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Phytonutrients Screening And Bioactivity Of Physalis Angulata's (Ground Cherry, Tapue) Roots And Stem Extracts

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Abstract: ***The phytonutrients screening of the roots and stems of Physalis angulata crude (tapue) extracts using solvent systems (ethanol, ethylacetate, acetone) and thin layer chromatographic method Antimicrobial activity were also carried out against some clinical isolates (Escherichia coli, Salmonella typhi and Pseudomonas aeruginosa). The results of the solvent systems extracts showed that alkaloids, phlobatinins, steroids, phenols, flavonoids, anthraquinones, glycosides and terpenoids are present in the roots and stems extracts of the plant and the absence of saponins in all the extracts. Terpenoid was found to be present in all the solvent systems in both the roots and the stems extracts while Alkaloids and flavonoids were positive in both ethyl acetate and ethanol solvent systems but were not detected in the acetone extract of the roots. The result also showed maximum positive (+) results for ethyl acetate and ethanol systems while acetone was the least system with few phytonutrients for both the roots and stems extracts. Saponnin was not detected in both stems and roots extracts. This was confirmed by the thin layer chromatographic result which showed that the ethyl acetate system has the highest movement of all the phytonutrients in the n-hexane/ethyl acetate(5:1) solvent ratio. The Antimicrobial activity of ethylacetate in roots extracts showed the maximum activity for salmonella typhi with 36mm zone of inhibition. The activity of 0 mm was observed in roots extracts in acetone and ethanol systems on Escherichia coli. In the stems extracts, ethyl acetate showed the highest activity against Salmonella typhi with 28mm zone of inhibition. It was also observed that the ethanol system of the stems extract showed the least activity with 10mm on Salmonella typhi. The result of this study provided the insight for the correct identification of the bioactive compounds and suitable solvent systems for separation of those compounds from the roots and stems extract of Physalis angulata.***

Keywords: ***Phytonutrients, Physalis angulata, antimicrobial activity, extracts, clinical isolates***

I. INTRODUCTION

Plants contain thousands of natural chemicals called phytonutrients or phytochemicals."Phyto" refers to the Greek word for plant. These are chemicals which help to protect plants from germs, fungi, bugs, and other threats are also responsible for a plant's organoleptic properties. Phytonutrients, however may help prevent diseases and keep the body working properly. *P. angulata* belongs to the family Solanaceae. It is known by different names, in Nigeria, it is commonly called "Koropo" in Yoruba, Tapue or pue in Tiv and in Hausa, the plant is called "Saadi Birii'. It is also called cut-leaf ground cherry in English and it is a small tropical annual herb that bears cream-coloured flowers and small edible orange-yellow fruits (Donker *etal.*,2012). Each fruit is like a yellow pearl a small lantern-shaped pod and very delicious to eat (Alves *etal.,2008*). The anti-microbial activity of zinc oxide ointment and *P. angulata* crude extracts against *Pseudomonas aeruginosa* and *Staphylococca aureus* were investigated by Donker and co-workers. Many of the species are reportedly used for treating asthma, urinary problems, rheumatism and tumor (Donker *eta.*, 2012).

Most of the species are herbaceous annual or perennial, native of tropical North and South America. Some species have edible fruits and are considered within popular medicine. It is a medically important plant used in traditional medicine as anti-rheumatic agent, and also for sore throat and abdominal pain treatment (Rengifo and Vargas, 2013). It is also considered as antipyretic, anti-diuretic, and also efficient for hepatitis and cervicitis treatment (Shravan *etal.*,2011). *Physalis angulata* is used in ethno medicine for treating sexually transmitted diseases, gastro-intestinal disorders, diabetes and leprosy (Ray and Gupta, 1994; Tomassini *et al.*, 1999).

