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Full Length Research Paper

The effect on potency of adding (-)-epicatechin to crude extracts of *Elephantorrhiza elephantina* and *Pentanisia prunelloides*

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***Elephantorrhiza elephantina* (*Ee*) and *Pentanisia prunelloides* (*Pp*) are two medicinal plants which are widely used by traditional healers to remedy various ailments including diarrhoea, dysentery, inflammation, fever, rheumatism, heartburn, tuberculosis, haemorrhoids, skin diseases, perforated peptic ulcers and sore joints in Southern Africa (South Africa, Swaziland, Botswana and Zimbabwe). Often, decoctions and infusions from these two plants are used in combination, specifically for stomach ailments. The following study was conducted to explore the possible mechanism underlying the synergistic interactions of the joint application of these two medicinal plant species.The checkerboard micro-dilution technique was used to determine the efficacy of (-)-epicatechin (EC): palmitic acid (PA) and (-)-epicatechin: *E. elephantina* or *P. prunelloides* combinations on five selected pathogenic bacteria. The results demonstrated that the combination of EC and PA exhibit either additive or synergistic but no antagonistic interactions. Of the 35 administered combinations, 11 were synergistic, 10 additive and 14 indifferent. The fractional inhibitory concentrations (FIC) indices for the combination of EC and *E. elephantina* for the three pathogens tested exhibited indifferent interactions with all FIC values above 1 while the FIC indices for the 1:1 combinations of EC and *P. prunelloides* exhibited additive interactions (FIC values between 1 and 0.50). This is the first report to explore the possible explanation underlying the synergistic interactions exhibited by the two medicinal plants.**

Key words: *Elephantorrhiza elephantina, Pentanisia prunelloides*, (-)-epicatechin, palmitic acid, efficacy, fractional inhibitory concentrations (FIC) index

INTRODUCTION

The use of plant extracts and mixtures is an ancient practice that has developed over thousands of years. It is referred to in Traditional Chinese Medicine (in the *Shen Nung Pen Tsao Ching* or *Divine Husbandman's Materia Medica,* ca. 3000 BC; Hamdard Pharmacopoeia of Eastern Medicine, 1970), Egyptian medicine (in the Ebers

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papyrus, 1550 BC; Chauncey, 1952), Ayurveda (based on the *Sushruta Samhita,* ca. 800 BC; Dwivedi and Dwivedi, 2007) as well as in *De Materia Medica* by Dioscorides (78 AD; Osbaldeston and Wood, 2000), to name a few. With recent emphasis on novel drug discovery, these age-old prescriptions are scientifically evaluated where efficacy is now being ascribed to possible synergistic interactions between extracts from different plants or components within the same plant extract, thus showing potential in multitarget therapy (Wagner*,* 2006). The driving hypothesis behind the idea of multi-drug therapy is to fight the pathogen via concerted action and not only through the direct destruction of the pathogen, but also by suppression, deactivation, interruption, diversion (or whatever the case may be) of various processes which are essential for the pathogen's survival. Potential benefits of using combination therapy include broad spectrum of efficacy, greater potency than either of the drugs used in monotherapy, improved safety and tolerability, and reduction in the number of resistant organisms (Lewis and Kontoyiannis, 2001). This multi-drug strategy is based on the proposition that many diseases have a multi-causal etiology and a complex pathophysiology, implying that it will be definitely advantageous to multiply targets in therapeutic efforts.

Bacterial multi-drug resistance efflux pumps (MDRs) are responsible for a significant level of resistance to antibiotics in pathogenic bacteria (Kumar et al., 2005). The mode of action for some antibiotics disrupts the capacity of these MDRs responsible for the extrusion of toxins across the permeability membrane barrier; hence, enhancing their efficacy. In southern Africa, plant extract combinations are also administered with the intention of attaining increased potency, as is implied with the term *imbiza* (that is, the generic Zulu name for plant mixtures that impart strength, health and vigour, normally as herbal preparations of a single plant or mixtures of plants which are administered orally for a purgative action, or as enemas) (Ngubane, 1977). One notable example of the combined administration of plant extracts to remedy stomach ailments and fevers comes from the traditional use of *Elephantorrhiza elephantina* together with *Pentanisia prunelloides* (Bryant, 1966). Such herbal mixtures may be obtained from muthi shops across South Africa, with a product by the name of '*Sejeso*' (Ingwe® brand) as a good example.

