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Original Research Article

Comparative Study of Antibacterial and Phytochemical Screening of Ethanolic Extracts of *Citrus aurentifolia* and *Psidium guajava* on Some Clinical Isolates (*Pseudomonas aeruginosa* and *Escherichia coli)* of Patients Attending General Hospital Damagum, Yobe State, Nigeria

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Abstract: The present study revealed the presence of some bioactive ingredients such as; Saponin, Flavonoids, Tannins, Alkaloids, Phenols, and Phyto Sterosl in both plants only tannin compound was absent in *Psidium guajava* extracts as slated in table 2. The antibacterial activity of ethanolic and aqueous extracts of leaves of *Citrus aurantifolia* and *Psidium guajava* were analyzed using standard procedure of Various concentrations of ethnolic and aqueous extracts viz; 50,40,30,20,10mg/ml respectively, as well as tested against the bacterial isolates.The bacterial isolates include ; *Escherichia coli* and *Pseudomonas aeruginosa* were both gram negetive bacteria. The highest zone of inhibitions of 20.0mm at 50mg/ml on *E. coli* and 15.0mm on *P. aeruginosa* at 50mg/ml were detected on aqueous leaves extracts, whereas, the ethanolic extract of *C. aurantifolia* showed zone of inhibitions on *E. coli* at 30, 40, 50mg/ml and no zone of inhibitions shown on *P.aeruginosa* except at 30mg/ml. Moreover, the aqueous extracts of *P. guajava* also showed the zone of inhibitions of 10mg/ml and 30mg/ml on *E. coli*, whilst on *P. aeruginosa* there was no zone of inhibitions, as same as on ethanolic extract of *P. guajava* at 10mg/ml on *E. coli* has no zone of inhibitions. The zones of inhibitions on both extracts (ethanolic and aqueous) were significant in only 1% to *E .coli* whereas, in *P. aeruginosa* there was no significant difference at 5%, as showed in table 4,5 and 6 as well as presented in the charts in elaborate.
Keywords: zone of inhibitions, significancy, extracts, *Escherichia coli* and *Pseudomonas aeruginosa*.

Introduction

Plant part have been reported to have various uses in folklore medical practice from ancient Assyria to the paranoiac Egypt records describe how medicinal plants use to cure illness (Ikenebomeh and Mettitira, 1988). The *C. aurantifolia* from reviewed literature described as anti-bacterial, anti-diabetic, anti-fungal, antihypertensive, antilipidemia, antioxidant and ant platelet activities. it is used to treatment of cardiovascular and urolithiasis disease and act as a fertility promoter as well as used as insecticide (Patil *et al*.,2013). Moreover, (Patil *et al*.,2013)., reported that *Citrus aurantifolia* fruit from taxa's, USA, consists at least 22 volatile compound and its major compound are limonene (30%) and dihydrocarvone (31%). About 100ug/ml of *Citrus aurantifolia* extract can inhibit the growth of colon SW-480 cancer cell in 78% after 48h of exposure. It showed the fragment of DNA and increased level of caspase-3-after a few years (Petil *et al*., 2013). Moreover, the citrus secondary metabolites were studied for anticancer activity for example flavonoid on skin cancer. Hespretin and limonoid on colon cancer (Chidanbara muthy *et al.*, 2013). *P. guajava* is a well known traditional medicinal plant and is used in various indigenous system of medicine. The fruits are often included among super fruits being rich in dietary fiber vitamins A and C, folic acid and dietary minerals such as potassium, copper and manganese. Having a generally broad, low-calorie profile of essential nutrients, a single common guava (*P. guajav*) fruit contains about four times the amount of vitamin C as an orange (Hassimotto *et al*., 2005). These constituents of *Psidium guajava* L. has made it possible to use traditionally for treatment of various ailmenst since a long time history. More recent ethno pharmacological studies showed that *Psidium guajava* is used in many part of the world for treatment of number of diseases such as anti-inflammatory, for diabetes, hypertension, carrier wound, analgesic and anti-pyretic effect (Gutierrez *et al*., 2008). The part of plant mostly used is the leaves, fruit bark and root. However, the decoction or infusion of the leaves is used as febrifuge, antispasmodic and for rheumatism in India (Hernandez, 1971). It is also used to treat diarrhea and

