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Research article

Antibiotic Activity of Wild Melon (*Adenopus Breviflorus* Benth) Fruit

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Wild Melon (Adenopus breviflorus benth) (ADB) matured whole fruits, its epicarp, pulp, and seed were respectively extracted with methanol, ethanol, ethyl acetate and n-hexane. The activity of each extract was examined at 10% concentration on four strains of bacteria isolates from poultry faeces, viz Escherichia coli, Klebsiella pneumoniae, Salmonella typhi and Streptococci enterococcus; the synergistic effect of seed and pulp extract were also examined on the test organisms at 10% concentration. The whole fruit and epicarp extracts were both screened for Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) ranging from 0.7-10% concentration. The results revealed that whole fruit and epicarp extract inhibited the growth of Klebsiella pneumoniae and Salmonella typhi at 10% concentration while Streptococci enterococcus and Escherichia coli were resistant to the same extract at the same concentration. The pulp and seed extract did not show antibacterial activity on any of the test organisms at 10% concentration the synergistic effect of the pulp and seed extracts also displayed no activity. The MIC of whole fruit and epicarp extract was 0.9-3% and 0.8 – 0.9% respectively. The results revealed that MBC of whole fruit and epicarp extract was between 9-10% and 10% respectively. It was concluded that the whole fruit and epicarp of wild melon have some phytochemicals which may be absent in the pulp and seeds or if present is at a significantly low level. These phytochemicals makes the whole fruit and epicarp extract to be capable of inhibiting the growth and the control of Klebsiella pneumoniae and Salmonella typhi. Further investigation is therefore suggested for the examination, identification, and quantification of phytochemicals in parts of ADB fruit.

Key words: Wild melon fruits, Antibiotic activity.

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INTRODUCTION

The dependence on plants and its products for health care needs has become a tradition in African medicine. Evidence shows that 70% of residents in rural communities make use of herbs in many ways to prevent or cure diseases (Odugbemi and Ayoola, 2010). Wild melon *(Adenopus breviflorus* benth) is one of the most locally exploited plant species in African traditional medicine (Ariwaodo *et al* 2011). The plant is a wild species of family cucurbitacae, it is a tendril climber, the leaves are simple, alternate and palmately veined, the pepo fruit is green with cream coloured narrow blotches (Dutta, 1995 and Bosa, 2008). Some members of the family like melon (*citrulus colocynthis*), pumpkin (*cucurbita maxima*), Cucumber (*Cucumis sativus*), water melon (*Citrulus lonatus*) and fluted pumpkin (*Telferia occidentalis*) have been widely cultivated for their edible fruits and oil obtained from the seeds while some have been reported to have anti sickling potentials (Nwaoguikpe *et al* 2013).

Traditional health practitioners in the tropics rely on selective harvesting of leaves, roots, bark, fruits and seeds in crude herbal medicine preparation, the wild being the reservoir of most of these plant materials. In South Africa for example, Cunningham (1988), Cunningham (1991), and Williams (1996) reported that of the 400-500 species of medicinal plants sold for traditional medicine care, 99% are harvested from the wild. Similar trend was observed in Nigeria by Fashola (2006), Obute (2007), and Ariwaodo *et al* (2011). Wild Melon fruit is claimed to be effective in preventing or curing some bacterial diseases affecting people (Umeire *et al* 2009 and Ariwaodo *et al* 2011). The people in the Middle Belt, South Western, and South Eastern Nigeria have thus been using wild melon fruit for the management of infections or ailments although scientific evidences for its antimicrobial effect is still lacking. In some cases, wild melon fruits are left around in the home as a disease prevention method. Some has been applied by some poultry farmers. Whether or not wild melon fruit is effective against disease organisms that are prevalent in poultry is not known. In view of these opinions and observations, this investigation was carried out to screen wild melon fruit for antibacterial activity.

MATERIALS AND METHODS

Collection of fruits and Identification

Wild melon (*Adenopus breviflorus benth* ADB) fruits were harvested from the range surrounding the communities of villages and towns in Kwara State South Senatorial District and from Moba LGA in Ekiti State of Nigeria. Some of these matured fruits were also purchased from their markets between December and March. The botanical identification of the fruits was done at the Department of Botany, Faculty of Life Sciences, University of Ilorin, Nigeria.