E. elephantina is known as elandsbean, *mupangara* (in Shona) or *intolwane* (in Xhosa and Zulu) (Phillips, 1917; Jacot, 1971). On its own, the root of this plant is known in Southern Africa for many traditional uses such the treatment of chest complaints and heart conditions (Watt and Breyer-Brandwijk, 1962), hypertension, syphilis, (Jacot, 1971) infertility in women, wasting in infants, fever, dysmenorrhea and haemorrhoids amongst others (Gelfand et al., 1985), and also as an aphrodisiac or emetic (to mitigate the anger of the ancestors or for fevers) (Hutchings et al., 1996). It is particularly known to be effective against stomach ailments such as abdominal pains, perforated peptic ulcers (bloody), diarrhoea and dysentery (Gelfand et al., 1985; Hutchings, 1989a; Pujol, 1990). *P. prunelloides* [syn. *P. variabilis* Harv. var. *intermedia* Sond, (Adeniji et al., 2000); common name: wild verbena (Van Wyk et al., 2009)] is an important traditional medicine in Southern Africa as a multi-purpose plant used for the treatment of several internal and external ailments (Rood, 1994; Maliehe, 1997; Grierson and Afolayan, 1999; Neuwinger, 2000). With stomach ailments in particular, the fresh root may be chewed and swallowed for the treatment of heartburn (Adeniji et al., 2000). Its vernacular names, that is, *setima-mollo* (Sotho) translated as "fire extinguisher" (Moteetee and Van Wyk, 2011), *icimamlilo* (Zulu) which means "putting out the fire" and *sooibrandbossie* (Afrikaans) translated as "little heartburn bush" (Van Wyk et al., 2009), emphasizes this longstanding traditional use. Root decoctions of *P. prunelloides* may also be taken orally as an emetic and for diarrhoea, dysentery, indigestion (Moteetee and Van Wyk, 2011).

The use of herbal remedies in the treatment of diarrhoeal diseases is a common practice in many communities of the world, including South Africa. A number of medicinal plants have been reported to be effective against diarrhoea and dysentery (Rouf et al., 2003). Diarrhoea, dysentery and cholera are some of the leading causes of morbitity and mortality in developing countries accounting for about 4.6 million deaths every year (Thaper and Sanderson, 2004). It is also alleged that about $4.3 million is spent every year on public and private direct health care costs due to diarrhoea alone (Pegram et al., 1998). It is against this background that rural dwellers rely on traditional medicine for their health care services due the prohibitive cost of orthodox medication. It is also reported that about 3 million South Africans use indigenous traditional plant medicine for primary health care purposes (Van Wyk and Gericke, 2000). It is therefore not surprising that 32 plant species derived from 26 families have been reported for the treatment of diarrhoea (Ippidii et al., 2008) in the Eastern Cape alone. Amongst the most frequently used plants for gastrointestinal problems are *E. elephantina* and *P. prunelloides.* Similar ethnobotanical studies have been reported in different South African provinces (Lewis et al., 2002; Mathabe et al., 2006) and other parts of the world (Mukharjee et al., 1998; Rahman et al., 2003). As a rule of the thumb, all these reports allude to the linkage of this disease to poor hygienic practices that are to a greater extent a function of poverty and poor service delivery (Obi et al., 2007).

In this study, we determined the antimicrobial activity of (-)-epicatechin (EC) and palmitic acid (PA) individually and in combination to probe the possible synergistic interactions between the two phytochemicals found in the two plant species as a validation of their possible contribution

to the enhanced potency of mixtures of *E. elephantina* and *P. prunelloides* especially for the remedy of stomach ailments in Southern African traditional medicine. Interaction between (-)-epicatechin with *E. elephantina* and *P. prunelloides* was also investigated to explore a possible explanation for the enhanced efficacy of the two plants administered in combination by traditional healers.

MATERIALS AND METHODS

Plant

Fresh plant rhizomes of *E. elephantina* and *P. prunelloides* were collected in June, 2010 from Kwazulu Natal Province, South Africa and were identified by Dr Anna Moteetee (Acting Dean of Faculty of Science University of Johannesburg). Voucher specimen numbers SJM-01 to SJM-2 were allotted and specimens were deposited in JRAU Herbarium, Department of Botany and Plant Biotechnology (Kingsway Campus) at the University of Johannesburg. Fresh plant rhizomes were washed with water, dried and marcerated and kept in the fumehood at room temperature. The dried plant materials were then ground into fine powders, extracted in solvent and water evaporated under reduced pressure and then stored in sample bottles and stored at -5°C until further use.

Plant extraction

Powdered material (100 g) of each plant was extracted with water and methanol, respectively. The methanol extracts were filtered under vacuum and evaporated to dryness under a stream of nitrogen at room temperature.The aqueous extracts were freeze dried then stored in tightly closed, sample bottles. Water was chosen especially as it is the solvent in which these medicinal plants are prescribed and administered by rural traditional healers while methanol is easier to dry apart from being a polar like water.

Determination of relative amounts of (-)-epicatechin in *E. elephantina* and *P. prunelloides* by Raman spectroscopy

Fine ground powders of fractions and extracts of *E. elephantina* and *P. prunelloides* were determined against (-)-epicatechin standard using the Raman instrument in Chemistry Department at the University of Johannesburg, Doornfontein Campus.

