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Interactive efficacies of *Elephantorrhiza elephantina* and *Pentanisia prunelloides* extracts and isolated compounds against gastrointestinal bacteria

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ABSTRACT

Elephantorrhiza elephantina (Burch.) Skeels (Fabaceae) and *Pentanisia prunelloides* (Klotzsch ex Eckl. & Zeyh.) Walp. (Rubiaceae) are two medicinal plants used extensively in southern Africa to treat various ailments. Often, decoctions and infusions from these two plants are used in combination specifically for stomach ailments. The antimicrobial activities of the methanol and aqueous extracts of the rhizomes of the two plants, as well as the two active ingredients from the plants [(−)-epicatechin and palmitic acid] have been determined apart and in combination against *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 8739) and *Bacillus cereus* (ATCC 11778). The minimum inhibitory concentration (MIC) values for the aqueous (0.50–16.00 mg/mL) and methanol (0.20–16.00 mg/mL) extracts independently demonstrated varied efficacies depending on the pathogen of study. When the two plants were combined in 1:1 ratios, synergistic to additive interactions (ΣFIC values 0.19–1.00) were noted. Efficacy for the two major compounds ranged between 0.13–0.63 mg/mL and mainly synergistic interactions were noted against *E. faecalis* and *E. coli*. The predominantly synergistic interactions noted between *E. elephantina* and *P. prunelloides* and major compounds, when tested in various ratios against these pathogens, provide some validation as to the traditional use of these two plants to treat bacterial gastrointestinal infections.

1. 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 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 being scientifically evaluated, with efficacy 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). In southern Africa, plant extract combinations are also administered with the intention of attaining increased potency, as is implied with the term *imbiza* (i.e. the generic Zulu name for plant mixtures that impart strength, health and vigour). These are normally prepared 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* (Pers. comm.: S. Mpofu with Chemist Ndlovu, a traditional healer practising in the Matabo area in the Midlands Province, Mberengwa district, Zimbabwe, 11 January 2008). Another example where *E. elephantina* and *P. prunelloides* are used together for the treatment of stomach ailments, is in a herbal mixture by the name of '*Sejeso*' (Ingwe® brand), which may be obtained from muthi shops across South Africa. This mixture is "indicated for symptomatic relief of heartburn, constipation, stomachache, loss of appetite, vomiting and indigestion. The recommended dosage is one-quarter of a cup taken three times daily after meals and the ingredients include (each 125 mg as listed per 125 mL) *Lesoko* (*Alepidea amatymbica*), *Monnamaledu* (*Hypoxis obtusa*, Moeng, 2010), *Poo-tshehla* (unknown ingredient), *Setimamollo* (*P. prunelloides*), *Mositsane* (*E. elephantina*),

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deionized water (92.5% m/v), and 1.2% m/v potassium sorbate as preservative." (Nair et al., 2012). Upon further investigation, it was found that this mixture is known as Sesjeso (Ingwe® brand) may contain varied ingredients since a bottle obtained from a muthi shop on Jeppe street, Johannesburg, 19 November 2013, under the same trade name, also include *Senna* Mill. as one of its listed ingredients.

E. elephantina is also known as elandsbean, eland's wattle, elephant's root, *baswortel*, *elandsboontjie*, *leerbossie*, *looiersboontjie*, *olifantswortel* (Afrikaans), *mositsane* (Sotho, Tswana) (Smith, 1966), *mupangara* (in Shona) or *intolwane* (in Xhosa and Zulu) (Phillips, 1917; Jacot Guillarmod, 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 Guillarmod, 1971), infertility in women, wasting in infants, fever, dysmenorrhoea and haemorrhoids amongst others (Bryant, 1966; Gelfand et al., 1985; Hutchings et al., 1996), 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 (Watt and Breyer-Brandwijk, 1962; Jacot Guillarmod, 1971; Gelfand et al., 1985; Hutchings, 1989a; Pujol, 1990; Mathabe et al., 2006; Appidi et al., 2008; Bisi-Johnson et al., 2010; Madikizela et al., 2012). *E. elephantina* has a reddish root. In the Sotho culture, "red medicines" are important in the sense that red is associated with blood and good health. Furthermore, the San people use red plant parts to treat anaemia, weakness (i.e. to strengthen the blood) and fevers (Laidler, 1928). These principles are evident in the uses mentioned.

