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COMPARATIVE ANALYSIS ON PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITIES OF THE LEAVES EXTRACT OF PSIDIUM GUAJAVA AND ITS MISTLETOE TAPINANTHUS GLOBIFERUS (EPIPHYTE)

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ABSTRACT
A comparative study on the phytochemical analysis and antibacterial activity of ethanolic and chloroform extracts of the leaves of psidium guajava and its mistletoe was carried out. Agar well diffusion was employed for the antibacterial activity against clinically gastrointestinal pathogens like E. coli, S. aureus and K. pneumonia. The phytochemical screening results revealed the presence of glycoside, alkaloid, flavonoid, terponoid, carbohydrate and saponins in the ethanol extract of psidium guajava leaves also the ethanol extract of the mistletoe leaves revealed the presence of alkaloid, flavonoid, terponoid, glycoside, carbohydrate and saponin. The chloroform extract of psidium guajava leaves revealed the presence of tannins, terponoid, flavonoids, carbohydrate, alkaloid and glycoside while the chloroform extract of the mistletoe leaves revealed the presence of flavonoid, terponoid, carbohydrate and tannins. For the antibacterial activities screening the result revealed the ethanol and chloroform extract of psidium guajava leaves to have greater zone of inhibition than the ethanol and chloroform extracts of mistletoe leaves. MIC and MBC were determined on both extract. Data obtained was subjected to statistical analysis using ANOVA.

Keywords: *Phytochemical Screening, Bacteria, Psidium Guajava, Mistletoe, Tapinanthus Globiferus (epiphyte)*

INTRODUCTION
Traditionally used medicinal plant, have recently attracted the attention of the biological scientific communities. This has involved the isolation and

identification of secondary metabolites produced by plants and their use as active principles in medicinal preparation.

Plant, have limitless ability to synthesize aromatic secondary metabolites most of which are phenols or their oxygen substituted derivatives (Rajendra P. et al., 2013)

Extraction involves separation of medicinally active constituents in plant tissue from inactive/inert components by using selective solvents and the most appropriate extraction technology. Solvents diffuse into the solid tissue and solubilize compounds of similar polarity. (Joonak, Sowmia et al., 2013).

The world health organization reported that 80% of the world population relies chiefly on traditional medicine and a major part of the traditional therapies which involve the use of plant extract and their constituent. (WHO 2014).it gave emphasis on the need to include traditional remedies within national drug policies as these plants serves as the best sources of a variety of drug? It is important to study plants so that a better understanding of their properties, sefty and efficacy is derived for improved benefit. The presence of phytochemicals constituent in medicinal plants made them useful for healing as well as for curing human diseases. (Nostro A, et al.,2000). The beneficial medicinal effect of plant materials typically result from the combination of secondary products presents in the plant. Today, nearly 50% of the thousand of drug commonly used and prescribe are either derived from plants sources or contains chemical imitation of plant compounds. (Ayodele, 2003).

Aims & Objective

This research was aimed at studying the antibacterial and photochemical properties of the extract leaves of psidium guajava and its mistletoe against clinically gastrointestinal isolate.

Objective of this research work are:

- To compare the photochemical compound in leaves of psidium guajava and its mistletoe
- To determine the antibacterial action of psidium guajava and its mistletoe
- To compare the antibacterial activity of psidium guajava and its mistletoe against some gastrointestinal isolate.

Scope and limitation

The scope and limitation of this research is to provide a medication that will cure gastrointestinal pathogens from psidium guajava leaves and compare the activity effect with its epiphyte (mistletoe).

MATERIAL AND METHOD

Sample collection

Fresh leaves of Psidium guajava and its epiphyte (mistletoe) were collected from Lamido road, No.26 G.R.A Kano, Nigeria. Using a polyethene bag to avoid any contamination.

Sample identification

The leaves were identified and authenticated by in the herbarium unit of the Biological Science Department of Bayero University Kano, Nigeria. Voucher number were given for the purpose of the study, (BUKHAN 336) for Psidium guajava and (BUKHAN 624) for its epiphyte (mistletoe) respectively.