Microbiological testing

The minimum inhibitory concentrations (MIC) microdilution method was adopted from that reported in the literature (Eloff, 1998). All microbiological techniques, media and culture preparations were adopted in line with the CLSI/NCCLS (2003) guidelines. The antimicrobial activity was evaluated against two Gram-positive bacteria, *Bacillus cereus* (ATCC ll778) and *Staphylococcus aureus* (ATCC 6538) and three Gram-negative bacteria, *Escherichia coli* (ATCC 8739,) *Klebsiella pneumoniae* (ATCC l3883) and *Enterococcus faecalis* (ATCC 292l2). The bacteria were cultured in Tryptone soya broth (TSB) for 24 h. The yeast (*Cryptococcus neoformans*) was incubated for 48 h. Cultures were prepared for micro-dilution assays using 1:100 dilution, yielding an approximate inoculums size of 1×10^6 colony forming units (CFU)/ml (Van Vuuren and Viljoen, 2009). The microplates were sealed with seal-plate films and incubated at 37°C overnight to stimulate bacterial growth. A 40 µl volume of 4×10^{-1} mg/ml *p*-Iodonitrotetrazolium (INT) was added to all inoculated wells and left to stand for 6 h before plates were examined for bacterial growth.

MIC and FIC determination for palmitic acid and (-)-epicatechin combinations against five pathogens

Combinations of the stock solutions were prepared to represent the following ratios of EC/ PA, respectively: 9:1; 7:3; 6:4; 5:5; 4:6; 3:7; 1:9. The antimicrobial activities of the combinations of the two compounds against five pathogens selected on the bases of their susceptibility are shown on Table 1. This experimental procedure was undertaken to probe the effect of the two compounds (EC and PA) on the selected pathogenic agents especially as they were identified in *E. elephantina* and *P. prunelloidesl,* respectively. The corresponding FIC values from this experimental procedure were derived from the templates shown in Table 1 for *B. cereus* (ATCC ll778), *S. aureus* (ATCC 6538), *K. pneumoniae* (ATCC l3883), *E. faecalis* (ATCC 292l2) and Table 2 for *E. coli* (ATCC 8739,).

The templates used for the determination of MIC values for palmitic acid and (-)-epicatechin against the five pathogens.

Two different starting concentrations (1 mg/ml) for *E. coli* and (5 mg/ml) for the remaining four pathogens were used (Tables 1 and 2). The starting concentration for mixtures was adjusted to 1 mg/ml due to the high susceptibility of these pathogen higher concentrations.

Determination of MIC and FIC indices of 1:1 combinations of (-)-epicatechin against either crude extracts of *E. elephantina* or *P. prunelloides*

Stock solutions of 1:1 by mass of (-)-epicatechin with either crude *E. elephantina* or *P. prunelloides* were prepared and tested against three selected pathogens. The respective antimicrobial activities were probed starting with an effective concentration of 1.25 mg/ml then the MIC values recorded (Table 6). The corresponding FIC indices were calculated as shown in brackets in order to evaluate the effect of (-)-epicatechin on either of the crude extracts. The FIC index (FICI) is defined as the interaction of two agents where the concentration of each agent in combination is expressed as a fraction of the concentration that would produce the same effect when used independently (Berenbaum, 1977; Climo et al., 1999; Meletiadis, 2005; Guo et al., 2007). It is determined as the correlation between the two combined substances and can be classified as either synergistic when FICI (≤ 0.50), additive (> 0.5 to ≤ 1), independent (> 1 to ≥ 4) or antagonistic (> 4.00). The dose combinations are represented by geometric points with co-ordinates matching the dose rates of the separate components in combination (Van Vuuren, 2007; Hemaiswarya, 2008).

RESULTS

Comparative analysis of catechins in *E. elephantina* and *P. prunelloides* against (-)-epicatechin standard

Qualitative relative amounts of catechins in both *E. elephantina* and *P. prunelloides* as determined by Raman are shown on Figure 1. Spectra 1 is for catechin fraction form *E. elephantina* (Zimbabwe sample), spectra 2, *P. prunelloides* extract (KZN sample), spectra 3, (-)-epicatechin standard and spectra 4, *E. elephantina* extract (KZN sample). Considering absorption peaks 3196, 3071.8 and 2808.8 for (-)-epicatechin, the corresponding

Table 1. Template for palmitic acid (PA)/(-)-Epicatechin (EC) at 5 mg/ml (used against all the other pathogens).

