Original Research Article

Phytochemical Screening and Antibacterial Activity of *Prunus avium* Extracts Against Selected Human Pathogens

ABSTRACT

Aim: This research was carried out to determine the phytochemical properties and antimicrobial activities of leaf and stem bark ethanol extracts of *Prunus avium* L. against selected human pathogens.

Methodology: The methods used included mechanical pulverization of the air-dried plant materials and solvent percolation extraction for 72 hrs. The resulting crude extracts were stored in sterile airtight McCartney bottles and stored in the refrigerator until use. After, they were screened for the presence of phytochemicals. Furthermore, the plant leaf and stem bark extracts were assayed for antibacterial activities against *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae,* and *Salmonella typhi*. The minimum inhibitory concentrations as well as time of kill of the extracts against the test pathogens was also determined.

Results: The results showed that flavonoid, saponins, alkaloids, tannins and phenols were present in the stem bark extracts while saponin was absent in the leaf extract. Furthermore, in the antimicrobial activity test, the plant extracts revealed varied activities along concentration gradient as higher concentration was observed to correspond to wider zones of inhibition. *E. faecalis* showed the highest susceptibility to both extracts at all the concentrations tested showing 11.00±0.00 and 16.33±0.01mm zone of inhibition for leaf and stem bark extracts respectively at 200mg/ml while *S. typhi* showed the least susceptibility to the extracts recording no inhibition against leaf extract at all the concentration used albeit showing 7.00±0.00mm inhibition zone against stem bark extract of the plant. The lowest MIC was found in stem bark extract against *K. pneumoniae* (3.125mg/ml), while the highest was recorded in leaf extract against *S. pneumoniae* (75mg/ml). The stem bark extracts showed the least time required to completely kill the pathogens, taking 15 minutes to completely inhibit *K. pneumoniae* followed by *E. coli* and *E. faecalis* which took 25 minutes each to be killed. However, the times recorded for the leaf extract to kill these organisms were higher than that recorded for stem bark extracts with *S. pneumoniae* recording the highest (100min) exposure time to be killed. The stem bark extract of the plant was more potent against the pathogens than the leaf extract.

Conclusion: The results of this study revealed that *Prunus avium* extracts contain biologically active constituents like saponins, alkaloids, tannins, flavonoids and phenols which may be responsible for the observed antibacterial activities of the plant against human pathogens.

Keywords: *Prunus avium; Phytochemical; Antibacterial; Pathogens*

Introduction

Throughout the world, plants have been identified and used as sources of therapy in traditional medicine for different purposes, including the treatment of bacterial and fungal infections. The use of traditional medicine for the maintenance of health has been on the rise in recent decades and it is gaining popularity among various groups of people around the world [1]. Its usage has not been limited to the use only by the poor in developing countries for the provision of primary health care but it has also taken up more importance in the health care delivery system of countries where orthodox medicine is predominant in the national health care system [2].

Medicinal plants have been described as plants with at least one of its parts containing metabolites which can be used for healing of diseases or can be used to synthesize useful drugs [3]. Inherent in medicinal plants are many biologically active secondary metabolites referred to as phytochemicals such as saponins, tannins, essential oils, flavonoids, alkaloids, and others with ability to prevent diseases and even cure them especially the infectious ones. These substances are generally synthesized by plants as a means of defense against their natural predators and disease causing agents, however, they have been found useful for the management of several diseases of man and his livestock [4]. Recently, there have been several reports of multiple drug resistance among various strains pathogenic microorganisms [5]. The rise in the reports of such antibiotic resistant microorganisms have search for more potent antimicrobials with broad spectrum activities by several researchers in recent times [6, 7]. The search light has been beamed on plants in the last decade for potential antimicrobials to be used in the management of the plethora of diseases affecting the human race.

