PHYTOCONSTITUENTS SCREENING AND BIOACTIVITY OF *Jatropha curcas* LINN (PHYSIC LEAF) AND *Sarcocephalus latifolius* (AFRICAN PEACH) LEAVES EXTRACTS

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Abstract: The phytoconstituents screening of *Jatropha curcas* Linn **(**physic leaf**)** and *Sarcocephalus latifolius* (African peach) leaves extracts was carried out using solvent systems (aqueous extracts, n-hexane extracts and ethanol). Quantification by thin layer chromatographic and antimicrobial activity against some clinical isolates (*Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*) was also carried out. The results of the solvent systems extracts showed that alkaloids, tannins, glycosides, steroids, phenols, terpenes, flavonoids, saponins and anthraquinones were present in the leaves extracts of the plants but absence of alkaloids, tannins, glycosides, steroids, phenols, terpenes, flavonoids, saponins and anthraquinones in hexane and water extracts of *Sarcocephalus latifolius* and absence of saponins in all the extracts. The quantitative analysis result showed that saponins were more abundant in the two plant samples with the highest quantity in *Jatropha curcas* Linn (80.0%) and *Sarcocephalus latifolius* (48.18%). This was followed by flavonoids with *Jatropha curcas* Linn (25.18%) and *Sarcocephalus latifolius* (13.00%) while Alkaloids were the least phytochemicals with the percentage composition of 5.24% in the *Jatropha curcas* Linn leaves extract and 5.50% in the *Sarcocephalus latifolius* leaves extracts. The antimicrobial analysis showed a zone of inhibition ranging from 15.67±1.15 to 18.33±0.58 against *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*.

Keywords: Phytonutrients, bioactivity, *Jatropha curcas*, *Sarcocephalus latifolius*

Introduction

Natural product chemistry is a branch of chemistry which deals with the identification, isolation, structural elucidation and study of the chemical characteristics of the chemical substances produced by living things. Within the field of organic chemistry, the definition of natural product is usually restricted to mean purified organic compounds isolated from natural sources that are produced by the pathways of primary and secondary metabolism (Hanson, 2013). Natural products have provided biologically active compounds for many years and many of today's medicines are obtained directly from natural source or were developed from a lead compound originally obtained from a natural source (Graham, 2001). Recently, there has been a renewed interest in natural product research due to the failure of alternative drug discovery to deliver many lead compounds in key therapeutic areas such as immune suppression, anti-infective and metabolic diseases (Mark, 2004). Secondary metabolites, also known as phytochemicals, natural products or plant constituents are responsible for medicinal properties of plants to which they belong. Secondary metabolites are a wide range of organic compounds that are not essential for cell structure and maintenance of life but are often involved in plant protection against biotic or abiotic stresses (Weisshar and Jenkins, 1998; Hattenschwiler and Vitousek, 2000). Plants produce a wide diversity of secondary metabolites which serve them as defense compounds against herbivores, and other plants and microbes but also as signal compounds. In general, secondary metabolites are often restricted to a single species or a narrow set of species within a group, whereas primary metabolites are typically found throughout the plant kingdom (Kennedy and Wightman, 2011). Examples of secondary metabolites are terpenoids, alkaloids, phenolic, flavonoids, tannins and saponin. Secondary metabolites exhibit a wide array of biological and pharmacological properties. Because of this, some plants or products isolated from them have been and are still used to treat infections, health disorders or diseases (Michael, 2015).

The antimicrobial ability of these plant extracts and oils has established a platform for the processing and transformation of plant products into pharmaceuticals, preservatives and medicines. The World Health Organization (WHO, 1999) define medicinal plant as any plant which in one or more of its organs, contain substances that can be used for therapeutic purposes, or which are precursors for chemical-pharmaceutical semi-synthesis. Medicinal plants have been used to treat human diseases for thousands of years because they have vast and diverse assortment of organic compounds that can produce a definite physiological action on the human body. Most important of such compounds are alkaloids, tannins, flavonoids, terpenoids, saponins and phenolic compounds. Scientists are interested in these compounds because of their medicinal (therapeutic) performance and low toxicity and harm (Inyayatulah *et al.*, 2012). A number of such compounds have been isolated from plants which could be used for the development of new drugs to inhibit the growth of bacterial and fungal pathogens and to quench reactive oxygen species (ROS) with possibly novel mechanisms of action and low toxicity to the host cell (Ahmad and Aquil, 2007).