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stomach ache in Columbia, Mexico, Maya, Nahuatl, Zapotec, USA and Mozambique. The leaves are used in USA as an antibiotic in form of poultice or decoction for wounds, ulcer and tooth ache (Heinrich, 1998; Leonti *et al*., 2002). In south Africa and Caribbean, extract of the leaves is used in management of diabetes and hypertension, Latin America central and west Africa south east Asia used decoction of the leaves as gargle for sore throats, swelling of the mouth, laryngitis, external ulcers on the skin and vaginal irritation (Ojewole,2005; Rouseff *et al*.,2008; Yang *et al*.,2007). The objectives of this study include the following: To identify the bioactive ingredients present in both plants; to isolate the *P.aeruginosa* and *E.coli* from the samples collected; to determine the antibacterial activity of the prepared extracts of different diluents as well as to ascertain the MIC of bacterial isolates on the extracts.

MATERIALS AND METHOD

Ethical approval

Ethical approval for the study was obtained from ministry of health Damaturu, Yobe State, Nigeria.

Preparation of plant materials

The leaves were indoor dried and grounded with the aid of pestle and mortar into a coarse powder, sieved with 1mm and store in a plastic container as described by (Fatope *et al*.,1993). Moreover, 50grams of the powderd leaves of *Psidium guajava* and *Citru aurantifolia* were weighted, which have been mixed with 500mills of the required diluents (ethanol and water) for some days. The mixture were filtered and the filtrate, were collected separately in a labelled beaker (Abdallah *et al*., 2017).

Phytochemical screening

The phytochemical analysis has been divided into; qualitative and quantitative method of plant extracts.5grams of leaves extracts of *Psidium gaujava* and *Citrus aurantifolia* powder was separately mixed with 50ml of distilled water and ethanol, to test the presence bioactive ingredients that may contribute to the activity of the plant extracts (Abdallah *et al*., 2016).

Test organisms

The isolates were obtained from the stool samples of patients from General Hospital Damagum. The organisms include; *Pseudomonas aeruginosa* and *Escherichia coli*. The isolates were identified using the schemes of Cheesbrough and then sub cultured into nutrient agar (Cheesbrough, 2006).

Culturing and isolation of the test organisms

A sterile wire loop has been used to inoculated stool samples on eosine methylene blue (Merck) that is differential media for *E. coli* and blood agar for *P. aeruginosa*. the culture has been inoculated at 37°c for 24hours . Where both organism were gram negative bacteria (Cheesbrough, 2006). However, during inoculation, the plates were dried because of easier growth and identification of the colonies. The wire loop was also flamed and sterilized. The plates were placed invertedly overnight, to prevent falling of condensed water vapor on plate surface (Cheesbrough, 2006).

Gram staining technique

Thin smear of about 200mm in diameter was made on grease free slides which were also fixed over a burning flame. A crystal violet solution was used to cover the smears for 60 second and after it was wash with distilled water .Secondly, lugol's iodine was also used to the surface for good 60 seconds. Acetone was used to decolorize the stain and lastly, the safranin solution was applied for counter stain on the surface for a minute, which has been washed and allowed to dry at room temperature. Then, the stains have been observed under microscope with oil immersion consequently red stain indicate gram negative bacteria (Cheesbrough, 2006).

Biochemical identification of bacterial isolates

This is done to make sure of an accurate test results so as to confirm the bacterial isolates working on. The test carried out include; oxidase test, indole test, citrate test and urease test as adopted by (Abdallah *et a*l., 2016).

Sensitivity testing

Mueller Hinton agar (fluka) was prepared based on the manufacturer's guide and suspended into a clean conical flask containing 1 liter of sterilized distilled water and allowed to sock and dissolved for some minute, boiled for some minutes and then autoclaved at121°c for 15 minutes, furthermore each organism (culture) was inoculated on plates using wire loop. A 6mm cork borer was used to bore holes on the medium. Six holes were made on each Petri plate, adequantely spaced-out. About 0.2 ml of the different concentration (10,20,30, 40, 50 mg/ml) were introduced into well. The petri plates were incubated at 37°c for 24 hours after which the zones of inhibitions were measured using a meter ruler (Geidam *et al*., 2007).