One of such plant is *Prunus avium* popularly called cherry which is a member of the *Rosaceae* family, subfamily *Prunoideae*. It occupy the *Cerasus* subgenus within *Prunus*, being fairly distinct from their stone fruit relatives; plums, apricots, peaches and almonds. *Prunus avium* L. is the sweet cherry and *Prunus cerasus* L. the sour, pie, or tart cherry [8]. The fruit of this plant has been widely studied and has been reported to contain potent bioactive substances among which are polyphenols. It is reportedly used for medical purposes due to some inherent phytochemicals in its various parts such as fruit, stem bark and roots [9, 10]. The leaves and seed of the plant are used as pharmaceuticals in the treatment of various diseases. The tree is also valuable for

ornamentation as an ever-green broadleaf plant [11]. Many studies have been reported on the physical, chemical, pomological and nutritional properties of the fruit of this plant but little have been done to scientifically establish the phytochemical constituents and antimicrobial activities of the leaf and stem bark of the plant [12, 13]. Therefore, this study was designed to determine the phytochemical constituents and antimicrobial activities of leaf and stem bark extracts of *Prunus avium* against selected human pathogens.

Materials and Methods

Collection, Identification and Preparation of Plant materials

Fresh leaves and stem bark of *P. avium* were harvested from a fruit orchard in Iyere, Ondo State, Nigeria in July, 2017. The plant was then authenticated at the Herbarium section of the Department of Forest Resources Technology and voucher specimen (X-PA7124L) was deposited in the same department, Rufus Giwa polytechnic, Owo. The authenticated plant materials were washed and cleaned thoroughly under running tap and then air-dried under shade for 4 weeks. The dried samples were then pulverized into powder with the use of a mechanical grinder and were stored in clean air- tight containers, and kept in a cool, dry place until required for use.

Extraction of the samples

One hundred gram (100g) of the powdered sample was soaked in 200ml of different ethanol for 48hr with intermittent stirring using sterile spatula. The plant extracts were then filtered through muslin cloth into sterile McCartney bottles and then dried invacuo using rotary evaporator at a temperature of 50^0C to yield crude extracts [14]. From the crude extract four concentrations were prepared for the assay by diluting 5.0g, 10.0g and 20.0g of the extracts in 100ml of 0.01% DMSO to obtain concentrations of 50mg/ml, 100mg/ml and 200mg/ml respectively.

Test microorganisms

The bacteria used in this study include *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae,* and *Salmonella typhi.* They were obtained from the Microbiology and Pathology Laboratory of Federal Medical Center, Owo, Nigeria.

Qualitative phytochemical screening

The extracts of the plant were subjected to qualitative phytochemical screening for the presence of tannins, saponin, flavonoids, alkaloids and phenol using standard procedures as described by Sofowora [15].

Test for tannins

1ml of extract was boiled in 20ml of water in a test and then filtered. A few drops of 0.1% ferric chloride was added and observed green or a blue – black coloration which confirmed the presence of tannin.

Test for saponin

About 5ml of the extract was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion which confirmed a positive presence of Saponins

Test for flavonoids

A 3ml portion of 1% Aluminum chloride solution was added to 5ml of each extract. A yellow coloration was observed indicating the presence of flavonoids. 5ml of dilute ammonia solution were added to the above mixture followed by addition of concentrated H_2SO_4. A yellow coloration disappeared on standing. The yellow coloration which disappeared on standing indicating a positive test for flavonoids.

Test for alkaloids

A 1ml portion of the extract was stirred with 5ml of 1% aqueous HCl on a steam bath and filtered while hot. Distilled water was added to the residue and 1ml of the filtrate was treated with a few drops of either Mayer's reagent (Potassium mercuric iodide- solution gave a positive test for alkaloids.

Test for phenol

A 5ml portion of the extract was pipetted into a 30ml test tube, and then 10ml of distilled water was added to it. 2ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added and left to react for 30min.The development of bluish-green colour was taken as a positive presence of phenol.

Antibacterial activities test

The extracts obtained from the plants leaf and stem bark were screened against the bacteria by agar well diffusion method [16]. A 25ml of Nutrient agar was poured into each Petri dish and after the agar solidified, the pathogenic test organisms were inoculated on the surface the plates (1×10^6 *cfu*/ml) using a sterile glass spreader and allowed to sink properly. Subsequently, the surface of the agar was punched with 6mm diameter cork borer into wells and a portion of 50µl of each of the extract concentrations was filled into the wells. Control wells containing the same volume of Dimethyl sulphoside (DMSO) served as negative control, while Chloramphenicol (50µg) was used as positive control for the plates respectively and the plates were incubated at 37^0C for 24 h. The diameter of the zones of inhibition was then measured in millimeters.