Processing of ADB Fruits

The fruits were properly washed under running tap water, rinsed with distilled water, air dried and preserved at room temperature for 72 hours in a well pre-cleaned processing room. The fruits were manually separated into the epicarp, pulp, and seed while some were left whole. These were further chopped into pieces and dried at room temperature and under shade, each were grounded separately.

Procedure of Extraction

For optimal yield of extract from different parts of plant, different solvents were used for the extractions (James, 2012; Halibur *et al* 2013). The epicarp, pulp, seed, and whole fruit were respectively extracted using ethanol, ethyl acetate, n-hexane and methanol, each part was soaked in the solvent for 72 hours after which each content was decanted, filtered, and concentrated.

Bacterial strains used

The bacterial strains used for this study were *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Streptococci enterococcus*. They were isolated from the poultry faeces at the Department of Microbiology, Faculty of Life Sciences, University of Ilorin, Nigeria.

Confirmation and identification of Bacterial Isolates

The bacterial isolates were cultured on nutrient agar and incubated at 37^0c for 24 hours and subsequently characterized by examination of colonial morphology by sub-culturing onto differential selective media such as desoxycholate citrate agar, brilliant green agar, Eosin methylene blue agar and Maconkey agar. The colonies from the various media were gram strained and further characterized by some biochemical tests which included catalase, coagulase, indole-oxidase and citrate utilization tests.

Standardization of Microorganisms

9.9ml of 1% H_2SO_4 was mixed with 0.1ml of 1% BaCI the mixture was then reconstituted into 10ml of sterile distilled water to make 0.5 McFarland standards. The inoculum was made up to 0.5 McFarland equivalents and a loopful of the standardized culture was used for antimicrobial assay.

Screening of extracts for antibacterial activity

To test for the antibacterial activity of whole fruit extracts, agar dilution method of Babayi *et al*, 2007 was employed with little modification. 2ml of extract + 1ml of Di-methyl sulfur oxide (DMSO) was added to Petri dishes containing 17ml of sterile Muller Hinton agar (oxoid) (1:9) to make a final concentration of 10%. The plates were prepared in duplicates and allowed to set at room temperature (28 ± 2^0c). A loopful of standardized test organism was streaked on the solidified agar with the incorporated extract and incubated for 24hours at 37^0c. Control agar plates were made in parallel and included Organism Viability Control (OVC), Medium Sterility Control (MSC), and Extract Sterility Control (ESC). Epicarp extract, pulp extract and seed extract were equally treated.

Screening of combined extracts of seed and pulp for antibacterial activity

To test for the antibacterial activity of combined seed and pulp, dilution method of Babayi *et al*. 2007 was employed with little modification, lml of seed extract + 1 ml of pulp extract + 1ml of DMSO was added to Petri dishes containing 17ml of sterile Muller Hinton agar (oxoid) (0.5:0.5:9) to make a final concentration of 10%. The plates were prepared in duplicates and allowed to set at room temperature (28 ± 2^0c). A loopful of standardized test organism was streaked on the solidified agar with the incorporated extracts and incubated for 24 hours at 37^0c. Control agar plates were made in parallel and included OVC, MSC and ESC.

Screening of Extracts for Minimum Inhibitory Concentration (MIC)

