after treatment.

Diclofenac

Qualitatively, the anti-inflammatory response rates followed a pattern similar to that of Dexamethasone. In all cases, drug-induced decreases in inflammation were greatest at 6h p.t. Peak inhibition

of 95% was recorded by the 100mg/kg, 6h after ingestion. Time course of diclofenac-induced inhibition of inflammation at 4, 5 and 6h post treatment shows a regression of inflammation over the 4-6h duration (Figure 2).

Concluding Comment: Time Course of Anti-Inflammation

While *Combretum molle* (stem bark) extract elicited peak inhibition at 2h with 300mg/kg dose, *Xylopia aethiopica* (fruit) extract evoke maximum inhibition at 3h with the same 300mg/kg dose. The comparable dose of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract (300mg/kg) was far more effective, and although efficacy waned to 80% after 3h after treatment, efficacy was nonetheless better than that of the *Combretum molle* (stem bark) alone and *Xylopia aethiopica* (fruit) extract alone (Figure 1).

Dose-Response: *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extracts and of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract mixture

To assess further whether or not the net relative inhibitory activities of the individual *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extracts and of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extracts are dose-dependent, calculated oedematous volumes were linked to their respective doses of utilized test samples after 24h in a method described in the Materials and Methods section. Calculated inhibition percentage and its representation in a net dose response curve for test samples [*Combretum molle* (stem bark), *Xylopia aethiopica* (fruit), *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract, control drugs] are shown in Figures 3 & 4.

Combretum molle (stem bark)

Combretum molle (stem bark) extract modulated the inflammatory response induced by carrageenan via a dose-dependent shrinkage of the swollen chick foot (Figure 3). Minimization of the foot oedema by *Combretum molle* (stem bark) at 30mg/kg was 2-fold lower than that observed with the 100mg/kg *Combretum molle* (stem bark) extract. The strength of the efficacy of inhibition for the 300mg/kg dose surpassed that of the 100mg/kg dose by 27% and that of the 30 doses by 50% (Figure 3).

Xylopia aethiopica (fruit)

Suppressive action on inflamed chick feet showed a direct relationship with the amount of ingested *Xylopia aethiopica* (fruit) extract (Figure 3). Administration of *Xylopia aethiopica* (fruit) extract at 30mg/kg produced a 1.4-fold higher anti-inflammatory effect on chick feet oedema compared to the 100mg/kg of the same extract. *Xylopia aethiopica* (fruit) extract was similarly effective at lower concentrations as its 100mg/kg administration yielded a 1.6-fold higher anti-inflammation relative to that of the highest dose of 300 mg/kg. Although efficacies of outcomes for both extracts are moderate, the contrast in anti-inflammatory responses between *Combretum molle* (stem bark) extract and *Xylopia aethiopica* (fruit) extract is appreciable (compare Figure 3a with Figure 3b). Overall, *Xylopia aethiopica* (fruit) extracts induced anti-inflammation to a larger quantitative degree than the suppressive effects evoked by *Combretum molle* (stem bark).

Combretum molle (stem bark)-*Xylopia aethiopica* (fruit) co-extract

A direct dose-response relationship between ingested co-extract concentration and triggered anti-oedematous effect was displayed by the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract (Figure 3c). The highest dose (300mg/kg) of *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract induced the highest proportionate anti-inflammatory effect of 63% inhibition. Chicken treated with a lower dose of *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extracts (100mg/kg) had a proportionally mean reduction of swelling of 48% while the lowest dose of 30mg/kg triggered an equivalently lowest suppressive effect of 22%.

The anti-inflammatory effect exerted by the 300mg/kg of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract represents an 0.97-fold higher suppressive effect relative to the individual *Combretum molle* (stem bark) extract at an analogous 300mg/kg dose and a 2.33-fold higher inhibitory effect relative to *Xylopia aethiopica* (fruit) extract at the same dose of 300mg/kg (Figure 3). *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract at 100mg/kg, significantly reduced mean foot oedema of chicks, to levels comparable to that of the steroidal positive control drug dexamethasone at 10mg/kg and to the nonsteroidal anti-inflammatory drug Diclofenac at 100mg/kg (compare Figure 3 with Figure 4).

