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ANTIBACTERIAL ACTIVITIES OF DRIED LEAF EXTRACTS OF *CARICA PAPAYA, PTEROCARPUS SOYAUXII*, AND *VERNONIA AMYGDALINA* ON CLINICAL ISOLATES OF *ESCHERICHIA COLI, KLEBSIELLA PNEUMONIAE, STAPHYLOCOCCUS AUREUS* AND *BACILLUS SUBTILIS*

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ABSTRACT

The antibacterial activities of cold and hot ethanol extracts of air-dried leaves of Carica papaya, Pterocarpus soyauxii, and Vernonia amygdalina on clinical isolates of Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus and Bacillus subtilis were investigated. The cup-plate agar method was used to determine bacterial susceptibility. All the plant extracts screened were potent on the entire clinical isolates tested. However, there was no significant difference in the inhibition zone diameters of the plant extracts screened (on all the test isolates). There was also no significant difference in the inhibition zone diameters between the cold and hot ethanol extracts of each plant. Phytochemical analysis revealed the presence of alkaloids, anthroquinone, flavonoids, saponins, steroids, tannins, terpenoids and glycosides in all leaf samples. The results obtained here reveal the antibacterial potentials of the leaf extracts of Carica papaya, Pterocarpus soyauxii, and Vernonia amygdalina, and suggests their possible exploitation for the development of novel herbal-based antimicrobials.

KEY WORDS: *Carica papaya, Pterocarpus soyauxii, Vernonia amygdalina, sensitivity, clinical isolates*

INTRODUCTION

The exploitation of plants for medicinal purposes is an age-long practice common to all societies. It is on this basis that researchers continue working on medicinal plants with a view to developing novel and improved therapeutic products (Usman & Osuji, 2007). Studies have it that plants play an indispensable role in the maintenance of healthy living (Edeoga & Eriata, 2001). Plants have been extensively applied for the treatment of ailments worldwide, and according to WHO, 80% of world population depend on plant based medicine for their healthcare (Osuagwu & Akomas, 2013; WHO, 2001).

The renewed interest in herbal medicine and the growing preference for herb-based medicine over synthetically produced medicine may not be unconnected with: high cost, adulteration, and increasing toxic side effects of synthetic drugs; multiple drug resistance due to indiscriminate use of commercial antimicrobial drugs; and the safety and cost-effectiveness of using plant based medicines (Shariff, 2001; Koche *et al,* 2001; Gupta *et al,* 2008). Apart from

the broad therapeutic potency of plant based medicine, antimicrobials from plants are not only highly effective in treating infectious diseases; they on the other hand mitigate many of undesirable side effects associated with synthesized antimicrobials (Werner *et al,* 1999; Perulmalsamy & Ignacimuthus, 2000).

According to researchers, the medicinal property of plants is due to the presence of secondary metabolites, such as: alkaloids, tannins, flavonoids, phenolics, steroids, resins, and others (Lohiya *et al,* 2008; Ianovici *et al.,* 2010). Antimicrobial activities are either due to single or combined effects of these bioactive substances (Frel *et al,* 1998).

The antimicrobial potentials of a wide array of plants have been investigated by numerous workers. *Carica papaya*, *Pterocarpus soyauxii*, and *Vernonia amygdalina* are common plants of the tropics. While the leaves of *P. soyauxii* and *V. amygdalina* are commonly used as vegetables in food preparation, the fruit of *C. papaya* is a soft fruit commonly used as a snack or desert (Osuagwu, 2008). For therapeutic purposes: *C. papaya* leaves are made into tea for the treatment of malaria, indigestion, obesity, and high blood pressure, while the fresh leaf poultice is used to treat sores (Verpoorte *et al,* 2002; Alabi *et al,* 2012). The leaves of *V. amygdalina* are employed in the treatment of malaria, hiccups, stomach discomforts, and kidney diseases (Hamowia & Saffaf, 1994). This study explored the phytochemistry of the crude ethanol leaf extracts of *C. papaya, P. soyauxii* and *V. amygdalina* and measured the *in vitro* effects of these crude extracts on bacteria of medical importance, namely: *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus*, and *Bacillus subtilis*.