Historically, *Physalis* species have been taxonomically classified based on characters such as habit, hair type, and number of calyx angles. The typical *Physalis* species is an herb with axillary yellow flowers that are solitary and bee pollinated (Shravan *etal*., 2011). Different phytosterols, carbohydrates, vitamins, minerals and lipids are contained by the genus *Physalis* and lead to the formation of the withanolides type structure (Whitson and Manos 2005). The study of Hsue *etal.*, (2011) had demonstrated in vitro genotoxic effect of aqueous extract on human lymphocytes using the comet and micronucleus assays. The name *Physalis* in Greek for 'a bladder' reference to the inflated calyx (Lawal *etal.*, 2010). The genus *Physalis* is a member of Nightshade family, Solanaceae. *Physalis* species are annual or perennial, erect or decumbent, sometimes rhizomatous, glabrous pubescent, and with variously toothed or lobed leaves (Kelly *etal.*, 2012).

In spite of the reported rich medicinal potentials of *Physalis angulata* there is paucity of literature on its chemistry and therapeutic applications in Nigeria. Phytochemical screenings are now seen as the first step towards the discovery of useful drugs that nature has been endowed with as potential source of drugs in diverse plants. The challenges of the claims of the traditional medicines lies in the lack of scientific proof of its efficacy and in order to understand the medicinal value and ascertain their rightful role in contributing to affordable healthcare, these plants must be accessed through scientific point of view. This entails predictable chemical consistency, therapeutically benefits and proof of safety based on well designed ,controlled studies using phytochemical screening , antimicrobial activities properties.

II. METHODOLOGY

SAMPLE COLLECTION AND IDENTIFICATION

The *P. angulata L.* whole plant specimen was collected from its natural habitat at Tse-Ugye village in Ukum local government of Benue state, Nigeria. (DMS coordinates- $7^{0}38^{1}38.15^{11}$N, $9^{0}33^{1}48.82^{11}$E) for its stem and roots .The sample was thoroughly washed and air dried for two weeks and finally milled into powder form using milling machine.

IDENTIFICATION

The plant was identified by Mr Waya of the Dept of Biological Sciences Benue State University, Makurdi. The sample specimen was deposited at their herbarium.

Microorganisms used in the study: The following organisms: *pseudomonas, aeruginosa*, *Salmonella typhi* and *Escherichia coli* were used and were obtained from the Microbiology Department, Federal University Wukari, Taraba State.

Kingdom	Plantae
Super division	Angiosperms
Division	Eudicots
Class	Asterids
Order	Solanale
Family	Solanaceae
Genus	Physalis
Species	Angulata

Table 1: Taxonomy of P. angulata L.

STERILIZATION OF MATERIALS

All glass wares used in this study were washed with detergent, rinsed and sterilized in a dry ventilated oven at 160 ^{o}C for 2 hours. All media were sterilized by autoclaving at a temperature of 121 ^{o}C and 15 psi for 15 minutes. The scalpel, cork borer, inoculating needle were sterilized by dipping them into 70% ethanol and passing them over a Bunsen burner flame until red hot.

MEDIA PREPARATION

The medium used was Mueller Hinton Agar (MHA) and was prepared according to the manufacturer's instruction. About 38 g of powdered MHA medium was dissolved in 1 L of sterile distilled water and sterilized by autoclaving at 121°C at 15 psi for 15 min and allowed to cool before pouring carefully into 100 sterile Petri dishes. The Petri dishes that contained the medium were incubated for 24 h at room temperature (37°C) to check for sterility before use (Bauer *etal.*, 1966).

EXTRACTION METHOD

The method used was cold maceration method despite the so many other methods such as serial exhaustive extraction, plant tissue hominization, soxhlet extraction, decoction, infusion, digestion, percolation and sonication methods.

COLD MACERATION METHOD

In maceration (for fluid extract), whole or coarsely powdered plant is kept in contact with the solvent in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermo labile drugs.

The roots and stems extract were prepared by soaking 20g the sample in 100ml of etyhlacetate, acetone and ethanol each for 24 hours with frequent agitation. The resulting mixture was filtered using filter paper and the filtrate were

concentrated by evaporation using rotary evaporator, kept in a vacuum oven overnight at room temperature to remove all the solvent and weighed .The extract was kept in the refrigerator until required for testing (Ncube *etal.*, 2008).