Dose-Response: Control Drugs Dexamethasone, Diclofenac

Dexamethasone

Dexamethasone was the strongest anti-inflammatory agent per ingested dose with relatively higher average reductions in volume of swelling in chicks at comparatively lower doses than those of individual extracts at higher doses. The strongest anti-oedematous response occurred at the highest dose (10mg/kg) of Dexamethasone where the drug-induced decrease in inflammation was greatest (49%) (Figure 4). Administration of the 3mg/kg dose of Dexamethasone led to about 1.3-fold lower anti-inflammatory effect relative to the 100mg/kg dose of individual extracts. Anti-inflammatory response of the lowest dose (1mg/kg) was marginally lower than that of the 30mg/kg of the individual extracts and of the co-extract by about 1.2% (Figures 3 & 4).

Diclofenac

Analogous to the dose-response curves of dexamethasone, diclofenac also showed stronger anti-inflammatory activities at lower doses relative to extracts but its efficacy was slightly lower than that of dexamethasone. But average anti-inflammatory responses were lower in diclofenac than they were in dexamethasone (compare the two end-point anti-inflammatory responses in Figures 3 & 4). The 10mg/kg of Diclofenac resulted in 20% inhibition of inflammation; the 30mg/kg of Diclofenac reduced inflammation by 42% while 100mg/kg of Diclofenac yielded 54% anti-oedematous effect. These results confirm that lower concentrations of Diclofenac induced anti-inflammatory responses commensurate with higher concentrations of both individual botanical extracts.

Comparative dose-response with EC50 values

ED50 values show that although the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract had the highest anti-inflammatory effect (1.40 fold higher than *Combretum molle* (stem bark); 1.69-fold higher than that of *Xylopia aethiopica* (fruit) and 3.9-fold higher than that of Diclofenac), its phytochemical pool failed to induce synergistic anti-oedematous effect. All extracts [*Combretum molle* (stem bark) extract and *Xylopia aethiopica* (fruit) extract and *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract], however, showed anti-inflammatory effects more potent than Diclofenac but less potent than Dexamethasone as demonstrated by sample ED50s (Table 6).

Discussion

This report presented evidence indicating a strong antioxidant synergism and a modest anti-fungal synergism resulting from the complementary bioactivities of the co-extract phytochemicals of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit). Based on its historical ethnomedicine use and on the strength of this experimental evidence, *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) co-extract mixture may be regarded not only as suitable botanical phytochemicals with which to initiate and sustain healthy wound healing but also to elucidate some of the primary molecular events that occur mechanistically during healthy wound-healing.

The polyphenol-rich content of *Combretum molle* (stem bark) (Tannins and Flavonoids) augments the alkaloid-rich, terpenoid-rich and anthraquinone-rich content of *Xylopia aethiopica* (fruit) leading to expanded and heightened bioactivities of the co-extract. Anthraquinones have proven anti-inflammatory potential [18,19]. Polyphenol-rich (Tannins) botanical mixtures are known to confer antioxidant and anti-inflammatory health benefits [18,19]. Alkaloid-induced suppression of microbial growth is also well known [18,19]. Taken together, the phytochemical composition suggests that the phytochemical constituent pool of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract constitute one of the most potentially strong biologically active constituents with capabilities that span a broader scope of bioactivities than that of individual extracts. The mechanistic mode of action of co-extract phytochemicals in wounds might therefore be multiple and might even encompass other biochemical activities distinct from the assessed anti-microbial, antioxidant and anti-inflammation effects.

The synergy mixture contains lower discrete extract concentrations relative to individual extracts. Although lower than individual extracts, as the effects of varying doses on the biochemical assays show, utilized/administered doses of co-extracts contain clinically relevant concentrations of the phytochemical biomolecules capable of inducing bioactivity. As a consequence, co-extracts dosed concurrently with lower concentrations of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) delivers the full complement of bioactive phytochemicals capable of evoking additive, synergistic or antagonistic effects in its therapeutic action. The use of lower concentrations of both extracts is also expected to lower the potential dose-induced toxicity of phytochemicals to levels at which the co-extract exhibits little undesirable side-effects and is well tolerated by users.