Determination Minimum Inhibitory Concentration (MIC)

The MIC of the plants extracts were determined by double dilution broth methods of Ghosh et al. [17]. Twofold dilutions of the extracts were prepared in Nutrient broth to achieve a decreasing concentrations ranging from the least concentration that produced clear zone of inhibition (50mg/ml to 0.156mg/ml). All tubes with the controls were labeled accordingly. Each dilution was seeded with 1ml of standardized inoculums (1.0×10^6 *cfu*/ml) and incubated at 37^0C for 24 hr. A tube containing only seeded broth (i.e. without plant extract) was used as the positive control while the un-inoculated tube was used as negative control. Aliquot of 0.1 ml from the tubes showing clear inhibition when compared with the controls were spread on fresh nutrient agar plates and the lowest concentration of the extract that prevent formation of colonies after 24 hr incubation at 37^0C was considered as the MIC [18].

Determination of the killing time of plant extracts

The MIC of each test organism was used for this assay. Each organism was exposed to the respective concentration for different time. A 0.1ml of each concentration was added to test tube containing 10ml of standardized inoculum, then it was centrifuged at 1000rpm for 2 hr. At 5 min interval, an aliquot of 1ml from the test tube is cultured on fresh Nutrient agar and incubated, the time at which there was no visible colony formation on agar plate was taken as the killing time of the extract against the organisms [19].

Data Analysis

Data were presented as mean±standard error (SE). Significance difference between different groups was tested using one-way analysis of variance (ANOVA) and treatment means were

compared with Duncan's New Multiple Range Test (DNMRT) using SSPS window 7 version17.0 software. The significance was determined at the level of $p \leq 0.05$.

Results and Discussion

3.1 Qualitative phytochemical composition of *P. avium*

The results of the qualitative phytochemical composition screening of the *P. avium* leaf and stem bark ethanol extracts is shown in Table 1 where it was revealed that all the tested phytochemicals (i.e. flavonoid, saponins, alkaloids, tannins and phenols) were present in the stem bark extracts while saponin was absent in the leaf extract. Also, it was observed that the reactions of these compounds were more intense in stem bark extracts compared with the leaf extracts suggesting that they may be present in more abundance in the plant stem bark. These phytochemicals have been reported to possess wide range of pharmacological activities such as antioxidant, antihelminthic and antimicrobial activities [20] and this suggests that *P. avium* leaf and stem bark may be explored for the development of possible pharmaceutical products.

Table1: Qualitative phytochemical composition of *P. avium* leaf and stem bark

Phytochemical	Leaf	Stem bark
Flavonoid	++	++
Saponin	-	+
Tannin	+	+
Alkaloid	+	++
Phenols	++	+++

Key: +++ = strong reaction, ++= moderate reaction, += mild reaction, - = not detected.

3.2 Antibacterial Activities of *P. avium*

The antibacterial activities of the leaf and stem bark extracts of *P. avium* revealed varied activities along concentration gradient as higher concentration was observed to correspond to wider zones of inhibition (Tables 2 and 3). The inhibitory activities of the extracts were more pronounced against Gram negative bacteria compared to the Gram positive ones. This corroborates the earlier reports of Bella et al. [21] and Nikita et al. [22] who had similar results. Further, the stem bark extract exhibited more potency against the test organisms than the leaf extract. *E. faecalis* showed the highest susceptibility to both extracts at all the concentrations tested showing 11.00±0.00 and 16.33±0.01mm zone of inhibition for leaf and stem bark extracts

respectively at 200mg/ml. Interestingly, *S. typhi* showed the least susceptibility to the extracts recording no inhibition against leaf extract at all the concentration used albeit showing a meager 7.00±0.00mm inhibition zone against stem bark extract of the plant.

