Trad. Med. J., May-August 2022
Vol. 27(2), p 153-158
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 17-05-2022
Revised : 30-07-2022
Accepted : 03-08-2022

Phytochemical Quantification and Efficacy of *Persea Americana* Extracts on Some Selected Pathogens

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ABSTRACT

Medicinal and aromatic plants are increasingly becoming the focus of many researchers in the search for alternative antimicrobial agents due to their large number of diverse bioactive compounds called phytochemicals. *Persea americana* (Avocado) is an evergreen tree plant known for its healthy fruit and has been used in the management of disease and infections. The stem bark of *P. americana* was investigated for its phyto-constituents and antimicrobial activity using standard methods. The stem bark was preponderant in polyphenolic compounds of which flavonoid was found to be abundant (480.01 ± 0.071 mg/g), the antimicrobial activity study of the extracts also showed good inhibitory activity on all the tested strains with *K. oxytoca* (27.50 ± 0.67 mm) and *A. flavus* (88.89%) mostly susceptible in a concentration-dependent manner. The study revealed that *P. americana* extracts could be explored in the search for alternative antimicrobial agents.

Keywords: *Persea americana;* phytochemical screening; antimicrobial study; alternative antimicrobial agents.

INTRODUCTION

Persea americana also known as avocado pear or alligator pear, belongs to the family *Lauraceae*. It is a tree native to tropical and subtropical regions of the world, the tree grows up to a height of 20 m (Bertin et al., 2020). The leaves are of variable shapes (oval, elliptic, and lanceolate) and sizes ranging between 7 and 41 cm in length (Ejiofor et al., 2018). The fruit is a large spherical green fleshy berry with a single seed at the centre. *P. americana* has been used in traditional folk medicine for the treatment and management of hypertension, urinary tract infections, tumours, bronchitis, diabetes, inflammation, rheumatism, sore throat, dysentery haemorrhage, diarrhoea, etc. (Falodun et al., 2013; Park et al., 2019). This research seeks to evaluate the phyto-constituents and investigate the antimicrobial potential of *P. americana* extracts.

METHODOLOGY

Persea americana stem bark was collected in Ogbomoso, Nigeria in January 2020, the sample was identified in the Department of Pure and Applied Biology, Ladoke Akintola University of Technology. The sample was rinsed with distilled water and dried at room temperature, after which it was pulverized and defatted with n-hexane and further extracted successively with ethyl acetate and ethanol (one week each) using the cold maceration method. The extracts were filtered with Whatman No 1 filter paper, and the filtrate was concentrated *in vacuo.* The concentrated extracts were kept in an air-tight container for further analysis.

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Qualitative and quantitative phytochemical screening

The presence of plant's secondary metabolites were tested for in the *P. americana* stem bark extracts using standard methods as reported by Harborne (1998). Phyto-chemicals tested include alkaloids, steroids, glycosides, terpenoids, carbohydrates, cardiac glycosides, saponins, tannins, phenols, flavonoids, and anthocyanins. Characteristic colour change or formation of precipitate was observed for identification. Tannin and phenol content of the extracts were quantified using the *Folin – Ciocalteus method* as reported by Tambe and Bhambar (2014), saponins and alkaloids content were determined using gravimetric as described by Harborne (1998) and Obadoni and Ochuko (2002) respectively, while flavonoids content in the extracts was quantified using Aluminum chloride colourimetric assay according to Mallaiah et al (2015).

Collection of test organisms

Clinical isolates of the test bacteria (Escherichia coli, Proteus vulgaris, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, and Klebsiella oxytoca) and fungi strains (Aspergillus niger, Aspergillus fumigatus, Fusarium poae, Aspergillus flavus, and Fusarium solanis) were obtained from Bowen Teaching Hospital, Ogbomoso, Oyo State, Nigeria.

Antibacterial susceptibility

The susceptibility of the bacteria isolates to *P. americana* extracts was evaluated using Agar well diffusion method as previously discussed by Soniya et al., (2013). Inoculum of the test bacteria (*Salmonella typhi, Pseudomonas aeruginosa, Klebsiella oxytoca, Staphylococcus aureus, Proteus vulgaris, and Escherichia coli,*) were used to seed Mueller–Hinton Agar (Lab M Ltd.) plates with sterile swab stick, after which five wells of diameter 7 mm each was created into which 100 µl of different concentration of *P. americana* extracts were added. The negative control is an additional well at the center of the plate containing DMSO. Standard antibiotic disk (Abtek Biologicals Ltd., UK) containing tetracycline 30 µg, ceftriaxone 30 µg, amoxicillin 25 µg, ciprofloxacin 5 µg, gentamycin 10 µg, ofloxacin 5 µg, augmentin 30 µg, and pefloxacin 10 µg were used for the gram-negative bacteria while gentamycin 10 µg, ciprofloxacin 5 µg, ofloxacin 5 µg, cephalothin 30 µg, amoxicillin 25 µg, pefloxacin 10 µg, streptomycin 30 µg, chloramphenicol 30 µg, cotrimoxazole 25 µg, and erythromycin 5 µg were used for the gram-positive bacteria. The plates were incubated at 37 °C for 24 hours after the inhibitory activity of the extracts and the standards were recorded as visible zones in diameter in mm.

