

## **Antimicrobial, antioxidant and cytotoxicity properties of selected wild edible fruits of traditional medicinal plants**

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## **Abstract**

The fruit pulp extracts of 12 selected ethnobotanical wild edible fruits were investigated for their antimicrobial, antioxidant and cytotoxicity properties. Methanol extracts of the pulps were prepared and tested against five micro-organisms (*Salmonella typhi*, *Streptococcus pyogenes*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Prevotella intermedia*). The fruit pulp extracts of the wild edible plants exhibited different degrees of antimicrobial activity, with *Adansonia digitata* exhibiting a considerable level antimicrobial activity against *Salmonella typhi*. The fruit pulp extracts of *A. digitata* exhibited the best antioxidant activity. None of the fruit pulp extracts were toxic to humans. The results show the possibility of using these fruit pulps for the development of functional foods with medicinal benefits.

**KEY WORDS:** Pulp extracts, wild fruits, methanolic extracts, minimum inhibitory concentration, cytotoxicity

## **INTRODUCTION**

Wild edible plants play an important role in human lives considering that, among their many uses, they are often used as food and medicine and for shelter (14). A wild edible plant is defined as an indigenous plant with one or more parts that can be used as food if gathered at an appropriate stage of growth and properly prepared (23). Wild edible fruits are consumed by many people in rural communities in many developing nations, as they cannot afford commercialised fruit and vegetables. Wild edible fruits are vital sources of essential nutrients, including vitamins and minerals, that are necessary for the proper functioning of the body (25).

Many of these wild edible plants and fruits have been found to possess high in-vitro antimicrobial activity, hence they are a promising source of antimicrobial ingredients for the food industry (22). In recent years, many traditional edible plant species have been studied for their

potential biological and health benefits, including their antioxidant, anticancer, anti-aging, anti-atherosclerotic, antimicrobial and anti-inflammatory activities (28,31,32).

Many wild fruits contain polyphenols which are known for their anti-oxidant activities – hence their role in the prevention of various diseases associated with oxidative stress, such as cancer, cardiovascular disorders and neurodegenerative diseases (2).

The antioxidants present in wild fruits have been associated with beneficial health effects, such as protecting biomolecules from oxidative damage by scavenging free radicals and inactivating other pro-oxidants (16).

Evidence from previous studies indicates that phenolic phytochemicals found in wild fruits can exhibit antimicrobial activity against some foodborne pathogens responsible for foodborne disease outbreaks (18,21). Thus, it is of great importance to screen wild edible fruit pulp of traditional medicinal plants for their medicinal properties the possibility to be used for the production of functional foods. The objective of this study was therefore to investigate the antioxidant, antimicrobial and cytotoxicity properties of the wild edible fruits of 12 selected ethnobotanical medicinal plants.

## MATERIALS AND METHODS

Collection of plant materials and preparation of fruit pulp extracts

Mature edible wild fruits of 12 traditional medicinal plants: *A. digitata*, *Berchemia discolor*, *Manilkara mochisia*, *Ximenia caffra*, *Strychnos madagascariensis*, *Strychnos pungens*, *Strychnos spinosa*, *Landolphia kirkii*, *Boscia albitrunca*, *Xanthocercis zambesiaca*, *Englerophytum megalismontanum megalismontanum* and *Garcinia livingstonei* (Table 1) were collected from the Mutale Local Municipality, which is located in the north-eastern part of the Limpopo Province of South Africa. The selection and collection of a particular fruit was based on consumption and usage information obtained from discussions with rural community members. From each plant, two

**Table 1.** Minimum inhibitory concentrations (MIC) and maximum bactericidal concentrations (MBC) of extracts of selected traditional plants on selected foodborne bacteria