P. prunelloides [syn. *Pentanisia 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 in that this multi-purpose plant is used for treatment of several internal and external ailments including boils, burns, snakebite, swelling, rheumatism, fever, chest pains, toothache, blood impurities, haemorrhoids, internal tumours, ulcers, venereal diseases, influenza and tuberculosis (Phillips, 1917; Watt and Breyer-Brandwijk, 1962; Bryant, 1966; Jacot Guillarmod, 1971; Hutchings, 1989a,b; Pujol, 1990; Rood, 1994; Hutchings et al., 1996; 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 (Watt and Breyer-Brandwijk, 1962; Adeniji et al., 2000). Its vernacular names, i.e. *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), emphasize 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). Leaves may be used for the treatment of diarrhoea where they are ground and soaked in warm water, followed by boiling with alum. This decoction is taken three times daily (Madikizela et al., 2012). For the treatment of vomiting, grated dried root is boiled and taken orally with one spoon for children or one "wine shot" for adults thrice per day (Bisi-Johnson et al., 2010). For swelling of the stomach, decoctions of bruised and boiled root are mixed with sour milk and taken orally (Smith, 1895).

The phytochemistry of neither *E. elephantina* nor *P. prunelloides* has been extensively studied. In a recent electrochemical study (Mpofu et al., 2014), (−)-epicatechin was quantified in both *P. prunelloides* [2.29 μg/g dry methanol (MeOH) extract and 0.26 μg/g dry aqueous extract] and *E. elephantina* (1.9 mg/g dry MeOH extract and 4.41 μg/g dry aqueous extract). Other compounds known from *E. elephantina* are 5.8–22.3% tannins (Watt and Breyer-Brandwijk, 1962), explaining the redness of the root, several phenolic compounds, i.e. flavonoids such as kaempferol, dihydrokaempferol, ethyl β-D-galactopyranoside, quercetin 3-*O*-β-D-glucoside, trans-3-*O*-galloyl-3,3′,5,5′,7-pentahydroxyflavan and taxifolin 3′-*O*-glucoside (Mthembu, 2007), as well as (+)-catechin, ethyl gallate, methyl gallate and gallic acid (Mthembu, 2007). Other compounds identified in the roots include sugars (16.8%; Watt and Breyer-Brandwijk, 1962) and β-sitosterol (Mthembu, 2007). The roots of *P. prunelloides* are reported to contain high levels of alcohol precipitable solids (0.7–7.0%, i.e. polysaccharides and glycoproteins), amino acids (α-aminobutyric acid, valine, allo-isoleucine, serine, aspartic acid, asparagine and alanine, of which the latter is most abundant) as well as several unidentified terpenes of medium and low polarity (Ndlovu, 2007). Palmitic acid was further identified as a major non-polar compound in *P. prunelloides* (Yff et al., 2002).

In this study we determined the antimicrobial activity of the two species both individually and in combination to probe the possible synergistic interactions between the two plants as a validation of their combined use in southern African traditional medicine specifically for stomach ailments. Furthermore, previous studies have shown that palmitic acid is active against various bacterial strains (Hashem and Saleh, 1999) including *Escherichia coli* (Yang et al., 2010), while (−)-epicatechin is an effective treatment for diarrhoea (Abhilash, 2010) and exhibits moderate antimicrobial activity (Pretorius et al., 2003). Hence, the interaction between these two compounds [palmitic acid and (−)-epicatechin] within *E. elephantina* and *P. prunelloides* was also investigated as a possible explanation for the efficacy of the two plants.

2. Materials and methods

2.1. Plant material

The tubers of *E. elephantina* and *P. prunelloides* were purchased from the Faraday "Muthi" Market in Johannesburg and authenticated by Prof A Moteetee in the Department of Botany and Plant Biotechnology at the University of Johannesburg. Voucher numbers were assigned as SJM00 for *P. prunelloides* and SJM01 for *E. elephantina*.