The spectrum of phytochemicals in assayed co-extracts allows certain operational hypothesis to be propounded regarding the net functional phytochemical mechanism for optimum chemotherapeutic action. Emerging scientific consensus, with ample literature support, posit that the net biological effect of the crude natural product mixtures is the result of the combined bioactivities of multiple constituents at multiple bio-molecular target sites in mechanisms that are either additive, synergistic or antagonistic in functional outcome [18,19]. Different permutations and combinations of additivity/synergy/antagonism are therefore summoned functionally and differentially by the individual constituents of the phytochemical pool to evoke the depth (potency) and the breadth (diversity) of the net bioactivity via biochemical responses that are either sequential or simultaneous [20]. And even where similar classes of phytochemicals are present in both plants, the enrichment of the phytochemical pool of the co-extract with likely structurally unique compounds of the same functional group will more likely facilitate synergistic activation of one or more bioactivities [21].

Co-administration of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extracts containing a diverse mixture of phytochemicals (structurally and functionally) will boost bioactivity either through pharmacokinetic interactions that result from inactivation of phytochemical elimination pathways or through phytochemical-receptor pharmacodynamic interactions leading to the observed antioxidant or anti-microbial or anti-inflammation effects that are additive or synergistic or antagonistic [18,19]. At the molecular level, it is likely *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract phytochemical interactions will induce a differential gene expression profile distinct from profiles activated by either extract alone. And at the physiological level, such gene

expressions may find complementary expression in the initiation, restoration and maintenance of healthy wound healing.

Uniform display of broadly modest anti-bacteria efficacy against all tested pathogenic human bacteria is in line with a diverse species-specific mechanism of action of unique phytochemicals. The results also suggest that the co-extract exhibited enhanced bacteria cell growth inhibitory activity over that of individual polant extracts and that co-use of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extracts should provide either intermittent or sustained bactericidal and/or bacteriostatic effect. Molecular level attempts to correlate the apparent low/non-susceptibility of some bacteria to the anti-bacterial effects of the co-extract phytochemicals appear contradictory and may well be easily explained as a bacteria variant-specific trait. Administered either orally or topically, *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extracts suppression of the proliferative growth of a wide range of bacterial pathogens in infected wounds represents an attractive appeal of its ethnomedicinal utility and provide a molecular support for its enduring ethnomedicinal efficacy [22].

Co-extract demonstration of a modest *in vitro* synergistic anti-fungal efficacy against the two pathogenic fungal cell lines, *Candida albicans* and *Taenia corporis*, suggests that co-use of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extracts can provide strong and sustained inhibition of fungal cell proliferation and/or potentially suppress fungal cell survival and virulence. The effects of co-extract phytochemicals observed with *Candida albicans* and *Taenia corporis* fungi is likely mediated through interactions with fungal-specific receptors for two reasons: the failure of the phytochemicals to reduce bacterial growth in a complementary synergistic manner and the successful execution of identical synergistic results with the two molecularly distinct fungal cell lines [23]. It could be surmised that embedded in the broad anti-proliferative effect on bacteria are phytochemicals whose activities draw upon additivity and/or antagonism while rooted in the modest synergy in anti-fungal effects are phytochemical constituents whose activities functionally co-operate to potentiate a net synergistic effect.

The efficacy of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract to suppress both bacterial and fungal growth provides important insight into therapeutic options besides the use of conventional fungicidal drugs to manage such unique fungal infections such as the formation of cross kingdom polymicrobial biofilms. Cross-kingdom polymicrobial biofilms are formed from the synergistic interaction of fungi with bacteria in an infectious microbial association that has a distinctive quorum-sensing capability within a molecularly unique polymicrobial biofilm [24]. This polymicrobial association enhances microbial virulence and increases microbial drug resistance. Taken together with the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract-mediated suppression of bacteria growth, the synergistic inhibition of fungal growth is ideally suited to chemotherapeutic mechanistic activities that mitigates the formation of cross kingdom polymicrobial biofilms and prevents quorum-sensing in the biofilm and can be exploited in healthy wound healing interventions. Thus, bacteria and fungi attracted to wound sites are strongly inhibited in their proliferative growth by *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract phytochemicals. Future studies can examine bioactive botanical compound modulation of cellular growth, cell-cycle checkpoints, fungal quorum-sensing/quenching effects and its biomarker(s) signaling, its associated fungal virulence and its efflux pump-mediated inhibition *in vivo*.