Antifungal activity study

Antifungal activity of *P. americana* extracts was evaluated using the mycelia inhibition method as reported by Lateef et al., (2016). This involves incorporation of 2 ml of 50 mg/ml of the crude extract into sterile Potato Dextrose Agar (PDA) plates, followed by inoculation of the plate with 6 mm agar plugs of fungi (*A. fumigatus, A. niger, A. flavus, F. poae, and F. solani)* at the center of the plate and incubated at 28 °C for 72 hrs. The control experiment consists of fungi plugs on PDA plates without *P. americana* extracts. Fungi growth was measured in diameters (mm) and percentage growth inhibitions of the extracts was calculated using the formula below:

$$\% \text{ Growth Inhibition} = \frac{Dcontrol - Dtest}{Dcontrol} \times 100$$

D: fungal growth on PDA plates (mm).

RESULTS AND DISCUSSIONS

Extraction yield

Solvent extraction is one of the important methods of separation of secondary metabolites from pulverized plant materials. The nature of the solvent employed in the extraction plays a significant role in the type, quantity, as well as pharmacological activities of the phytochemicals that will be extracted from plant materials (Nguyen et al., 2016). Temperature, duration of extraction, the polarity of solvent, and extraction method are other determinants of extraction yield.

Cold maceration method was used in the extraction of phytochemicals from *P. americana* stem bark and percentage yields were 0.93, and 1.37 % using ethyl acetate and ethanol respectively. The extraction yield increased with increasing polarity of the solvent. This observation is probably due to the increased dipole moment within the ethanol molecule compared to ethyl acetate which gives rise to increased polarity. A strong relationship has been found to exist between polarities of solvent and dielectric constants values of the solvents (ethyl acetate: 6.02, ethanol: 24.6) and their individual ability to break through the plant cell wall and hence extract phytochemicals (Moldoveanu and David, 2013). This result clearly showed that more of the phytochemicals were extracted with ethanol, mainly due to its higher polarity compared to ethyl acetate and its ability to break through the cell wall of *P. americana* stem bark.

Phytochemical composition of *P. americana*

Results of the phytochemical composition of *P. americana* stem bark crude extracts are shown in Table I. Data from the table revealed the presence of saponins, alkaloids, flavonoids, tannins, steroids, glycosides, carbohydrates, and phenols in both extracts, coumarin, and anthocyanins were however absent in the ethyl acetate extract while terpenoid was absent in the ethanolic extract. Similarly, results obtained from the quantitative screening of the extracts are presented in table 2, data from the table showed a clear trend of increase in the quantities of the phytochemicals (in the extracts) with increasing polarities of the solvent. The observed increase in quantity could be attributed to the principle of like dissolves like, i.e., more of the polar component of

Table I. Qualitative phytochemical screening of *P. americana*

	Parameters	Ethyl acetate	Ethanol
1	Saponin	+	+
2	Flavonoid	+	+
3	Alkaloid	+	+
4	Steriod	+	+
5	Coumarin	-	+
6	Tannin	+	+
7	Terpenoid	+	-
8	Anthocyanin	-	+
9	Glycosides	+	+
10	Carbohydrate	+	+
11	Phenol	+	+

Table II. Quantitative phytochemical composition of *P. americana* extracts

Parameters	Ethyl acetate	Ethanol
Saponin (%)	3.50±0.033	6.50±0.025
Alkaloid (%)	0.80±0.020	2.13±0.015
Flavonoid (mg/g)	62.22±0.005	480.01±0.071
Phenol (mg/g)	23.98±0.006	39.95±0.057
Tannin (mg/g)	16.05±0.002	32.24±0.001

the plant material were extracted in the more polar ethanol.

Tannins, flavonoids, and phenols content were exceptionally higher compared to other phytochemicals quantified in this study with flavonoids found to be preponderant in the ethanolic extract (480.01±0.071), significant positive correlation has been found to exist between phenolic contents of plant extracts and their ability to trap free radicals, chelate metal ions, quench singlet oxygen and act as antioxidants (Zain and Omar, 2018). Phenolic compounds are naturally occurring compounds characterized by one or more hydroxyl groups attached to the aromatic ring(s). Their antioxidant properties are largely due to the phenolic ring, hydroxyl groups, presence, and length of conjugation as well as the different types of substitution on the aromatic rings. They ranged from simple phenolics to the highly complex and polymerized polyphenols (Minatel et al., 2017). Flavonoids are polyphenolic, the basic skeleton consist of two benzene rings which are linked by a hetero-cyclic pyran ring (Kumar and Pandey, 2013). They have been reported to possess several health-promoting and disease preventing properties including antioxidant, anti-inflammatory, antiviral, anticancer, and antimicrobial properties (Karak, 2019). Alkaloids and saponins were also found in appreciable quantities in the ethanolic extract (2.13±0.015 and 6.50±0.025 respectively). Alkaloids have been reported to possess antimicrobial, analgesic, antitumor, antiviral, and anti-inflammatory (Arpita Roy, 2017), while saponins are important adjuvants that help in enhancing and improving the effectiveness of vaccines. They also play a vital role in maintaining the immune system (Guclu-Ustundag and Mazza, 2007).