The tubers obtained for both species were cut into smaller pieces and dried in a fume hood. These were ground to fine powder and stored in the refrigerator until further use. Powdered material (100 g) of each tuber was defatted with hexane and further extracted, first with MeOH and then with water. The extract mixtures were filtered under vacuum and the filtrates dried to yield 26 g and 25 g for MeOH extracts, as well as 25 g and 17 g for the aqueous extracts of *P. prunelloides* and *E. elephantina*, respectively. By following this extraction procedure, the aim was to extract medium polar to polar compounds as would be done by using water and/or alcohol for traditional preparations.

2.2. Antimicrobial testing

The minimum inhibitory concentration (MIC) microdilution method was used (Eloff, 1998). All microbiological techniques, as well as the preparation of media and culture were conducted as prescribed by CLSI/NCCLS (2003) guidelines. Three bacterial strains [*Bacillus cereus* (ATCC 11778), *E. coli* (ATCC 8739) and *Enterococcus faecalis* (ATCC 29212)] were cultured in Tryptone Soya broth (TSB) for 24 h. The selection of microbial strains was based on their prevalence in gastrointestinal infections.

A standard microplate technique was used where 100 μL of sterile water was added to each well of a 96 well microplate. Aliquots of 100 μL *E. elephantina* and *P. prunelloides* extract (64 mg/mL — MeOH extract reconstituted in DMSO (dimethylsulfoxide) for complete solubility; 64 mg/mL — aqueous extract reconstituted in water) as well as (−)-epicatechin (95% pure, HPLC grade, Sigma-Aldrich), palmitic acid (ANALAR grade, Sigma-Aldrich) were added to the first row of the wells. Serial dilutions were made until all wells contained extract or standard at concentrations ranging between 0.13 mg/mL and 8 mg/mL for extracts. In cases where inhibition was observed lower than 0.13 mg/mL, lower starting concentrations of 6.40 mg/mL were used to determine the end-points. Concentrations ranged between 1.95 and 1 250 μg/mL for the compounds tested. After serial dilution, each well

was inoculated with 100 μL of the prepared culture. Cultures yielding an approximate inoculum size of 1×10^6 colony forming units (CFU)/mL were prepared for micro-dilution assays by diluting 1:100 to a 0.5 Mc Farland standard. The positive control used was ciprofloxacin (0.01 mg/mL), while water and DMSO were used as negative controls. The microplates were sealed and incubated at 37 °C overnight to stimulate bacterial growth. A 40 μL volume of 0.4 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. The MIC results for the extracts and compounds investigated independently are given in Table 1 (Please note that the solvent controls had no antimicrobial effect and was consequently not included as results).

2.3. Determination of synergistic interactions

Several in vitro techniques have been developed to determine the possible synergistic drug interactions, i.e. disc diffusion, checker board MICs, time kill and others (Hemaiswarya et al., 2008). In this study, the MIC assay was performed on the plant extracts (*E. elephantina* and *P. prunelloides*) and active compounds [(−)-epicatechin and palmitic acid] independently and in various combinations with the sum of the fractional inhibitory concentration (ΣFIC) calculated. This method has been described and validated by Van Vuuren et al. (2009) and Van Vuuren and Viljoen (2008, 2011). The interaction between the samples, in this case the two extracts and compounds, is determined algebraically by calculating the ΣFIC. The calculation is based on the concentration of each extract in combination with the other and expressed as a fraction of the concentration that would achieve the same effect when the extract is used independently (Eq. (1)) (Hemaiswarya et al., 2008). The outcome of the interaction is categorized based on the value obtained for the FIC index (FICI), i.e. synergism (when FICI ≤ 0.50), additivity (FICI > 0.50 to ≤ 1.00), non-interactive or indifferent (FICI > 1 to ≤4) and antagonism (FICI > 4.00) (Berenbaum, 1977, 1978, 1980; Van Vuuren and Viljoen, 2011).