Sample preparation

The collected sample were washed thoroughly with running water and finally with sterile distilled water and then air dried under shade for 2 weeks. The dried leaves was grinded into powder.

Sample treatment

The powdered leaves of Psidium guajava and its mistletoe were extracted using soxhlet extraction according to the method demonstrated by Vasanthipadamanabhan et al., (2013). Ethanol and chloroform were used as the extraction solvent. The powdered leaves (25g) of psidium guajava and Tapinanthus globiferus was transferred into a soxhlet flaks in a 250ml of absolute ethanol and extracted for six hours. The extraction solvent was recovered using rotary evaporator. The extract was further concentrated by evaporation using water bath. The extraction was repeated using chloroform as the extraction solvent.(Uzama D, Envuladu PE. 2017).

Phytochemical Analysis

Test for Tannins

To 1ml of the extracts few drops of alcoholic solution of 0.1% FeCl3 was added, dark blue, greenish black soluble compounds indicate the presence of tannins (Aiyegoro et al., 2010).

Test for Glycoside

Salkowski's Test: 2ml of concentrated H2SO4 were added to the whole aqueous plant crude extracts. A reddish brown color formed which indicated the presence of steroidal aglycone part of the glycoside. (T.S. Roopashree et al., 2008).

Test for steroids

2ml of chloroform and concentrated H2SO4 were added with the 5ml aqueous plant crude extracts. In the lower chloroform layer red color appear that indicate the presence of steroids. (T.S. Roopashree et al., 2008).

Test for saponnins

Foam test- the extracts were mixed with 5ml of distilled water and shaken vigorously. The formation of stable foam indicated the presence of saponins (Abubakar S. et al., 2015). Test for Alkaloids

A few drops of mayer's reagent were added to 1ml of the extracts. White precipitate indicated the presence of alkaloids. Also few drops of dragendorf's reagent give orange precipitate with alkaloids. (Javier, et al., 2014).

Test for flavonoids

A few drops of 10% sodium hydroxide solution were added to 2ml of the extracts. This produced a yellow coloration, a change in color from yellow to colorless on addition of dilute HCl acid indicated the presence of flavonoids. (Syamsuding Abdillah et al., 2015).

Test for terpenoid

2ml of chloroform and few drops of concentrated H2SO4 were added to each extracts. A reddish brown coloration formed in the interface indicates the presence of terpenoids. (Evans WC, Trease GE. 2002)

Test for carbohydrate

1g of extracts was weighed and diluted with 2ml of distilled water. Fehling's solution (A and B) was added and warmed the mixture. A brick-red precipitate at the bottom of test tube was formed which indicates the reducing sugars. (Igbinosa O.O. et al., 2009).

Antibacterial Screening

Source of Test organism

A pure culture of clinical isolate of bacteria was used for the bioassay studies. The isolates include

Escherichia coli, Staphylococcus aureus and Klebsiella pneumonia. The isolates were obtained from microbiology laboratory of Bayero University Kano (BUK) , And the experiment was carried out in microbiology laboratory at School of Technology (S.O.T), Polytechnic, Matan fada Road, Kano, Nigeria.

Preparation of Extract Concentrations

This was carried out using standard method described by Cheesbrough (2002). Stock solution of the ethanol and chloroform extracts of Psidium guajava and its mistletoe (epiphyte) was prepared by weighing 0.2g of each and dissolved in 5ml of Dimethyl sulfoxide (DMSO) in glass vial bottles. This gave an extracts concentration of 20mg/ml (stock solution). Three different extracts concentrations (10, 5 and 2.5mg/ml) was prepared from the stock solution (20mg/ml) using double serial dilution for each extract.