To test for minimum inhibitory concentration of whole fruits extract, Babayi *et al*., 2007 method was employed with little modification, 1.8ml of extract + 1ml of DMSO, 1.6ml of extract + 1ml of DMSO, 1.4ml of extract + 1ml of DMSO, 1.2ml of extract + 1ml of DMSO, 1ml of extract + 1ml of DMSO, 0.8ml of extract + 1ml of DMSO, 0.6ml of extract +1ml of DMSO, 0.4ml of extract +1ml of DMSO and 0.2ml of extract +1ml of DMSO was added to Petri dishes containing 17.2ml, 17.4ml, 17.6ml, 17.8ml,, 18ml, 18.2ml, 18.4ml, 18.6ml and 18.8ml of sterile Muller Hinton Agar to final concentrations of 9%, 8%, 7%, 6%, 5%,4%, 3%, 2% and 1% respectively. The plates were prepared in duplicates. Then the plates were allowed to set after which they were labeled with appropriate test organisms. The organisms were standardized as described for antimicrobial assay. Each plate was then streaked with a loopful of standardized sensitive test organisms (organisms that were sensitive during determination of antibacterial activity). Control agar plates were made in parallel and included OVC (Organism Viability Control)) MSC (Medium Sterility Control) and ESC (Extract Sterility Control). The plates were then incubated at 37^0c for 24 hours. Epicarp extract was equally treated.

Screening of Extracts for Minimum Bacteriocidal Concentration ((MBC)

To test for minimum bacteriocidal concentration of whole fruits extract, Babayi *et al*., 2007 method was employed with little modification. Fresh 19ml of Muller Hinton agar containing no extract were prepared and allowed to solidify. These plates (without incorporated extracts) were subsequently inoculated with all plate of extracts showing no visible growth from minimum inhibitory concentration (MIC) assay using sterile swab stick. Control plate included medium sterility control (MSC) was also made in parallel. The plates were then incubated at 37^0c for 24hours.

RESULTS

Antibacterial activity of the extracts

The results of the antibacterial activity of whole and parts of the fruit extract on test organisms are shown in Table 1. The results revealed that extracts of whole fruit and epicarp inhibited the growth of *Klebsiella pneumoniae* and *Salmonella typhi* at 10% concentration while *Streptococcus enterococcus* and *Escherichia coli* were resistant to both extracts at the same concentrations.

The results of the antibacterial activity of pulp and seed extracts on test organisms as shown on Table 1 revealed that both extracts did not display antibacterial activity on any of the test organisms at 10% concentration.

Antibacterial activity of combined extracts of pulp and seed

The results of the antibacterial activity of combined extract of pulp and seed on test organisms are shown on Table 2. The results revealed that combined extracts of pulp and seed did not display antibacterial activity on any of the test organisms at 10% concentration.

Minimum inhibitory concentration of extracts

The results of the minimum inhibitory concentration of whole fruit extract on test organisms are shown in Table 3. The results revealed that the minimum inhibitory concentration of whole fruit extract was between 0.9-3% concentrations.

The results of the minimum inhibitory concentration of epicarp extract on test organisms are shown on Table 4.

The results revealed that the minimum inhibitory concentration of epicarp extract was between 0.7-0.8% concentrations.

Minimum Bacteriocidal Concentration (MBC)

The results of the minimum bacteriocidal concentration of whole fruit extract on test organisms are shown in Table 5. The results revealed that the minimum bacteriocidal concentration of whole fruit extract was between 9-10% concentrations.

The results of the minimum bacteriocidal concentration of epicarp extract on test organisms are shown in Table 6. The results revealed that the minimum bacteriocidal concentration of epicarp extract was 10%.

TABLE OF RESULTS

Table 1: Antibacterial activity of whole fruit, Epicarp, pulp, and seed extracts at 10% concentration each.

Test Organisms	Whole Fruit	Epicarp	Pulp	Seed
Escherichia Coli	-	-	-	-
Klebsiella pneumoniae	+	+	-	-
Salmonella typhi	+	+	-	-
Streptococci enterococcus	-	-	-	-

Key
+ = activity
– = no activity

Table 2: Antibacterial activity of combined extracts of pulp and seed at 10% concentration

Test Organisms	Combined extract of pulp and seed
Escherichia Coli	-
Klebsiella pneumoniae	-
Salmonella typhi	-
Streptococci enterococcus	-

Key
+ = activity
– = no activity

Table 3: Minimum Inhibitory Concentration of Whole Fruit Extract

Extract Concentration (%)												
Test organisms	10	9	8	7	6	5	4	3	2	1	0.9	0.8
Klebsiella pneumoniae	+	+	+	+	+	+	+	+	+	+	+*	-
Salmonella typhi	+	+	+	+	+	+	+	+*	-	-	-	-