Statistical tool

The package used for the data analysis was statistix (SAS). version 8.0 so as to know the level of significances among the variables.

RESULTS

Table 1 Physical characteristics of both ethanolic and aqueous extracts of *Psidium guajava* and *Citru aurantifolia*

S/N	EXTRACTS	WEIGHT CONC (g)	% YIELD	APPEARANCE	CHARACTERISTIC TEXTURES
1	Ethanolic extracts of citrus leaves	60	66.6	Light green	Powder
2	Ethanolic extracts of guava leaves	60	58.3	Grayish in color	Powder
3	Aqueous extracts of citru leaves	60	33.3	Pale green	Powder
4	Aqueous extracts of guava leaves	60	75	Grayish in color	Powder

Formula for percentage yield = initial weight of sample /weight of extracts×100

Table 2. Qualitative analysis of phytochemical screening of *P.guajava* and *C. aurantifolia*

S/N	Phytochemical ingredients	STATUS	
		P. guajava leaves	*C. aurantifolia* leaves
1	Saponins	+	+
2	Flavonoid	+	+
3	Tannin	-	+
4	Alkaloid	+	+
5	Phenol	+	+
7	Phytosterol	+	+

Key : + = present, - = absent.

Table3.Morphological and biochemical test for identification of the isolates

S/N	Biochemical test	*E. coli*	*P. aeruginosa*
1	Colony morphology		
2	Nutrient agar	Cream pinpoint colonies	Cream coloured, opaque, colonies
3	Selective medium	EMB agar, greenish metallic sheen	Blood agar, large colonies, whitish mucoid rough surface, translucent and rough edge.
4	Motility	Motile	Motile
5	Grams nature	Gram negative	Gram negative
6	Cellular morphology	Cocci in cluster	Cocci in cluster
7	Indole	+ve	-ve
8	Urea	-ve	-ve
9	Voge's proskauer	-ve	-ve
10	Citrate	-ve	+ve
11	Oxidase	-ve	+ve

Key: -ve=negative, +ve=positive

Table 4 Showing the zone of inhibition in various extract against the test organisms

Treatment	**EC**	**PA**
Extract (ml)		
C(AQ)E	6.4000	9.2000
C(eth)E	7.0000	1.8000
P(AQ)E	6.8000	6.6000
P(eth)E	6.8000	7.4000
S.E	3.8678	3.1385
Sig.	NS	NS
Conc. Levels (mg)		
10	0.0000	6.5000

20	1.0000	3.7500
30	9.5000	7.7500
40	10.000	8.2500
50	13.250	5.0000
S.E	2.8853	4.0301
Sig.	**	NS

Means within a column followed by the same letters are statistically not significant at 5% level of probability using Duncan's multiple range test (DMRT)

KEY: ** = Significant at 1%, * = Significant only at 5% and Ns = Not significant at 5%. EC = *E-Coli*, PA = *P. aeruginosa* C(AQ)E= Citrus aqueous leaves extracts; C(eth)E= Citrus ethanolic leaves extracts; P(AQ)E= Guava equeous leaves extracts and P(eth)E= Guava ethanolic leaves extracts

Table5 . Showing the minimum inhibitory concentration (MIC) of ethanolic extracts of *Psidium guajava* on test organisms

Treatment	**Cex**
Test Organism	
EC	0.0500[a]
PA	0.0140[a]
S.E	0.0306
Sig.	NS
Conc. Levels (mg)	
10	0.0000[a]
20	0.0350[a]
30	0.0400[a]
40	0.0750[a]
50	0.0100[a]
S.E	0.0550
Sig.	NS

Means within a column followed by the same letters are statistically not significant at 5% level of probability using Duncan's multiple range test (DMRT)