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Preliminary phytochemical screening of the extract were done for alkaloid, tannin, saponin, steroid, terpenoid, phenolics, flavonoid ,anthraquinone and glycoside using standard chemical screening method (Evans *et al 2003*, Siddiqui *et al*.,1997, Earnsworth *et al*., 1974, Harbone *et al*.,2009).

BIOASSAY

This is the study of antimicrobial activity of the crude extracts against microorganism. It was used as a guide to determine the active component of the roots and stems of *Physalis angulata*. The extract were tested for antimicrobial activities.

ANTIMICROBIAL SUSCEPTIBILITY TEST

Susceptibility test were carried out using the well diffusion method to detect the presence of anti-bacterial activities of the plant samples (Evans *etal.*, 1997). A sterile swab was used to evenly distribute bacterial culture over the appropriate medium (Siddiqui *etal.*, 1997). Mueller Hinton agar was prepared as per the instructions by the manufacturer. The plates were allowed to dry for 15 minutes before use in the test. Once the media solidified then it was then inoculated with the bacteria species. The media was then punched with 6 mm diameter hole and was filled with extract; a pipette was used to place 30ul of the extract into the well. A total of six extracts was used on a particular bacterial species; with a total of three plates used for each extract. The positive control was the same on all isolate. The plates were incubated at 37^0C for 24 hours after which they were examined for inhibition zones. A ruler was used to measure the inhibition zones.

III. DETECTION OF BIOACTIVE COMPONENTS

DETECTION OF ALKALOIDS

Extracts were dissolved individually in dilute Hydrochloric acid and filtered based on the method of [19].

Mayers test: Filtrate were treated with mayers reagent (potassium mercuric iodide) formation of a yellow coloured precipitate indicate the presence of alkaloids.

Wagners test: Filtrate were treated with wagners reagent (iodine in potassium iodide).Formation of brown/reddish precipitate indicate the presence of alkaloids.

DETECTION OF GLYCOSIDES

Extracts were hydrolyzed with HCl and then subjected to test for glycosides

Modified Borntragers test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volume of benzene .The benzene layer was separated and treated with ammonia solution. Formation of rose-pink in the ammmoniacal layer indicates the presence of anthranol glycosides (Earnsworth *etal.*, 1987).

Glycoside test was carried: 0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

To test the cardiac glycoside phytochemicals presence, in a test tube 5 ml of extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride ($FeCl_3$) solution. Afterwards it was underplayed with 1 ml concentrated sulphuric acid (H_2SO_4). A brown ring of the interface indicates a de-oxy sugar characteristic of cardenolites. 50 mg of extract is hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered, to 2 ml of filtered 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia is added to it. Pink color indicates the presence of glycosides.

DETECTION OF SAPONINS.

Saponins were tested variously as follows:

Froth test: Extracts were diluted with 20ml distilled water and this was shaken in a graduated cylinder for 15 minutes. Formatiom of 1cm layer of foam indicates the presence of saponins.

Foam test: Approximately 0.5g of the extract was shaken with 2ml of water. If foam produced persist for 10 minutes, it indicate the presence of saponins.

Heat test: About 0.2 g of the extract was shaken with 5mlof distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

TEST FOR PHENOLS

To test the Phenol phytochemical presence, in a test tube 1ml of extract and 2 ml of distilled water were added followed by few drops of 10% ferric chloride ($FeCl_3$). Appearance of blue or green colour indicates presence of phenols. The extract (50 mg) is dissolved in distilled water and to this 3ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

TEST FOR THE CARBOHYDRATES (REDUCING SUGARS)

Two methods were used to test for reducing sugars. First, the ethanol extract (1 ml) was added to 1ml of water and 20 drops of boiling Fehling's solution (A and B) in a test tube was added too. The formation of a precipitate red-brick in the bottom of the tube indicates the presence of reducing sugars. Second, To a 2 ml of aqueous solution, 5-8 drops of boiling Fehling's solution were added. A red-brick precipitate showed the presence of reducing sugars. The extract (100 mg) is dissolved in 5 ml of water and filtered. To 0.5ml of filtrate,

0.5ml of Benedict's reagent is added. The mixture is heated on boiling water bath for 2 minutes. A characteristic colored precipitate indicates the presence of sugars.