Key
* =Minimum Inhibitory Concentration (MIC)
+= Activity
- =no activity

Table 4: Minimum Inhibitory Concentration of Epicarp Extract

Extract Concentration (%)													
Test organisms	10	9	8	7	6	5	4	3	2	1	0.9	0.8	0.7
Klebsiella pneumoniae	+	+	+	+	+	+	+	+	+	+	+*	+	-
Salmonella typhi	+	+	+	+	+	+	+	+	+	+	+*	-	-

Key
*=Minimum Inhibitory Concentration (MIC)
+ = activity
- = no activity

Table 5: Minimum Bacteriocidal Concentration of Whole Fruit Extract

Extract Concentration (%)													
Test organisms	10	9	8	7	6	5	4	3	2	1	0.9	0.8	0.7
Klebsiella pneumoniae	+	+*	-	-	-	-	-	-	-	-	-	-	-
Salmonella typhi	+*	-	-	-	-	-	-	-	-	-	-	-	-

Key
*= Minimum Bacteriocidal Concentration (MBC)
+ = activity
- = no activity

Table 6: Minimum Bacteriocidal Concentration of Epicarp Extract

Extract Concentration (%)												
Test organisms	10	9	8	7	6	5	4	3	2	1	0.9	0.8
Klebsiella pneumoniae	+*	-	-	-	-	-	-	-	-	-	-	-
Salmonella typhi	+*	-	-	-	-	-	-	-	-	-	-	-

Key
*=Minimum Bacteriocidal Concentration (MBC)
+ = activity
- = no activity

DISCUSSION

Indigenous knowledge of people have played a significant role in understanding the biodiversity of livestock diseases and in the application of principles of managing them, such knowledge has been shown, in certain cases, to be effective, sustainable, environmentally friendly and practical (Mooga and Harrison, 2010), this explains why ethno/veterinary or biological importance of commonly available indigenous herbs and shrubs cannot be excluded in the prevention and control of livestock diseases in Africa especially in the poor rural farming communities that don't have adequate access to modern and or conventional livestock management skills. Also, the advent of organic farming in the developed countries of the world and the resultant push for the use of more environmentally friendly and humane methods of raising animals gives a lot of hope for the utilization of traditional knowledge and ethnoverterinary medicine in Africa (Adedeji *et al* 2012). The roots, barks, leaves, fruits, seeds of many herbs, shrubs, and trees have been claimed to be effective in the prevention or control of diseases in man and such experience advanced for use in livestock. Gidey *et al* (2012) collected twenty-two species of ethnoveterinary medicinal plant and were identified for treating eighteen (18) different livestock ailments in Northern Ethiopia. Evidences of ethnoveterinary practices have been documented in Kenya [Njoronge and Bussmann, 2006], Tanzania (Amy *et al* 2001), Ethiopia (Gidey *et al* 2012), Nigeria (Nwude and Ibrahim, 1980, Sonaya *et al*, 1992), Ghana (Williams 1990] and many other parts of Africa [Havunduka 1976). Among the many species of ethno/veterinary or biological importance is the wild melon (*Adenopus breviflorus benth*), (Igolie *et al* 2011, Borokini *et al* 2013 and Nwauzona and Dappa 2013).

Many herbal extracts have been seen to exhibit antibacterial activities and further pharmacological

investigations suggested for their possible use as antidote for many human and livestock diseases, among these are *Anthocephalus cadamba* fruits (Mishra 2011), bark of *Asmina triloba (*Abalaka and Oyewole 2011), fruits of *Manilkara hexandra* and *Mimusops elongi* (Patel and Rao 2012), *Treculia africana* Deone bark (Ogbonnia *et al* 2008), leaves of *ocimum gratissimum* (Odoemelam *et al* 2012), *Jatropha curcus* fruit parts (Rachana *et al* 2014), fruits of *Thymus vulgaris phoenix dactylifera, Crataegus azarolus* (Ayachi *et al* 2009). In the family of Cucurbitacae are seed extracts of *Cucurbita maxima* Duchense (Sharma *et al 2013*), oil seeds from pumpkin (C*ucurbita moschata*) (AbdEl-Aziz and AbdEl-Kalek 2011), and whole fruit of *Lagenaria brexiflora* Roberts (Tomori *et al* 2007) and many other medicinal plants of tropic and temperate origin (Shale *et al* 1999; Ahmad and Beg, 2001; Nair and Chanda, 2007).