Antibacterial activity using agar well diffusion assay

The agar well diffusion technique as described by Biradar et al., (2007) was employed to test the antibacterial effect of ethanolic and chloroform extract leaves of Psidium guajava and its mistletoe. The Muller-Hinton agar was poured into sterilized 90mm petri-dishes, allowed to solidify for 30minutes. The test organism was inoculated onto the sterile agar plates using a sterile swab stick. After 15minutes of the inoculation, five wells of 6mm in diameter each were aseptically bored using a sterile cork borer on each agar plate. On each agar plate, about 0.3ml of the extract of varying concentration (10mg/ml, 5mg/ml and 2.5mg/ml) were dispensed into 3

wells, then 30µg/ml of chloramphenicol solution of about 0.1ml dispensed in another well which served as positive control and about 0.1ml of DMSO dispensed to the last well which served as negative control. The plates were allowed on flat bench to allow diffusion into the agar and then incubated at 370C for 18-24 hours. These experiments were replicated for each of the test bacteria. Effects of the extracts was assessed by measuring the diameters of zones of inhibition to the nearest millimeter as described by National Committee for Clinical Laboratory Standards (2003), and were then compared with the standard chloramphenicol.

Determination of minimum inhibitory concentration (MIC)

The broth dilution method using serially diluted plant extracts according to the National Committee for Clinical Laboratory Standard protocol (National Committee for Clinical Laboratory Standard, 2003) as described by Lar et al. (2011). Six tubes labeled 1-6 were used for the determination of MIC of the both extract. The six tubes contained 5ml of Muller-Hinton broth. One ml of the crude extract in the concentration (2.5mg/ml, 1.25mg/ml, 0.625mg/ml and 0.315mg/ml) were introduced into tube 1-4 by serially dilution and were mixed thoroughly. To each of the test tube (1-5), 0.1ml of the test organism (E. coli, S. aureus and Klebsiella) were added with the last tube serving as broth control for each respectively. All the tubes were incubated at 370C for 24 hours, after which they were examined for bacterial growth. The minimum inhibitory concentration (MIC) of the crude extract is the lowest concentration of the extract that is capable of inhibiting the growth of specified inoculum of the test organisms.

Determination of minimum bactericidal concentration (MBC)

Prepared Muller-Hinton agar was poured into sterile petri dishes equivalent to the number of culture tubes that showed no visible growth, it was allowed to solidify. Using a sterile pipette, 0.1ml were transferred from each tube to the surface of the agar for each respectively. The plates were incubated at 370C for 24 hours and the bacteria growth colonies were observed on the solid medium plate Vollekova et al.,(2001).

Result

Table 1: Revealed the result of phytochemical screening of chloroform and ethanol leaves extracts of psidium guajava and its mistletoe.

Phytocompounds	Ethanol extract of psidium guajava	Chloroform extract of psidium guajava	Ethanol extract of mistletoe	Chloroform extract of mistletoe
Tannins	_	+	_	+
Glycosides	+	+	+	_
Steroids	_	_	_	_
Alkaloids	+	+	+	_
Flavonoids	+	+	+	+
Terpenoids	+	+	+	+
Carbohydrate	+	+	+	+
Saponins	+	_	+	_

Key: (+) = present, (-) = absent

Table 2: Revealed the antibacterial activity of Chloroform and Ethanol leaves extract of psidium guajava against test organisms.

Isolate	Concentration (mg/ml)/zone of inhibition (mm)				
	10	5	2.5	Control(30µg/ml)	Extracts
Escherichia coli	15	14	13	19	PCE
	16	15	14		PEE
Staphylococcus aureus	15	12	10	21	PCE
	14	13	12		PEE
Klebsiella pneumonia	13	12	11	22	PCE
	13	12	10		PEE

Key: PCE= psidium guajava chloroform extract, PEE= psidium guajava ethanol extract.

Table 3: Revealed the antibacterial activity of chloroform and ethanol leaves extract of mistletoe

Isolate	Concentration (mg/ml)/ zone of inhibition (mm)				
	10	5	2.5	Control (30µg/ml)	Extract
Escherichia coli	13	12	11	20	MCE
	13	13	10		MEE
Staphylococcus aureus	12	10	9	22	MCE

	11	10	9		MEE
Klebsiella pneumonia	13	12	11	21	MCE
	10	9	9		MEE

Key: MCE= mistletoe chloroform extract, MEE= mistletoe ethanol extract.