Polyphenol phytochemicals in *Combretum molle* (stem bark) extracts are likely responsible for much of the antioxidant effects of the co-extract mixture. But lack of direct correlations between phenols and DPPH-based antioxidant activity argues also for the involvement of non-phenolic antioxidant phytochemicals in the mediation of the observed antioxidant response. That the *in vitro* antioxidant synergy is so potent argues for the involvement of non-covalent interactions between *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) phytochemicals that potentiates an overall synergistic antioxidant scavenging of DPPH free radicals *in vitro*. Mechanistically, *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) co-extract phytochemical-mediated decreases in oxidative stress is likely attained through the activation/deactivation of the endogenous antioxidant genes expression patterns in response pathways leading to the regulation of the cellular redox status and guiding the eventual enhancement of healthy wound healing (Hsieh and Wu, 2008). In this context, *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) co-extract mixture's phytochemical pool can also limit free radical-mediated damage via the induction or upregulation of various endogenous antioxidant enzymes present in wound surfaces such as catalase and superoxide dismutases [25].

Dysregulation of NF-kB activation/deactivation is the established mechanism leading to induction of inflammation by carrageenan [26]. Whether inflammation is of the short- or long-term duration, proinflammatory and anti-inflammatory stimuli engages genes of the NF-kB pathway leading to selective induction/de-activation of multiple pathway targets and the eventual evocation of inflammatory/anti-inflammatory responses. But aberrant NF-kB pathway activation/deactivation are also observed during oxidative stress induced dysregulation of antioxidant response pathways. Antioxidant phytochemicals including polyphenols can, thus, play a dual role in mechanisms specific for anti-inflammation effects and in processes specific for antioxidant effects. Inhibition of ROS by extract polyphenols does not only lead to a decrease in associated inflammatory responses but might directly inhibit oxidative stress through the attenuation of NF-kB activation/deactivation [27,28]. Consequently, the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract phytochemicals specifically block/unblock NF-kB signaling pathways to mitigate vascular and cellular inflammation

and simultaneously block/unblock NF-kB pathway activation to evoke synergistic neutralization of reactive oxygen species (ROS). Co-extract antioxidant synergy constituents therefore represents promising candidates for healthy wound healing interventions.

Contextually, the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract mixture also plays a protective anti-inflammatory role as demonstrated by its robust inhibition of the carrageenan-induced inflammation of chicken feet. The hypothesis that the anti-inflammatory activities of the potentially structurally diverse phytochemicals particularly flavonoids are due, in part, to their structure-dependent interactions at multiple targets appears promising Individual constituents of the botanical extracts may interact with multiple specific receptors on immune, on endothelial, and on antioxidant receptors to regulate downstream genetic and epigenetic signaling pathways, modulate global gene expression that leads to the amelioration of inflammation [25]. Future studies can identify the mechanisms through which such botanical phytochemicals suppress NF-kB-mediated inflammation, specifically looking at phytochemical targets and assessing relative global gene expression levels following ingestion/topical treatment. Together, such future studies can identify pathways through which *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) botanicals co-extract suppress inflammation thereby paving the way for better treatment modality against inflammatory responses in wounds.

The lack of synergy in anti-inflammation effects demonstrated here by the *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) co-extract opposes the general findings of previous studies that showed significant functional synergism in the anti-inflammatory activity of the co-extract of *Strophanthus hispidus* (roots) and *Aframomum meleguta* (seeds) [15]. The apparent different anti-inflammatory response observed here for the co-extract of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) may well be attributable to variations in the number, molecular structures and functionalities of its phytochemical pool and in the eventual collective mechanism-specific bioactivity they engender.

Future additional studies can be used to define the breadth of antifungal and antioxidant activities *in vivo* as well as to determine the mechanistic specificities of the different bioactivities. In this context, future research of high priority can, therefore, encompass testing the fungicidal efficacy of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extracts against a broader array of clinically-relevant fungi such as *Cryptococcal meningitis*, *Cryptococcus neoformans*, *Cryptococcus gattii, Coccidioides immitis* and *Coccidioides posadasii*. *In vivo* efficacy of the co-extracts' fungicidal activities can also be tested in animal models of three of the major invasive fungal infections: invasive aspergillosis, pulmonary cryptococcosis and mucormycosis. Given its anti-fungal phytochemical synergy, anti-fungal efficacy of the *Combretum molle* (stem bark)-*Xylopia aethiopica (fruit)* co-extracts can also be tested in combination with other classes of antifungal agents (such as echinocandins, polyenes, flucytosine and azoles) to assess for multi-drug synergistic activity capable of mitigating multi-drug resistance pathogenic fungal infections [23]. To expand the structure-based studies leading to the development of novel synergistic, broad-spectrum antifungals, synergy-directed fractionation can be used to identify compounds with potential synergy to potentiate bioactivity. Synergy studies can also be employed to explore the ability of identified co-extract constituents to promote sensitivity of multi-drug resistant fungi to established anti-fungal agents such as fluconazole and amphotericin B [23,29].