100%	90 : 10		70 : 30		60 : 40		50 : 50		40 : 60		30 : 70		10 : 90		100%
PA	PA	EC	PA	EC	PA	EC	PA	EC	PA	EC	PA	EC	PA	EC	EC
1.25	1.125	0.125	0.875	0.375	0.750	0.500	0.625	0.625	0.500	0.750	0.375	0.875	0.125	1.125	1.25
0.63	0.563	0.063	0.438	0.188	0.375	0.250	0.313	0.313	0.250	0.375	0.188	0.438	0.063	0.563	0.63
0.313	0.282	0.031	0.219	0.094	0.188	0.125	0.156	0.156	0.125	0.188	0.094	0.219	0.031	0.282	0.313
0.156	0.141	0.016	0.105	0.047	0.094	0.0625	0.078	0.078	0.0625	0.094	0.047	0.105	0.016	0.141	0.156
0.078	0.705	0.0079	0.053	0.024	0.047	0.0313	0.039	0.039	0.0313	0.047	0.024	0.053	0.0079	0.705	0.078
0.039	0.353	0.0039	0.027	0.012	0.0235	0.0157	0.0195	0.0195	0.0157	0.0235	0.012	0.027	0.0039	0.353	0.039
0.0195	0.177	0.0020	0.014	0.006	0.0118	0.0078	0.0098	0.0098	0.0078	0.0118	0.006	0.014	0.0020	0.177	0.0195
0.0098	0.0089	0.0010	0.007	0.003	0.0059	0.0039	0.0049	0.0049	0.0039	0.0059	0.003	0.007	0.0010	0.0089	0.0098

Table 2. Template for palmitic acid (PA)/(-)-epicatechin (EC) at 1 mg/ml (used against *E. coli* only).

100%	90:10		70:30		60:40		50:50		40:60		30:70		10:90		100%
PA	PA	EC	PA	EC	PA	EC	PA	EC	PA	EC	PA	EC	PA	EC	EC
0.250	0.225	0.025	0.175	0.075	0.150	0.100	0.125	0.125	0.100	0.150	0.075	0.175	0.025	0.225	0.250
0.125	0.113	0.013	0.088	0.038	0.075	0.05	0.063	0.063	0.050	0.075	0.038	0.088	0.0125	0.113	0.125
0.063	0.057	0.007	0.044	0.019	0.038	0.025	0.032	0.032	0.025	0.038	0.019	0.044	0.0063	0.057	0.063
0.0313	0.029	0.0035	0.022	0.0095	0.019	0.013	0.016	0.016	0.013	0.019	0.0095	0.022	0.00315	0.0285	0.0313
0.0156	0.015	0.0018	0.011	0.00048	0.0095	0.0065	0.008	0.008	0.007	0.0095	0.0048	0.011	0.00158	0.0143	0.0156
0.0078	0.008	0.0009	0.0055	0.0024	0.00475	0.0033	0.004	0.004	0.0035	0.0048	0.0024	0.0055	0.00079	0.00715	0.0078
0.0039	0.004	0.0005	0.0028	0.0012	0.0024	0.00165	0.002	0.002	0.0018	0.0024	0.0012	0.0028	0.000395	0.00358	0.0039
0.00195	0.002	0.0003	0.0014	0.0006	0.0012	0.00083	0.001	0.001	0.0009	0.0012	0.0006	0.0014	0.000198	0.00179	0.00195

peaks for the three extracts of samples of *E. elephantina* and *P. prunelloides* showed less intensity with the *E. elephantina* peaks being more pronounced. The same trend was exhibited for the following sets of peaks with respect to standard (-)-epicatechin, (1616.3, 1341.7 and 1069.9) and (839.9, 723.4 and 547.4). If the intensities of peaks are related to the concentrations of the respective compounds in the referred extracts, it can be inferred that *P. prunelloides* extracts contain a higher concentration of catechins. Taking the KZN samples for the two medicinal plants, it can also be proposed that *E. elephantina* contains a greater concentration of catechins.

The MIC and FIC values for all the combinations of palmitic acid and (-)-epicatechin agsinst five tested pathogens

The MIC values for both EC and PA and the different combinations of the two compounds individually are shown in Table 3. Generally, most MIC values for the individual compounds were greater than the values for the corresponding mixtures (Table 3). The different combinations of palmitic acid and (-)-epicatechin exhibited predominently additive and synergic interactions. Of all the 35 possible interactions, 11 were synergistic, 10 additive and 14 indifferent (Figure 2). There were no antagonistic interactions observed for the combinations tested. The distribution of the synergistic interactions of the two compounds against a set of five pathogens is shown in Figure 2. Another notable enhanced efficacy of the combination of *E. elephantina* and *P. prunelloides* is the susceptibility of *B. cereus*. All the palmitic acid/epicatechin combinations exhibited indifference against this pathogen while the

Table 3. MIC values for different combinations of palmitic acid (PA) and (-)-Epicatechin (EC) at 5 mg/ml against 4 pathogens.

