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PHYTOCHEMICAL STUDIES AND ACTIVITIES OF LEAF EXTRACTS OF *JUSTICIA SECUNDA* AND *PERSEA AMERICANA* AGAINST SELECTED CLINICAL MICROBIAL ISOLATES

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ABSTRACT

The phytochemical compositions and antimicrobial activities of methanol leaf extracts of *Justicia secunda* and *Persea americana* on selected clinical isolates were investigated using standard agar well diffusion method at concentrations of 50,150, 200, 250 and 300mg/ml. Qualitative and quantitative phytochemical revealed the presence of saponins (0.58±0.008) %, flavonoid (0.07± 0.0 12) %, tannins (0.96± 0.0 13) %, steroids (2.66± 0.006) %, cardiac glycoside (0.24±0.008) %, alkaloid (12.25±0.013)%, for *Justicia secunda*. That of *Persea americana* were; saponin (0.18±0.012)%, flavonoid (O.73±O.012)%, tannins (2.74±0.006)%, steroids (O.89±O.013)%, cardiac glycoside(O.75.±O.OlO)%, Alkaloid (10.24±0.008)%. The highest zone of inhibition for *Justicia secunda* leaves against *E. coli*. *Staphylococcus aureus*, *pseudomonas aeruginosa* and *Bacillus spp* were 19.0mm, 17.2mm, and 9.3mm, whereas the highest zone of inhibition for *Persea americana* leave against the microorganisms were 14.2mm, 13.3mm and 9.6mm respectively. There were resistances of *Bacillus spp* and *pseudomonas aeruginosa* against *Persea americana* methanol extract. However, the tube dilution method was employed for determining the minimum inhibitory concentration (MIC) of the extracts. The MIC values on *E.coli*, *staphylococcus aereus*, *pseudomonas aeruginosa* and *Bacillus spp* for methanol extract of *Justicia secunda* were 3.06mg/lml, 12.50mg/ml, 6.13mg/ml and 12.5mg/mI. MIC for *Persea americana* extract were 1.53mg/mI, 6.13mg/mI and 0.76mg/mI respectively. The presence of phytochemical constituents in the leaves supports their use against some pathogenic microorganisms.

KEYWORDS: Antimicrobial, phytochemical, *Justicia secunda*, *Persea americana*.

1.0 INTRODUCTION

Medicinal plants are those plants that are used in treating and preventing diseases that are generally considered to be deadly to humans, (Anselem, 2004).

According to Doherty *et al*. (2010), a medicinal plant is any plant in which one or more of its organ contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs and pharmaceutical properties. Plants make many chemical compounds that are for biological functions, including defense against insects, fungi and herbivorous animals. Medicinal plants are mostly used in traditional medicine to treat various ailment including diarrhea, constipation, convulsion, stomach upset etc.

According to Olutayo *et al.* (2011), medicinal plants are essential for the health of individuals and communities in developing countries such as Nigeria where most rural dwellers lack finance for medicinal treatment. The use of herbs has been their source for treating common diseases.

Phytochemicals are chemical compounds formed during the plants normal metabolic processes (Eleazu *et al*., 2012). The phytochemicals found in these plants help in preventing harm from microorganisms because of the presence of Bio-active active compounds in plants that has a toxic effect on the cell wall of microorganisms (Konsam *et al*., 2015).

Studies have shown that plants with phytochemicals have antimicrobial potentials. However, the activity of plants against microorganisms depends mostly on the various constituents of the plants as well as type of microorganisms being targeted.

Justicia secunda is a native tropical herbaceous plant, originating from south- America which belongs to the *Acanthaceae* family. The plants are commonly known as blood root in Nigeria. *Justicia secunda* leaf decoction is used in treating wound, anemia and abdominal pain (Nguessan *et al.,* 20110). According to Dai *et al.* (2010) a decoction of the plant is used as treatment for menstrual pain and for dilation after miscarriage.

Persea americana is a tropical herbaceous plant, from the family of *Lauraceae*. The leaves of the plant are oval having a shape of 3 to 10 inches long. The flowers are small and greenish in

colour. The fruit is an oval or pear shaped drupe, with a fleshy outer covering surrounding the single seed (Hogan, 2008).