Table2: Antibacterial activity of *P. avium* leaf ethanol extract on selected pathogens

Organisms	Conc. (mg/mL)				
	50	100	200	DMSO	Chl(100µg/ml)
	Zones of inhibition (mm)				
S. aureus	3.00±0.00[a]	6.00±0.00[b]	8.33±0.58[c]	NI	25.00±0.00[d]
S. pneumoniae	NI	4.67±0.58[a]	7.33±0.58[b]	NI	20.00±0.00[c]
E. coli	4.00±0.00[a]	8.67±0.58[bc]	10.00±0.00[c]	NI	24.33±0.58[d]
E. faecalis	5.67±0.58[a]	8.33±0.58[b]	11.00±0.00[c]	NI	21.00±0.00[d]
K. pneumoniae	6.67±0.58[a]	9.00±0.00[b]	10.67±0.58[b]	NI	22.00±0.00[c]
S. typhi	NI	NI	NI	NI	22.67±1.00[c]

Values are Mean±S.E.M (mm), Values followed by different alphabet along the rows are significantly different at p=0.05, NI= no inhibition, Chl=Chloramphenicol.

Earlier reports have shown that most antimicrobial agents' activity correlates positively with concentration of the agent [23] and the results obtained in this study supports this submission. The difference in the susceptibility pattern of Gram positive and Gram negative bacteria recorded in this study may be due to the differences in their cell wall structures. Since Gram positives cell wall are thicker than that of Gram negatives and are rigid because of the reinforcement with peptidoglycan although this has not translated to antibiotic resistance. Gram negatives are known to be more antibiotic resistant and this has been alluded to their impenetrable cell wall [24] as well as possession of high level of lipopolysaccharides in their outer membrane [25]. Therefore their pronounce susceptibility to *P. avium* extracts suggests that the plant may contain some active chemicals that may be exploited for the development of novel antimicrobial agents against these troublesome pathogens that are very active in circumventing most of the known antibiotics.

Comment [C1]: Change ml for mL

Table3: Antibacterial activity of *P. avium* stem bark ethanol extract on selected pathogens

Comment [C2]: Change like 2

Conc. (mg/ml)	50	100	200	DMSO	Chl(100µg/ml)
Organisms	**Zones of inhibition (mm)**				
S. aureus	6.33±0.01[a]	10.00±0.10[b]	12.67±1.15[c]	NI	28.33±0.00[d]
Streptococcus pneumoniae	2.67±0.00[a]	6.33±0.05[b]	9.33±0.02[c]	NI	24.67±0.01[d]
Escherichia coli	6.33±0.00[a]	11.67±0.08[b]	15.00±0.15[c]	NI	20.33±0.58[d]
Enterococcus faecalis	6.33±0.00[a]	10.67±0.02[b]	16.33±0.01[c]	NI	21.67±0.00[d]
Klebsiella pneumoniae	7.33±0.11[a]	11.00±0.10[b]	15.33±0.12[c]	NI	21.00±1.00[d]
Salmonella typhi	NI	3.67±0.58[a]	7.00±0.00[b]	NI	22.67±0.01[c]

Values are Mean±S.E.M (mm), Values followed by different alphabet along the rows are significantly different at p=0.05, NI= no inhibition, Chl=Chloramphenicol.

The observed disparity in the antibacterial activities of the leaf and stem bark extract of the plant may be linked to the number and quantity of phytochemicals present in them. The stronger reactions in the tests for these compounds in the stem bark extract is an indication that they are present in higher quantity than in the leaf. Adeshina et al. [26] reported in their work that plant rich in phytoconstituents like alkaloid, flavonoids, tannins, terpenoids and steroids have antibacterial properties. Moreover, plants rich in flavonoids and tannins are reported for their antibacterial activities which are accomplished by inactivating enzymes while tannins and other compounds of phenolic nature are also classified as active antimicrobial compounds [27].

3.2.1 Minimum Inhibitory Concentration of *P. avium* against selected pathogens

The minimum inhibitory concentration (MIC) of an antimicrobial agent has been described as the smallest concentration of the substance that inhibits the growth of test microorganisms [28]. It is usually adopted in confirming the resistance of microorganisms to antimicrobials. The

lowest MIC was found in stem bark extract against *K. pneumoniae* (3.125mg/ml), while the highest was recorded in leaf extract against *S. pneumoniae* (75mg/ml) as presented in Table 4. These observations differ from the report of Rovcanin et al. [29] who obtained lower MIC of 0.25mg/ml for *P. avium* petiole ethanol extract against *E. coli* ATCC 25922. However, the MIC recorded for stem bark extract against *S. aureus* (6.25mg/ml), *E. faecalis* (6.25mg/ml) and *E. coli* (12.5mg/ml) are also encouraging since they suggests that these organisms may not be resistant to the extract whereas, *S. pneumoniae* and *S. typhi* used in this study may be resistant to the extracts. These results suggest that this plant may be useful in the management of intestinal pathogens especially the *Enterobacteriaceae* and to treat some related microbial infections.