$$\mathrm{FICI} = \mathrm{FICA} + \mathrm{FICB} = \frac{\mathrm{MIC_A\ in\ combination}}{\mathrm{MIC_A\ tested\ alone}} + \frac{\mathrm{MIC_B\ in\ combination}}{\mathrm{MIC_B\ tested\ alone}} \quad (1)$$

The FICI for plant interactions was calculated as the sum of the FIC values of *P. prunelloides* and *E. elephantina* and, where MIC_A is the minimum inhibitory concentration of *P. prunelloides*, and MIC_B is the minimum inhibitory concentration of *E. elephantina* either independently or in combination. Where the interaction between palmitic acid and (−)-epicatechin was investigated, MIC_A represented the MIC of palmitic acid and MIC_B that of (−)-epicatechin.

For varied ratio combinations of extract or compound mixtures where initial concentrations being 1 or 5 mg/mL, depending on the activity observed, were combined in ratios of 9:1; 7:3; 6:4; 5:5; 4:6; 3:7; and 1:9 and tested using the MIC methods as described previously. Isobolograms (Loewe and Muischnek, 1926; Suliman et al., 2010; Van Vuuren and Viljoen, 2011; York et al, 2012) are used to graphically demonstrate the interaction between *P. prunelloides* and *E. elephantina*, or between palmitic acid and (−)-epicatechin (Fig. 1), with respect to the pathogens studied. The rectangular co-ordinates (x and y) on the graph are representative for *P. prunelloides* and *E. elephantina*, or palmitic acid and (−)-epicatechin, respectively. Straight lines connecting the points provide visual discrimination between synergism (points on and below the 0.50 line), additivity (points between the 0.50 and including 1.00 lines), non-interactive or indifference (points between 1.00 and including 4.00) and antagonism (points above 4.00) (Suliman et al., 2010; Van Vuuren and Viljoen, 2011; York et al., 2012).