MATERIALS AND METHODS

Collection and authentification of plant samples. Leaves of *Carica papaya*, *Pterocarpus soyauxii*, and *Vernonia amygdalina* were collected from a household backyard garden in Mbaise, (Imo State, Nigeria) at midday. The leaves were authenticated and labeled by the Forestry Department, Ministry of Agriculture and Natural Resources, Owerri (Imo State, Nigeria).

Preparation of plant extracts. The leaves were thoroughly rinsed in running tap water and then in sterile distilled water. After which they were air-dried at room temperature (29 ± 2^0C) to constant weight and then pulverized into fine powder with an electric mill. The pulverized leaves were labeled and stored in clean sealed containers at 4^0C till required. 100g of each powdered plant sample was soaked and thoroughly mixed in 500mL of 70% ethanol and left to stand for 48 hours for the cold extraction, while for the hot extraction a similar set-up was maintained at 60^0C in a water-bath for 3 hours. The slurries were then aseptically filtered using folded white cotton handkerchiefs. The filtrates were evaporated to dryness using a rotary evaporator at 40^0C. The dried residues were dissolved in sterile distilled water using two-fold serial dilution method to obtain 100mg/mL, 50mg/mL, 25mg/mL, and 12.5mg/mL concentrations.

Collection and preparation of test organisms. Stock cultures of clinical isolates of *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus*, and *Bacillus subtilis* were obtained from the Microbiology Diagnostic Laboratory of Imo State University Teaching Hospital (IMSUTH) Orlu (Imo State, Nigeria). Viability test was carried out for each isolate by resuscitating the organism in buffered sterile peptone broth and then sub-cultured into soybean casein digest broth and incubated at 37^0C for 24 hours.

Antimicrobial activity test. Plant extracts were screened for antimicrobial activity on the test isolates using a modified method of the cup-plate agar diffusion method (Ebi & Ofoefule, 1997). 0.2mL of 24 hour broth culture of the test organism was used to aseptically seed 20mL of sterile molten Mueller Hinton agar in a sterile Petri dish. The contents were thoroughly mixed by rotating the Petri dish slowly, to ensure even distribution of the test organism, and then left to solidify. Cups of 4mm diameter were made in the solidified agar using a sterile cork borer. 0.04mL of each concentration of each plant extract screened was aseptically introduced into the cup and labeled accordingly. The dishes were left to stand for one hour at room temperature for proper diffusion of the extracts before incubating at 37^0C for 24 hours. Control experiments were also set up using sterile soybean casein digest broth. After incubation the inhibition zone diameters (IZD) were measured using a micrometer caliper. All tests were done in triplicate and the results presented as mean±SEM.

Minimum inhibitory concentration (MIC) test. The MIC of each extract was computed by plotting the logarithm of concentration ($\log_{Conc}$) against the square of the inhibition zone diameters (IZD^2). The antilogarithm of the intercept on the $\log_{Conc}$ axis gave the MIC value (Osadebe & Ukwue, 2004).

Phytochemical analysis. The powdered plant samples were used to carry out phytochemical tests using standard methods (Agomuo, 2002).

Statistical analysis. All statistical analyses were determined using Microsoft® Excel 2003.

RESULTS AND DISCUSSIONS

The sensitivities of test organisms to different plant extracts at various concentrations are displayed in Table 1. Inhibition zone diameters were directly proportional to the concentration of plant extract tested, however ANOVA revealed that among all the plants screened there was no significant difference in the IZD of the plant extracts per concentration per test isolate; and also no significant difference between the cold and hot ethanol extracts per concentration per test isolate. The IZD values obtained here for air-dried leaf samples appear to be lower than those obtained for fresh green leaves, but higher than those obtained for dried leaves with cold aqueous extraction as reported by Khan *et al.* (2012). This variation may be due to differences in the concentrations of bioactive substances in fresh and dried leaves, and the fact that the bioactive components may be more soluble in ethanol than in cold water (Doughari & Sunday, 2008). On the other hand values obtained here for *P. soyauxii* were higher than those obtained by Osuagwu and Akomas for ethanol extracts of dried leaves (Osuagwu & Akomas, 2013). This variation may be accounted for by the ages of the leaves tested. Younger leaves contain a relatively higher concentration of secondary metabolites than older leaves (Osuagwu *et al,* 2007).

