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Phytochemical Screening and Antimicrobial Activity of the Aerial Part of Three Selected Plants

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ABSTRACT
We investigate the antimicrobial activity and phytochemical screening of the aerial parts *of Annona senegalensis*, *Petiveria alliacea* and *Secamone afzelii* that have been used as folk medicines. The methanolic extracts were tested on various microorganisms for the antibacterial and antifungal activity using agar well diffusion and poisoned food technique respectively. The length of the inhibition zone was measured in millimetres from the edge of the well to the edge of the inhibition zone. The extracts were assessed in an effort to validate the potential activity of the plants against microbes. The result showed the extracts possess considerable antimicrobial potentials. The phytochemical screening of the plants revealed the presence of alkaloids, flavonoids, tannins, steroids, terpenoids, saponins and anthraquiones. The phytochemicals attributed to the antimicrobials activity of the plants extracts
Keywords: antibacterial, antifungal, *Annona senegalensis*, *Petiveria alliacea*, *Secamone afzelii*

1. Introduction

Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements and cosmetics. Since ancient times, people have been exploring the nature particularly plants in search of new drugs which has resulted in the use of large number of medicinal plants with curative properties to treat various diseases (Verpoorte, 1998). According to WHO survey, 80% populations living in the developing countries rely exclusively on traditional medicine for their primary health care needs of which most involve the use of plant extracts (Sandhya *et al*., 2006). The studies of plants continue principally for the discovery of novel secondary metabolites or phytochemical which is the non-essential nutrients derived from plants exhibiting a number of protective functions for human beings (Neethu and Neethu, 2016). The medicinal value of plants depends on chemical substance present in them which possess distinct physiological action on the human and animal system. The microorganisms have developed resistance against many antibiotics due to the indiscriminate use of antimicrobial drugs (Ahmad *et al*., 1998). Antibiotics are sometimes associated with side effects (Cunha, 2001) whereas there are some advantages of using antimicrobial compounds of medicinal plants, such as often fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature (Vermani and Garg, 2002). The most important classes of these bioactive constituents of plants are alkaloids, flavonoids, tannins, saponins, terpenoids and phenolic compounds (Hill, 1952). Generally referred to as phytochemicals or metabolites (Okwu, 2005; Iwu MM *et al*., 1999).

Petiveria alliacea is a plant from the family Phytolaccaceae, known by different names in different countries of Central and South America, the Caribbean and Africa. For hundreds of years it has been used for pain relief, and as an anti-influenza, anti-inflammatory, anti-tumor, anti-bacterial, anti-fungal, anti-hyperlipidemia, and anti-diabetic drug (Tropical Plant Database-Anam, 2011). This plant also grows in Indonesia, but it has not been used extensively. It is traditionally used in Indonesia as an analgesic, anti-inflammatory and for treatment of hemoptysis (Mulyani *et al*., 2012). Widowati, (2007) and Weniger *et al*., (1986) reported that *P. Alliacea* can reduce the length of therapy with standard drugs in tuberculosis patients. Several volatile compound like; benzyl-2-hydroxyethyl trisulfide (Szczepanski *et al*., 1972), cis-3,5-diphenyl-1,2,4-trithiolan (trithiolaniacin), benzaldehyde, benzoic acid, elemental sulfur, and trans-stilbene (Adegosan, 1974), dibenzyltrisulfide (Sousa *et al*.,1990) and benzaldehyde, benzyl alcohol, cis- and trans-stilbenes, benzyl benzoate, dibenzyldisulfide, and dibenzyltrisulfide (Ayedoun *et al*., 1998) had been isolated from *P. alliacea. P. Alliacea* is also known as skunk weed due to its characteristic odour resulting from the presence of sulfurate compounds (De Sousa *et al*., 1990). It has been reported that *P. Alliacea* exhibited antirheumatic, anticarcinogenic, antiflu, antitussive, analgesic and antiinflammatory (Villar *et al*., 1997).

*Annona senegalensis*is a subtropical plant (Okoli *et al*., 2010) that has been implicated for the treatment of chest pain, coughs, anaemia, urinary tract infection (Burkill, 1985 and Muanze *et al*., 1994], cancer treatment

[Durodola *et al*., 1975 and Fatope *et al*., 1993], diarrhoea, dysentery (Ekpenda *et al*., 1998 and Kudi and Myint, 1999), anthritis and rheumatism (Dalziel, 1937 and Audu, 1989).The isolation of monotetrahydrofuran and bis-tetrahydrofuranacetogenins (Sahpaz *et al*., 1994) and two cytotoxic monotetrahydrofuranacetogenins (Sahpaz *et al*., 1996) from this plant are also documented.