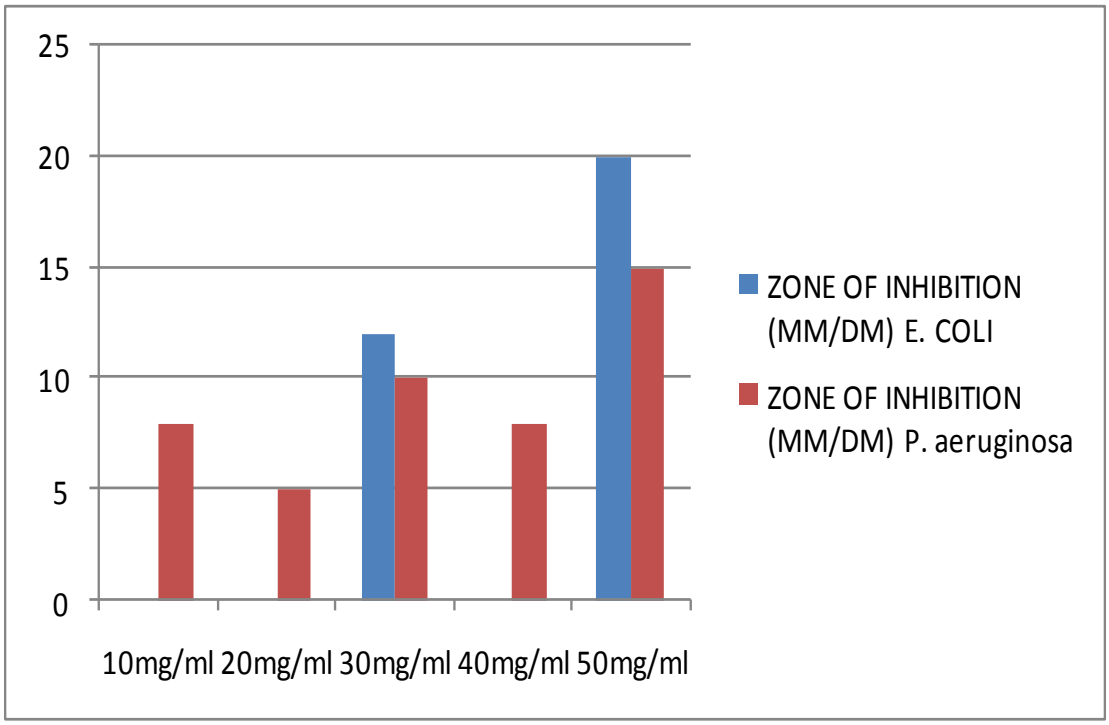
** = Significant at 1%, * = Significant only at 5% and Ns = Not significant at 5%. EC = *E-Coli*,PA = *P. aeruginosa*.

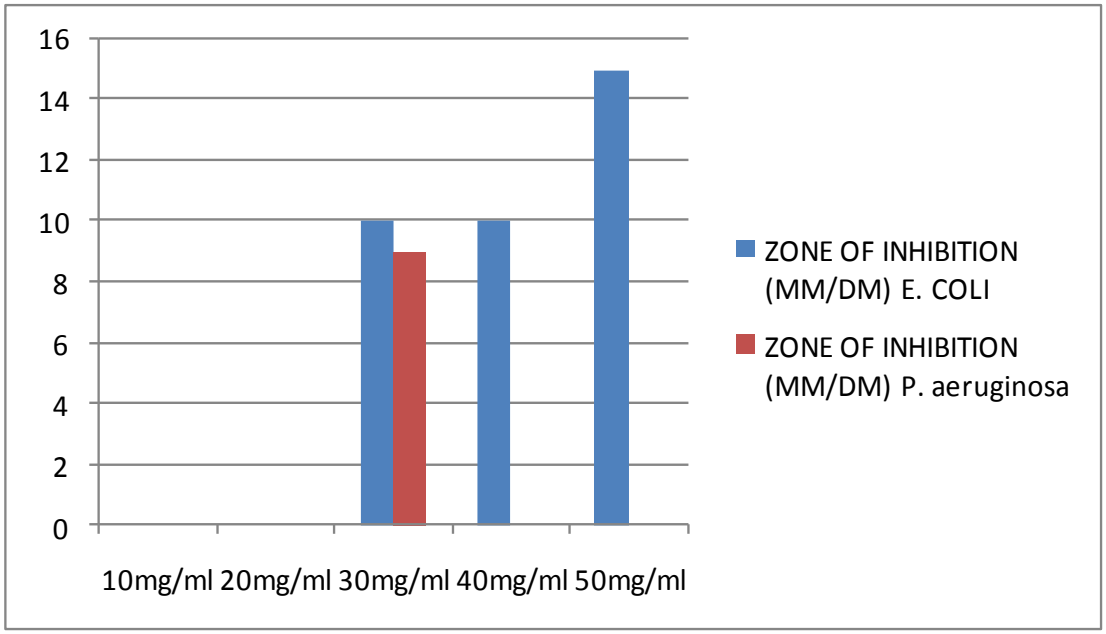
Table 6: Showing the minimum inhibitory concentration (MIC) of ethanolic extracts *of Citrus aurantifolia* on test organisms