How does the antimicrobial, anti-inflammatory and antioxidant activities evoked by the phytochemical pool of *Combretum molle* (stem bark)-*Xylopia aethiopica (fruit)* co-extracts interact to trigger healthy wound healing? The data presented here, together with reports in the extant literature [30-32], allows the proposition of a sketchy mechanism of action of the *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) co-extract phytochemicals. Interaction with multiple targets may explain the prevalence of multiple bioactivities with both individual *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) extracts and with the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extracts. Co-extracts' capability of synchronizing its multiple activation of targets in lockstep with each other is critical for the potentiation of a synergistic response in anti-fungal and well as in antioxidant effects.

In view of the continual accrual of evidence from ethnomedicinal sources supporting the evocation of multi-mechanism at multisites by crude extract phytochemicals [18,19], *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) co-extract may well be regarded not only as a suitable botanical extract with which to elucidate the primary events that occur in the healthy wound-healing but can also be used to explore phytochemical synergy as a mechanistic affirmation of its ethnomedicinal remedy. Since use of multiple plants is a mainstay operational method for ethnomedicinal remedies, this study provides a generalized mechanism for the elucidation of phytochemical interactions in crude medicinal extracts and offers additional scientific evidentiary support for traditional medicinal practices that are rooted in historical precedents but are often steeped in religiosity [18,19].

Summary/Conclusion

Combretum molle (stem bark)-*Xylopia aethiopica* (fruit) co-extract have a long history of traditional use for the management of wounds yet the basic scientific support base for their co-use of their aqueous extracts are yet to be established. The goal of understanding, in mechanistic terms, how co-use of the extracts of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) botanicals contribute to wound healing therefore formed the scientific basis of this study. The experimental investigation concentrated on the assessment of the bioactive effects of *Combretum molle* (stem bark) in combination with *Xylopia aethiopica* (fruit) on the mitigation of

microbial proliferation, on the attenuation of oxidative activities and on the suppression of the inflammation response in well-established biochemical assays.

The *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract mixture constitutes a pool of chemically distinct phytochemical compound classes that included tannin, steroid and flavonoid, all of which are known to inhibit inflammation-driven cellular activities; are reported to attenuate resident microbial activities; and are previously described in the literature to mitigate cellular oxidative stress through the evocation of multi-mechanisms at multi-target sites. Compared to individual plants, the relatively higher efficacy of the *Combretum molle* (stem bark)-*Xylopia aethiopica* (fruit) co-extract in managing wound healing can be putatively attributed to the larger pool of and variable composition of molecularly distinct phytochemicals that work in synergy in some biochemical mechanisms to alleviate some of the symptoms.

Consequently, the most notable results of this study are the observation that co-extract mixture stimulates healthy wound healing by attenuating bacteria growth nominally; by mitigating fungal growth synergistically; by alleviating oxidative stress synergistically and by inhibiting inflammation-driven cellular activities nominally.

Taken together, these results demonstrate that *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) co-extract can be designed to target fungal systems with a good level of specificity and efficacy. Isolation of and synthetic elaboration of distinct phytochemical synergists can lead to the development of compounds that can more strongly suppress cellular ROS generation and effectively suppress oedematous response via the activation/deactivation of NF-kB genes and gene products. Together, these results also suggest that the co-use of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) is beneficial for the healing of acute and chronic skin, epithelia and mucosal wound conditions and that the therapeutic efficacy of the co-extract to some facets of healthy wound healing effects resides in synergy of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) phytochemicals. This conclusion dovetails with emerging scientific consensus, with ample literature support, that posit that the net biological effect of crude botanical extracts is the result of the combined bioactivities of multiple constituents that are either additive, synergistic or antagonistic in functional outcome. Phytochemical synergy could be a mechanism by which *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) co-extract phytochemicals exerts its antioxidant-protective actions and its anti-fungal effects on wounds. Future studies will allow the focused screening and optimization of *Combretum molle* (stem bark) and *Xylopia aethiopica* (fruit) phytochemical synergists as new antifungals and as potentially novel antioxidants and will also explore their development as efficacious and healthy wound-healing prophylaxis.

