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Secondary Metabolite Constituents and Antibacterial Potency of *Physalis angulata* against some Clinical Isolates

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Abstract: *Physalis angulata* is a specie of the Solanaceae family, in which edible fruit is used in several countries of tropical and subtropical regions of the world as medicinal and fruit-tree. In this study, the secondary metabolite screening of the crude extracts consisting of (ethanol, ethylacetate, chloroform and n-hexane) of the whole plant and antibacterial activity was investigated against some clinical isolates. The screening of the secondary metabolites revealed some active components such as alkaloids, tannins, terpenoids, phenolics and flavonoids. The antimicrobial screening of the ethanolic extract of the plant at concentrations of 25, 50, 100 and 125 mg/ml revealed various zones of inhibition from 8.2 to 17.2 mm for the different organisms. In conclusion, the extract of *Physalis angulata* exhibited antibacterial activity made possible by the presence of the secondary metabolite constituents and therefore could be used as antibiotic against the bacteria strains investigated in this study.

KEYWORDS: *Physalis angulata*, Solanaceae, Antibacterial, Clinical isolates

1.0 Introduction

The use of natural products in the treatment of a variety of diseases has increased due to the considerable number of medicinal plants with proven biological activities applicable to the treatment of some diseases.

Physalis angulata L., (family: Solanaceae), known in the Peruvian, Amazon as mullaca, is a much branched annual shrub distributed in many tropical countries and subtropical regions of the world. It can grow until it reaches 1.0 m. The flowers are bell shaped but the most distinctive feature is the fruiting calyx which enlarges to cover the fruit and hangs downward like a lantern. Each fruit is like a yellow pearl into a lantern shaped pod (Mejia and Rengifo, 2000).

Physalis angulata is used in many part of the world to treat several diseases such as cancer, bacteriemia, tumors, gonorrhea, diabetes

(macerated roots), infertility (fruits), malaria (Ruiz *et al.*, 2011), diuresis, inflammation of the bladder, asthma (leaf infusion), hepatitis (root infusion) Mejia and Rengifo, 2000), rheumatism, stomach ache, prostrate and kidney stones, (Coelho-Ferreira, 2009), anaemia, to prevent abortion, and in reducing fever. In Peru, the decoction of the leaves and fruits is used for "Tertian" due to postpartum infections (Jovel *et al.*, 1996). Their anti-inflammatory and antispasmodic properties are also known (Silva *et al.*, 2005).

Phytochemical studies on *P. angulata* revealed that it contains flavonoids, alkaloids and many different types of plant steroids. The main component are Physalins B, G, D, E, F, H, I, K, U and V isolated from root, stems and leaves of *P. angulata* (Damu *et al.*, 2007; Chen *et al.*, 2011); withanoloides A, B, C and D were obtained from the methanolic extract of the fresh leaves, stems and aerial parts of *P. angulata* (Shingu *et al.*, 1991, 1992; He *et al.*, 2007) and carotenoids (De Rosso and Mercadante, 2007).

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Its biological properties include antimicrobial, anticancerous, antitumorous, antimalarial, anti-inflammatory, antileishmanial, hypotensive and immunosuppressive activity (Helvacı *et al.*, 2010; He *et al.*, 2007; Ferreira *et al.*, 2006; Sa *et al.*, 2011; Pinto *et al.*, 2010; Guimaraes *et al.*, 2010; Yu *et al.*, 2010).

Due to the dearth of information on the antibacterial activity of the ethanolic extract of *Physalis angulata* whole plant, this study therefore reports on the secondary metabolite constituents of some solvent extracts of the whole plant and the effects of the ethanolic extract on some clinical isolates.

2.0 Materials and Methods

2.1 Plant Material

Fresh whole plant of *Physalis angulata* was collected from Odo-Isuti Egan, Lagos State, Nigeria in December, 2013. The plant was identified at the Plant Biology Department of the University of Ilorin, Nigeria.

2.2 Test Organisms

The test organisms used for this research, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were gotten from the Microbiology and Parasitology Laboratory of the University of Ilorin Teaching Hospital, Ilorin, Nigeria.

2.3 Preparation of Ethanolic Extract

This was air-dried for two weeks and grounded into powder. Two litres of ethanol was added to 250 g of powdered *Physalis angulata* and was cold extracted for a week. This was filtered using Whatman No. 2 filter paper and concentrated using rotatory evaporation to obtain the crude ethanol extract. The crude ethanol extract was successively macerated with n-hexane, chloroform and ethylacetate respectively.

2.4 Screening of Secondary Metabolites

The crude extracts was screened for the presence of alkaloids, tannins, saponins,

steroids, terpenoids, phenolics, flavonoids and quinones according to the standard procedures (Harbone, 1978; Sofowora, 1993; Trease and Evans, 1989).

2.5 Antimicrobial Assay

2.5.1 Preparation of Media

All media were prepared according to research specifications following the manufacturers' instructions.

2.5.2 Maintenance of the Test Organisms

The organism was inoculated into nutrient agar slants, incubated and kept at 4°C prior to sub-culture.