TEST FOR STEROIDS

Five drops of H_2SO_4 was added to 1ml of each extract in the test tube. The solutions were observed for a red colouration indicating the presence of steroids in the extract.

TEST FOR FLAVONOID

ALKALINE REAGENT TEST

An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids Harborne (Evans *etal.*, 2009).

Extract of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless, indicates the presence of flavonoids.

TEST FOR PROTEINS

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Watch man No.1 filter paper and the filtrate is subjected to test for proteins.

Millon's test: To 2 ml of filtrate few drops of Millon"s reagent are added. A white precipitate indicates the presence of proteins (Dermarderosian and Liberti, 1997).

Biuret test: 2 ml of filtrate is treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour ethanolic layer indicates the presence of protein (Rasch and Swift 1960).

DETECTION OF ANTHRAQUINONES

Borntrager's test: About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink color indicates the presence anthraquinones.

DETECTION OF OILS AND RESINS

Test solution was applied on filter paper a transparent appearance was observed on the filter paper. It indicates the presence of oils and resins.

TEST FOR PHLOBATANNINS

A portion of each extract was boiled with 1% aqueous HCl. The solution was observed for a red deposit of precipitate taken as evidence for the presence of phlobatannins

TEST FOR TERPENOIDS

The extract (0.2g) was mixed with 2ml chloroform, and 3ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown interface was formed which indicated the presence of terpenoids.

THIN LAYER CHROMATOGRAPHY

Phytochemical screening of secondary metabolites namely, alkaloids, terpenoids, steroids, flavonoids etc was done by thin layer chromatography (TLC). Chromatography was performed on 2x5 cm silica gel TLC plates (0.2 mm thickness, 60F254 Merck) plates. The lipophilic extracts of samples of concentration 10 mg/ml were spotted 2 drops per spot on TLC plates. The TLC plates were developed on solvent system n-hexane-ethyl acetate-methanol 1:1, 5:1,7:1 (v/v). For complete development of TLC plates the solvent was allowed to move a distance of 15cm from start of extract spots. The developed TLc plates were dried and sprayed by reagent colour detection method. Dragendroff's reagent was used to screen alkaloids, orange colour spot indicates the presence of alkaloids in Merck (10x10cm) plates. Anisaldehyde sulphuric acid reagent detected terpenoids and steroids. Violet, blue, red, grey or green spot indicates the presence of terpenoids, and steroids [24). The general organic substances were detected by spraying 20% H_2SO_4 in ethanol. Yellow, grey, or black colour of spot indicates the presence of the general organic substances. The TLC plate was sprayed with 20% H_2S0_4 in ethanol. The result showed that roots and stems detected the general organic substances in the range of Rf values between 0.18 to 0.86. The phytochemical profiles of lipophilic extracts in this study were separated using RP -18 Merk. (10x 10 cm plates) plates. The (Rf) value which is the ratio of the distance moved by the solute against the distance moved by the solvent was calculated to obtain various the relative front (Rf) values (Halpaap and Krebs 1980).

$$R_f = \frac{\text{Distance moved by the solute (cm)}}{\text{Distance moved by the solvent (cm)}} \quad \ldots\ldots\ldots\ldots(1).$$

IV. RESULTS AND DISCUSSION

Roots

S/N	Solvent	Colour of extract	Yield of extract	%Yield of extract
1	Ethyl acetate	Brownish green	7.3g	3.65
2	Acetone	Brownish green	6.2g	3.10
3	Ethanol	Brownish green	5.7g	2.85

Table 2: Percentage yield of the extracts Physalis angulata

Stems

1	Ethylacetate	Green	7.0g	6.08
2	Acetone	Green	2.0g	2.35
3	Ethanol	Green	4.0g	4.90

Table 3: Percentage yield of the extracts Physalis angulata

Roots

S/N	Secondary metabolite	Ethylacetate	Acetone	Ethanol
1	Alkaloid	+	-	+
2	Phlobatanin	-	-	+
3	Saponin	-	-	-
4	Steroid	-	+	+