References

1. Adaramoye OA, Erguen B, Nitzsche B, Höpfner M, Jung K, et al. (2016) Antioxidant and antiproliferative potentials of methanol extract of *Xylopia aethiopica* (Dunal) A. Rich in PC-3 and LNCaP cells. J Basic Clin Physiol Pharmacol 28(4): 403-412.
2. Mulaw T, Wubetu M, Dessie B, Demeke G, Molla Y (2019) Evaluation of antimalarial activity of the 80% methanolic stem bark extract of *combretum molle* against plasmodium berghei in mice. J Evid Based Integr Med 24: 2515690 X19890866.
3. Osafo N, Obiri DD, Antwi AO, Yeboah OK (2018) The acute anti-inflammatory action of xylopic acid isolated from *Xylopia aethiopica*. J Basic Clin Physiol Pharmacol 29(6): 659-669.
4. Ekuadzi E, Biney RP, Benneh CK, Osei Amankwaa B, Jato J (2017) Anti-inflammatory properties of betulinic acid and xylopic acid in the carrageenan-induced pleurisy model of lung inflammation in mice. Phytother Res 32(3): 480-487.
5. Ojewole JA (2008) Analgesic and antiinflammatory effects of mollic acid glucoside, a 1- alpha-hydroxycycloartenoid saponin extractive from *Combretum molle* R. Br. ex G. Don (*Combretaceae*) leaf. Phytother Res 22(1): 30-35.
6. Simon MK, Ajanusi OJ, Abubakar MS, Idris AL, Suleiman MM (2012) The antihelmintic effect of aqueous methanol extract of *Combretum molle* (R. Br. x. G. Don) (*Combretaceae*) in lambs experimentally infected with *Haemonchus contortus*. Vet Parasitol 187(1-2): 280-284.
7. Biney RP, Benneh CK, Ameyaw EO, Boakye-Gyasi E, Woode E (2016) *Xylopia aethiopica* fruit extract exhibits antidepressant-like effect *via* interaction with serotonergic neurotransmission in mice. J Ethnopharmacol 184: 49-57.
8. Mohammed A, Islam MS (2017) Antioxidant potential of *Xylopia aethiopica* fruit acetone fraction in a type 2 diabetes model of rats. Biomed Pharmacother 96: 30-36.
9. Fankam AG, Kuiate JR, Kuete V (2015) Antibacterial and antibiotic resistance modifying activity of the extracts from *Allanblackia gabonensis*, *Combretum molle* and *Gladiolus quartinianus* against Gram-negative bacteria including multi-drug resistant phenotypes. BMC Complement Altern Med 15: 206.
10. Regassa F, Araya M (2012) *In vitro* antimicrobial activity of *Combretum molle* (*Combretaceae*) against *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from crossbred dairy cows with clinical mastitis. Trop Anim Health Prod 44(6): 1169-1173.
11. Asres K, Mazumder A, Bucar F (2006) Antibacterial and antifungal activities of extracts of *combretum molle*. Ethiop Med J 44(3): 269-277.
12. Mohammed A, Victoria Awolola G, Ibrahim MA, Anthony Koorbanally N, Islam MS (2019) Oleanolic acid as a potential antidiabetic component of *Xylopia aethiopica* (Dunal) A. Rich. (*Annonaceae*) fruit: bioassay guided isolation and molecular docking studies. Nat Prod Res 16: 1-4.
13. Mohammed A, Koorbanally NA, Islam MS (2016) Anti-diabetic effect of *Xylopia aethiopica* (Dunal) A. Rich. (*Annonaceae*) fruit acetone fraction in a type 2 diabetes model of rats. J Ethnopharmacol 180: 131-139.
14. Woode E, Ameyaw EO, Boakye-Gyasi E, Abotsi WK, Oppong Kyekyeku J, et al. (2016) Effects of an ethanol extract and the diterpene, xylopic acid, of *Xylopia aethiopica* fruits in murine models of musculoskeletal pain. Pharm Biol 54(12): 2978-2986.
15. Mensah JK, Ibrahim A, Jibira Y (2019) Co-extract mixture from *Strophanthus hispidus* (roots) and *Aframomum meleguta* (seeds) show phytochemical synergy in its antiinflammatory activity. Arch Pharm Pharma Sci 3: 089-100.
16. Mensah JK, Amarh MA (2019) Antioxidant, antimicrobial and anti-inflammation activities of *Telfairia occidentalis* seeds extract. Current Science Perspectives 5(2): 14-23.

