OPEN ACCESS

Original Article

Applied Life Sci.

Invitro Antimicrobial Activities of Extracts of *Magnifera indica, Carica papaya and Psidium guajava* Leaves on *Salmonella typhi* Isolates

Moses ALO [1*], Ukpai Agwu EZE [1], Chukwudi ANYIM [2]

ABSTRACT [ENGLISH/ANGLAIS]

The emergence of resistant strains of pathogenic microorganisms has continued to pose a major health concern about the efficacy of several antibiotics in current use. The search for never, more effective, affordable, and easily available drugs from sources such as medicinal plants has become a necessary approach towards overcoming this medical challenge. In this study, the in vitro antimicrobial activities of cold water and ethanolic extracts of *Magnifera indica*, *Carica papaya* and *Psidium guajava* leaves were investigated against clinical isolates of *Salmonella typhi* using the agar-well diffusion method. Both extracts showed antimicrobial activities against the isolates, which were dose dependent. The ethanolic extract of *Magnifera indica* leaves at a concentration of 0.8 g/dl had the highest effect on the test organism with 19 mm diameter of zone of inhibition while at 0.2 g/ml of the cold water extracts of *Carica papaya* had a higher antimicrobial activity compared to 0.2 g/ml concentration of the ethanol extracts. *Psidium guajava* showed antimicrobial activities in all the concentrations of cold water and ethanolic extracts of the leaves, and compared favourably with chloramphenicol positive control. There was decrease in antimicrobial activity when the extracts of the three leaves were combined. This study concludes that the plant extracts have extended activity against *S. typhi* and may be explored in the management of infections caused by the bacteria.

Keywords: Medicinal plants, pathogenic microorganisms, extended activity, *Salmonella typhi, management of infections*

RÉSUMÉ [FRANÇAIS/FRENCH]

L'émergence de souches résistantes de micro-organismes pathogènes a continué àposer un problème majeur de santé quant à l'efficacité de plusieurs antibiotiquesd'usage courant. La recherche de médicaments jamais, plus efficaces, abordables etfacilement disponibles provenant de sources telles que les plantes médicinales est devenue une démarche nécessaire en vue de surmonter ce défi médical. Dans cette étude, l'activité in vitro anti-microbiens de l'eau froide et extraits éthanoliques de Magnifera indica, Carica papaya et Psidium guajava feuilles ont été étudiés sur des isolats cliniques de Salmonella typhi en utilisant la méthode de diffusion agar-même.Les deux extraits ont montré une activité antimicrobienne contre les isolats, qui étaientdose-dépendants. L'extrait éthanolique de Magnifera indica laisse à une concentration de 0,8 g / dl avait la plus effet sur l'organisme d'essai avec 19 mm de diamètre de zone d'inhibition tandis moins 0,2 g / ml des extraits d'eau froide de Carica papaya une plus forte activité antimicrobienne par rapport à 0,2 g / ml de concentration des extraits d'éthanol. Psidium guajava ont montré une activité antimicrobienne dans toutes lesconcentrations d'eau froide et d'extraits éthanoliques des feuilles, et se compare favorablement avec contrôle positif au chloramphénicol. Il y avait diminution de l'activitéantimicrobienne lorsque les extraits des trois feuilles ont été combinées. Cette étude conclut que les extraits de plantes ont étendu l'activité contre S. typhi et peut êtreexploré dans la gestion des infections causées par les bactéries

Mots-clés: Les plantes médicinales, des micro-organismes pathogènes, l'activité élargie,Salmonella typhi, la gestion des infections

Affiliations:

[1]Department of Medical Laboratory Science, Ebonyi State University, P.M.B. 053, Abakaliki, NIGERIA.

[2]Department of Applied Microbiology, Ebonyi State University, P.M.B. 053, Abakaliki, NIGERIA

Email Address for Correspondence/ Adresse de courriel pour la correspondance: mosesalo@yahoo.co.uk

Accepted/Accepté: March, 2012

Full Citation: Alo M, Ukpai Agwu Eze U.A, Anyim C. Invitro Antimicrobial Activities of Extracts of Magnifera indica, Carica papaya and Psidium guajava Leaves on Salmonella typhi Isolates. World Journal of Public Health Sciences 2012;1:1-6.