Antimicrobial susceptibility of *P. americana* extracts

The result obtained for the inhibitory activity of *P. americana* extracts on the tested bacterial strains is presented in Table III. The table showed that the tested strains were susceptible to the extracts in a concentration-dependent manner, with moderate to high activities recorded. The highest inhibitory activity in this study was recorded against the growth of *K. oxytoca* at a concentration of 120 mg/ml (ethyl acetate extract). This study also found that *K. oxytoca, P. aeruginosa,* and *S. aureus* were most affected by ethyl acetate extract while *P. vulgaris, E. coli,* and *S. typhi* are more sensitive to ethanolic extract. Inhibitory activity of the extracts was comparable to those of the standard antibiotics used, which suggests that the extracts could be potent alternative with limited side effects compared to the standard drug. The results are in agreement with those obtained by (Osuntokun et al., 2017) where *P. americana* stem oil inhibited the growth

Table III. Antibacterial activity of *P. americana* extract

Organisms	Conc (mg/ml)	Ethyl acetate	Ethanol	DMSO	Standard
K. oxytoca	50	21.00± 0.76	15.50 ±0.86	-	TET:27.50
	70	22.00± 0.76	17.59±0.62	-	OFL:30.50
	100	25.75± 0.35	18.33±0.41	-	PFX:30.00
	120	27.50± 0.67	18.50±0.47	-	CPX:28.50
E. coli	50	16.50± 0.47	19.50± 0.80	-	TET:31.00
	70	17.50± 0.05	21.50± 0.72	-	CPX:28.00
	100	17.83± 0.10	25.00± 0.61	-	PFX:28.00
	120	22.83± 0.15	26.00± 0.21	-	
P. aeruginosa	50	12.33± 0.26	14.33± 0.72	-	CRO:25.50
	70	19.25± 0.15	16.17± 0.43	-	AUG:25.50
	100	23.50± 0.72	17.67± 0.57	-	GEN:22.50
	120	24.00± 0.22	18.00± 0.28	-	
P. vulgaris	50	11.00± 0.32	15.50± 0.19	-	TET: 26.50
	70	13.00± 0.13	19.00± 0.03	-	
	100	14.50± 0.52	19.83± 0.11	-	
	120	17.00± 0.26	21.33± 0.61	-	
S. aureus	50	14.00± 0.02	14.50± 0.74	-	CPX: 19.50
	70	17.50± 0.43	17.00± 0.09	-	OFL: 15.50
	100	19.00± 0.61	18.00± 0.55	-	
	120	21.50± 0.21	19.00± 0.62	-	
S. typhi	50	12.50± 0.47	15.50± 0.34	-	CRO:30.00
	70	13.50± 0.72	17.83± 0.33	-	
	100	14.50± 0.72	18.50± 0.27	-	
	120	17.50± 0.49	19.50± 0.44	-	

PEF= pefloxacin; GEN= gentamycin; TET= tetracycline; OFL= ofloxacin; AUG= augmentin,; CPX=ciprofloxacin; CRO= ceftriaxone; * The assay was done in triplicate

Table IV. Antifungal activity of *P. americana*

Organisms	Ethyl acetate		Ethanol		Control
	FG	GI	FG	GI	FG
A. niger	8.00	85.58	8.50	84.68	55.50
A. flavus	8.50	88.19	8.00	88.89	72.00
A. fumigatus	11.00	81.66	8.00	86.66	60.00
F. solani	10.00	80.58	9.50	81.55	51.50
F. poae	8.50	81.91	10.50	77.65	47.00

***FG = fungal growth, %GI = % growth inhibition**

of *E. coli, S. typhi*, *K. pneumonia,* and *S. aureus* in a concentration-dependent manner. The result of the antifungal activity study of the extracts is also presented in Table IV. The extracts had significant activity on all the tested fungi, with the growth of *A. flavus* mostly inhibited (88.89%), this observation showed that the extracts could be a useful alternative in the treatment of infections caused by the tested fungi strains. The observed antimicrobial activities of the extracts could be attributed to the presence of phytochemicals as shown in Tables I and II.

CONCLUSION

This study has been able to reveal the phyto-constituents of *P. americana* stem bark of which flavonoid was found to be abundant. Also the extracts displayed good antimicrobial activities which might be attributed to the phyto-constituents. It is also evident from the study that the plant could be a promising alternative in the treatment and management of disease and infections caused by the tested microbial strains and could be a potential nuclei in the drug discovery process.

ACKNOWLEDGMENT

Technical assistance rendered by Mr M.P. Obitokun and Mr S. B. Ogunsona is duly acknowledged

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