Plant extracts	MIC (mg/ml)					MBC (mg/ml)				
	S.t	S.p	B.c	K.p	P.i	S.t	S.p	B.c	K.p	P.i
<i>Adansonia digitata</i>	6.3	6.3	1.6	12.5	>25.0	6.3	25.0	6.3	25.0	>25.0
<i>Berchemia discolor</i>	6.3	6.3	3.1	6.3	>25.0	12.5	>25.0	12.5	>25.0	>25.0
<i>Manilkara mochisia</i>	12.5	12.5	1.6	0.4	25.0	12.5	>25.0	3.1	3.1	>25.0
<i>Ximenia caffra</i>	12.5	12.5	6.3	25.0	25.0	>25.0	>25.0	12.5	>25.0	>25.0
<i>Strychnos madagacariensis</i>	12.5	12.5	6.3	>25.0	>25.0	>25.0	>25.0	12.5	>25.0	>25.0
<i>Strychnos pungens</i>	12.5	12.5	>25.0	25.0	>25.0	25.0	>25.0	>25.0	>25.0	>25.0
<i>Strychnos spinosa</i>	25.0	>25.0	1.6	12.5	>25.0	>25.0	>25.0	6.1	>25.0	>25.0
<i>Landolphia kirkii</i>	25.0	25.0	6.3	6.3	>25.0	>25.0	>25.0	25.0	>25.0	>25.0
<i>Boscia albitrunca</i>	6.3	6.3	>25.0	12.5	>25.0	12.5	>25.0	>25.0	>25.0	>25.0
<i>Xanthocercis zambesiaca</i>	12.5	>25.0	6.3	6.3	>25.0	>25.0	>25.0	25.0	>25.0	>25.0
<i>Englerophytum megalismontanum</i>	12.5	6.3	12.5	12.5	>25.0	>25.0	>25.0	25.0	>25.0	>25.0
<i>Garcinia livingstonei</i>	25.0	12.5	3.1	6.3	>25.0	12.5	>25.0	25.0	25.0	>25.0

NB: **S.t**, *Salmonella typhimurium* ATCC 14028; **S.p**, *Streptococcus pyogenes* ATCC 21059; **B.c**, *Bacillus cereus* MTCC 430; **K.p**, *Klebsiella pneumoniae* ATCC 13883; **P.i**, *Providentia intermedia* ATCC 25611

mature fruits which were ready for consumption were harvested during the fruiting periods of each plant. These fruits were transported on ice block immediately after harvest to the laboratory for further processing within 12 hours. Voucher specimens of each fruit were prepared and deposited at the HGWJ Schweitkerdt Herbarium of the University of Pretoria in South Africa. The fruit pulps were air dried and ground into a fine powder, after which 20g of each powdered material was homogenised in 70% methanol and left on a shaker for five days to obtain fruit pulp solutions. The fruit pulp solutions were filtered (using Whatman filter paper No. 1 [110mm diameter] – Merck Chemicals [Pty] Ltd, Wadeville, South Africa) and concentrated (using the Büchi Rotavapor R200 – Labotec [Pty] Ltd, Halfway House, South Africa) to obtain fruit pulp extracts. The extracts were freeze dried, using a Virtis Bench Top Manifold Freeze Dryer (Sp Scientific [Pty] Ltd, New York, United States of America).

#### Preparation of test micro-organisms

The micro-organisms used in this study were *Salmonella typhimurium* (ATCC 14028), *Streptococcus pyogenes* (ATCC 21059), *Bacillus cereus* (MTCC 430) and *Klebsiella pneumoniae* (ATCC 13883) which were grown at 37°C for 24 h in nutrient broth and *Privotella intermedia* (ATCC 25611) which was grown at 37°C for 24 h in Casein-peptone Soy Agar medium (CASO) (Merck SA [Pty] Ltd) under anaerobic conditions in a jar with anaerocult A (Merck SA [Pty] Ltd). The subculturing of microbial cultures was done once weekly.