INTRODUCTION

Plants are the basis for the development of modern drugs and reports shows that medicinal plants have been used many years in the treatment of diseases worldwide [1,2].These medicinal plants have immensely contributed to the development of human health and welfare. Concomitantly, there is an increase in data and huge patronage to herbal products round the world [4]. Medical uses of plants range from the administration of the roots, barks, stems, leaves and seeds to the use of extracts and decoction from the plants [5]. Traditional medicine is still the predominant means of healthcare in developing countries where about 80% of their total population depends on it, for their wellbeing [6]. Traditional medicinal plants are a therapeutic resource used by the population of the African continent, specifically for healthcare, which may also serve as starting materials for drugs [7]. In Nigeria, herbs are distributed in many herbal clinics and most of which are taken in the form of concoctions in the treatment of commonly occurring infectious diseases, including sexually transmitted diseases, malaria, dysentery, diarrhea, pyogenic infections, dental caries, and many other diseases.

World Health Organization (WHO) defines medicinal plants as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration, or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products [8]. Medicinal plants are of great importance to the health of individuals and communities [9]. A medicinal plant is one whose one or more of its organs contain substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs [10, 2]. Medicinal plants are reservours of various metabolites and provide unlimited source of important chemicals that have diverse biological properties and represents a rich source from which antimicrobial agents can be obtained [11].

Emergence of resistant strains of pathogenic microorganism has continued to pose a major health concern about the efficacy of several antibiotics in current use [11]. This increasing rate of development of resistance to commonly used antibiotics has led to the search for newer, more effective, affordable and easily available drugs in particular from medicinal plants [12]. This study was designed to determine the antimicrobial activities of cold water and ethanol extracts of the leaves of *Magnifera indica*, *Carica papaya* and *Psidium guajava* against clinical isolates of *Salmonella typhi*.

MATERIALS AND METHODS

Collection of Plant Materials

The leaves of *Magnifera indica*, *Carica papaya* and *Psidium guajava* were collected from Aguogboriga Estate, Abakaliki in Ebonyi State, Nigeria and proper identification was done by a botanist in the department of Applied Biology, Faculty of Biological Sciences, Ebonyi State University, Abakaliki, Nigeria.

Preparation and Extraction of Plant Materials

The leaves of *Magnifera indica*, *Carica papaya* and *Psidium guajava* were washed with clean water and air-dried for 5 days. The dried leaves were stored in sealed and labeled containers for use. The bioactive compounds were extracted using the methods of Akerele et al. [13] with slight modification. 20 g of the ground leaves of *Magnifera indica*, *Carica papaya* and *Psidium guajava*, were suspended in 120 ml of 98% ethanol, and distilled cold water respectively and left for 24 hours. Thereafter, the suspensions were filtered into sterile universal containers separately using Whatman no.1 filter paper. The extracts were allowed to dry at a temperature of 40 ^{0}C into powder. The powder of the extracts obtained were stored in sealed bottles and kept in a refrigerator at -4 ^{0}C until further use.

Collection and Preparation of Inocula

The pure cultures of 5 clinical isolates of *Salmonella typhi* were collected from the Medical Laboratory of Ebonyi State University Teaching Hospital, Abakaliki, Nigeria. Three to five pure colonies of the *Salmonella typhi* isolates on nutrient agar were collected with a sterile wire loop and emulsified in 3-4 ml of sterile physiological saline. The dilution with the sterile physiological saline continued until the turbidity of the suspension matched with that of the McFarland's standard [14].

Determination of the Antimicrobial Activity

Different grams (0.2 g, 0.4 g, 0.6 g, and 0.8 g) of the powder of the leaf extracts were weighed separately and dissolved in 1ml of distilled water each, to give concentrations of 0.2 g/ml, 0.4 g/ml, 0.6 g/ml and 0.8 g/ml respectively. A sterile swab was dipped into the suspensions of the *Salmonella typhi* isolates, and excess fluid removed by pressing and rotating the swab against the side of the tube above the level of the suspension, one for each isolate. The swab was streaked evenly over a nutrient agar plates and allowed for 3-5 minutes so that the surfaces of the agar will dry, with their lids in place. Sterile cork borer was used to punch wells (4 mm in diameter) on the cultured plates. Each well and plate were appropriately labeled with the concentration of the extracts. With sterile Pasteur pipettes, 20 µl of each concentration of plant extracts were introduced into independent wells while 2.5 µg disc of chloramphenicol was placed on the plates to serve as positive control, and distilled water and ethanol without extracts were also introduced into independent wells to serve as a negative control. The plates were incubated at 37 ^{0}C for 24 hours. The active extracts showed zones of inhibition diameter, which were measured by using a meter rule to measure 2 points across the zone and the average diameter recorded. Three replicates were performed for each test.