Table 4: Revealed the minimum inhibitory concentration (MIC) of chloroform and ethanol leaves extracts of psidium guajava

	Extract concentration (mg/ml)											
	Chloroform						Ethanol					
Isolate	2.5	1.25	0.625	0.315	NC	PC	2.5	1.25	0.625	0.315	NC	PC
E. Coli	-	-	+	+	+	-	-	-	+	+	+	-
S. aureus	-	-	+	+	+	-	-	-	-	+	+	-
K. Pneumonia	-	-	+	+	+	-	-	-	+	+	+	-

Key: NC= negative control, PC= positive control

Table 5: Revealed the MIC of chloroform and ethanol leaves extract of mistletoe leaves (epiphyte)

	Extract concentration (mg/ml)											
	Chloroform						Ethanol					
Isolate	2.5	1.25	0.625	0.315	NC	PC	2.5	1.25	0.625	0.315	PC	NC
E. coli	-	+	+	+	+	-	-	+	+	+	-	+
S. Aureus	-	-	+	+	+	-	-	-	+	+	-	+
K. Pneumonia	-	-	+	+	+	-	-	-	+	+	-	+

Key: PC =positive control, NC= negative control.

Table 6: Revealed the minimum bactericidal concentration (MBC) of chloroform and ethanol extract of psidium guajava leaves

	Extract concentration (mg/ml)										
	Chloroform					Ethanol					
Isolate	2.5	1.25	0.625	NC	PC	2.5	1.25	0.625	NC	PC	
E. Coli	-	+	+	+	-	-	+	+	+	-	S.
Aureus	-	+	+	+	-	-	-	+	+	-	
K. Pneumonia	-	+	+	+	-	+	+	+	+	-	

Key: NC= negative control, PC= positive control.

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able 7: Revealed the minimum bactericidal concentration (MBC) of chloroform ethanol extract of mistletoe leaves

	Extract concentration (mg/ml)									
	Chloroform					Ethanol				
Isolate	2.5	1.25	0.625	PC	NC	2.5	1.25	0.625	PC	NC
E. Coli	+	+	+	-	+	+	+	+	-	+
S. Aureus	-	+	+	-	+	-	+	+	-	+
K. Pneumonia	-	+	+	-	+	-	+	+	-	+

Key: NC= negative control, PC= positive control.

DISCUSSION

From the results of Table 2 and 3 it was revealed that the psidium guajava and its mistletoe shows different degree of inhibition against different microorganisms. The activity of the extract was based on the zone of inhibition, the greater the zone of inhibition the more active the extracts was. Uzama D. et al., (2017). For the chloroform extracts of psidium guajava and that of the mistletoe leaves, the extract of psidium guajava has greater zone of inhibition in E. coli and S. aureus than the mistletoe leaves. For the ethanol extracts of psidium guajava and mistletoe leaves, the extracts of psidium guajava also has greater zone of inhibition against E. coli, S. aureus and K. pneumonia. This show that the psidium guajava leaves have more antibacterial action than the mistletoe leaves which support the finding of Uzama D. et al., (2017), in their comparative analysis of Albizia lebbeck leaves and its mistletoe. The ranged of inhibition of
ethanol extract was between 16mm to 10mm at 10mg/ml to 2.5mg/ml of concentration and chloroform extract was between 15mm to 10mm according to result reported by Adeleke et al., (2006) suggested that the diameter of zone of inhibition of 10mm were considered active. The zone of inhibition are higher at 10mg/ml this implied that Concentration and zone of inhibition thus have a direct relationship this means that the higher concentration the higher the zone of inhibition and vice versa which correlated the findings of Anani et al., (2000) and Doughari (2006).By statistical analysis using ANOVA it was observed that the hypothesis of equality of the treatment effect is rejected at both 5% and 1% level of

significant which indicate different among the average effect of the treatment.