#### Broth microdilution assays

The microdilution technique, using 96-well microplates (9), was used to obtain the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the crude extracts of the fruit pulps against the selected micro-organisms. The extracts were serially diluted in the 96-well plate with 48-hour-old micro-organisms culture which had been serially diluted to  $5 \times 10^6$  CFU/ml following incubation at 37°C. The final concentration of extracts and positive controls (CHX) ranged from 25.0mg/ml to 0.8mg/ml. Microbial growth was indicated by adding

40 µl (0.2 mg/ml) *p*-iodonitrotetrazolium violet (INT) (Sigma-Aldrich, South Africa) to micro-plate wells and incubated at 37°C for 48 h. MIC values were determined as the lowest concentration of plant extracts that inhibited the growth of micro-organism and for which there was no change in the colour change of INT. The MBC of the pulp extracts were determined by adding 50 µl of the suspensions from the wells, which did not show any growth after incubation during MIC assays, to 150 µl fresh broth. These suspensions were re-incubated at 37°C for 48 h and 40 µl (0.2 mg/ml) INT added. The MBC values were determined as the lowest concentration of plant extract that inhibited 100% growth of micro-organisms and for which there was no change in the colour change of INT (7).

#### Antioxidant assay

The free radical scavenging activities were measured using 1,1 diphenyl-2-picryl-hydraxyl (DPPH) assay (8,12) with slight modifications. The extracts and vitamin C (positive control), 1000 µg/ml (20 µl), were added in the first three wells of a 96-well plate containing 200 µl distilled water to make up a final concentration of 100 µg/ml. The remaining wells were filled with 110 µl distilled water. The 100 µg/ml extracts and vitamin C in the first rows were serially diluted by adding 20 µl to the wells (which had been dispensed with 110 µl distilled water), followed by 90 µl DPPH (90 mM) methanolic solution to obtain final concentrations of the extracts (which ranged from 100 to 0.8 µg/ml). The plates were incubated at 37°C for 30 minutes and the absorbance was measured at 517 nm, using the enzyme-linked immunosorbent assay (ELISA) plate reader. The percentage radical scavenging activity in the extracts was determined through comparison with ethanol (blank). The inhibition ratio was calculated as follows: % DPPH radical scavenging =  $(AC-AS)/AC \times 100$ , where AC is the absorbance of the control solution (containing only DPPH solution) and AS is the absorbance of the sample in the DPPH solution. The percentage of DPPH radical scavenging was plotted against the plant extract/compound concentrations (µg/ml) to determine the concentration of extract/compound required to scavenge DPPH by 50% (EC<sub>50</sub>).

## Cytotoxicity assay

Only the cytotoxicity of six selected fruits of traditional medicinal plants that exhibited considerable antimicrobial activities and which were mostly utilised locally in the Limpopo Province was analysed (*A. digitate*, *B. discolour*, *L. kirkii*, *M. mochisia*, *X. zambesiaca* and *G. livingstonei*). The cytotoxicity of the active methanol extracts was measured against human kidney cells (HEK 293) by means of the XTT (sodium 3'-[1-(phenyl amino-carbonyl)-3,4-tetrazolium]-bis-[4-methoxy-6-nitro] benzene sulphonic acid hydrate) method (36). In a microtiter plate, the outer wells were filled with 200  $\mu$ l of incomplete medium (without FBS or PS) and the inner wells were filled with 100  $\mu$ l cell suspension and incubated for 24 h in a humidified atmosphere, with 5% CO<sub>2</sub> at 37°C. The plant extracts were serially diluted, making up various concentrations with a range of 400 to 3.1  $\mu$ g/ml added to the microtiter plate containing human kidney cells, and incubated for 72 hours. Each extract was tested in triplicate. Medium control and DMSO control were included in triplicate for each sample that was tested. XTT reagent was prepared to make a final concentration of 0.3 mg/ml, which was added to the cells in the microtiter plate and incubated for two to three hours. Included in the assay was positive drug control actinomycon-D (at various concentrations, ranging from 0.05 to 0.0003  $\mu$ g/ml). After incubation, the absorbance of the colour was spectrophotometrically quantified using an ELISA plate reader, which measured the optical density at 490 nm with a reference wavelength of 690 nm.

## Statistical analysis

The statistical analysis was conveyed as means  $\pm$  SD using GraphPad Prism 4.0 with a significant difference of ( $P < 0.05$ ).