5	Phenol	+	-	-
6	Flavonoid	+	-	+
7	Anthraquinone	+	-	+
8	Glycoside	+	-	-
9	Terpenoid	+	+	+

Table 4: Preliminary phytochemical screening of extract of roots and stem of physalis angulate

Roots

1	Alkaloid	+	-	+
2	Phlobatanin	-	-	+
3	Saponin	-	-	-
4	Steroid	+	+	-
5	Phenol	-	+	+
6	Flavonoid	+	+	+
7	Anthraquinone	+	-	+
8	Glycoside	+	-	+
9	Terpenoid	+	+	+

KEY: + = positive - = negative

Table 5: Preliminary phytochemical screening of extract of roots and stem of physalis angulate

		Roots Extracts			Stems extracts		
	Phytochemicals/ reagents	Ethylacetate	Acetone	Ethanol	EA	AC	ET
1	Alkaloid Wagner test	+	-	+	+	-	+
2	Phlobatanin HCl test	-	-	+	-	-	+
3	Saponin Foam test	-	-	-	-	-	+
4	Steroid H_2SO_4 test	-	+	+	+	+	-
5	Phenol $FeCl_3$ test	+	-	-	-	+	+
6	Flavonoid NaOH+HCl test	+	-	+	+	+	+
7	Anthraquinone Borntragers test	+	-	+	+	-	+
8	Glycoside NaOH test	+	-	-	+	-	+
9	Terpenoid Chloroform + H_2SO_4 test	+	+	+	+	+	+

Key: + =positive, -= negative EA=ethyl acetate, AC=acetone, ET=ethanol

Table 6: Quantitative phytochemicals from roots and stems extracts of physalis angulata

		Micro Organisms (mm)		
S/N	Extract	*Escherichia Coli*	*Salmonella Typhi*	*Pseudomonas aeruginosa*
1	Acetone extract roots	0,0,0 M/SDZH=0 ±0.00	16,23,23 M/SDZH=20.67 ±3.11	27,25,24 M/SDZH=25.33±1.11
2	Ethylacetate extract roots	28,27,30 M/SDZH=28.33 ±1.11	32,35,36 M/SDZH=34.33 ±1.56	28,30,31 M/SDZH=29.67±1.11
3	Ethanol extract roots	0,0,0 M/SDZH=0 ±0.00	15,16,15 M/SDZH=15.33 ±0.44	25,26,26 M/SDZH=25.67±0.44
4	Acetone extract stems	22,23,20 M/SDZH=21.67 ±1.11	13,20,19 M/SDZH=17.33 ±2.89	25,26 ,23 M/SDZH=24.67±1.11
5	Ethylacetate extract stems	24,20,18 M/SDZH=20.67 ±.22	27,28,26 M/SDZH=27.00 ±0.67	28,23,24 M/SDZH=25.00±2.00
6	Ethanol extract stems	13,16,15 M/SDZH=14.67 ±1.11	11,13,10 M/SDZH=11.33 ±1.11	27 ,25 22 M/SDZH=24.67±1.78
7	Positivecontrol-Gentamicin	24,23,24 M/SDZH=23.67 ±0.44	24,25,26 M/SDZH=25.00 ±0.67	24 ,25,23 M/SDZH=24.00±0.67

Key: M/SDZH=mean and standard deviation zone of inhibition of crude extract of Physalis angulate

Table 7: Antimicrobial activities of Physalis angulate showing mean and standard deviation zones of inhibition of crude extracts of Physalis angulata.

Roots

Solvent system	Number of spot	Retention factor (RF)
n-hexane/ethylacetate (1:1)	4	0.20,0.25,0.35,0.40, 0.53
n-hexane/ethylacetate (5:1)	8	0.19,0.22,0.33,0.44,0.52,0.60, 0.70,0.76
n-hexane/ethylacetate (7:1)	3	0.52,0.62,0.72
Methanol/n-hexane (1:1)	3	0.10,0.24,0.32
Methano/n-hexane (5:1)	4	0.14,0.28,0.44,0.92
Methanol/n-hexane (7:1)	1	0.80

