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Pharmacological efficacy of local complementary and alternative medicines: *Psidium guajava, Vitex negundo, Moringa oleifera* and *Blumea balsamefira*

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Abstract

The marked increase of use and consumption of herbal remedies is mainly due to the beneficial uses it has been reported to have. Despite of its popular use as complementary and alternative medicines, there is limited scientific data available about the safety and toxicological evaluations of these herbal preparations. Therefore, it is the aim of the study to carry out a preliminary antibacterial and toxicological determination of both fresh and air-dried leaves of *Psidium guajava, Vitex negundo, Moringa oleifera* and *Blumea balsamifera*. The herbal preparations varied with its antibacterial activities and compared to the standard antibiotic chloramphenicol, the resulting zones of inhibition of the various extracts were significantly smaller. However, the results signifies promising possibility of being able to purify active agents against certain pathogenic bacteria. The non-inhibitive properties of other extracts also indirectly support some folkloric claims to help assuage some medical conditions that are not of bacterial origins. The determination of the low mutagenic potential of the herbal preparations infer that these are relatively free from possible carcinogens.

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Introduction

Herbal medicines are the oldest method for treating diseases and at the same time still the most commonly used worldwide (Dish *et al.*, 2017). It is estimated that 80% of the world population relies on herbal medicines spanning over all regions and all social classes (Ekor, 2014). The Philippines, being highly acclaimed as a biodiversity hotspot, has an abundance of plants that has been used as traditional herbal medicines for centuries. The four most commonly used herbal plants native to the Philippines, registered in the Bureau of Food and Drugs to have a number of practical and medicinal uses are *Psidium guajava, Vitex negundo, Moringa oleifera* and *Blumea balsamefira*(Santos, 2005). These herbal plants are generally accepted locally as alternative medicines for coughs, fever, body pains, asthma symptoms and other respiratory problems (Jawahar *et al.*, 2008).

Although much attention has been focused on the folkloric benefits of these four indigenous herbal plants, the scientific evidence is still lacking. These herbal remedies are not covered by prescription drug laws and therefore not a subject to the rigorous standards and testing. Thus, it is the aim of this study to evaluate the *in vitro* efficacy and potential pharmacological properties of the various extracts of the four common Philippine herbal plants. There is a definite need in the documentation of its safety and efficacy as well as the determination of its potential to be exploited and developed into healthcare formulations. Furthermore, this evidence-based study may also promote complementary and alternative medicine as a legitimate public health intervention.

Materials and methods

Plant sample collection

The shoots of *P. guajava, B. balsamifera, M. oleifera* and *V. negundo* were collected in various areas in Iligan City, Philippines. Collections of leaf samples were done at five to six o'clock in the morning to prevent the loss of water soluble constituents of the the plants.

A fraction of the leaf samples were used directly and the remaining leaves were air-dried. The air-dried leaves were cut inro pieces and were ground into powdery form.

Processing of leaves for various extractions

Each plant representative has fresh and air dried counterparts. Rapid extraction was done following the method of Zhang *et al.* (2006) where ground and fresh leaves weighing 2.5g were placed in a homemade tea bag. The bags were soaked for 15 minutes in 75ml of hot water. The samples were then stored in uniform bottles and refrigerated at 4°C.

The aqueous extraction (Chyu *et al.*, 2004) made use of 600 grams of both ground and fresh leaves which were then extracted by continuous soaking in 1000ml of sterile distilled water for 24 hours. Decoction, on the other hand, was done using the method of Houri *et al.* (2008) where 600g of ground and fresh leaves were boiled in 1000ml of distilled water for 15 minutes. The extracts from both methods of aqueous extraction and decoction were filtered using whatman filter paper and were stored in uniform bottles at 4°C prior to use.

Methanol extraction by Harbone (1994) made use of 600g of ground and fresh leaves soaked in 700ml of 95% methanol for 24 hours. The volatile oil obtained were concentrated using a rotary evaporator.

Determination of antibacterial properties

This study ascertained the antibacterial profiles of the extracts against four representative bacteria: *Staphylococcus aureus, Aeromonas hydrophila, Escherichia coli* and *Pseudomonas aeruginosa*. The standard Kirby Bauer Disc Diffusion method was used.