## RESULTS

### Inhibition activities of plant extracts

The fruit pulp extracts of *A. digitata*, *B. discolor* and *B. albitrunca* showed the highest antimicrobial activities against *Salmonella typhi*, with each having a MIC value of 6.3 ug/ml. This was followed by *M. mochisia*, *X. caffra*, *S. madagacariensis*, *S. pungens*, *X. zambesiaca* and *E. megalismontanum* (with each having a MIC value of 12.5 mg/ml) and *L. kirkii* and *G. livingstonei* (with each having a MIC value of 25 mg/ml). The fruit pulp extracts of *A. digitata*, *B. discolor*, *B. albitrunca* and *E. megalismontanum* showed the highest inhibition activities against *Streptococcus pyogenes*, with each having a MIC value of 6.3 mg/ml. This was followed by *M. mochisia*, *X. caffra*, *S. madagacariensis*, *S. pungens* and *G. livingstonei* (with each having a MIC value of 12.5 mg/ml) and *S. spinose*, *L. kirkii* and *X. zambesiaca* (with each having MIC values  $\geq$  25.0 mg/ml) (Table 1).

*Bacillus cereus* was most susceptible to the different plant extracts in which the fruit pulp extracts of *A. digitata*, *M. mochisia* and *S. spinose* showed the highest inhibition activities against *Bacillus cereus*, with each having a MIC value of 1.6 mg/ml. This was followed by the fruit pulp extracts of *B. discolor* and *G. livingstonei*, with each having a MIC value of 3.1 mg/ml; *X. caffra*, *S. madagacariensis*, *L. kirkii* and *X. zambesiaca*, with each having a MIC value of 6.3 mg/ml; *E. megalismontanum*, with a MIC value of 12.5 mg/ml; and *S. pungens* and *B. albitrunca*, with each having MIC values  $\geq$  25.0 mg/ml (Table 1).

The fruit pulp extracts of *M. mochisia* showed the highest inhibition activities against *Klebsiella pneumoniae*, with a MIC value of 0.4 mg/ml. This was followed by the fruit pulp extracts of *B. discolor*, *L. kirkii*, *X. zambesiaca* and *G. livingstonei*, with each having a MIC value of 6.3 mg/ml; *A. digitata*, *S. spinose*, *B. albitrunca* and *E. megalismontanum*, with each having a MIC value of 12.5 mg/ml; and *X. caffra*, *S. madagacariensis* and *S. pungens*, with each having MIC values  $\geq$  25.0 mg/ml. *Privotella intermedia* was the least inhibited of all the micro-organisms tested, with MIC values  $\geq$  25 mg/ml for all the plant extracts (Table 1).

Bactericidal activities of plant extracts



Most of the plant extracts showed very little bactericidal activities against the tested bacteria, at MBC values  $\geq 25.0$  mg/ml, with no plant extract having a MBC value of less than 25 mg/ml against *Streptococcus pyogenes* and *Privotella intermedia*. *Bacillus cereus* was most susceptible to six of the 12 extracts analysed (at MBC values of  $\leq 12.5$  mg/ml), compared to five of the 12 extracts for *Streptococcus pyogenes* and one of the 12 extracts for *Klebsiella pneumoniae*. *M. mochisia* showed the most bactericidal activity, with MBC values of 3.1 mg/ml against *Bacillus cereus* and *Klebsiella pneumoniae* and MBC values of 12.5 mg/ml against *Salmonella typhi*. This was followed by *A. digitata* with MBC values of 6.3 mg/ml against *Streptococcus pyogenes* and *Bacillus cereus*. *A. digitate* and *B. discolor* also showed some bactericidal activities against *Streptococcus pyogenes* and *Bacillus cereus*, with MBC values of 12.5 mg/ml.