Parameter		*B. cereus* ATCC 11778		*S. aureus* ATCC 6538		*E. faecalis* ATCC 29212		*K. pneumoniae* ATCC 13883	
Ratios	**100%**	**PA**	**EC**	**PA**	**EC**	**PA**	**EC**	**PA**	**EC**
10:0	1.25:0.000	0.313	0.000	1.250	0.000	0.625	0.000	1.250	0.000
9:1	1.125:0.125	0.563	0.063	0.563	0.063	0.282	0.313	0.563	0.063
7:3	0.875:0.375	0.438	0.188	0.438	0.188	0.219	0.094	0.438	0.188
6:4	0.750:0.500	0.380	0.250	0.750	0.500	0.188	0.125	0.750	0.500
5:5	0.625:0.625	0.313	0.313	0.625	0.625	0.156	0.156	0.625	0.625
4:6	0.500:0.750	0.250	0.375	0.500	0.750	0.125	0.188	0.125	0.188
3:7	0.375:0.875	0.188	0.438	0.188	0.044	0.094	0.219	0.188	0.438
1:9	0.125:1.125	0.063	0.563	0.125	1.125	0.0313	0.282	0.063	0.563
0 : 10	0.00:1.250	0.000	0.625	0.000	1.25	0.000	0.625	0.000	0.625

combined aqueous extracts of *E. elephantina* and *P. prunelloides* exhibited at least two synergistic interactions (result not shown). This again alludes to the notion that it is not necessarily the presence of palmitic acid and epicatechin in the two plant species used in combination that accounts for the various synergistic interactions observed especially considering *B. cereus*. There could be other interactions involving other phytochemicals underlying this disperity.

Of great interest as well was the susceptibility of *E. coli* with the lowest FICi of 0.041 to the PA:EC combination of 7:3 (Table 4). Of the seven PA:EC combinations six were synergistic and only one combination being additive (Table 4). This observation suggests that the PA:EC combinations from the two plant species is conspiuously effective against *E. coli*, justifying the traditional use for the treatment of stomach ailments by traditional healers. A similar trend was also exhibited for *E. faecalis* that is also associated with gastrointestinal ailments (Table 4). The combination also showed synergy (FIC = 0.40) for the PA:EC combination of 4:6 against *K. pneumoniae*, one of the drug resistant Gram negative pathogens. This pathogen is implicated for chest problems for which *E. elephantina* and *P. prunelloides* are also used in traditional phytotherapy. *S. aureus* also showed marked susceptibility (Figure 2). Of the seven combinations administered to this pathogen, five were synergistic with the remaing two being additive, FIC = 1 (Table 5). This pathogen is also implicated for gastrointestinal ailments for which *E. elephantina* and *P. prunelloides* are administered. The susceptibility of this pathogen to the combination of these two compounds may be proposed as a justification for the use of *E. elephantina* and *P. prunelloides* to remedy stomach ailments as well.

Comparative efficacy of 1:1 combinations of (-)-epicatechin with *E. elephantina* and *P. prunelloides.*

The MIC values for both EC and 1:1 combinations of EC and either *E. elephantina* or *P. prunelloides* are shown in Table 6. Generally, MIC values for the individual EC and crude extracts of the two plants were greater than the values for the corresponding 1:1 mixtures (Table 6). All FIC values for the 1:1 combinations of *E. Elephantina* and (-)-epicatechin for the three pathogens tested exhibited indifferent interactions that is, all values were below 1 (Table 6). On the other hand all FIC indices for the 1:1 combinations of *P. prunelloides* and (-)-epicatechin demonstrated synergy that is, all values are between 0.38 and 0.50 depending on the pathogenic strain tested and this suggested enhanced potency (Table 6).

DISCUSSION

Both palmitic acid and (-)-epicatechin are common dietary phytochemicals and have been evaluated for several biological indications both *in vitro* and *in vivo*. Palmitic acid [CH_3 $(CH_2)_{14}COOH$] is a medium-length saturated fatty acid and is present as a major lipid in leaves and some seed oils (Harborne and Baxter, 1993). Previous studies have shown that palmitic acid is active against various bacterial strains (Hashem and Saleh, 1999) including *E. coli* (Yang et al., 2010), while (-)-epicatechin is an effective treatment for diarrhoea (Abhilash, 2010) and exhibits moderate antimicrobial activity (Pretorius et al., 2003). The primary mode of action of fatty acids is suggested to target cell membrane, (Tsuchido et al., 1985) and the proposed fatty acid-induced autolysis rather than large-scale solubilisation of the cell membrane is alleged to be detergent-like in character. Such antibacterial action could be explained through the insertion of the non-polar moieties of the fatty acids into the phospholipid layer of the bacterial cell membrane, resulting in a change in membrane permeability, alteration in function of membrane proteins responsible for maintenance of cellular functions and an uncoupling of the oxidative phosphorylation system (Saito

Table 4. Calculated FIC showing synergism between palmitic acid (PA; A) and (-)-epicatechin (EC; B) (stock solutions used were prepared in DMSO, concentrations given with pathogens).