3. Results and discussion

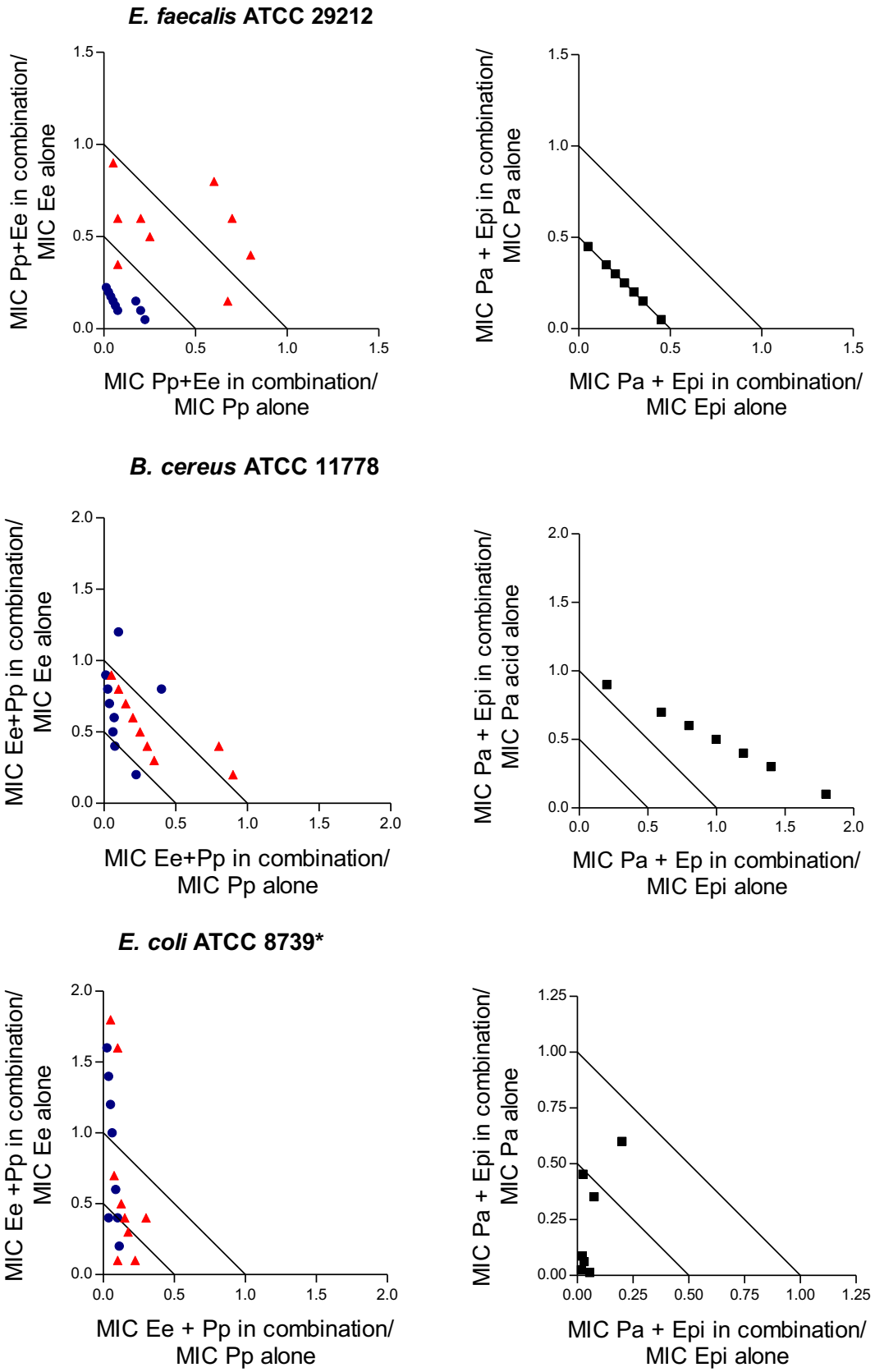
3.1. Independent studies

The MIC results (Table 1) are given for aqueous and MeOH extracts of *P. prunelloides* and *E. elephantina* as well as the tested compounds, palmitic acid and (−)-epicatechin, against two Gram-positive (*B. cereus* and *E. faecalis*) and one Gram-negative (*E. coli*) pathogen associated with gastrointestinal complaints. The MIC values for the aqueous (0.50–16.00 mg/mL) and MeOH (0.20–16.00 mg/mL) extracts independently demonstrated varied efficacy depending on the pathogen of study.

Previous antimicrobial studies with *E. elephantina* (Hedberg and Staugard, 1989; Aaku et al., 1998; Pretorius et al., 2003; Naidoo, 2004; Mathabe et al., 2006) have been conducted. Naidoo (2004) reported results specifically against stomach pathogens where the acetone leaf extracts had higher activities than acetone root extracts against *E. coli*, *E. faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* with the lowest MIC value reported as 0.625 mg/mL. Mathabe et al. (2006) assessed MeOH, EtOH (ethanol), acetone and aqueous *E. elephantina* extracts against eight different pathogens related to diarrhoea and observed inactivity for all extracts against *Salmonella typhi* as well as *E. coli*. MIC activities against *S. aureus* and *Vibrio cholerae* ranged between 0.156 and 0.312 mg/mL, while the highest inhibition was exhibited against *Shigella flexneri* (MIC 0.078–0.156 mg/mL). Yff et al. (2002) and Jäger (2003) reported poor antibacterial activity for aqueous extracts of *P. prunelloides*, but much higher activity was reported for EtOH and ethyl acetate root extracts [*Bacillus subtilis* (0.78–1.56 mg/mL), *Klebsiella pneumoniae* (0.39–1.56 mg/mL), *S. aureus* (0.39–1.56 mg/mL) and *E. coli* (0.78–3.13 mg/mL); Yff et al., 2002]. This trend was also observed in this study where MIC values against *B. cereus* and *E. faecalis*

Table 1
Minimum inhibitory concentration (MIC in mg/mL) and sum of the fractional inhibitory concentration (ΣFIC: given in brackets where applicable with interactive interpretation) results obtained for crude root extracts of *P. prunelloides* and *E. elephantina* as well as the 1:1 combinations.