Secamone afzelii is a creeping woody climber with pinnately compound leaves. It is often seen as a nuisance to other plants or crops because of its domineering spread wherever it grows (Tavs and Doris, 2012). The root is said to be poisonous but is used, more the less, by the Zulu medicine man as a remedy for spinal disease (Watt and Breyer–Brandwijk, 1962). *S. afzelii* is used by native people as an anti-inflammatory, anti-bacterial and tonic drug. Latex of *S. afzelii* is traditionally used in various skin diseases (boils, abscesses and eruptions) Bitter sap of stems and leaves of *S.afzelii* is used as a stomachic and purgative and diuretic drug. Crushed *S.afzelii* is used for cooking food for gonorrhoea patient (abbiw, 1990). Hervé *et al*., (2008) reported the presence of Flavonoids, Saponins, Reducing sugars, coumarines, alkaloids, proteins, tannins, steroids and polyterpenes and Quinones in the methanol extract of leave and stem of *S. afzelii.* The objective of this study was to evaluate the phytochemical screening and antimicrobial activity of the selected plants

2.0 Materials and Method

2.1 Sample Collection and Preparation

Fresh aerial parts of the plants were collected in their natural habitats. Locations where samples were collected include Akure in Ondo State, Ajilete in Ogun State, Iwo in Osun State and Ibadan in Oyo state. The samples were then air dried in an open ventilated room, to ensure they retain their natural feel and major constituents. After proper drying, they were pulverised into powdery form.

2.2 Extraction Process

700g of each of the dried and pulverized plant material were weighed and poured in 5ltr glass flasks. 2.5ltr N-Hexane was poured in each and left for 24hrs. The N-hexane was used to de-fat the plant materials. The solvent was decanted after 24hrs and 2.5ltr Methanol was thereafter poured in each flask and left for 72hrs. The extracts were taken to MCRL laboratory in University of Ibadan where they were concentrated using Rotary evaporator. The yield was 3.36% for *Anonna senegalensis*, 2.64% for *Petiveria alliacea* and 3.06% for *Secamone afzelli*

2.3 Phytochemical Screening

The phytochemical investigation of the methanol extracts of *Anonna senegalensis, Petiveria alliacea and Secamone afzelli* was carried out using standard protocol.

2.4 Test Microorganisms

The microorganisms of choice used for antibacterial activity are *Staphylococcus aureus, Bacillus subtilis, Sylmonela typhimurium, Enterococcus spp, Klebsiela aerogenes, Escherichia coli, Pseudomonas aeruginosa, Pseudomonas syringine, Xantomona saxonopodis, Shigella dysantrioue,* while the microorganism used for antifungal activity are *Erwinia herbicola, Fusariumoxyporium, Marcophomina phomoides, collectotrichum lindimathiumum, Ceratocystis paradoxa and Helminthoporuin toxicum.*

2.5 Antimicrobial Activity

The stock culture of these organisms had already been identified and typed. All bacteria were cultured aerobically at 37^{oc} for 24hrs on peptone water and antimicrobial testing were carried out on the nutrient agar (NA) plates. All fungi were also grown aerobically at 27^{oc} of Potato Dextrose Agar (PDA) plates Agar well diffusion methods of Murray *et al* (2000) modified by Olurinola (2004) was employed for this study. 20ml of nutrient agar was dispensed into sterile universal bottles. Then inoculated with 0.2ml of bacteria cultures mixed gently and poured into sterile petri-dishes. After setting, a number 3-cup borer (6mm diameter) was properly sterilized by flaming and used to make three to five uniform cups/wells in each petri dish. A drop of molten nutrient agar was used to seal the base of each cup. The cups/wells were filled with 50ul of the extract concentration and allowed to diffuse for 45minutes. The solvent used for reconstituting these extracts were similarly plated. The plates were incubated at 37^{oc} for 24hrs for bacteria. The zones of inhibition were measured with Digital Vernier Callipers in mm. The experiment was carried out in triplicate.

Antifungal activities of the samples were carried out by poisoned food technique. 5ml each of the samples was mixed with 20ml of PDA separately before pour plated and allowed to solidify at ambient temperature.A 5mm disc cut from the periphera of 7-day old culture of the test fungi was inoculated in the center of the PDA plates (mixture of PDA and sample). A negative and positive control experiment was set up separately containing distil water and reference antifungal fungicides (Koside). Both plates were incubated aseptically at 27^{oc} for 72 – 96 hrs. Mycelinl growth of the isolates were measured with the aid of Digital Venier Calipers and the mycelinl growth inhibition (in percentage) was calculated and recorded appropriately by the formula :

$$\frac{dc - dt}{dc} \times 100$$

Where dc = diameter of bacterial/fungal colony in negative control sets

dt = average diameter of bacterial/fungal colony in the set up containing experimental plants

The experiment was carried out in triplicate.