The closeness in the values of the MIC in Table 2 corroborates the no significant difference in the IZD of the plant extracts on the test organisms as earlier pointed out by ANOVA. The relatively high MIC for cold ethanol extracts of *Carica papaya* and *Pterocarpus soyauxii* on *Klebsiella pneumoniae* and *Escherichia coli* respectively attests to the claim that gram negative bacteria have higher resistance to plant extracts, which is said to be related to the thick murein layer in their outer membrane which prevents the entry of inhibitor substances

(Suresh *et al,* 2008). On the other hand disparity in MIC may also be due to variable sensitivity to chemical substances related to different resistance levels among strains (Cetin & Gurler, 1989).

TABLE 1. Antibacterial Properties of Dried Leaves Extracts

Plant extract	**Concentration (mg/mL)**	**Inhibition zone diameter (mm±SEM)**			
		E.coli	*K.pneumoniae*	*S. aureus*	*B. subtilis*
Cold extract					
C. papaya	100	11.2±0.29	14.4±0.18	11.21±0.05	11.34±0.75
	50	9.89±0.29	12.2±0.16	10.14±0.51	9.69±0.35
	25	8.68±0.50	10.1±0.24	8.0±0.48	6.71±0.41
	12.5	6.20±0.58	7.89±0.14	6.56±0.31	4.61±0.29
P. soyauxii	100	9.0±0.31	9.2±0.42	10.50±0.15	12.45±0.31
	50	7.2±0.12	7.1±0.15	8.6±0.15	9.43±0.22
	25	5.1±0.05	5.0±0.33	5.41±0.04	7.12±0.46
	12.5	4.2±0.44	4.12±0.21	4.69±0.35	4.72±0.05
V. amygdalina	100	7.51±0.22	12.24±0.12	11.12±0.12	12.21±0.03
	50	4.82±0.42	9.76±0.18	7.23±0.03	10.13±0.08
	25	-	6.12±0.21	4.12±0.05	8.81±0.12
	12.5	-	4.59±0.32	-	4.42±0.11
Hot extract					
C. papaya	100	16.12±0.01	15.12±0.36	12.91±0.55	16.65±0.49
	50	14.77±0.17	13.39±0.19	10.81±0.68	14.81±0.38
	25	12.29±0.25	11.55±0.41	8.51±0.20	12.80±0.04
	12.5	9.19±0.23	8.35±0.32	7.12±0.49	9.55±0.62
P. soyauxii	100	11.26±0.84	11.52±0.14	11.21±0.75	10.10±0.03
	50	9.16±0.59	8.89±0.1	9.12±0.50	8.12±0.46
	25	7.23±0.30	6.51±0.07	7.51±0.05	6.52±0.71
	12.5	4.55±0.19	4.45±0.12	5.55±0.25	4.89±0.67
V. amygdalina	100	9.11±0.34	12.46±0.03	9.42±0.86	10.42±0.23
	50	7.12±0.54	10.12±0.33	6.21±0.32	8.34±0.46
	25	5.44±0.34	7.42±0.49	5.89±0.05	5.59±0.52
	12.5	4.29±0.18	5.12±0.38	4.22±0.55	-

TABLE 2. Minimum Inhibitory Concentration of Dried Leaves Extracts

Plant extract	**Minimum inhibitory concentration (MIC) in mg/mL**			
	E.coli	*K. pneumoniae*	*S. aureus*	*B. subtilis*
cold				
C. papaya	0.65	0.92	0.61	0.62
P. soyauxii	0.80	0.61	0.65	0.65
V. amygdalina	0.64	0.63	0.62	0.64
hot				
C. papaya	0.67	0.63	0.66	0.55
P. soyauxii	0.65	0.64	0.63	0.62
V. amygdalina	0.63	0.62	0.67	0.63

From Table 3, all leaf samples analyzed appear to have similar phytochemical compositions. This may account for their analogous antimicrobial potencies as revealed in