CONCLUSION

From the result of this study, it can be concluded that the psidium guajava leaves extract appear to possess more antibacterial activity than the leaves extract of mistletoe. Based on the pharmacological results of the study, it could be confirmed that the extracts contain chemical constituents of pharmacological significance. The extract were effective against infection caused by clinical isolate like Escherichia coli, Staphylococcus aureus and Klebsiella pneumonia.

RECOMMENDATION

It is therefore recommended that further study should be conducted to ascertain the efficacy, toxicity and suitability of using extracts in-vivo.

Further research should be conducted to test the implication of taking the soaked plants part in water.

Proper labeling should be made on containers indicating dosage, date of manufacture, date of expiry and appropriate storage condition.

Unregulated herbal medicine should be manufactured according to standard as authorized by NAFDAC and standard organization of Nigeria (SON).

REFRENCES

Aiyegoro, O.A., Okoli, A.I. 2010.Priliminary phytochemical Screening and in vitro antioxidant activities of the aqeuos extract of Helichrysum longifolium DC. BCM Complement Altern. Med., 10:21

Bradar YS, Jagatap S, Khandewal KR, Singhania SS. 2007. Exploring of antimicrobial activity of Triphala Mashi- an Ayurvedic formulation. Oxf .,J 5 (1) : 107-113.

Cheesbrough , M. (2006) . Biochemical tests to identify bacteria in laboratory practice in tropical conutries. Cheesbrough, M (ed). Cambridge edition pp. 63-87.

Coyle MB. 2005. Manual of antimicrobial susceptibility testing American society for Microbiology.

Clinical Laboratory standard Institute (National Committee for Clinical laboratory Standard). 2003. Method for dilution antimicrobial susceptibility test for bacteria that grow aerobically; approved standard M7-A6. 6th ed. Wayne Pa, USA: national Committee for clinical laboratory standard. P.18-22.

Evans, W.C and Tease, G.E (2002). Parmacognosy, 15th Ed. London: Saunderso publishers. Pp 42-44, 246-249, 304-306, 331-332.

Ekeleme K, Tsaku p, Nkenel, Ufomadu U, Abimuku R, Oti V and Sidi M. [2017]. Phytochemical analysis and antibacterial activity of Psidium guajava L. leaf extracts GSC Biological and Pharmaceutical Science, (2), 13-19.

Geidam, Y., A. Ambali, A., G. and Onyeyili, P.A. 2007. Preliminary Phytochemical and antibacterial evaluation of crude aqueous Extract of Psidium guajava leaf. J. Applied Sci.

7: 511-514.

Gutierrez, R., M. Mitchell, S. and Solis, R., V. 2008. Psidium guajava. A review of it's traditional uses. Phytochemistry and Pharmacology. J. Ethnopharmacol. 47: 1-27.

Hassimotto, N., M.A Genovese, M., I and Lajolo, F., M. 2005. Antioxidant activity of dietery Fruits, Vegetables and Commercial Frozen fruits pulps. J. Agric.Food chem.. 53: 29282935.

HM Burkill, The useful Plants of Tropical Africa. Edition 2. Vol.4. Families M-R. Royal Botanic Gardens Kew, 1997.

Hoagy S. Harvesting Real Mistletoe for Christmas; 2008 © Hoagy Seoins Dec.

Igbinosa, O.O., Igbinosa, E.O. and Aiyegoro, O.A., (2009). Antimicrobial activity and Phytochemical screening of Stem bark extracts from Jatropha curcas (Linn). African Journal of pharmacy. 3 (2): 058-062.

IA Ross. Medicinal plants of the world: Chemical constituents, traditional and modern medicinal uses. New jersey; Humana press 1999.

Joonak., Sowmia, C., Dhanya, K.P., and Divya, M.J. 2013. Preliminary phytochemical investigation of Mangifera indica leaves and screening of antioxidant and anticancer activity. RJPBCS, 4 (1): 1112-1115.