Sample	*E. coli* (ATCC 8739)	*B. cereus* (ATCC 11778)	*E. faecalis* (ATCC 29212)
P. prunelloides (aq)	16.00	4.00	4.00
E. elephantina (aq)	1.00	0.50	2.00
P. prunelloides + *E. elephantina* (aq)	2.00 (1.00, additive)	0.50 (0.50, synergistic)	0.50 (0.19, synergistic)
P. prunelloides (MeOH)	16.00	0.50	0.40
E. elephantina (MeOH)	4.00	0.25	0.20
P. prunelloides + *E. elephantina* (MeOH)	4.00 (0.60, additive)	0.25 (0.70, additive)	0.20 (0.80, additive)
Palmitic acid	0.25	0.31	0.63
(−)-Epicatechin	0.13	0.63	0.63
Palmitic acid + (−)-Epicatechin	0.16 (0.09, synergistic)	0.63 (1.50, non-interactive)	0.31 (0.50, synergistic)
+ Control: Ciprofloxacin (μg/mL)	0.16	0.31	0.63



*One antagonistic interaction (aqueous extract for Ee:Pp at a ratio of 1:9) not shown due to graph scale.

Fig. 1. Interactions between varied ratios of *Pentanisia prunelloides* (Pp) and *Elephantorrhiza elephantina* (Ee) (● aqueous and ▲ MeOH extracts) and major compounds, (−)-epicatechin (Epi) and palmitic acid (Pa), when tested against three gastrointestinal pathogens. *One antagonistic interaction (aqueous extract for Ee:Pp at a ratio of 1:9) not shown due to graph scale.

were lower for aqueous extracts of *P. prunelloides* than those obtained for MeOH extracts (Table 1). The antimicrobial activity reported for *P. prunelloides* was suggested to be due to the presence of the major non-polar compound palmitic acid (Yff et al., 2002). (−)-Epicatechin and other flavonoids present in the extracts of these two plant species are also known to contribute towards antimicrobial activity (Binutu and Cordell, 2000; Cushnie and Lamb, 2011). Our results demonstrate that both palmitic acid and (−)-epicatechin show antimicrobial efficacy (MIC values ranging between 0.13 and 0.63 mg/mL) against all pathogens tested (Table 1).

Both palmitic acid and (−)-epicatechin are common dietary phytochemicals and have been evaluated for several biological indications both in vitro and in vivo (Scopus database, March 2013, MacDonald, 2000; Sánchez-Moreno, 2002). 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). Fatty acids rarely exist as free acids as they participate in non-specific binding to proteins resulting in inhibition of many enzymes (Gurr and James, 1980). Furthermore, cell membranes are important sources of fatty acid-derived molecules which act as intracellular mediators or extracellular signals important in interspecies communication and internal defence mechanisms (Weber, 2002). For fatty acids, the primary mode of action is suggested to be the targeting of the cell membrane, where Tsuchido et al. (1985) proposed fatty acid-induced autolysis rather than large-scale solubilisation of the cell membrane due to the detergent-like character of these fatty acids. Freese et al. (1973) reported that fatty acids are able to inhibit growth and oxygen consumption by *B. subtilis* in nutrient medium, supposedly by inhibiting transport of molecules, such as amino acids and keto acids through the membrane. 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 and Tomioka, 1988). The antibacterial mode of action exerted by flavan-3-ols such as (−)-epicatechin and it's gallated derivatives on the other hand, includes damaging the cytoplasmic membrane, as well as inhibiting nucleic acid synthesis, energy metabolism and cell membrane synthesis (Cushnie and Lamb, 2011).

These compounds also have other physiological effects which may contribute to the effective use of these species in the treatment of stomach ailments. Flavonoids are thought to be responsible for antidiarrhoeal activity by increasing colonic water and electrolyte reabsorption (Palombo, 2006). (−)-Epicatechin itself has been shown to be an effective treatment for diarrhoea (Abhilash, 2010), a phenomenon reiterated through experimental and computational studies (Velázquez et al., 2012). Furthermore, flavonoids, such as (−)-epicatechin, and terpenes are known to be bitter, and consequently cause an *amarum* effect, which is the stimulation of the taste buds leading to the promotion of gastric juices and bile secretion (Van Wyk and Wink, 2004). Bitter compounds are also suspected to be able to regulate metabolic and digestive functions not only through taste stimuli in the mouth, but also via gene expression in the gastrointestinal tract itself (Behrens and Meyerhof, 2010). According to Traditional Chinese Medicine, bitter substances "have draining and drying functions", i.e. they are used for purging fire (possibly also describing an affliction such as heart burn) and treating constipation, amongst other medicinal uses (Wu, 2005).