Tables 1 and 2. The presence of bioactive substances has been reported by different authors to confer antimicrobial activity to plant extracts. Draughon have specifically attributed antibacterial effects of plant extracts on the presence alkaloids, tannins and flavonoids.[24] Despite the fact that the mechanism of action of these bioactive agents are not thoroughly understood, Hassan *et al*. (2007) suggested that the antimicrobial activity of these secondary metabolites may be due to the disruption of cell wall formation which consequently causes the leakage of cytoplasmic constituents.Tannins coagulate wall proteins; flavonoids inhibit enzyme activity, thus disrupting cell wall or cell membrane integrity at very low concentrations; while saponins disrupts cell wall porosity hence facilitating the entry of toxic components or the leakage of vital constituents (Dathak & Iwu, 1991; Kurta *et al,* 1994; Tsuchiya *et al,* 1996; Onwuliri & Wonang, 2005).

TABLE 3. Phytochemical Analysis of Leaf Samples

Phytochemicals	Carica papaya	Pterocarpus soyauxii	Vernonia amygdalina
Alkaloids	+	+	+
Anthroquinone	+	+	+
Cathecols	-	-	-
Flavonoids	+	+	+
Phenols	-	-	-
Saponins	+	+	+
Steroids	+	+	+
Tannins	+	+	+
Terpenoids	+	+	+
Glycosides	+	+	+

CONCLUSIONS

Each plant extract screened recorded antibacterial potencies on the entire test organisms surveyed in this work. Data obtained here also showed that the hot ethanol extracts showed more predictable and stable results than the cold extracts, thus suggesting hot ethanol extraction as a more efficient process especially when considered alongside the time saved in carrying out hot ethanol extraction. The results obtained here reveal the antibacterial potencies of these plants commonly found in the tropics. Hence, suggesting possible exploitation of these plants for their antimicrobial active principles for the development of novel herbal-based antimicrobials.

REFERENCES

- Agomuo EN, Amadi BA, Chikezie PC, Ibegbulem CO. 2002. Basic Analytical and Research Methods in Biochemistry. 1st edn. Owerri (Nigeria): Supreme Publishers.
- Alabi OA, Haruna MT, Anokwuru CP, et al. 2012. Comparative studies on antimicrobial properties of extracts of fresh and dried leaves of *Carica papaya* (L) on clinical bacterial and fungal isolates. *Adv Appl Sci Res*, 3(5): 3107-3114.
- Cetin TE, Gurler N. 1989. Bacterilerin antibiyotiklere duyaarlilik deneyinin yapilmasi. *Kukem Dergisi*, 12: 2 – 5.
- Dathak P., Iwu S. 1991. Inhibition of xanthine oxidase activity by some flavonoids. *Filoterapia*, 63: 385.
- Doughari JH, Sunday D. 2008. Antibacterial activity of *Phyllanthus muellerianus*. *Pharm Biol,* 46(6): 400 – 405.
- Draughon FA. 2004. Use of botanicals as bio-preservatives in foods. *Food Tech*, 58(2): 20 -28.
- Ebi GC, Ofoefule SI. 1997. Investigation into the Folkloric antimicrobial activities of *Landolphia owerriance. Phytotherap Res*, 11: 147 – 151.