3. Result

3.1 Phytochemical screening

The result of phytochemical screening of the methanolic extract of the selected plant were presented in Table1

3.2 Antibacterial Activity

The result of antibacterial activity omethanolic extract of the selected plants were presented in Table 2

3.3 Antifungal Activity

The result of antifungal activity of methanolic extracts of the selected plants were presented in Table 3

4. Discussion

The yield of the extract was as follows 3.36% for *Anonna senegalensis*, 2.64% for *Petiveria alliacea* and 3.06% for *Secamone afzelli*.

The methanol extracts of *Secamone afzelli, Anonna senegalensis and Petiveria alliacea* showed the presence of alkaloids, flavonoids, tannins, steroids, terpenoids, anthraquinones and saponins. Cardiac glycosides was absent in all the plants

Antibacterial and antifungal activities of methanol extract of *Petiveria alliacea, Secamone afzelii* and *Annona senegalensis* were tested against ten bacteria and five fungi. The results of antibacterial and antifungal activities are presented in table 2 and 3. The extracts recorded promising activities against the tested organisms. The highest activity of *Secamone afzelii* was 9.99±2.00mm against *Bacillus subtilis* and its lowest activity was 3.63±0.41mm against *Escherichia coli*. 7.62±0.45mm was the highest diameter of inhibition recorded by *Petiveria alliacea* and 2.20±1.00mm was its lowest diameter of inhibition.

For the antifungal activities of the extracts, *Secamone afzelii* recorded highest activity against *Fusarium oxyporium* with percentage mycelinl growth of inhibition of 69.35±2.61nm. *Annona senegalensis* recorded highest activity against *Marcophomina phomoides* with percentage mycelinl growth of inhibition of 61.77±1.53mm, percentage mycelinl growth of inhibition of 70.86±1.20nm against *Ceratocystis paradoxa* and 70.31±1.37mm percentage mycelinl growth of inhibition against *Collectotrichum lindimutianum*. *Secamone afzelii* recorded percentage mycelinl growth of inhibition against *Helminthosporium toxicum with the value of 76.97±0.93%.* The antibacterial and antifungal activities can be attributed to the presence of phytochemicals such as flavonoids, tannins, alkaloids, steroids, coumarines, cardiac glycosides, quinines and terpenoids in the plants extract (Rocha and Silva, 1969; Jolad *et al*., 1984; Adzu *et al*., 2005;Tavs and Doris, 2012). The antimicrobial properties exhibited by the extracts may be associated with the presence of tannins, saponins and alkaloids found in the plant extracts. A large number of flavonoids have been reported to possess antimicrobial properties (Bastista *et al.,* 1994; Tsuchiya *et al*., 1996; Boris, 1996; Olowusulu and Ibrahim, 2006; Akinjobi *et al*., 2006). Tsuchiya *et al.,* (1996) attributed the antimicrobial activities of flavonoids to their ability to complex with extracellular and soluble proteins as well as their ability to complex with bacterial cell walls. They suggested that more lipophylic flavonoids exert antimicrobial activity by disrupting microbial cells (Flora and Folasade, 2008). Herbs that have tannins as their component are astringent in nature and are used for the treatment of gastrointestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003; Asguith and Butler, 1986). Saponins believed to be responsible for numerous pharmacological properties (Estrada *et al*., 2000) and have been shown to have immense significance as anti hypercholesterol, hypotensive and cardiac depressant properties (Price, 1987). Waterman (1992) reported that alkaloids and flavonoids were useful as antimicrobial, anti-inflammatory and anti-oxidant agents.

5. Conclusion

The result obtained from this study has shown that phytochemical screening of the methanolic extract of *Petiveria alliacea, Secamone afzelii* and *Annona senegalensis* reveal the presence of alkaloids, Flavonoids, saponins, tannins, anthraquinones, terpenoids, and Steroids. The extracts also demonstrated significant antimicrobial and antifungal activities against the tested organisms.Therefore more detailed studies are needed to isolate, characterized and evaluate the active components and the mechanism of action.