3.2. Combination studies

The two plants (*P. prunelloides* and *E. elephantina*) in a 1:1 combination demonstrated synergistic to additive effects for both aqueous and methanolic extracts (Table 1). It was noted in a previous study (Mabona et al., 2013), that traditional plant use is not an exact science, and quantities of plants are not measured accurately to microgram levels. Plants may be combined as one handful with another and differences in chemical composition may exist due to geographical variation. The active compounds may thus not always be present in the same ratio, hence the need to investigate various ratios. The results obtained for the two plants where the pathogens were subjected to various different ratios within the combination revealed that 51% of all ratios for the aqueous extracts were synergistic. Compared to the methanolic extracts, showing 18% of the ratios demonstrating synergy, a valid justification for combining *P. prunelloides* and *E. elephantina* as traditionally prepared (in water) over organic extracts is provided (Fig. 1; Table 2). Interestingly, a similar synergistic effect was observed between the aqueous extracts of these two plants where combinations were exposed to the skin pathogens *S. aureus*, *S. epidermidis*, as well as *Candida albicans* (Mabona et al., 2013). Aqueous extracts very often show poor activity and the synergistic interactions presented for these two plants presented here, and in previous studies (Mabona et al., 2013), validate the use in traditionally prepared mixtures. Only one occurrence of antagonism (for *E. coli* with a 1:9 combination of *P. prunelloides* with *E. elephantina* aqueous extracts) was observed in this study and no accounts of antagonism observed against other pathogens (Mabona et al., 2013). These observations demonstrate the favourable approach to combining these two plant species once again. The best examples of synergistic enhancement of activity were observed for *E. faecalis* and *E. coli*. Both the aqueous and MeOH extract combinations exhibited synergism against *E. coli* when higher concentrations of *P. prunelloides* were present. Even though two instances of synergism were reported for *B. cereus*, most results showed additivity.

Table 2
ΣFIC showing interaction between extracts of *P. prunelloides* and *E. elephantina* and major compounds [palmitic acid and (−)-epicatechin].

Combination ratio	ΣFIC		
	P. prunelloides: *E. elephantina* Aqueous extract	*P. prunelloides*: *E. elephantina* Methanol extract	Palmitic acid: (−)-epicatechin
E. faecalis (ATCC 29212)			
09:01	**0.275**	0.825	**0.501**
08:02	**0.300**	1.200	ND[b]
07:03	**0.325**	1.300	**0.501**
06:04	**0.175**	1.400	**0.501**
05:05	**0.188**	0.750	**0.501**
04:06	**0.200**	0.800	**0.501**
03:07	**0.213**	**0.425**	**0.501**
02:08	**0.225**	0.675	ND
01:09	**0.238**	0.950	**0.501**
B. cereus (ATCC 11778)			
09:01	**0.425**	1.100	1.897
08:02	1.200	1.200	ND[b]
07:03	0.775	0.650	1.698
06:04	**0.475**	0.700	1.598
05:05	0.563	0.750	1.498
04:06	1.300	0.800	1.399
03:07	0.738	0.850	1.299
02:08	0.825	0.900	ND
01:09	0.913	0.950	1.100
E. coli (ATCC 8739)			
09:01	**0.313**[a]	**0.325**	**0.069**
08:02	**0.500**	**0.200**	ND[b]
07:03	0.688	**0.475**	**0.041**
06:04	**0.438**	0.550	**0.044**
05:05	1.063	0.625	**0.094**
04:06	1.250	0.700	0.800
03:07	1.438	0.775	**0.428**
02:08	1.625	1.700	ND[b]
01:09	7.250	1.850	**0.479**

[a] Values given in bold where they correspond with the points below or on the 0.50 synergism line in Fig. 1.
[b] ND = not determined. The 08:02 and 02:08 ratios were not undertaken for the interactive compound study.