Table4: MIC of *P. avium* leaf and stem bark ethanol extract on selected pathogens

Organisms	Leaf (mg/ml)	Stem bark (mg/ml)
Staphylococcus aureus	50	6.25
Streptococcus pneumoniae	75	50
Escherichia coli	50	12.5
Enterococcus faecalis	25	6.25
Klebsiella pneumoniae	25	3.13
Salmonella typhi	ND	75

Key: ND= not detected

Comment [C3]: MIC values are too high … please see this reference : Aligiannis, N., Kalpoutzakis, E., Mitaku, S., & Chinou, I. B. (2001). Composition and antimicrobial activity of the essential oils of two Origanum species. Journal of agricultural and food chemistry, 49(9), 4168-4170. The best value of MIC is 0.6 to 1.5 mg/mL

3.2.2 Killing Time of *P. avium* against selected pathogens

The minimum exposure time for the test organisms against the extracts to achieve complete inhibition of growth is presented in figure 1. Here, the stem bark extracts showed the least time required to completely neutralize these pathogens recording a time of 15 minutes to completely inhibit *K. pneumoniae* followed by *E. coli* and *E. faecalis* which took 25 minutes each to be inhibited. However, it took 75 minutes for *S. typhi* to be completely inhibited. Moreover, the times recorded for the leaf extract to kill these organisms were higher than that recorded for stem bark extracts with *S. pneumoniae* recording the highest (100min) exposure time to be killed. These observations are in line with earlier reports [30] and they suggests that the stem bark

extracts may be used to formulate new first line drugs in the management of infectious diseases especially those caused by the susceptible bacteria.

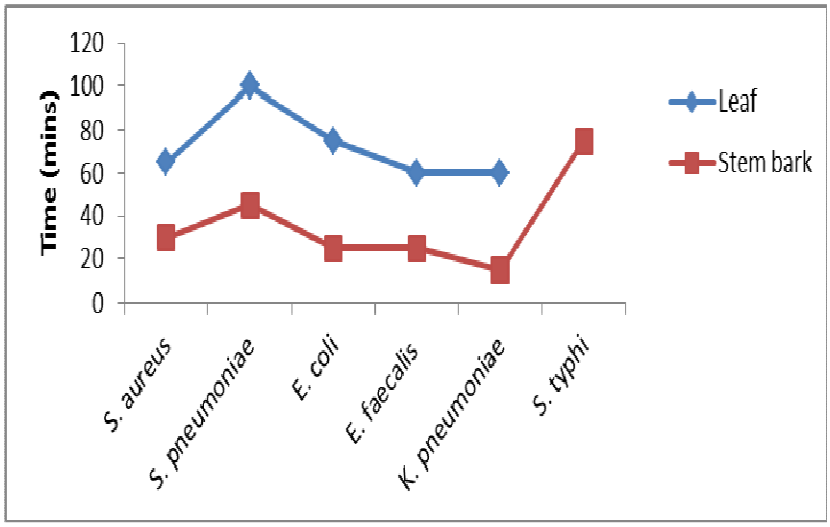

Figure1: The time of kill of *P. avium* extracts against selected pathogens

Conclusion

From the foregoing, the extracts of *P. avium* contain alkaloids, tannins, flavonoids and phenols whereas saponin is only present in stem bark and absent in the leaf of the plant. Moreover, the extracts possess antibacterial activity at higher concentrations against the test bacteria. Furthermore, the extracts were more potent against Gram negative organisms than the Gram positives. Finally, stem bark extracts of the plant needs lesser time to achieve total neutralization of the test bacteria compared with the leaf extracts.

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