According to Ortiz *et al.* (2004), the pulp of *Persea americana* is used as baby food. In Nigeria, the leaves of *Persea americana* are used for treating cough and digestive disorder. *Persea americana* has been reported to have antibacterial effect against gram negative bacteria (Aime *et al.*, 2014).

3.0 MATERIALS AND METHODS

3.1 Collection of Plant Samples And Identification

Fresh leaf samples of *Justicia secunda* and *Persea americana* were collected from J.C Nwaegwugwu's compound in World Bank Housing Estate, Abayi, Aba in Osisioma Local Government area of Abia state. The leaves were identified by a botanist at Federal University of Technology (FUTO) Owerri Imo state.

3.2 Sample Preparation and Extraction

The fresh leaf samples of *Justicia secunda* and *Persea americana* were properly washed with distilled water to remove debris. Thereafter, the leaves were air dried for 3 weeks under regular turning to enhance even drying. The dried leaf samples were separately grounded into fine powder using a mechanical grinder. The cold extraction method was adopted for extraction with little modification (Kigigha *et al.*, 2015).

50g of each powdered plant material of *Justicia secunda* and *Persea americana* were separately weighed and soaked in 500ml of methanol at ambient room temperature for 72hours under regular shaking condition. Each extract was filtered using Whatmann filter paper No. 1. The filtrates were concentrated in a rotary evaporator till the volume was reduced to about 100ml.

3.3 Phytochemical Screening Of The Plants

Preliminary phytochemical contents of the plant extracts (*Justicia secunda and Persea americana*) were carried out according to Okwu (2005), Doherty *et al.* (2010), Sofowora (1993) and Chiegina and Ukeh (2012). Quantitative phytochemical tests were carried out according to the method of Harbon (1973).

3.4 ANTIMICROBIAL SCREENING

3.4.1 Source and Preparation of Organisms

The microorganisms used in this study were obtained from the stock culture in the Department of Medical Microbiology and Parasitology of Federal Medical Centre (FMC) Owerri. The purity of the bacteria was checked by sub-culturing in a freshly prepared Mueller-Hinton broth. *Staphylococcus aureus* was characterized by plating on Mannitol salt agar which gave yellow pigmentation. The other microorganisms were collected in slants in McCartney bottlers containing Mueller- Hinton agar and appropriately stored until needed (Cheesbrough, 2004). Biochemical tests were also conducted for proper confirmation.

3.5 Antimicrobial Screening of The Extract

A known weight of each extract of *Justicia secunda* and *Persea americana* leaves were reconstituted in sterile distilled water to give the desired concentration in milligram (mg). 0.2ml of 10^6 cfu/ml was used as inoculum size for all organisms. Each inoculum was mixed with 20m1 of Mueller-Hilton agar in Petri dishes. 6mm in diameter wells were punched in the agar medium using sterile glass cork borer before being filled with 0.1ml of plant extracts on each plate. The plates were incubated for 24hrs at 37°C and the diameter of zone of inhibition was measured. The experiment was carried out in duplicates. Agar well diffusion method was employed for the antimicrobial testing using the scheme of Lino and Deogracious (2006) with slight modification by Kigigha and Atuzie (2012).

3.6 TEST FOR MINIMUM INHIBITORY CONCENTRATION (MIC) OF THE METHANOL EXTRACTS

The Kirby-Bauer method of MIC was adopted for the determination of MIC of the methanol extract of *Justicia secunda* leaves and *Persea americana*. Mueller Hinton broth was prepared according to the manufacturers' instruction. It was autoclaved at 121°C and allowed to cool. The cooled broth was dispensed into 10 test tubes containing 9m1 of broth and was labeled from 1- 10 appropriately; tube 10 was labeled sterility control while tube 9 was labeled growth control. 1m1of the pure extract (50mg/ml was pipetted into tube I and was serially diluted up to tube 8 with concentration of 50 mg/ml - 300mg/ml. A sterile wire loop was used to pick a colony of the test organism *E. coil* and placed into tube 1 - 9. They were then incubated at 37°C for 24hrs. After incubation, the tubes were observed for turbidity. The procedure was also repeated for other organisms using the other extracts.

4.0 RESULTS

Table 1: Phytochemical Constituents of *Justicia Secunda* Leaves.