All nine aqueous extract combinations showed strong synergism against *E. faecalis* where ΣFIC values ranged between 0.175 and 0.325 (Fig. 1, Table 2).

It is often assumed that when two plants are combined, a combination of the active compounds contribute towards a synergistic effect. Thus a preliminary 1:1 combination of the isolated active compounds [palmitic acid and (−)-epicatechin] from each plant was undertaken. Synergistic efficacies of ΣFICs 0.09 and 0.50, for *E. coli* and *E. faecalis* respectively (Table 1) were observed. This prompted the idea to vary the concentration of the two compounds. When examined in varied ratios, synergistic interactions were noted for most of the combinations against *E. faecalis* and *E. coli*, irrespective of the ratio. Complete indifference was observed for all combinations against *B. cereus* (Fig. 1; Table 2).

The ΣFIC values for the two combined plants and their respective major compounds (Table 2) show that at a ratio of 9:1 (where *P. prunelloides* and palmitic acid is in majority) a synergistic effect is observed for both aqueous and MeOH extracts against *E. coli*. Palmitic acid is a major compound present in *P. prunelloides* and these results may confirm the role of the major compound in this combination. For studies against *E. faecalis*, the synergistic interactions of the two major compounds correspond with those obtained for the aqueous extracts. Since (−)-epicatechin and palmitic acid are both medium polar compounds, this may show a possible synergistic interaction amongst other more polar compounds present in these extracts. However, the various combinations of (−)-epicatechin and palmitic acid show higher efficacy than many of the MeOH extract combinations against *E. faecalis*. This effect is even more pronounced against *E. coli* (Fig. 1). In this instance, conjugation between (−)-epicatechin and palmitic acid could afford a synergistically improved activity where the lipophilic moiety of palmitic acid may not be able to penetrate the polar, negatively charged cell membrane of *E. coli*, but when conjugated with (−)-epicatechin (Matsubara et al., 2007), penetration could be possible, affording autolysis of the membrane by palmitic acid together with efflux inhibition by (−)-epicatechin. Furthermore, esterification of fatty acids to polyhydric alcohols is also reported to increase their antimicrobial effectiveness (Kabara, 1980). While this is true with glycerol or sucrose (Kabara, 1980), it may also be the case with flavan-3-ols such as (−)-epicatechin, where 3-O-decyl-(+)-catechin was found to be 64–128 fold more active than the parent, (+)-catechin, against *S. aureus* (MIC 1 μg/mL) and *E. faecalis* (MIC 2 μg/mL) (Stapleton et al., 2004; Park and Cho, 2010).

4. Conclusions

Combination (multidrug) therapy as opposed to the reductionist (single) therapy can be used not only to obtain synergistic antimicrobial activity, but also to expand the antimicrobial spectrum, prevent the emergence of resistant mutants and minimise toxicity (Williamson, 2001). This approach is common and somewhat intrinsic in traditional phytotherapy, as seen with the concomitant use of *E. elephantina* and *P. prunelloides*. It has been demonstrated for the first time that there is effective synergy in the use of both *P. prunelloides* and *E. elephantina* against stomach pathogens. Furthermore, the two moderately active compounds isolated from these plants, i.e. (−)-epicatechin and palmitic acid have shown synergistically enhanced activity especially against *E. coli* and *E. faecalis*. Apart from these findings, *E. elephantina* is known to exhibit anthelmintic (Mølgaard et al., 2001), antiprotozoal (Maphosa and Masika, 2012), anti-ehrlichial (Naidoo et al., 2006), anti-babesial (Naidoo et al., 2005), anti-inflammatory and antinociceptive (Maphosa et al., 2009) properties. *P. prunelloides* has also been shown to have anti-inflammatory and antiviral properties apart from being antibacterial (Yff et al., 2002). All these properties provide scientific rationale for the traditional use of these plants in stomach ailments.

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