#### Antioxidant and cytotoxicity activities of plant extracts

Of the 12 extracts tested, only the methanol extract of *A. digitata* showed significant antioxidant activity, with an  $IC_{50}$  value of 16.18  $\mu$ g/ml – up to 10 times larger than that of vitamin C (10.67  $\mu$ g/ml); all the other extracts had  $IC_{50}$  values above 100  $\mu$ g/ml (Table 2). The fruits of the six selected fruits of traditional medicinal plants that exhibited considerable antimicrobial activities were found to be non-toxic to human kidney cells, considering that the  $IC_{50}$  of all the plant extracts were above 400  $\mu$ g/ml (Figure 1).

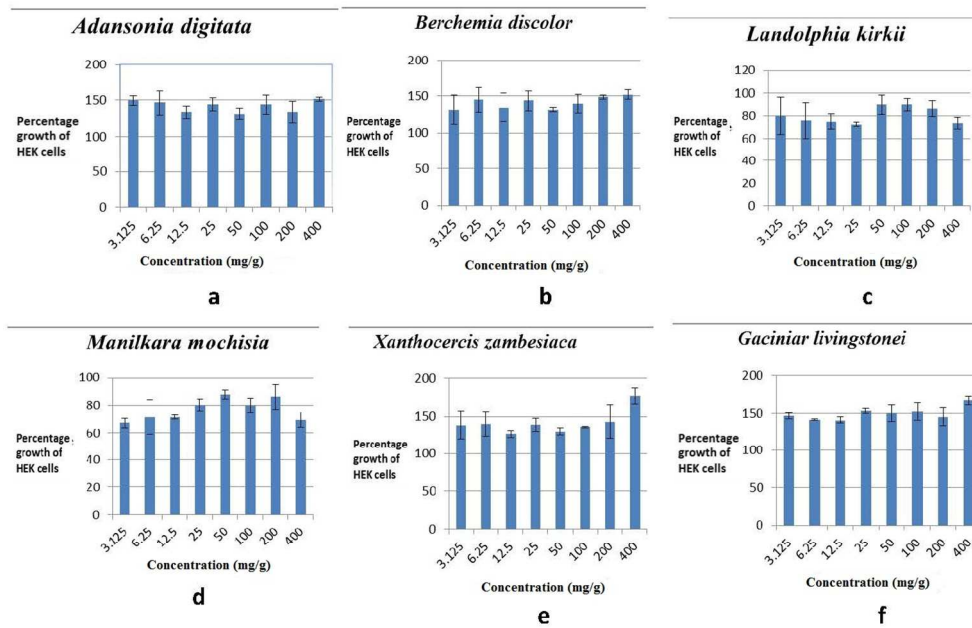
## DISCUSSION

#### Antimicrobial activities of plant extracts

The root, fruit and leave extracts of many medicinal plants have been found to possess significant antimicrobial activity against many micro-organisms, including foodborne pathogens such as *Salmonella typhi*, *Streptococcus pyogenes*, *Bacillus cereus* and *Escherichia coli* (15). In this study, the fruit pulp extracts of *A. digitata*, *B. discolor* and *B. albitrunca* showed the highest antimicrobial activities against *Salmonella typhi*, *Streptococcus pyogenes* and *Bacillus cereus*. Surprisingly, the

**Table 2.** The free radical activity of extracts of selected traditional plants

<b>Plant extract</b>	<b>IC<sub>50</sub> (µg/ml)</b>	<b>STD</b>
Vitamin C	1.67	± 1.95
<i>Adansonia digitata</i>	16.18	± 2.14
<i>Berchemia discolor</i>	>100	±1.85
<i>Manilkara mochisia</i>	>100	±1.82
<i>Ximenia caffra</i>	>100	±2.1
<i>Strychnos madagacariensis</i>	>100	±2.85
<i>Strychnos pungens</i>	>100	±1.82
<i>Strychnos spinosa</i>	>100	±3.1
<i>Landolphia kirkii</i>	>100	±2.22
<i>Boscia albitrunca</i>	>100	±2.1
<i>Xanthocercis zambesiaca</i>	>100	±1.85
<i>Englerophytum megalismontanum</i>	>100	±3.52
<i>Garcinia livingstonei</i>	>100	±1.1



**Figure 1.** The cytotoxicity effects of the six selected active extracts (*Adonsonia digitata*, *Berchemia discolor*, *Manilkara mochisia*, *Xanthocercis zambesiaca* and *Garcinia livingstonei*) on the growth of human kidney cells (HEK293)

254x190mm (145 x 149 DPI)

fruit pulp extracts *A. digitata* presented good antimicrobial activity against *Salmonella typhi* and *Streptococcus pyogenes*, considering that various extracts of *A. digitata* have previously been found not to present significant antibacterial activity against a vast array of tested bacteria strains (19,29).