- Edeoga HO, Eriata DO. 2001. Alkaloids, tannins, and saponins contents of some Nigerian medicinal plants. *J Med and Arom Plant Sci*, 23: 244 – 249.
- Frel B, Bork P, Heinrich M, et al. 1998. Multiple screening of medicinal plants from Mexico – ethnobotany and bioassay as basis for phytochemical investigation. *Phytomed.* 5(3): 177 – 186.
- Gupta C, Amar P, Ramesh G, Uriya C, Kumari A. 2008. Antimicrobial activity of some herbal oils against common food-borne pathogens. *Afr J Microb Res,* 2: 258 – 261.
- Hamowia AM, Saffaf AM. 1994. Pharmacological studies on *Vernonia amygdalina* (Del) and *Tithonia diversifola* (Gray). *Vet Med Giza,* 2: 91 -97.
- Hassan SW, Umar RA, Ladan MJ, et al. 2007. Nutritive value, phytochemical and antifungal properties of *Pergularia tomentosa* L. (*Asclepiadaceae*). *Int J Pharmacol*, 3(4): 334 – 340.
- Ianovici N., Ţărău G., Todosi A. L., Iriza E., Danciu A., Ţolea L., Tudosie D., Munteanu F., Bogdan D., Ciobănică V.. 2010. Contributions to the characterization of *Plantago* species from Romania. Review, *Annals of West University of Timişoara, ser. Biology,* 13: 37-76
- Khan JA, Yadav J, Srivastava Y, Pal PK. 2012. *In vitro* evaluation of antimicrobial properties *Carica papaya*. *Int J Biol Pharm Allied Sci (IJBPAS),* 1(7): 933 – 945.
- Koche DK, Bhadange DG, Kamble, KD. 2001. Antimicrobial activity of three medicinal plants. *Bios Disc,* 2(1): 69 -71.
- Kurta MB, Heath IB, Marrinam J., Dreikorn S, Onishi J, Douglas C. 1994. How Morphological effects of lipopeptides against *Aspergillus fumigatus* correlates with activities against (1, 3) – Beta D-glucan synthase. *Antimicrob Agents Chem*, 38(7): 1480 – 1489.
- Lohiya NK, Manivannan B, Goyal S, Ansari, AS. 2008. Reversible contraception with chloroform extract of *Carica papaya* Linn. seeds in male rabbits. *Asian J Androl,* 10(2): 298 – 306.
- Onwuliri FC,Wonang DL. 2005. Studies on the combined antibacterial action of ginger (*Zinziber officinale* L.) and garlic (*Allium sativum*) on some bacteria. *Nigerian J of Bot,* 18: 224 – 228.
- Osadebe PO, Ukwue SE. 2004. A comparative study of the phytochemical and antimicrobial properties of the Eastern Nigerian specie of African mistletoe (*Loranthus micranthus*) from different host trees. *J Biol Res Biotech*, 2(1): 18 – 23.
- Osuagwu GC, Akomas CB. 2013. Antimicrobial activity of the leaves of three species of Nigerian *Pterocarpus* (Jacq.). *Int J Med and Arom Plants*, 3(2): 178 -183.
- Osuagwu GG, Okwulehie IC, Emenike JO. 2007. Phytochemical and mineral content of the leaves of four Nigerian *Pterocarpus* species. *Int J Mol Med Adv Sci*. 3(1): 6 -11.
- Osuagwu GG. 2008. Proximate and vitamin contents of four Nigerian *Pterocarpus* species. *Nigerian Food J,* 26(1): 21 -26.
- Perulmalsamy R., Ignacimuthus S. 2000. Antibacterial Activity of some folklore medicinal plants used by tribals in Western India. *J Ethnopharm*, 69: 68 – 71.
- Shariff ZU. 2001. Modern Herbal Therapy for Common Ailments. Nature Pharmacy Series (vol.1). Ibadan (Nigerian): Spectrum Books Limited. 9 – 84.
- Suresh K, Deepa P, Harisaranraj R, Vaira A. 2008. Antimicrobial and phytochemical investigation of the leaves of *Carica papaya* L., *Cynodon dactylon* (L.) Pers., *Euphorbia hirta* L., *Melia azedarach* L., and *Psidium guajava* L. *Ethnobot Leaflets*, 12: 1184 – 91.
- Tsuchiya H, Sato M, Miyazaki T. 1996. Comparative study of the antibacterial activity of phytochemical flavones against methicillin-resistant *Staphylococcus aureus*. *J Ethnopharmacol*,50(1): 27 -34.
- Usman H., Osuji JC. 2007. Phytochemical and *in vitro* antimicrobial assay of the leaf extract of *Newbouldia laevis. Afr J Trad* , 4(4): 476 – 480.
- Verpoorte R, Contin A, Memelink J. 2002. Biotechnology for the production of plant secondary metabolites. *Phytochem Rev*. 1(1): 13 – 35.
- Werner F, Okemo P, Ansorg R. 1999. Antimicrobial activity of East African medicinal plants. *J Ethnopharm,* 60: 79 – 84.
- World Health Organization (WHO). 2001. General guidelines for methodologies on research and evaluation of tradition medicine. World Health Organization, Geneva. 9(8): 1378 – 1382.