Combination ratio	mg/ml extract contribution to combination		MIC contribution in combination[a]		Calculated FIC fractions		Calculated FIC[b]
	PA	EC	PA (MIC_A)	EC (MIC_B)	FIC_A	FIC_B	
***E. coli* (ATCC 8739) at 1 mg/ml**							
10:00	0.250	0.000	0.250	0.000	1.000	0.000	1.000
09:01	0.225	0.013	0.0140	0.0016	0.0562	0.0125	0.069
07:03	0.175	0.038	0.0055	0.0023	0.0218	0.0187	0.041
06:04	0.150	0.050	0.0047	0.0031	0.0187	0.0250	0.044
05:05	0.125	0.063	0.0078	0.0078	0.0312	0.0624	0.094
04:06	0.100	0.075	0.0500	0.0750	0.2000	0.6000	0.800
03:07	0.075	0.088	0.0189	0.0441	0.0756	0.3528	0.428
01:09	0.025	0.113	0.0063	0.0567	0.0252	0.4536	0.479
00:10	0.000	0.125	0.000	0.125	0.000	1.000	1.000

Table 5. FIC values for different combinations of palmitic acid and (-)-epicatechin against five pathogens.

PA	EC	*B. cereus* ATCC 11778	*S. aureus* ATCC 6538	*E. faecalis* ATCC 29212	*K. pneumoniae* ATCC 13883	*E. coli* ATCC 8739
10	0	1.00	1.00	1.00	1.00	1.00
9	1	1.92^{I}	0.50^{S}	0.49^{S}	0.55^{A}	0.069^{S}
7	3	1.81^{I}	0.50^{S}	0.49^{S}	0.65^{A}	0.041^{S}
6	4	1.62^{I}	1.00^{A}	0.51^{A}	1.40^{I}	0.044^{S}
5	5	1.49^{I}	1.00^{A}	0.51^{A}	1.50^{I}	0.094^{S}
4	6	1.41^{I}	0.25^{S}	0.51^{A}	0.40^{S}	0.800^{A}
3	7	1.30^{I}	0.50^{S}	0.49^{S}	0.85^{A}	0.428^{S}
1	9	1.08^{I}	0.25^{S}	0.49^{S}	0.95^{A}	0.479^{S}
0	10	1.00	1.00	1.00	1.00	1.000

PA represents parts of palmitic acid and EC represents parts of (-)-epicatechin. Interpreting values: synergy (≤0.5), additive > 0.5 - 1.0), no interaction [>1.0 to ≤ (4.00) or antagonistic (> 4.0)].

and Tomioka, 1988). The antibacterial mode of action exerted by flavan-3-ols such as (-)-epicatechin and it is gallated derivatives on the other hand, including damaging the cytoplasmic membrane, as well as inhibiting nucleic acid synthesis, energy metabolism and cell membrane synthesis (Cushnie and Lamb, 2011).

The synergistic interactions of palmitic acid and (-)-epicatechin were demonstrated against the five pathogens (Table 4). Of particular interest was the demonstration of synergism towards both Gram positive and Gram negative bacteria, K. *pneumoniae*

Table 6. MIC and FIC values for 1:1 concentrations of crude extracts of *P. prunelloides* and *E. elephantina* against (-)-epicatechin at 1.25 mg/ml.

Crude *E. elephantina*/*P. prunelloides*	*B. cereus*	*E. faecalis*	*E. coli*
Crude *E. elephantina*	0.313	0.625	0.625
50:50 (Ee/EC)	1.25(2.5)	1.25(2.0)	1.25(2.0)
(-)-epicatechin	1.25	0.625	0.625
Crude *P. prunelloides*	1.25	0.625	0.625
50:50 (Pp/EC)	0.625(0.38)	0.625(0.50)	0.625(0.50)
(-)-epicatechin	0.625	0.625	0.625