17. Mensah JK, Golomeke D (2015) Antioxidant and antimicrobial activities of the extracts of the Calyx of *Hibiscus Sabdariffa* Linn. Current Science Perspectives 1(2): 69-76.

18. Williamson EM (2001) Synergy and other interactions in phytomedicines. Phytomedicine 8(5): 401-409.

19. Liu RH (2004) Potential synergy of phytochemicals in cancer prevention: mechanism of action. J Nutr 134(12): 3479S-3485S.

20. Caesar LK, Cech NB (2019) Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2. Nat Prod Rep 36(6): 869-888.

21. Zhou JR, Yu L, Zhong Y, Blackburn GL (2013) Soy phytochemicals and tea bioactive components synergistically inhibit androgen-sensitive human prostate tumors in mice. J Nutr 133(2): 516-521.

22. Asres K, Mazumder A, Bucar F (2006) Antibacterial and antifungal activities of extracts of *combretum molle*. Ethiop Med J 44(3): 269-277.

23. Essid R, Hammami M, Gharbi D, Karkouch I, Hamouda TB, et al. (2017) Antifungal mechanism of the combination of *Cinnamomum verum* and *Pelargonium graveolens* essential oils with fluconazole against pathogenic Candida strains. Appl Microbiol Biotechnol 101(18): 6993-7006.

24. Wolcott R, Costerton J W, Raoult D, Cutler S J (2013) The Polymicrobial Nature of Biofilm Infection. Clin Microbiol Infect 19(2): 107-112.

25. Hsieh TC, Wu JM (2008) Suppression of cell proliferation and gene expression by combinatorial synergy of EGCG, resveratrol and gamma-tocotrienol in estrogen receptor-positive MCF-7 breast cancer cells. Int J Oncol 33(4): 851-859.

26. Borthakur A, Bhattacharyya S, Anbazhagan AN, Kumar A, Dudeja PK, et al. (2012) Prolongation of carrageenan-induced inflammation in human colonic epithelial cells by activation of an NFκB-BCL10 loop. Biochimica et Biophysica Acta 1822(8): 1300-1307.

27. Chikara S, Nagaprashantha LD, Singhal J, Horne D, Awasthi S, et al. (2018) Oxidative stress and dietary phytochemicals: Role in cancer chemoprevention and treatment. Cancer Lett 413: 122-134.

28. Zhang L, Virgous C, Si H (2019) Synergistic anti-inflammatory effects and mechanisms of combined phytochemicals. J Nutr Biochem 69: 19-30.

29. Ayaz M, Ullah F, Sadiq A, Ullah F, Ovais M, et al. (2019) Synergistic interactions of phytochemicals with antimicrobial agents: Potential strategy to counteract drug resistance. Chem Biol Interact 308: 294-303.

30. Komape NP, Bagla VP, Kabongo-Kayoka P, Masoko P (2017) Anti-mycobacteria potential and synergistic effects of combined crude extracts of selected medicinal plants used by Bapedi traditional healers to treat tuberculosis related symptoms in Limpopo Province, South Africa. BMC Complement Altern Med 17(1): 124-128.

31. Brahmbhatt M, Gundala SR, Asif G, Shamsi SA, Aneja R (2013) Ginger phytochemicals exhibit synergy to inhibit prostate cancer cell proliferation. Nutr Cancer 65(2): 263-272.

32. Malongane F, McGaw LJ, Mudau FN (2017) The synergistic potential of various teas, herbs, and therapeutic drugs in health improvement: a review. J Sci Food Agric 97(14): 4679-4689.