Key: Ace= Acetone extract

Table 8: Result of thin Layer chromatography(TLC) in Acetone extract roots (AcE)

Roots

Solvent system	Number of spot	Retention factor (RF)
n-hexane/ethylacetate (1:1)	3	0.14,0.28,0.32
n-hexane/ethylacetate (5:1)	9	0.06,0.18,0.20,0.38,0.54,0.60,0.64,0.72
n-hexane/ethylacetate (7:1)	4	0.16, 0.52, 0.66, 0.72
Methanol/n-hexane (1:1)	5	0.14,0.30,0.42, 0.56,0.98
Methano/n-hexane (5:1)	4	0.50,0.77,0.93,0.95
Methanol/n-hexane (7:1)	4	0.10,0.18,0.32,0.56

Key: EaE= ethylacetae extract

Table 9: Result of thin Layer Chromatography (TLC) in Ethyl acetate extract (EaE)

Roots

Solvent system	Number of spot	Retention factor (RF)
n-hexane/ethylacetate (1:1)	4	0.14,0.28,0.37,0.46
n-hexane/ethylacetate (5:1)	4	0.28,0.38,0.57, 0.77
n-hexane/ethylacetate (7:1)	2	0.29, 0.77
Methanol/n-hexane (1:1)	4	0.25,0.44,0.63,0.75
Methanol/n-hexane	2	0.70, 0.86

(5:1)		
Methanol/n-hexane (7:1)	4	0.29,0.37,0.56,0.74

Key: EtA: Ethanol extract

Table 10: Result of thin Layer chromatography (TLC) in Ethanol extract (EtA)

Stem

Solvent system	Number of spot	Retention factor (RF)
n-hexane/ethylacetate (1:1)	4	0.13,0.16,0.33,0.40, 0.52
n-hexane/ethylacetate (5:1)	3	0.54,0.72,0.77
n-hexane/ethylacetate (7:1)	3	0.20,0.32,0.50
Methanol/n-hexane (1:1)	4	0.17,0.22,0.36,0.67
Methano/n-hexane (5:1)	4	0.19,0.26,0.35,0.52
Methanol/n-hexane (7:1)	4	0.52,0.77,0.92,0.96

Key: AcE= acetone extract

Table 11: Result of thin Layer chromatography (TLC) in Acetone extract roots (AcE)

Stem

Solvent system	Number of spot	Retention factor (RF)
n-hexane/ethylacetate (1:1)	4	0.20,0.24,0.36,0.42, 0.56
n-hexane/ethylacetate (5:1)	8	0.19,0.23,0.34,0.45,0.53,0.60,0.72,0.77
n-hexane/ethylacetate (7:1)	3	0.53,0.63,0.75
Methanol/n-hexane (1:1)	3	0.12,0.26,0.36
Methano/n-hexane (5:1)	4	0.14,0.27,0.43,0.95
Methanol/n-hexane (7:1)	1	0.78

Key: EaE: Ethyl acetate extract

Table 12: Result of thin Layer chromatography (TLC) in ethyl acetate extract (EaE)

Stem

Solvent system	Number of spot	Retention factor (RF)
n-hexane/ethylacetate (1:1)	3	0.12,0.27,0.34
n-hexane/ethylacetate (5:1)	9	0.7,0.19,0.20,0.37,0.57,0.62,0.64,0.74
n-hexane/ethylacetate (7:1)	4	0.16, 0.55, 0.68, 0.74
Methanol/n-hexane (1:1)	5	0.12,0.31,0.44, 0.58,0.97
Methano/n-hexane (5:1)	4	0.50,0.74,0.92,0.96
Methanol/n-hexane (7:1)	4	0.11,0.17,0.33,0.57

Key: EtA: Ethanol extract

Table 13: Result of thin Layer Chromatography (TLC) in Ethanol (EtA)