Ames test for determination of extract mutagenicity

This standard biological assay was employed to determine the mutagenic potenatial of the various leaf extracts.

Screening for phytochemical compounds

The methanol extacts of the leaf samples were used in the phytochemical testing. Presence or absence of saponins, alkaloids, tannins, steroids, flavonoids, cyanogenic glycosides and anthraquinone were determined.

Results and discussion

Antimicrobial spectrum of the selected herbal plants

The antibacterial activities of the different infusions (and its subsequent dilutions) against *E. coli, P. aeruginosa, S. aureus* and *A. hydrophila* were determined with chloramphenicol as the positive control given its broad efficacy against broth gram-positive and gram-negative bacteria.

Table 1. Revertant colonies of histidine-negative *Salmonella typhimurium* from the various extracts of the selected herbal plants.

Type of Extract	Number of revertant colonies of his-*S. typhimurium*				
	P. guajava extract	*V. negundo* extract	*M. oleifera* extract	*B. balsamefira* extract	Controls/ Commercial Teas
Control A					195
Control B					254
Comm TA					52
Comm TB					30
FAE	65	100	19	11	
FDE	60	82	32	13	
FRE	48	72	91	115	
DAE	14	11	12	15	
DDE	26	37	21	88	
DRE	18	23	13	44	
DME	0	0	0	0	

P. Guajava leaves, as most medicinal plants used in folk medicine, lack any systematic methodology needed to demonstrate genuine efficacy (Rouseff *et al.*, 2008). It has a long history of folk medicinal uses worldwide and is an important food crop in tropcial and subtropic countries. The fresh leave samples (rapid extracts, aqueous extracts and decoctions) showed minimal activity to the bacterial strains used with zones of inhibition ranging from (8-11millimeters). The results obtained may be because the leaves contain an essential oil rich in cineol, tannins and triterpenes that are capable of damaging enteric bacteria. Preparations from dried leaves (rapid-aqueous-methanol extracts and decoctions) revealed consistently higher antibacterial activities ranging from8-13 mm. The results obtained are indicative of its potency and thus dried guava leaves must be consumed in moderation. Such antibacterial characteristic would be more suited if it is applied directly to the infected areas to effectively destroy bacterial cells rather than damaging other tissues in the body.

Vitex negundo is is an important medicinal plant of which all of its parts are used folkloric medicine with (Lal *et al.*, 2006). A number of pharmacological activities have been attributed to *V. negundo* (Tasduq *et al*, 2008). All preparations of both fresh and dried leave yielded negative antibacterial results except for the methanolic extracts of dried *V. negundo* leaves which significantly antagonized the growth of all four test organisms. Zones of inhibition ranged from 7-9mm in all four test strains which is indicative of the presence of potent antibacterial molecules, thus confirming the use of methanolic extraction as an efficient method of extracting antibacterial compounds (Mbata *et al.*, 2008).

Moringa oleifera quite well-known in developing countries in Southeast Asia as an important nutritional supplement as the leaves contain nutrients especially essential amino acids, vitamins, minerals, and beta-carotene (Sharma *et al*., 2012; Sabale *et al*., 2008). To date, a variety of biological activities of parts of *M. oleifera* have been reported (Charoensin, 2014; Mishra *et al*., 2011; Anwar *et al*., 2007). The *M. oleifera* extracts for both fresh and dried samples for both rapid extracts and decoctions showed no zones of inhibition against all bacterial samples. However, the aqueous extracts of fresh leaves exhibited clearing zones on cultures of *S. aureus* (8 mm) and *A. hydrophila* (14mm), and the dried leaves extracts had zones on inhibition again *A. hydrophila* (11mm) and *E. coli* (15mm) The methanol extracts from fresh leaves showed absence antibacterial activity while, the methanol extracts of the dried leaves had zones of inhibition against *S. aureus* (11mm), *A. hydrophila* (8mm) and *E. coli* (9mm). *M. oleifera* may inhibit bacterial growth due to its phenolic components which may act as antioxidants (Oduro *et al*., 2008) and in some conditions as prooxidants (Amagloh and Benang, 2009).

Table 2. Secondary metabolites screened from the methanolic leaf extracts.