Furthermore, 0.2 g/ml concentrations of cold water extracts of *Magnifera indica, Carica papaya,* and *Psidium guajava* leaves were pooled together to test the effect of the combined extracts. The same was applied to the 0.4 g/ml, 0.6 g/ml and 0.8 g/ml concentrations of the cold water extracts of the leaves, as well as for the ethanol extracts of the leaves.

RESULTS

Different concentrations of the ethanolic and cold water extracts of the leaves of *Magnifera indica, Carica papaya,* and *Psidium guajava* showed antimicrobial activities against the isolates of *Salmonella typhi.*

The 0.8 g/ml concentration of both the cold water and ethanol extracts of *Magnifera indica* had the highest zones of inhibition compared to the other different concentrations. Also, 0.2 g/ml of the ethanol extracts of *Magnifera indica* showed antimicrobial activity on the isolates tested while there was no antimicrobial activity of the same concentration of cold water extract (Table 1).

In table 2, all the concentrations of the cold water extracts of *Carica papaya* showed higher antimicrobial activity against the isolates while all the concentration of the ethanol extracts showed reduced or no antimicrobial activity against the isolate. The zones of inhibition of cold water extracts and ethanol extracts were statistically significant (P<0.05).

Both the cold water and ethanol extracts of *Psidium guajava* showed antimicrobial activity against the isolates except in the third isolate in which 0.2g/ml concentration of cold water extract did not show any inhibition (table 3). The combination of the different concentrations of ethanol and cold water extracts of *Magnifera indica, Carica papaya and Psidium guajava* showed reduced antimicrobial activity compared to the effects of each single plant extracts (Table 4).

Table 1: This table shows inhibition zones of different concentrations of cold water and ethanol extracts of *Magnifera indica* on *Salmonella typhi* isolates

Isolates	Extracts concentrations (g/ml) and diameter of zone of inhibition (mm)							
	Cold water extracts				Ethanol extracts			
	0.2	0.4	0.6	0.2	0.8	0.2	0.4	0.6
1	0.00	0.00	12.00	0.00	16.00	11.00	14.00	17.00
2	0.00	7.00	15.00	0.00	18.00	12.00	14.00	17.00
3	0.00	0.00	7.00	0.00	11.00	5.00	7.00	10.00
4	0.00	5.00	7.00	0.00	10.00	5.00	9.00	13.00
5	0.00	0.00	10.00	0.00	15.00	6.00	9.00	12.00

Table 2: This table shows inhibition zones of different concentrations of cold water and ethanol extracts of *Carica papaya* on *Salmonella typhi* isolates

Isolates	Extracts concentrations (g/ml) and diameter of zone of inhibition (mm)							
	Cold water extracts				Ethanol extracts			
	0.2	0.4	0.6	0.2	0.8	0.2	0.4	0.6
1	5.00	8.00	10.00	16.00	0.00	0.00	0.00	10.00
2	7.00	12.00	15.00	17.00	0.00	0.00	5.00	11.00
3	5.00	7.00	10.00	16.00	0.00	0.00	0.00	0.00
4	5.00	9.00	11.00	15.00	0.00	0.00	0.00	10.00
5	9.00	12.00	15.00	17.00	0.00	0.00	9.00	12.00

DISCUSSION

The increased frequency of resistance to commonly used antibiotics led to the search for newer, cheap, and easily affordable drugs in the management of infectious diseases. Although orthodox drugs are popular, however, herbal medicine continued to be practiced due to richness of certain plants in varieties of secondary metabolites such as alkaloids, flavonoids, tannins, and terpenoids which have been reported to have antibacterial activities [12, 15, 16]. The phytochemical analysis of aqueous extract of *Magnifera indica* has been reported to contain tannins, phlebotanins, cardiac glycosides, saponins and polyphenols [17, 18, 19]. Doughari et al. [20] reported that aqueous extracts of *C. papaya* has alkaloids, tannins, phenols, saponins and glycosides.