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Table1: Phytochemical screening of methanol extracts of *Secamoneafzelli, Anonna senegalensis*and *Petiveriaalliacea*

Test		Plant extracts		
		Secamoneafzelli	*Anonnasenegalensis*	*Petiveriaalliacea*
Alkaloid	Dragendorff's reagent	+ve	+ve	+ve
	Mayer's reagent	+ve	+ve	+ve
	Wagner's reagent	+ve	+ve	+ve
Flavonoids	Ammonia/H_2SO_4	+ve	+ve	+ve
	Aluminium solution	+ve	+ve	+ve
	Ethyl acetate/Ammonia	+ve	+ve	+ve
Saponins	Frothing	+ve	+ve	+ve
Tannins	Ferric chloride	+ve	+ve	-ve
Anthraquinones	Chloroform/ammonia	+ve	+ve	+ve
Terpenoids	Chloroform/H_2SO_4	+ve	+ve	+ve
cardiac glycosides	Keller- Kiliani test	-ve	-ve	-ve
Steroids	Chloroform/acetic anhydride/H_2SO_4	+ve	+ve	+ve

Table 2: Antibacterial activity of the methanol extracts of *Secamone afzelli, Anonna senegalensis* and *Petiveria alliacea*

Test organisms	Diameter Zone of Inhibition in mm			
	Secamone afzelii	*Petiveria alliaecea*	*Annona senegalensis*	*Streptomycine sulphate* (control)
Staphylococcus aureus	$7.91^{b}\pm0.61$	$7.62^{c}\pm0.45$	$6.58^{abc}\pm1.27$	$23.89^{a}\pm4.42$
Bacillus subtilis	$9.99^{b}\pm2.00$	$7.53^{c}\pm0.76$	$8.46^{bc}\pm1.67$	$22.13^{a}\pm4.99$
Sylmonelatyphimurium	$8.57^{b}\pm0.41$	$6.74^{bc}\pm0.87$	$9.35^{bc}\pm1.15$	$23.85^{a}\pm4.11$
Enterococcus spp	$8.63^{b}\pm0.81$	$6.87^{bc}\pm0.58$	$9.80^{bc}\pm0.79$	$28.45^{a}\pm1.93$
Klebsiela aerogenes	$8.90^{b}\pm1.11$	$6.40^{bc}\pm0.76$	$6.97^{c}\pm0.49$	$29.00^{a}\pm3.21$
Escherichia coli	$3.63^{a}\pm0.41$	$3.77^{ab}\pm1.21$	$10.10^{a}\pm0.52$	$17.88^{a}\pm0.65$
Pseudomonas aeruginosa	$8.64^{b}\pm0.07$	$4.65^{abc}\pm0.90$	$4.96^{a}\pm1.19$	$28.84^{a}\pm1.11$
Pseudomonas syringine	$3.74^{a}\pm0.41$	$2.20^{a}\pm1.00$	$6.27^{a}\pm0.14$	$29.25^{a}\pm0.32$
Xantomona saxonopodis	$7.49^{ab}\pm0.25$	$7.13^{bc}\pm0.43$	$5.23^{a}\pm0.28$	$29.39^{a}\pm0.65$
Shigella dysenteriae	$7.09^{ab}\pm1.23$	$5.07^{abc}\pm0.13$	$5.56^{a}\pm0.33$	$26.43^{a}\pm1.23$
Erwinia herbicola	$6.13^{ab}\pm0.49$	$5.29^{abc}\pm0.23$	$4.98^{a}\pm0.59$	$21.82^{a}\pm 2.04$

Values are means of duplicate ± standard error. Column means followed by the same superscript letters are not significantly different at $P<0.05$.

Table 3: Antifungal activity of methanol extracts of *Secamone afzelli, Anonna senegalensis* and *Petiveria alliacea*

Test organisms	Mycelinl Growth Inhibition in Percentage			
	Secamone afzelii	*Petiveriaalliae cea*	*Annona senegalensis*	*Koside* (control)
Fusarium oxyporium	$69.35^{c}\pm2.61$	$67.34^{d}\pm1.34$	$68.86^{b}\pm2.10$	$86.89^{b}\pm0.95$
Marcophomina phomoides	$34.11^{a}\pm0.91$	$13.88^{a}\pm0.09$	$61.77^{a}\pm1.53$	$73.71^{a}\pm0.90$
Ceratocystisparadoxa	$53.41^{b}\pm0.56$	$69.60^{d}\pm0.54$	$70.86^{b}\pm4.20$	$87.40^{b}\pm0.33$
Collectotrichumlindimutian um	$57.52^{b}\pm0.47$	$50.74^{b}\pm1.05$	$70.31^{b}\pm1.37$	$88.64^{b}\pm2.00$
Helminthosporiumtoxicum	$76.97^{d}\pm0.93$	$63.37^{c}\pm0.33$	$74.59^{b}\pm0.56$	$90.74^{b}\pm2.33$

Values are means of duplicate ± standard error. Column means followed by the same superscript letters are not significantly different at $P<0.05$.