V. DISCUSSION

The percentage yield of the different extracts of *physalis angulata* obtained from ethylacetate, acetone and ethanol were: 3.65g, 3.10g and 2.85g respectively for root extract and 6.08g, 2.35g and 4.90g respectively for stem. The results obtained from the extracts of both the roots and the stems of *Physalis angulata* using ethylacetate, acetone and ethanol as presented in Tables 2and 3. The Ethylacetate, acetone and ethanol extracts of both the roots and stems were screened for the presence of some phytochemicals such as alkaloids, phlobatanins saponins, steroids, phenols, flavonoids, anthraquinones, glycosides and terpenoids. The results as presented in Tables 4-6 revealed the absence of saponin in all the extracts. Terpenoid was found to be present in all the extrats of both the roots and the stems. Alkaloid was detected in ethylacetate and ethanol of both the roots and stem, but absent in the acetone extract of both roots and stem. Flavonoid was also present in ethylacetate and ethanol but absent in acetone extract. Anthraquinone was detected in ethylacetate and ethanol but absent in acetone extract. The result also shows that phlobatanin was only present in ethanol extract but absent in ethylacetate and acetone. The result of antimicrobial activity as shown in Table 7 obtained from roots and stem extracts of *Physalis angulata* revealed that all the extracts of stems of *Physalis angulata* exhibited or inhibited anti-bacterial activity against *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa*. The same result was shown in the roots extracts except that acetone and ethanol extracts showed no result on *Escherichia coli.* .Ethylacetate extracts show active result against the three laboratory isolates as follows: In roots it shows the highest activity for *Salmonella typhi* test (36mm zone diameter of inhibition) followed by *Pseudomonas aeruginosa* with 31mm zone diameter inhibition and then on Escherichia coli with 30mm zone diameter of inhibition.In the stem extracts ethylacetate extract also show the highest activity against *Salmonella typhi* with 28mm zone diameter of inhibition and *Pseudomonas aeruginosa* with 28mm zone diameter of inhibition . Lastly ethylacetate show inhibition against *Escherichia coli* with 24mm zone diameter. The result of acetone in roots extracts show 27mm zone diameter inhibition on *Pseudomonas aeruginosa*,23mm zone diameter inhibition on *Salmonella typhi* and 0mm on *Escherichia coli*. Ethanol showed 26mm zone diameter inhibition on *Pseudomonas aeruginosa*, 16mm zone diameter inhibition on *Salmnella typhi* and 0mm on *Escherichia coli*. For stems extracts of *Physalis angulata* the result of acetone showed 26mm zone diameter inhibition on *Pseudomonas aeroginosa*, 23mm zone diameter inhibition on *Escherichia coli*, 20mm zone diameter inhibition on *Salmonella typhi*. Again ethanol extract showed 27mm zone diameter inhibition on *Pseudomonas aeroginosa*, 16mm zone diameter inhibition on *Escherichia coli* and 13mm zone diameter inhibition on *Salmonella typhi*. Positive control here refers to the reference point or a guide where the obtained results are compared. Gentamicin was used as a positive control. The results of the thin layer chromatography as presented in Tables 8-13 show that ethyl acetate system has the highest movement of all the phytonutrients , this implies that hexane/ethyl acetate(5:1) is the best system a good solvent for acetone. Chloroform, ethyl

acetate, ethanol, hexane and acetone extracts show equal movement in the solvent system of hexane/ethylacetae (5:1), Hexane/methanol (7:3), Hexane/ethylacetae (7:3) respectively. The choice of compound and the solvent system hence has a special role to play in the extraction of phytonutrients. Polar solvents extracts polar compounds whereas non polar solvent extracts non polar compounds. Again, the quantity of active components required for a particular inhibition of anti-microbial also account the non-effectiveness of Acetone extract and Ethanol extracts on the inhibition of *Escherichia coli*. These classes shows medicinal activity such as anticancer, antibacterial, diabeties, treatment of malaria, anaemia (Gahan,1984).They are also known to show curative activity against several bacteria and it is not suprising that those plant extract are used traditionally by herbalist to cure bacteria related ill health. Mejia and Rengifo, (2000) reported that Tannin act by iron deprivation, Hydrogen bonding or specific interaction with vital protein such as enzyme in microbial cells. The presence of terpenoid that have carboxylic acid group could also be responsible for the activity of organic extracts (Luckins,1998). *Physalis angulata* can be used in the treatment of certain illness because it contain glycosides which have long been employed as important ingredient for arrow poison and drug (Scalbert,1991).The presence of glycoside was detected in *physalis angulate*. Glycosides have been known to lower blood pressure and this effect could be attributed to the presence of steroidal nucleus and deoxy-sugar both of which are present in glycoside and has demonstrated a blood anticoagulant activity (Luckins, 1998). People with blood disorders such as haemophilia, those taking heart medications, or those with other heart problems such as low blood pressure should not use this plant without supervision and advice of a qualified health care practitioner .Alkaloid were detected in all the extract except acetone .Hence *Physalis angulata* can be used as an analgesic, anaesthetic agent and social since it contain alkaloid (Casario,1985). It has been reported that alkaloid has contributed to the majority of the poison neurotoxins and traditional psychedelics and social drugs eg nicotine, caffeine, methamphetamine(ephedrine),cocaine and opiates consumed by human (Cox,1993).