Jatropha curcas Linn and *Sarcocephalus latifolius* (African peach) have been used as a folkloric medicine by the local inhabitants of Taraba state, particularly among the Tiv and Jukun communities. *Sarcocephalus latifolius* species has medicinal uses that are much more known in sub Saharan Africa in the traditional pharmacopoeia (Badiaga, 2011). It is used in the treatment of certain major diseases such as diabetes (Karou *et al.*, 2011) HIV (Lamorde *et al.*, 2010) and malaria (Benoit-Vical *et al.*, 1998). *Jatropha curcas* species belong to the family *Euphorbiaceae* and are used in the traditional folkloric medicine to cure various ailments in Africa, Asia and Latin America (Burkill, 1994). *Jatropha curcas* has been reported to be effective in the cure of fever, mouth infections, jaundice, guinea worm, sores and joint rheumatism (Fagbenro-Beyioku, 1998). Many *Jatropha* species possess antimicrobial activity (Aiyela-Agbe *et al.*, 2000). The aqueous extract of the branches have been

reported to strongly inhibit HIV induced cytopathic effects with low cytotoxicity (Matsuse *et al.*, 1999).and herbal medicines for their primary care. In Africa up to 90% and in India 70% of the population depends on traditional medicine to help meet health care needs. Some of the pharmaceutical products currently available to physicians are derived from plants that have a long history of use as herbal remedies, including aspirin, digoxin, quinine and opium (Swain *et al.*, 1968).

Materials and Methods

Sample collection

The leaves of the plants analyzed were collected from their natural habitat in Wukari Local Government of Taraba State, Nigeria with coordinates 8^0 22' 00"N, 12^0 4' 60" E. The samples were dried for two weeks then milled into fine powder using milling machine.

Extraction

Cold maceration using one solvent: this was done by soaking 20 g of each of the samples in 200 ml of solvent (n-hexane, ethanol and water) for four days with frequent agitation in order to get the leaves extract. The resulting mixture was filtered using a filter paper and concentrated by allowing the solvent (n-hexane) evaporate in an open air and ethanol was concentrated using the rotary evaporator and water extract was concentrated in a water bath at 60 ˚C. The extract was kept in a refrigerator until required for testing.

Phytochemical screening

A preliminary screening of each extract (aqueous extracts, n-hexane extracts and ethanol extracts) of the two plant samples (*Jatropha curcas* and *Sarcocephalus latifolius*) was performed following the standard phytochemical analysis protocol described by Ushie *et al*. (2016), Egwaikhide and Gimba (2007) and Geetha *et al*. (2014) to identify the types of secondary metabolites present in them.

Antimicrobial activity

Antimicrobial susceptibility was carried out using three clinical isolates (*Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli*) based on the well diffusion method to detect the presence of antibacterial activities of the plant samples (Perez *et al.*, 1990). A sterile swab was used to evenly distribute bacterial culture over the appropriate medium (Muller Hinton Agar). Muller Hinton Agar was prepared as per the instructions by the manufacturer. The plates were allowed to dry for 15 min 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 30 µl of the extract into the well. A total of two 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 37ºC for 24 h after which they were examined for inhibition zones.

Results and Discussion

The hexanic, aqueous and ethanolic extracts of *Jatropha curcas* and *Sarcocephalus latifolius* were screened to determine the presence or absence of secondary metabolites like terpenoids, alkaloids, flavonoids, phenols, tannins, anthraquinons, steroids and glycosides, saponins. The results of the analysis carried out on the above listed four plant extracts are shown in the Tables 1 – 7, respectively.

The phytochemical analysis of the bioactive components in the leaves extracts of *Sarcocephalus latifolius* and *Jatropha curcas* are as shown in Tables 1 – 3. The results provide evidence for the presence of alkaloid, tannins, glycosides, steroids, phenols, terpenes, flavoids, saponins and anthraquinones in selected extracts and absence of alkaloid, tannins, glycosides, steroids, phenols, terpenes, flavoids, saponins and anthraquinones in hexane and water extracts of *Ocimum gratissimum*. The presence of tannins in all the plants extracts could attribute to the use of such plants as antiseptic and as astringents against diarrhea (Fujiki *et al*., 2012). Glycosides found in these plants encouraged the use of those plants in the treatment of arrow poison pharmacologically (Trease and Evans, 1989).