Phytochemicals	Qualitative result	Quantitative Conc. In (%)
Saponins	++	0.58±0.008
Flavonoids	+	0.07±0.012
Tannins	++	0.96±0.013
Steroids	+	2.66±0.006
Cardiac glycoside	+	0.24±0.008
Alkaloids	+	12.25±0.013
Terpenoids	+	0.13±0.008

++ = highly present, + = moderately present.

Values are mean standard deviation of duplicate determinations.

Table 2: Phytochemical Constituents of *Persea Americana* Leaves.

Phytochemical	Qualitative result	Quantitative Conc. In (%)
Saponins	+	0.18±0.012
Flavonoids	+	0.73±0.012
Tannins	+	2.74±0.006
Steroids	+	0.89±0.013
Cardiac glycoside	+	0.75±0.010
Alkaloids	+	10.24±0.008
Terpenoids	+	0.61±0.020

++ = Highly present, + = moderately present.

Values are mean standard deviation of duplicate determinations.

Table 3: Zones of inhibition (mm) of methanolic extracts of *Justicia secunda.*

Plant used	Conc. Of crude extract (%w/v (mg/ml)	*E.coli*	*S. aureus*	*P.aeruginosa*	*Bacillus spp*
Justicia					
***secunda* Leaves**	50	9.50	11.10	6.50	2.00
	100	11.60	13.20	7.00	2.20
	150	12.50	13.00	7.50	4.30
	200	13.80	14.60	7.30	5.60
	250	16.50	16.00	9.40	8.80
	300	19.00	17.20	12.20	9.30
Persea					
Americana Leaves	50	-	-	-	-
	100	-	-	-	-
	150	-	-	-	-
	200	11.20	8.30	-	-
	250	11.80	13.30	-	-
	300	14.20	13.30	-	-

Table 4: The minimum inhibitory concentration (MIC) of the extracts of *Justicia secunda* and *Persea Americana* leaves on test organisms.

Test organisms	Methanol leaf extract of *Justicia secunda* (mg/ml)	Methanol leaf extract of *Persea americana* (mg/ml)
Escherichia coli	3.060	1.53
Staphylococcus aureus	12.50	6.13
Pseudomonas aeruginosa	6.130	0.76
Bacillus spp	12.50	-
Proteus spp	-	-

DISCUSSIONS

The phytochemical constituents of *Justicia secunda* leaves is presented in Table one. The result revealed that Tannin and Saponin were highly present with concentrations of 0.96 ± 0.013% and 0.58 ± 0.008% respectively.

Flavonoids, steroid, cardiac glycoside, alkaloids, terpenoids were moderately present with concentrations ranging from 0.13 ± 0.008% to 12.25 ± 0.013%.

Table 2 shows the phytochemical constitutents of *persea americana* leaves revealing the presence of Saponins, Flavonoids, Tannins, Steroids, Cardiac glycoside, Alkaloids and Terpenoids in moderate forms. Meanwhile the concentration ranged between 0.61 ± 0.020% to 10.24 ± 0.008% respectively.

Table 3 shows the zones of inhibition (mm) of methanolic extracts of *Justicia secunda* and *Persea americana* leaves on selected isolates at concentrations of 50-300mg/ml. *Escherichia coli* increased from 9.50mm-19.00mm, *Staphylococcus aureus* also increased from 11.10mm-17.20mm including *Pseudomonas aeruginosa* and *Bacillus spp.*

In *Persea americana* leave there was an inhibition from 11.20mm-14.20mm in *Escherichia coli* form 200-300mg/ml. Also *Staphylococcus aureus* increased from 8.30mm-13.30mm in concentrations ranging from 200-300mg/ml respectively. There were absence inhibition of *Escherichia coli Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus spp* from 50-150mg/ml concentrations. Whereas *Pseudomonas aeruginosa* and *Bacillus spp* were not inhibited in all the concentrations.

Table 4 shows the minimum inhibitory concentrations (MIC) of the extract of *Justicia secunda* and *Persea americana* leaves on test organisms. The result indicates that

Escherichia coli.Staphyloccocus aureus,Pseudomonas aeruginosa and Bacillus spp had 3.060,12.50,6.130 and 12.50 mg/ml as MIC in ethanol extract of *Justicia secunda* leaves. Also the MIC of methanol leave extract of *Persea Americana* were 1.53mg/ml in Escherichia coli,6.13mg/ml in Staphylococcus aureus and 0.76mg/ml in *Pseudomonas aeruginosa. Bacillus spp* was absent in *Persea americana* extract.