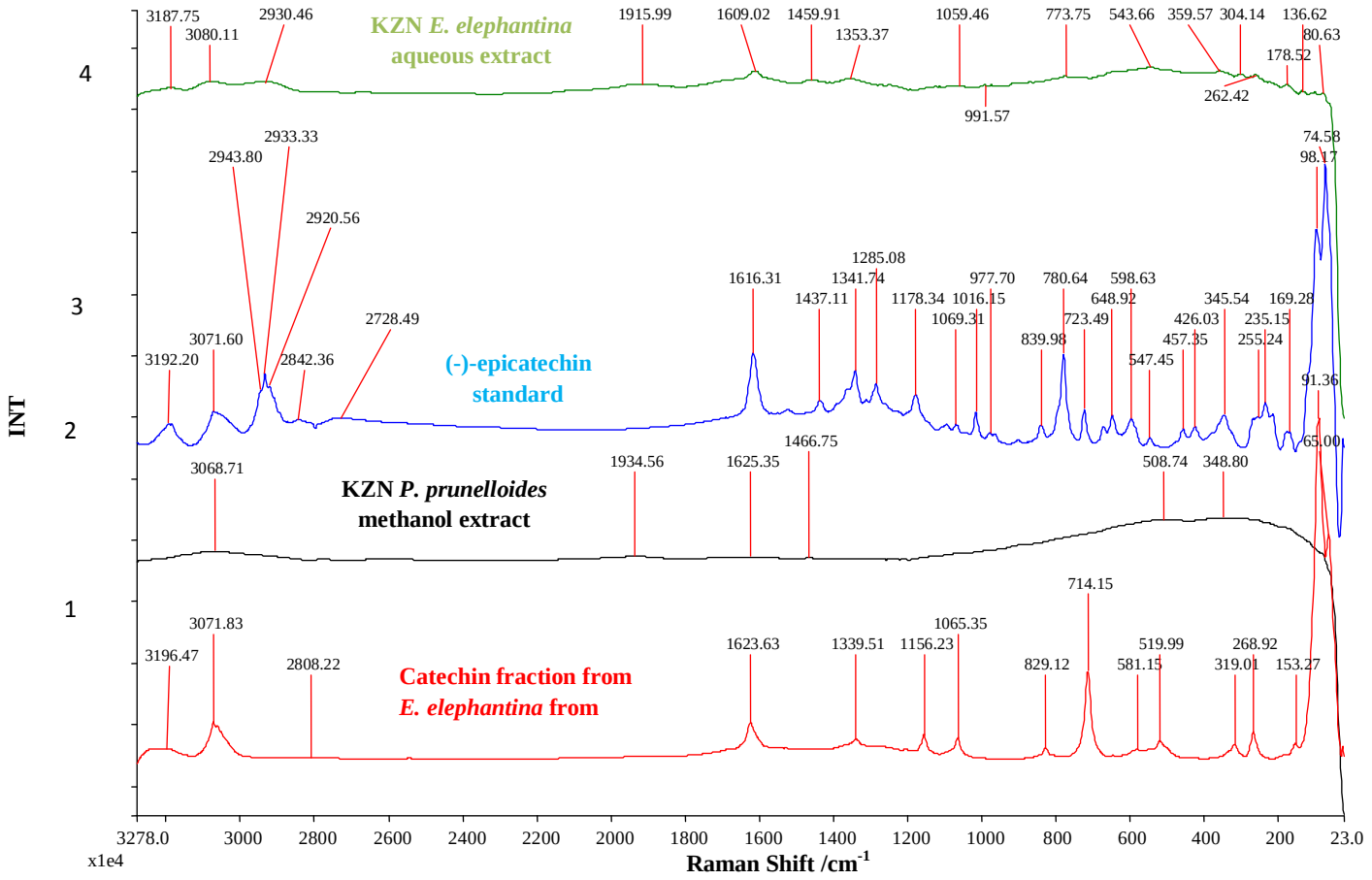

Figure 1. Determination of relative amounts of (-)-epicatechin in *E. elephantina* and *P. prunelloides* by Raman spectroscopy.

(0.40), S. *aureus* (0.25,), *E. faecalis,* (0.49), *E. coli*, (0.041). The results of this combination study show that *P. prunelloides* and *E. elephantina* display synergism or additive interactions subject to the test pathogens and the specific ratio in which the extracts were combined (Table 4). Since these two compounds have been identified in the two medicinal plants under study, it could be proposed that the synergistic interactions demonstrated in this study could also be effected by these two compounds among other undetected interactions. So interesting and conspicuous is the increased susceptibility of *E. faecalis* to the combinations of *E. elephantina* and *P. prunelloides* relative to that of palmitic acid and (-)-epicatechin administered individually (results not shown). The effects of different combinations of palmitic acid and (-)-epicatechin are just maginally synergistic with FIC indices approximately 0.5 (Figure 2) while most combinations of *E. elephantina* and *P. prunelloides* have been reported to have FIC values ranging between 0.18 and 0.33. This therefore suggests that there is far much more