2.5.3 Standardization of Inoculum

Colonies of each organism growing as a pure culture in the nutrient agar plate were inoculated into 10mls of peptone water in different test tubes respectively for each organism aseptically. The mouth of the tubes were covered with cotton wool and wrapped with aluminum foil followed by incubation at 37°C for 24hrs.

2.5.4 Dilution Methods

Colonies of each organism growing as a pure culture in the nutrient agar plate were inoculated into 10mls of peptone water in different test tubes respectively for each organism aseptically. The mouth of the tubes were covered with cotton wool and wrapped with aluminum foil followed by incubation at 37°C for 24hours.

2.5.5 Agar Dilution

Tests were carried out according to the Farmacopéia Brasileira (1988) and USP XXIII Pharmacopoeia (1995) procedures using impregnated disks of different concentrations. The plates were incubated at 35 °C for 18 h in an ambient air incubator. The zone diameters were measured.

3.0 Results and Discussion

The use of natural products to treat variety of diseases has increased due to large number of

medicinal plants that have shown biological activity.

The preliminary screening of the secondary metabolites in *Physalis angulata* whole plant revealed a wide array of phytoconstituents depending on the solvent used. For instance, among all chemical compounds like alkaloids, tannins, saponins, steroids, terpenoids, phenolics, flavonoids and quinones that were screened for, all except saponins were present in the ethanolic, ethyl acetate and n-hexane extracts (Table 1). Apart from saponins that were not detected in the chloroform extract, the extract did not also show detectable quantities of quinones (Table 1). Several of these secondary metabolites have been reported to be responsible for the diverse medicinal properties of these plants. Some of these compounds have also been associated with antibacterial activities and thus have curative properties against pathogens.

The ethanolic extract of the whole part of *Physalis angulata* inhibited the growth of all the bacterial species (*S. aureus*, *E. coli*, *K. pneumonia*, *P. aeruginosa*) investigated in the present study as evidenced by the absence of turbidity, although at different concentrations. The zones of inhibition produced by the ethanolic extract of the whole plant of *Physalis angulata* ranged from 8.2 mm to 17.2 mm and were concentration dependent (Table 2). Infectious disease of microbial origin caused by *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* constitute major causes of morbidity and mortality in several countries of the world today (Kloos and Keim, 1993). Such microbial infection can result in diarrhoea, pneumonia, typhoid etc. The zones of inhibition and the absence of turbidity in the extract treated colonies of the bacteria confer antibacterial activity on the ethanolic extract of *Physalis angulata* whole plant. The finding is similar to

the report by Hwang *et al* (2004), where the bactericidal test showed that methanol extract of *P. angulata* conferred fast killing effect against *S. mutans* in 2 min at 50 mg/ml concentration.

Even though the exact antimicrobial mechanism of phenolic compounds is not clear, phenolic compounds are commonly known for their antimicrobial effects (Ciocan and Ioan, 2007; Tiwari *et al.*, 2009). The ability of phenolic compounds to alter microbial cell permeability, thereby permitting the loss of macromolecules from the cell interior, could help explain some of the antimicrobial activity (Bajpai *et al.*, 2008). In addition, the concentration dependent effects of the extract in the present study could also be attributed to the effect of phenolic compounds; at low concentration, phenols affect enzyme activity while at high concentrations they cause protein denaturation. Again, Flavonoid rich plant extracts from different species have been reported to possess antibacterial activity (Mishra *et al.*, 2011, Mishra *et al.*, 2013). Antibacterial flavonoids might be having multiple cellular targets, rather than one specific site of action. One of their molecular actions is to form complex with proteins through nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, and so forth. Lipophilic flavonoids may also disrupt microbial membranes (Cowan, 1999; Mishra *et al.*, 2009). Therefore, the presence of phenolics and or flavonoids in the extract of the plant in this study may account for the antibacterial activity of *Physalis angulata*.

In conclusion, the extract has shown antibacterial activity against the test organisms. Therefore, it could be explored as an antibiotic.

Table 1: Secondary metabolite constituents of the crude extracts of whole plant of *Physalis angulata*

Secondary Metabolites	Ethanol	Ethyl acetate	n-henxane	Chloroform
Alkaloids	+	+	+	+
Tannins	+	+	+	+
Saponin	-	-	-	-
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Phenolics	+	+	+	+
Flavonoids	+	+	+	+
Quinones	+	+	+	-

Key: + = Present

- = Absent

Table 2: Zones of inhibition of the bacteria by the ethanolic extract of the whole plant of *Physalis angulata***Concentration/Zones of Inhibition (mm)**

Bacterial Species	25mg/ml	50 mg/ml	100 mg/ml	125 mg/ml
<i>S. aureus</i>	8.2	9.6	10.6	11.6
<i>E. coli</i>	10	11.2	12.6	13.0
<i>K. pneumonia</i>	14.0	14.8	16.2	18.0
<i>P.aeruginosa</i>	12.0	15.0	16.5	17.2

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