However, extracts of *B. discolor* are reputable for their antimicrobial activities against different strains of bacteria, including strains of drug resistant *Mycobacterium tuberculosis* isolates (11). Various extracts of *B. albitrunca* have been found to possess significant antimicrobial activities against some bacteria, including *B. subtilis* and *K. pneumonia* (27).

The fruit pulp extracts of *E. megalismontanum* exhibited good antibacterial activity against *Streptococcus pyogenes*, despite previous findings where various extracts were found to show moderate antimicrobial activity against selected micro-organism. Furthermore, Gram-negative bacteria were more resistant to the extracts in comparison to Gram-positive bacteria (24). *X. caffra* (which showed moderate antimicrobial activity in this study) was found to have considerable in-vitro activity against *Candida* species (10), while the extracts *Strychnos pungens* and *X. zambesiaca* have been found to exhibit good to moderated antimicrobial activities against bacteria and fungi (13,30).

The fact that *Bacillus cereus* was most susceptible to the different plant extracts confirms the finding of another study where Gram-positive bacteria, such as *B. cereus*, were found to be more susceptible to other medicinal plant extracts than other bacteria (26). Surprisingly, the fruit pulp extracts of *Manilkara mochisia* showed very good antimicrobial activities against *Klebsiella pneumoniae*, considering that very little information is available in the literature about the antimicrobial activities of the extract of this plant. The fact that *Privotella intermedia* showed the least antimicrobial activity came as surprise, as it was previously found to possess moderate antimicrobial activity (20).

Based on the MBC results of the plant extracts, it is clear that the antibacterial activity exhibited by the extracts are mostly bacteriostatic, considering that only extracts of *M. mochisia*, *A.*

*digitata* and *B. discolor* exhibited significant bactericidal activity. Extracts of medicinal plants often exhibit better bacteriostatic properties than bactericidal properties for the same micro-organisms (33;35). The antimicrobial activity of the *M. mochisia* extract against *Salmonella typhi* is one of the best that have so far been registered for traditional medicinal plant extracts, considering that most authors found moderate MBC activities (1).

#### Antioxidant and cytotoxicity activities of plant extracts

The fact that all the fruit extracts exhibited antioxidant activity is not surprising, since different fruit parts have often been found to exhibit antioxidant activities (17). The fact that the fruit pulp extract of *A. digitata* showed significant antioxidant activity does not come as surprise, as another author also found that it exhibited high antioxidant activity compared to other fruits (34). The finding that none of the fruit pulp extracts were toxic to human kidney cells is in line with previous studies where medicinal plants were proven to be non-toxic to humans at the levels in which they are naturally consumed (3,5). It should be noted that in sub-Saharan Africa the fruits of many of the plant used in this study are often used traditional as food while the leaves, backs, roots and seeds extract are often used for medicinal purposes (6).

### CONCLUSION

The pulp extracts of the wild edible fruits from traditional medicinal plants exhibited different degrees of antimicrobial activities, with *A. digitata* exhibiting a substantial antimicrobial activity against *Salmonella typhi* a Gram-negative bacteria. Only the pulp extracts of *A. digitata* and *B. discolor* exhibited substantial bactericidal activity against *B. cereus*, while that of *M. mochisia* exhibited substantial bactericidal activity against *K. pneumoniae*. When compared to the other pulp extracts, the extract of *A. digitata* exhibited the best antioxidant activity. These results show that these fruit pulp extracts possess some bioactive properties that can be exploited for medical purposes.

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