The antimicrobial activities of family Cucurbitacae as reported by many authors is broad, extracts of some members have been shown to demonstrate antibacterial, antiviral, and antifungal properties (Tomori *et al* 2007, AbdEl-Aziz and AbdEl-Kaleek 2011), antiulcer (Kaushik *et al* 2011), antioxidant, antidiabetic and antiflammatory (Sivannarayar *et al* 2013, Sharma *et al* 2012, Essien *et al* 2013). Extracts of whole fruit of Lagenria *breviflora* inhibited the growth of colonies of Gram positive bacteria (*Bacillus subtilis* and *Staphylococuss aureus*) and that of Gram negative bacteria (*Salmonella gallinarium, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus* and *Esherichia coli*) (Tomori *et al* 2007). Results of this research agreed with the literature on the antibacterial activities of phytochemicals in extracts of Cucurbitacae fruits. The extracts of the whole fruit in this study was active on *Klebsiella pneumoniae* and *salmonella typhi* as observed in the reports of Tomori *et al* (2007). The seed extracts of *Cucurbita moschata* was reported to be active on *Staphylococcus aureus* and *Bacilus subtilis* (AbdEl-Aziz and AbdEl-Kaleek 2011), the seed extract of wild melon in this study is not active on the four test organisms, however, the extracts were not tested on any *Staphylococcus* species or *Bacillus* species. The pulp for extract was not also active on the four test organisms like the seed extract.

Bacterial diseases of poultry are many and some could cause nightmare for keepers if not prevented or adequately controlled when the flock is infected, these diseases as discussed by Atteh (2004), Berry and Whitenack (2014), Jammat and Morton (2010) include colibacillosis caused by E*schericha coli*, salmonella infections i.e. paratyphoid and pullorum diseases caused by *Salmonella typhi,* Stapylococcus infections caused by S*taphylococcus aureus,* infections *coryza* by *Haemophillus gallinarium,* Chronic Respiratory diseases by *Mycoplasma gallisepticum*, fowl cholera by P*asteurella multocida and tuberculosis by* mycobacterium avium just to mention a few. The literature reports and results of this study indicate that, in the absence of toxic materials in the cucurbit fruit and seed, a level of well processed whole fruit or the epicarp of the wild melon incorporated in a balanced ration of poultry might prevent or control some of these bacterial diseases e.g. Salmonella infections.

Reports of Sonaiya *et al* (1992), Nwachukwu *et al* (2010), and Adedeji *et al* (2012) have substantiated the importance of some cucurbits used in ethno/veterinary or biological medicine to prevent or control some diseases of poultry or man although very little document suggests their application in poultry feed formulation.

CONCLUSION

The fruit of wild melon as widely claimed could be medicinal, most especially the whole fruit or its epicarp, the effectiveness of the whole fruit as antibacterial is likely due to the presence of the epicarp i.e. the antibacterial activities of the whole fruit on the test organisms in this research is probably due to the phytochemicals in the extract of the epicarp, since neither the pulp or seed extracts exhibited any activity. The epicarp extract is at low as 3% concentration inhibited the growth of *Klebsiella pneumonia* and *salmoniella typhi*, this result has advanced reasons for a more intensive investigations of the epicarp of the wild melon and its ethnoveterinary advantage in poultry. The whole fruit, epicarp, and seed could be further examined for their likely nutritional and prophylactic advantage in monogastric livestock. The phytochemical compounds and their quantitative analysis in the fruit of wild melon and its parts should be screened for probable exploitation in commercial pharmacological studies. The seed extracts could also be investigated for its fatty acid composition to discover its domestic and industrial relevance to man.

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