Table 3: This table shows inhibition zones of different concentrations of cold water and ethanol extracts of *Psidium guajava* on *Salmonella typhi* isolates

Isolates	Extracts concentrations (g/ml) and diameter of zone of inhibition (mm)							
	Cold water extracts				Ethanol extracts			
	0.2	0.4	0.6	0.2	0.8	0.2	0.4	0.6
1	5.00	8.00	10.00	12.00	6.00	8.00	13.00	16.00
2	5.00	9.00	15.00	18.00	7.00	10.00	13.00	15.00
3	0.00	7.00	11.00	14.00	5.00	9.00	12.00	15.00
4	7.00	11.00	13.00	16.00	5.00	7.00	10.00	12.00
5	7.00	9.00	15.00	18.00	7.00	10.00	15.00	17.00

Table 4: This table shows antimicrobial activities of the combination of the different concentrations of the leaf extracts on *Salmonella typhi* isolates

Isolates	Extracts concentrations (g/ml) and diameter of zone of inhibition (mm)							
	Cold water extracts				Ethanol extracts			
	0.2	0.4	0.6	0.2	0.8	0.2	0.4	0.6
1	0.00	4.00	7.00	10.00	0.00	4.00	7.00	12.00
2	0.00	5.00	9.00	15.00	0.00	5.00	8.00	12.00
3	0.00	4.00	7.00	11.00	0.00	0.00	0.00	7.00
4	0.00	6.00	10.00	12.00	0.00	0.00	0.00	9.00
5	5.00	7.00	11.00	14.00	0.00	4.00	6.00	13.00

Among the extracts of *Magnifera indica* tested, ethanol extracts of the leaves showed a higher activity on *Salmonella typhi* isolates, while the cold water extracts of *Carica papaya* leaves showed higher antibacterial activity to *Salmonella typhi* isolates compared to ethanol extracts. This agrees with the work done by Doughari and Monzara [19] and Doughari et al. [20] who reported that the ethanolic extracts of *Magnifera indica* and *Carica papaya* leaves had more activity than its cold water extracts. The relative high proportions of alkaloids, tannins and saponins in ethanol extracts may be responsible for this higher activity of the ethanol extracts [21].The activity of cold water and ethanolic extracts of *Psidium guajava* were proportional. Reports showed that *Psidium guajava* contains alkaloids, phenols and tannins and this might be the reason for its antimicrobial activity in both ethanol and water extracts [22]. The variation of antibacterial activities of the different extracts can be rationalized in terms of the polarity of the solvents used, polarity of the compounds being extracted from each solvent and, in addition to their extrinsic bioactivity and by their ability to dissolve or diffuse in the media used in the assay [23].

Furthermore, it is seen that the diameter of the zones of inhibition of the leaf extracts increases as their concentrations increases, with exception of some concentrations of *Magnifera indica* and *Carica papaya* leaf extracts which inhibited no zones of inhibition at all. For instance, the 0.2 g/ml concentration of cold water extracts of *M. indica* showed no zones of inhibition on the 5 clinical isolates of the test organism, likewise the 0.2 g/ml concentration of the ethanolic extract of *Carica papaya*. This may be due to the presence of the bioactive ingredients of the extracts in the particular concentration is in small quantity.

There was decrease in the diameter of zones of inhibition when the leaf extracts from the three plants were combined according to cold water and ethanolic extracts respectively. This could be as a result of dilution effects of the extracts or inhibition action of some bioactive ingredients by other ingredients, that is, antagonistic effects of some of the active ingredients. Demonstration of antibacterial activity against the test isolates is an indication that there is possibility of sourcing alternative antibiotic substances in these plants for the development of newer antibacterial agents.

CONCLUSION

This study concludes that *M. indica, C. papaya* and *P. guajava* leaves have extended antibacterial activity against *Salmonella typhi*. These plant leaves provides opportunity for the production of new synthetic drug for the treatment of *Salmonella typhi* infection. The result of this study tends to corroborate the local use of these plants for the treatment of typhoid fever. The pharmaceutical companies should research more on these plants as a basis for use in developing antimicrobial and other agents. Furthermore, toxicological studies can also be carried out to determine the reliance of these herbs without any side effects

REFERENCES

[1] Ates DA, Erdogrul OT. Antimicrobial activities of various medicinal and commercial plant extracts. Turkish Journal of Biology 2003;27:157-62.

[2] Ogieodia EA, Oluowo EF. Assessment of some therapeutic plants of the Abbi people in Ndokwa West L.G.A. of Delta State, Nigeria. Ethnobotanical Leaflets 2009;13:989-1002.