Plant Sample	Phytochemicals						
	Alkaloids	Flavonoids	Saponins	Tannins	Anthraquinone	Steroids	Cyanogenic glycosides
Psidium guajava	++	--	+++	+	--	+	--
Vitex negundo	++	--	+	+	--	+	--
Moringa oleifera	--	+	+++	+	--	+	--
Blumea balsamifera	+	--	++	+	--	+	--

Legend: --absent
++30%-50% of the total extracts
+10%-30% of the total extracts
+++50%-80% of the total extracts.

Blumea balsamifera have been reported to have quite wide use in the Philippine systems of traditional medicine. Decoction of the dried leaves yielded positive antibacterial activity against *S. aureus* (12mm), *A. hydrophila* (11mm) and *P. aeruginosa* (9mm), all of which are reported to be causative agents of gastrointestinal diseases supporting the claims that *B. balsamifera* have antidiarrhetic and antigastralgic properties. Methanolic extracts of the dried leaves exhibited antibacterial activities against *S. aureus* (12mm) and *A. hydrophila*.

Mutagenic potential of the various extracts of the four local herbal plants

The mutagenic potential of the various extracts are shown in Table 1.

All extracts from the four selected herbal plants showed off drop off numbers of isolated colonies compared to the control plates. The negative results on Ames test were all indicative of non mutagenicity. These results are in support of previous researches indicating the lack of mutagenic effects of the four herbal plants (Prashith *et al*., 2013; Awodele *et al*., 2012; Teixeira*et al*., 2003)**.** The collective absence of genotoxic probability of all extracts from the four selected plants infer that these CAMs are relatively free from possible carcinogens.

Phytochemical screening of various methanolic leaf extracts

The analysis showed variations in the presence of phytochemicals found in the different plant samples. Saponins were the dominant compound in *P. guajava* (in ~50-80%).

These are thought to have anti-inflammatory effect (Hassan *et al*., 2012). Next were alkaloids (30%-50%) which according to studies possess antimicrobial activity against both gram-positive and gram-negative bacteria (*Ayandele and Adebiyi,* 2007). Moreover, tannins and steroids were only about 10% - 30% of the extract. Tannins have anti-enzymatic and astrigent properties while steroids are important for the development of body's natural sex hormone. However, flavonoids, anthraquinone and cyanogenic glycosides were absent.

The phytochemicals found in *B. balsamifera* were saponins (30% - 50%), alkaloids (10% - 30%), tannins (10% - 30%) and steroids (10% - 30%). But flavonoids, anthraquinone and cyanogenic glycosides showed negative results in phytochemical screening. On the other hand, *M. oleifera* possess flavonoids (10% - 30%), tannins (10% - 30%), saponins (10% - 30%) and steroids (10% - 30%). Only *M. oleifera* contain flavonoids which according to studies, possess strong antioxidants and can therefore protect plant cells and tissues against free radicals. Flavonoids are phenol derivatives synthesized in substantial amounts and widely distributed among plants which have shown to have beneficial effects on human health because it fights viruses and slow aging (Cabrera *et al*., 2006). Moreover, the results also shows that saponins, tannins, and steroids were present. The phytochemical screening of *V. negundo* revealed that alkaloids (30% - 50%), saponins (10% - 30%), tannins (10% - 30%) and steroids (10% - 30%). Flavonoids, anthraquinone and cyanogenic glycosides were markedly absent.

Table 2 illustrates that saponins were found in highest concentration in all four plant samples, of which *M. oleifera* and *P. guajava* had exhibited the highest amount of saponins. Some experiments i*n vitro* and *in vivo* demonstrate the beneficial effects of saponins such as being antimutagenic and anticarcinogenic properties (Reza *et al*., 2007).

Legend.

COMM TA	-	Commercial Tea A	DDE	-	Dried sample, Aqueous extract
COMM TB	-	Commercial Tea B	DDE	-	Dried sample, Decoction extract
FAE	-	Fresh sample, Aqueous extract	DRE	-	Dried sample, Rapid extract
FAD	-	Fresh sample, Decoction extract	DME	-	Dried sample, Methanol extract
FRE	-	Fresh sample, Rapid extract	his^-	-	Histidine negative

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