The result was positive for steroid in all extracts indicating that the plants are of importance in the pharmacy due to sex hormones (Okwu, 2001). The presence of terpenoids that have carboxylic acid groups could also be responsible for the activity of the organic extract (Njoku and Obi, 2009). These phytochemicals are biologically active and can be responsible for the antimicrobial activity of the plants (Oyama *et al.*, 2016).

Table 1: Phytochemical analysis of *Sarcocepalus latifolus* leaves extracts

Phytochemicals	Aqueous extract	Hexane extract	Ethanol extract
Alkaloids	+	+	+
Saponins	+	-	+
Flavonoids	++	+	+
Tannins	++	+	+
Glycosides	++	+	++
Steroids	+	-	+
Anthraquinones	-	-	-
Phenols	+	+	+
Terpenes	+	-	-

(-) = Indicate the absence of a phytochemical, (+) = indicates the presence of a phytochemical, (++) = indicate that phytochemical is highly/intensively present

Table 2: Phytochemical analysis of *Jatropha curcas* leaves extracts

Phytochemicals	Aqueous extract	Hexane extract	Ethanol extract
Alkaloids	+	+	-
Saponins	+	-	-
Flavonoids	++	+	+
Tannins	++	+	+
Glycosides	+	+	+
Steroids	+	+	+
Anthraquinones	-	-	-
Phenols	-	-	-
Terpenes	++	+	+

Table 3: Quantitative phytochemical analysis of *Jatropha curcas*, and *Sarcocephalus latifolius* leaves extracts

Phytochemicals	*Jatropha curcas,*	*Sarcocephalus latifolius*
Alkaloids	5.24	5.50
Flavonoids	25.18	13.00
Saponins	80.0	48.18

Table 4: Antimicrobial sensitivity testing of hexane, aqueous and ethanol extract of *Jatropha curcas* and *Sarcocephalus latifolius*

Extracts	*E. coli*	*S. typhi*	*P. aeroginosa*
AQSLE	10.00±1.00	18.67±1.15	11.67±1.5
AQJCE	0.00	0.00	0.00
HEJCE	8.00±3.79	0.00	0.00
HESLE	9.67±1.53	0.00	0.00
ETJCE	0.00	17.33±4.16	0.00
ETSLE	12.33±2.52	0.00	20.67±3.05
Erythromycin (control)	15.67±1.15	16.00±1.00	18.33±0.58
PEF (control)	26.67±1.53	28.33±0.58	25.33±2.52

AQSLE= Aqueous *Sarcocephalus latifolius* extract, AQJCE= Aqueous *Jatropha curcas* extract, HEJCE= hexane *Jatropha curcas* extract, HESLE=Aqueous *Sarcocephalus latifolius* extract, ETJCE= Ethanol *Jatropha curcas* extract, ETSLE = Ethanol *Sarcocephalus latifolius* extract. Value represents mean±standard deviation of three replicates. The significant difference between means was measured at P(< 0.05).

The antimicrobial analysis was carried out using erythromycin as a positive control drug to compare the results of the plants extracts as presented in Table 4. Erythromycin showed a zone of inhibition ranging from 15.67±1.15, 16.00+1.00 and 18.33±0.58 against *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*. *Salmonella typhi* recorded the highest zone of inhibition with hexane extract of *Lavandula angustifolia* followed by aqueous or water extract of *Sarcocephalus latifolius* and ethanol extract of *Jatropha curcas* with zones of inhibition of 20.67±1.15, 18.67±1.15, 17.33±4.16, respectively. This finding indicates a possible use of such extracts as an alternative remedy for *Salmonella typhi* associated diseases such as typhoid, malaria, fever. These support the report of Odama *et al*. (2016) which found that *Pseudomonas aeruginosa* was actively inhibited in ethanol extract of *Sarcocephalus latifolius* with inhibition zone of 20.67±3.05 more than erythromycin drug (18.33±0.58) which was used as control indicating the possible use of this plant extract as remedy against nosocomial infections. These findings indicates that nosocomial infections such as urinary tract infections, respiratory system infections, dermatitis, gastrointestinal infections can be effectively treated with these plants extracts since they exhibit the growth of this bacteria especially ethanolic extract of *Sarcocephalus latifolius* whose antimicrobial activity could be attributed to the presence of phenols, tannins and flavonoids (Alsiddig *et al*., 2017). Aqueous extract of *Sarcocephalus latifolius* and ethanolic extract of *Jatropha curcas* can effectively serve as a substitute to erythromycin drug.