[3] Okoli RI, Aigbe O, Ohaju-Obodo JO, Mensah JK. Medicinal plants used for managing some common ailments among Esan people of Edo State, Nigeria. Pakistan Journal of Nutrition 2007;6(5):490-6

[4] Omoseyindemi BX.Plants as natural medicine. Paper presented at the Annual Conference of Botanical Society of Nigeria (BOSON) University of Lagos 2003.

[5] Ogbulie JN, Ogueke CC, Nwanebu FC. Antimicrobial properties of Uvarichamae, Congrenemalatifolium, Garcina kola, Vernoniaamygdalina and Aframomum melegueta. African Journal of Biotechnology 2007;6(13):1549-53.

[6] WHO. The promotion and development of traditional medicines. Technical report Series, Geneva 1978; 622

[7] Sofowora A. Medicinal plants and traditional medicines in Africa. 2nd edition. Spectrum Books, Ibadan, Nigeria. 1993. p.289.

[8] WHO. Legal status of traditional medicine and complementary/Alternative medicine: A worldwide Review. WHO Publishing, Geneva. 2001.

[9] Edeoga HO, Okwu DE, Mbachie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 4(7):685-6.

[10] Junaid SA, Olabode AO, Okworiu AE. The antimicrobial properties of Ocimum gratissimum extracts on bacterial gastrointestinal isolates. African journal of Biotechnology 2006;5(22):2315-7

[11] Timothy O, Idu M. Preliminary phytochemistry and in vitro antimicrobial properties of aqueous and methanol extracts of Icacinatrichantha Olive leaf. International Journal of Medicinal and Aromatic Plants. 2011;1(3):184-8

[12] Adenkunle AS, Adekunle OC.Preliminary assessment of antimicrobial properties of aqueous extract of plants against infectious diseases. Research Article Biology and Medicinal 2009; 1(3):20-4.

[13] Akerele JO, Obasuji O, Ebomoyi MI, Oboh IE. Uwumarongie seeds of Garcinia kola heckle (Guttiferae). African Journal of Biotechnology 2008; 7(2):162-72.

[14] Cheebrough M. Antimicrobial susceptibility testing in vitro. In: Medical laboratory practice in tropical countries, part 2. Educational low price edition. Cambridge University Press, Cambridge, UK 2000. p.135-7.

[15] Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews 1999;12(14):564-82.

[16] Lewis K, Ausubel FM. Prospects for plant-derived antibacterial. Nature Biotechnology 2006; 24(12):1504-7.

[17] Madunagu BE, Ebana RUB, Ekpe ED. Antibacterial and antifungal activity of some medicinal plants of Akwa-Ibom State, Nigeria. African Journal of Biology and Applied Chemistry 1990;35:25-30.

[18] Ross MST, Brain KR. An introduction of phytopharmacy. Pitman Medical Publishing Company, Kent 1977;PP.17-49

[19] Doughari JH, Manzaara S. Invitro antibacterial activity of crude leaf extracts of Magnifera indica Linn. African Journal of Microbiology Research 2008;2:067-72.

[20] Doughari JH Elmahmood AM, Manzara S. Studies on the antibacterial activity of root extracts of Carica papaya L. African Journal of Microbiology research 2007;2:037-41.

[21] Kathad HK, Shah RM, Sheth NR, Patel KN. Invitro antioxidant activity of leaves of GarujapinnataRoxb. International Journal of Pharmaceutical Research 2010;2(3):9-13.

[22] Buvaneswari S, Raadha CK, Krishnaveni N, Jayashree S. In vitro antimicrobial activity of Psidiumguajava against clinically important strains. E-Journal of Life Science 2011;1(1):14-22.

[23] Anjana S, Rani V, Podmini R. Antibacterial activity of some medicinal plants used by tribal against UTI causing pathogens. World Applied Sciences Journal 2009;7(3):332-9.

ACKNOWLEDGEMENT / SOURCE OF SUPPORT

Authors want to acknowledge Dr. Iroha, I.R., Applied Microbiology Department, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria for his academic encouragement. The authors are thankful to the entire staff of Eston Medical Laboratory, Abakaliki, Ebonyi State, Nigeria. Support was also provided by Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

CONFLICT OF INTEREST

No conflict of interest was declared by authors.