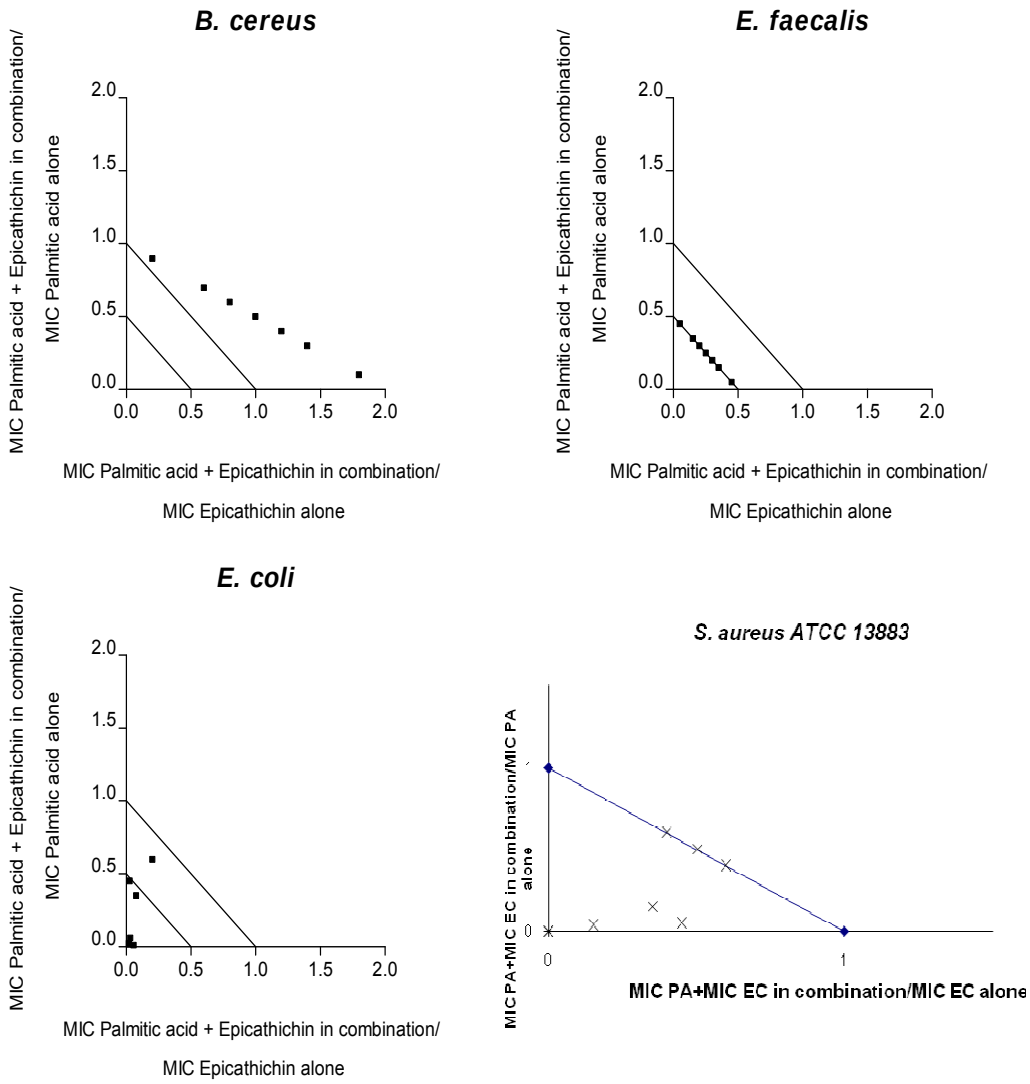

Figure 2. Isobolograms of interactions of palmitic acid and (-)-epicatechin.

to the potency of *E. elephantina* and *P. prunelloides* other than the mere presence of palmitic acid and (-)-epicatechin in the two species.

Synergy or additivity in most combinations of EC and PA appeared in anti-bacterial activity against both Gram + Gram positive and Gram negative bacteria. Gram-negative bacteria have an effective permeability barrier composed of the outer phospholipidic membrane with lipopolysaccharide components which restricts penetration of amphipathic compounds (Tegos et al., 2002). Gram positive bacteria have an outer peptidoglycan layer which does not form a permeability barrier making them more susceptible to antimicrobial agents (Tadeg et al., 2005). Contrary to the structural differences of the pathogens tested, the combinations of palmitic acid and epicatechin or *P. prunelloides* withepicatechin exhibited activity against both strains of pathogens. The appearance of synergy in the activity against both Gram negative and Gram positive bacteria suggests that mixtures of components of *P. prunelloides* and *E. elephantina* can strongly enhance a sufficiently high bioavailability of anti-bacterial components within the cells effectively enhancing their potency.

The results of this study demonstrated a relatively greater content of (-)-epicatechin in *E. elephantina* compared to *P. prunelloides* (Figure 2) which also confirms reports in literature (Arotiba et al., 2013). *P. prunelloides* on the other hand has been reported to contain palmitic acid which is a known anti-microbial compound (Yff, 2002). The enhanced synergistic and additive effects that were observed with various ratios of plant administered imply that the phytochemicals from *P. pruneloides* and possibly some from *E elephantina* play different roles from a direct antibiotic one. It is most likely that the combination of *E.*

elephantina and *P. prunelloides* would result in the epicatechin from *E. elephantina* enhancing the efficacy of phytochemicals in *P. prunelloides* resulting in synergistic interactions as reflected by the FIC indices below 0.50. On the other hand, the addition of (-)-epicatechin to *E. elephantina* that already contains a lot of this compound has no effect on the efficacy of the mixture (indifferent) as reflected by the FIC indices greater than 1 but less than 4. Of course, more combinations could have been carried out apart from the 50:50 combinations administered as a probe of the trend of interactions. More work is underway in our laboratories to further explore various combinations.

Conclusion

This study has demonstrated that the addition of (-)-epicatechin to crude *E. elephantina* has no effect but has a notable enhancement of the efficacy on crude *P. prunelloides* extracts. It could therefore be proposed that *E. elephantina* that contains a greater quantity of (-)-epicatechin enhances phytochemicals, especially palmitic acid in *P. prunelloides* when the two medicinal plants are jointly administered. Hence, justifying the synergistic and additive interactions exhibited by the two medicinal plants in this study.

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Conflict of interest

The authors declare that they have no competing interests.

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