A quantitative examination was carried out and the result shown in Tables 5 – 7, to determine the amount of three different phytochemicals including alkaloid, flavonoid and saponins. The quantification of the phytochemicals The result showed that saponins was more abundant in all the plant samples with the highest quantity recorded in *Sarcocephalus latifolius* (80% saponin) followed by *Lavandula angustifolia* (56.14% saponin), *Jatropha curcas* (48.18% saponin) then *Ocimum gratissimum* (24.78% saponin). The abundant nature of saponins in this plant could be responsible for the phytoanticipins or phytoprotectant nature of the plant alongside the antimicrobial activity observed in these plants (Yoshiki *et al*., 1998; Lacaille *et al*., 2000).

Table 5: Results of thin layer chromatography for aqueous extracts

Sample	Solvent system	Number of component	Retention factor
Sarcocephalus latifolius	n-hexane : ethyl acetate (1:1)	Nil	……
	n-hexane : ethyl acetate (5:1)	5	0.22, 0.88, 0.79, 0.95, 0.98
	n-hexane : ethyl acetate (7:3)	3	0.35, 0.87, 0.97
Jatropha curcas	n-hexane : ethyl acetate (1:1)	2	0.15, 0.35
	n-hexane : ethyl acetate (5:1)	4	0.10, 0.16, 0.38, 0.69
	n-hexane : ethyl acetate (7:3)	7	0.18, 0.48, 0.52, 0.58, 0.80, 0.84, 0.90

Table 6: Results of thin layer chromatography for hexane extracts

Sample	Solvent system	Number of component	Retention factor
Sarcocephalus latifolius	n-hexane : ethyl acetate (1:1)	2	0.62, 0.97
	n-hexane : ethyl acetate (5:1)	5	0.14, 0.23, 0.31, 0.46, 0.53
	n-hexane : ethyl acetate (7:3)	6	0.10, 0.20, 0.32, 0.68, 0.77, 0.94
Jatropha curcas	n-hexane : ethyl acetate (1:1)	3	0.82, 0.98, 0.98
	n-hexane : ethyl acetate (5:1)	2	0.11, 0.96
	n-hexane : ethyl acetate (7:3)	6	0.13, 0.47, 0.56, 0.66, 0.78, 0.91

Table 7: Summary of the thin layer chromatography comparing different solvent systems

Extracts	Number of components Per solvent system (Hexane:Ethyl acetate) 1:1	Number of components Per solvent system (Hexane:Ethyl acetate) 5:1	Number of components Per solvent system (Hexane:Ethyl acetate) 7:3
HELAE	2	7	7
AQLAE	6	5	8
HEJCE	3	2	6
AQJCE	2	4	7
HESLE	2	5	6
AQSLE	0	5	3
HEOGE	1	3	7
AQOGE	0	5	5

All the extract from *Sarcocephalus latifolius* (i.e. aqueous or water, hexane and ethanol) activity worked against the growth of *Escherichia coli* with highest zone observed in ethanolic extract 12.33±2.52, 10.00±1.00 mm for aqueous water extract and 9.67±1.53 mm for hexane extract respectively. This was in agreement with the work carried out by Shafaghat *et al*., 2011. The ability of these plant extracts to inhibit the growth of *Escherichia coli* indicates that this plant drug can be used in the treatment of gastroenteriti (Burkill, 1994) as been observed in Table 7. All the aqueous extracts of *Sarcocephalus latifolius* and *Jatropha curcas* showed no activity against the test organisms. Also ethanol and hexane extract of *Jatropha curcas* showed no activity against *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*, respectively. The inability of most of the aqueous extract to show antimicrobial activity could be because water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolics are only important as antioxidant compounds. Though, traditional healers use primarily water but plant extracts from organic solvents give more consistent antimicrobial activity compared to water extracts (Zhang *et al*., 2008).

Conclusion

This present study found different phytochemicals such as alkaloid, flavonoids, tannins, glycosides, steroids, phenols, terpenes, saponins, anthraquinones among others in the two plants studied. The quantification of some of those phytochemicals was also evaluated using thin layer chromatography The result obtained indicates that *Sarcocephalus latifolius* and *Jatropha curcas* have high potential as drug candidates especially against *Salmonella typhi* associated diseases since they showed more activity than the commercial drug (erythromycin) which was used as a control. *Sarcocephalus latifolius* exhibited strong antibacterial activity against *Pseudomonas aeruginosa* and generally all the plants showed strong antibacterial property. Thus, this study indicates that these plants show much promise in the development of phytomedicines due to their antibacterial properties.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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