Treatment	**Cex**
Test Organism	
EC	0.0640[a]
PA	0.0440[a]
S.E	0.0395
Sig.	NS
Conc. Levels (mg)	
10	0.0750[a]
20	0.0000[a]
30	0.0550[a]
40	0.0800[a]
50	0.0600[a]
S.E	0.0694
Sig.	NS

Means within a column followed by the same letters are statistically not significant at 5% level of probability using Duncan's multiple range test (DMRT)

** = Significant at 1%, * = Significant only at 5% and Ns = Not significant at 5%. EC = *E-Coli*, PA = *P. aeruginosa*.





DISCUSSION.

In the present study the result of phytochemical analysis revealed the presence of some bioactive ingredients such as; Saponins, Flavonoids, Tannins, Alkaloids, Phenols, and Phyto Sterols in both plants only tannin compound was absent in *Psidium guajava* extracts as slated in table 2. Similarly the Flavonoids extracted from *P. guajava* leaves were found to be effective against several strains of pathogenic bacteria (Abdallah *et al*., 2016). In the present study, the physical characteristic of both leaves extracts were 60g was taken as weight concentration of both extracts, so the aqueous extracts of guava leaves contain high percentage yield 75% and appear grayish in color, followed by ethanolic extracts of citrus about 66.6 %, appear light green and ethanolic extracts of guava 58.3%, appear grayish in color, then aqueous extracts of citrus have least percentage yield which was 33.3% appeared pale green and powder in texture as shown in table 1.

However, the percentage yield of medicinal plant extract which contain the bioactive metabolites very considerable with plant spacies and the method or solvent used for extraction, Also factors like age of the plant may have affected the percentage yield (Yahaya *et al.,* 2012). The isolates were cultured, sub cultured as well as biochemically identified as shown in table 3 which wer both gram negative bacteria. However in the present study the zone of inhibitions of various extracts against isolated organisms were active on aqueous extract of *C. aurantifolia* which showed the highest zone of inhibitions 20.0mm at 50mg/ml on *E. coli* and 15.0mm on *P. aeruginosa* at 50mg/ml, where ethanolic extract of *C. aurantifolia* showed zone of inhibitions on *E.coli* at 30, 40, 50mg/ml and no zone of inhibition were shown on *P. aeruginosa* rather on 30mg/ml. Moreover, the aqueous extracts of *P. guajava* showed zone of inhibitions of 10mg/ml on *E. coli*, and 30mg/ml whereas, on *P. aeruginosa* there was no zone of inhibitions, the same as that of ethanolic extract of *P. guajava* at 10mg/ml on *E. coli* which showed no zone of inhibitions surfaced as showed in table 4,5 and 6 as well as presented by the charts above. Moreover, guava leaf extracts also inhibited the growth of *Streptococcus aureus* in a study carried out by disc diffusion method (Abdulrahim *et al.,* 2002) as well as ethanolic and aqueous extracts produced the MIC value a range from 0.15-50mg/ml against tested organisms.

Conclusion

The study revealed the present phytoconstituents found in both plants extracts as well as isolated bacteria were both negative in gram reactions as well as inhibited by the plants extracts worked on. The level of significances were also presented in bar charts.

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