The medical uses of *Physalis angulata* are numerous and a wide variety of species are used for asthma, urinary problems, rheumatism, and tumors are reported. Their anti-inflammatory and anti-spasmodic properties are also known. Shravan *etal.,*(2011), and Ray AB and Gupta (1984) reported some data on therapeutic applications and describe the pharmacological activity of the Physalis species as anti-parasitic, anti-viral, and anti-neoplasic. The presence of the above mentioned phytonutrients and the antimicrobial activity of these compounds in *physalis angulata* is conformity with the reported literature information and represent an important potential source for new effective health care delivery system in Nigeria.

VI. CONCLUSION

The phytochemical screening of *Physalis angulata* crude extracts yielded alkaloids, phlobatannins, steroids, phenols, flavonoids, anthraquinones, glycosides and terpenoids are were found to be present in both the roots and stems extracts except saponin which was not actively present in both the roots and stems extracts of the plant. Flavonoids are known to be potent water soluble super antioxidants and free radical scavengers which prevent oxidative cell damage, inhibit tumor growth and strong anticancer activity. Alkaloids were also detected in ethylacetate and ethanol extracts, hence *Physalis angulata* can be used as an analgesic, anesthetic and social drugs. Terpenoid was detected in all the extracts of both the roots and stem of *Physalis angulata*. The presence of terpeniods that have carboxylic acid group could be responsible for the activity of the organic extracts. Glycosides were also detected in *Physalis angulate* and are known to lower blood pressure and antidiarrheal. This effect could be attributed to the presence of steroidal nucleus and deoxy-sugar. The fact that the plant *Physalis angulata* was active against laboratory isolates is also an indication that it can be a source very of potent antibiotic substance that can be used against drug resistant organisms prevalent in hospital environments. These findings have also confirmed the reported antiflammatory, antispasmodic, an analgesic and diuretic properties which are attributed to terpenoids, anthraquinones, steroids, phenols, flavonoids and glycosides.

CONTRIBUTIONS OF AUTHORS

Asso. .Prof. Egwakihide Peter and Dr. Imarenezor, Edobor Kenneth designed, carried out the analysis and as well proof read the research proposal and draft manuscript of this research. Dr. Ugye Joseph and Ama Shedrack also conducted the research work and wrote the research proposal and the findings draft of this study.

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REFERENCES

[1] Roy, Pannalal. (2009). Rajmalar Tripura. Agartala: Nava Chandana Prakashani.
[2] Das, Nirmal. (2015). Tripuraya Pujaparban: In Anthropological and Folk Cultural Perspective. Agartala: Tripura Bani Prakashani.
[3] Chowdhury, Vasanta., & Ray, Parimal. 'Representation of the Chaturdasa Devatas on a coin of Ratnamanikya deva of Tripura,' JNSI, Vol. XXXVII, 1975, Parts I-II, pp. 121-13.
[4] Das, Ratna. (1985). Socio-Religions Background of Art Heritage. In Jagadis Gan Chaudhuri (Ed.), An Anthology of Tripura (pp. 31-34). New Delhi: Inter – India Publication.

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[5] Tripura, The Land of Fourteen Gods and million statues; http://www.tripura.org.in/hindu.htm