Javier E., Ramirez, Ricardo Zambrano, Beatriz Sepulveda and Mario, J. 2014. Simirgiotics. Antioxidant properties and Hyphenated HPLC-PDA-MS profiling of Chilean pica mango fruits (Mangifera indica L. CV.). mol., 19: 438-458.

Judd, WS; Campbell, CS; Kellogg, EA; Stevens, PF; Donoghue, MJ (2002). Plant systematic, a philogenetic approach. Sinauer Associates, inc. pp 398-399.

KM Nadkarni, AK Nadkarni, Indian Materia Medica- With Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic and Home remedies. Vol.1. Popular Prakashan Private Ltd., Bombay, India, 1999.

Kubmara D, Ajoku GA, Enwerem NM, Okorie DA. 2007. Preliminary phytochemical and

antimicrobial screening of 50 medicinal plants from Nigeria. Afric. J. Biotechnol., 6: 16901696.

Lar PM, Ojile EE, Dashe E, Oluona JN. 2011. Antibacterial activity of Moringa oleifera seed extracts on some gram negative bacterial isolates. Africa. J. Nat. Sci., 14: 57-62.

Nikaido H. (2009) Multidrug resistance in bacteria. Annual Review of Biochemistry,78, 119-146. Oliver- Bever, Bep: Medicinal plants in tropical West Africa. Cambridge University press.

Cambridge. 1986.

Ojewole, J., A.O. 2003 Antiinflammatory and analgesic effects of Psidium guajava Linn. Leaf. J.

Ethnopharmacol. 85: 61-71.

Qa'dan, E; Thewaini, A.; Ali, D.A.; Afifi,R.; E lkhawad, A,; Matalka K.Z. The antimicrobial activities of Psidium guajava and Juglants regia leaf extracts to acne-developing organisms.AMJ. Chin. Med. 2005, 33, 197-204.

Rajendra Prasad Bharti 2013. Studies on Antimicrobial Activity and Phytochemical profile of Mangifera indica leaf extract.IORS J. Environ. Sci. Toxicol. Food Technol., (IORS-JE ST FT) 7 (3): 74-78.

Rajeswar, Y., Maleya, G. and Upal K., M. 2005. "In vitro Lipid peroxadation and antimicrobial activity of Mucuna prariens seeds". Iranian J. pharma. Therapeutics. 4: 32-35.

Syamsudin Abdillah, Risma Marisi Tanbunan, Yunahara Farida, Ni Made Dwisandhiutami, Rita Maleta Dewi. 2015. Phytochemical Screening and antimalarial activity of some plants traditionally used in Indonesia. Asian pac. J. Trop. Dis. 5 (6): 454-457.

Salazar, D.M.; Melgajero, P.; Martinez., R.; Martinez, J.J.; Hernandez, F.; Burguera, M. Phenological Stages of the guava tree (psidium guajava L.). Sci. Hortic. 2006, 108, 157161.

Tsaku PA, Ehinmidu JO and Muhammad SA. (2017). Antibiotics susceptibility and plasmid profile of Escherichia coli from door handles in two tertiary institutions in Nasarawa State, Nigeria Journal of advance in microbiology, 3 (3), 1-13.

"Thai guava forming gets momentum." Dhaka Tribune. August 28, 2016.

Uzama Danlami, Envuladu Patience Elisha. A Comparative Study on the Phytochemicals and Antimicrobial activities of the Ethanol and Petroleum Ether Extracts of the Leaves Albizia lebbeck and its mistletoe. International Journal of pharmacy and chemistry vol.3, No.2, 2017, pp. 13-18. doi: 10. 11648 ij. Ijpc. 20170302.11.

Vasanthipadamanabhan, Manimekalai Ganapathy and Vasthkennedy Evan jelene. (2013). Preliminary phytochemical and antibacterial studies on flowers and pods of Albizia lebbeck (Benth).3 (9).