The result of Qualitative phytochemical analysis of methol extract of *Justicia secunda* and *Persea americana* leaves revealed the presence of Saponins, Flavonoids, Tannins, Steriods, Cardiac glycoside, Alkaloids and Terpenoids. Quantitative phytochemical constituents in *Justicia secunda* leaves were: Saponin 0.58±0.008%, flavonoids 0.07±0.012%, Tannin 0.96±0.013%, steroids 2.66 ±0.006%, Cardiac glycoside 0.24 ± 0.008%, Alkaloids 12.25 ± 0.013% and Terpenoids 0.13±0.008%, In table 1.

In Table 2, Quantitative phytochemical screening of *Persea americana* leaves were as follows; Saponins 0.18±0.012%, flavonoids 0.73±0.012%, Tannins 2.74± 0.006%, Steroids 0.89±0.013%, Cardiac glycoside 0.75 + 0.010%, Alkaloids 10.24 ±0.008% and Terpenoids 0.61±0.020%. The presence of these phytochemicals indicates that the plants can serve as antimicrobial agents (Doherty *et al.*, 2010).

Alkaloids can be used to attack pests and insects in plants. Synthetic Derivatives of alkaloids can as well be used as antimicrobial agents, antispasmodic and bactericidal effects (Kigigha *et al.*, 2015).

Flavonoid as an antioxidant agents in both plant can serve as anticaricinogenic, antibacterial and antitumor agent (Okwu, 2005). Saponins and tannins could also serve as suppressant and treatment of wounds, ulcer and burns, including anti-inflammatory problems. The result of these phytochemical study is also in line with the work of Kigigha *et al* ., 2005, on activities of *Aframomum melegueta* seed against *Escherichia coli, Staphylococcus aureus* and *Bacillus species*.

Zones of inhibition (mm) of methanolic extract of *Justicia secunda* and *Persea americana* leaves is shown in Table 3.

At higher concentrations, there were inhibiton of both plants against the tested clinical isolates from *Escherichia coli* to *Bacillus spp*. As the concentration increases the trends in Inhibition increases significantly in both gram positive and gram negative organisms.

aIt is worthy to note that the highest zone of inhibition for justicia secunda leaves was found in *Escherichia coli* having 16.50mm whereas the least was seen in *Bacillus spp* with 2.00mm. Methanol extract has shown a higher level of resistance against the organism tested. The result of this study in terms of zone of inhibiton is comparable to a previous report by Doherty *et al*., Chijina and Ukah (2012).

There were actually no zones of inhibiton of methanol extract of *Persea amaericana* leave in *Pseudomonas aeruginosa* and *Bacillus spp* at all concentrations. This may be attributed to the cell wall and production of beta-lactamase (Cheesbrough, 2004).

The highest zone of inhibiton was seen in *Escherichia coli* having 14.20mm at 300mg/ml. A study conducted by Agu and Thomas (2012) on Antibacterial activities of ethanol and Aqueous Extract of five Nigerian Medicinal plants on some wound pathogens also reported zones of inhibition of both gram positive and gram negative organisms at 21 and 20mm is also in line with the study of this work.

The antimicrobial potential of extract of methanol on both plants has been shown to possess some broad spectrum activities against gram negative and positive organisms. This is similar to the work of Agu and Thomas, (2012).

Minimum inhibitory concentration of the Extract of *Justicia secunda* and *Persea americana* leaves in table 4 on the tested organisms were found to be effective at the various concentrations. The result of this work shows that antimicrobial activites and phytochemical analysis of *Staphylococcus aureus Pseudomonas aeruginosa* and *Bacillus spp* had 3.060, 12.50, 6.130 and 12.50mg/ml as MIC in methanol extract of *Justicia secunda* leaves.

Also the MIC of methanol leave extract of *Persea amaericana* were 1.53mg/ml in Eschericia coli, 6.13mg/ml in *Staphylococcus aureus* and 0.76mg/ml in *Pseudomonas aeruginosa*. *Bacillus spp* was absent in *Persea amaericana*.

CONCLUSION

The plants have some antimicrobial potency and should be exploited for economic gain.

RECOMMENDATION

It is therefore recommended that Gas chromatography should also be used to screen the bioactive components of both plants and also other gram positive and gram negative organisms should also be used to confirm their antimicrobial effect as well.

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