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Extraction, phytochemical composition and antimicrobial inhibition efficiency of the methanol extracts of the crude seed sample and the basic metabolites from crude seed and leaf samples of *Datura Stramonium*(Thorn Apple)

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ABSTRACT

The seed of Datura Stramonium was defatted with petroleum ether and the marc cold extracted with methanol. The methanol extract was partitioned in dilute sodium hydroxide: hydrochloric acid: chloroform (NaOH:HCl:$CHCl_3$) and treated to obtain the basic metabolite. The dried leaf was also extracted using the batch method with methanol and was also partitioned in the same medium to obtain the basic portion or basic metabolite of the extract. The phytochemical analysis of the crude seed sample and its basic metabolite revealed the presence of alkaloids, tannins, phenols, terpenoids and saponins and absence of steroids and glycosides. The basic metabolite obtained from the leaf extract showed the presence of all the phytochemicals except flavonoid. The antibacterial sensitivity carried out on Muller Hinton agar using the agar well diffusion method (MHA) showed that the crude seed extract had no inhibition against the four pathogens used while the basic metabolite from the seed extract at a concentration of 50 mg/10 mL(milligrams/ milliliter) showed inhibition zone diameter (IZD) values of 10 mm for Escherichia coli, 20 mm for Salmonella typhi and no activity for Staphylococcus aureus and Klebsiella pneumonia. The basic portion of the plant leaf extract at the same concentration of 50 mg/10mL showed IZD of 28 mm for salmonella typhi, 8 mm for Klebsiella pneumonia and 8 mm for Staphylococcus aureus; this basic portion was not active at all against Escherichia coli. The standard drug ampiclox which was used as control drug at 50 mg/mL showed IZD values of 18 mm for the four pathogens respectively. This indicated that the basic metabolites from both extracts would serve as potential antimicrobial compounds against typhoid fever; even more potent than the control drug ampiclox.

Key words: antibacterial, extracts, leaf, inhibition, phytochemical, seeds.

INTRODUCTION

Medicinal plants are plants having their parts containing substances that have useful therapeutic functions or serve as precursors for the synthesis of antimicrobial, antivirus, antifungal, antitumour, antimalarial, anti-inflammatory, and analgesic, antitumour drugs, etc[1-2]. There have been reports of multiple drug resistance and the persistence of this challenge has led to the development of more potent synthetic antibiotics and other drugs which are expensive, with their attendant and serious side effects. Therefore local medicinal plants provide the link for new possible antimicrobial as well as other drugs [3].

Datura stramonium was probably cultivated in the Caspian Sea and spread to Europe in the 1st Century. At present it grows in waste places in Europe, America and South Africa and in Nigeria as well [4]. It is also known commonly as jimson weed or devil's trumpet [5]. *Datura stramonium* belongs to the family of *solanaceae* and has great pharmacological potential with great utility in folk medicine [6]. In folklore medicinal uses, *Datura stramonium* has shown astringent, anti-respiratory and decongestive properties. The infusion of the leaf is used to relieve the pain of rheumatism and gout, the poultice of the leaf is used on wounds and sores [7-8]. The leaf was mixed with mustard

oil for skin eruptions/infections; the juice from the flowers was used for otitis media. The oil from the seed was used for skin infection; it has antitussive and purgative activities while the seeds marc was used as purgative, expectorant, antipyretic compounds and in the treatment of asthma [8-11]. Nain, Bhatt, Dhyani and Joshi 2003 reported that the extracts from the leaf and branches of this plant species showed high antibacterial and antifungal activities [12]. The seeds have narcotic effect and were smoked for its narcotic effect and also used as anodyne and as sleep inducer [13-15]. Some tropane alkaloids were reported to have been isolated from the aerial parts of the plant [16-17]. The seeds were also was used as anti-inflammatory, antispasmodic, and astringent agents and for the treatment of hair loss in a work carried out by some researchers [18-20]. This paper looked at the plant chemicals present in the methanol extracts of the crude seed sample, basic metabolite of crude seed sample and the basic metabolite of the dry leaf sample and also at their antimicrobial inhibition efficiency.

MATERIALS AND METHODS

All reagents used were of analytical grade purchased from BDH, Poole England, and weighing was done on Mettler P2010.

2.1. Sample Collection and Preparation of Extract

Fresh *Datura Stramonium* plant was collected from Oja Oba Market Shagamu in Ogun State, Nigeria in June, 2014 and authenticated by a Taxonomist Dr. C.V. Nnamani of Applied Biology Department, Ebonyi State University Abakaliki as *Datura* Stramonium seed and leaf belonging to the family *solanaceae*. The leaf and seed were separated, washed to remove dirt and sand and then sun dried and ground into powder separately. About 175 g of each sample was cold extracted with 2 x 250 cm^3 of petroleum ether (60-80^oC) for 72 h. The marcs were soaked subsequently in 2x 250 cm^3 methanol for another 72 h. The solutions of the extracts were allowed to evaporate on a sand bath to recover 11.0 g and 5.0 g of green viscous solids respectively.

2.2. Test Organisms

The test organisms were obtained from Applied Microbiology Department, Ebonyi State University Abakaliki. The tests organisms were *Salmonella typhi*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Escherichia coli.*

2.3. Phytochemical Screening of the Crude Seed Sample

To 2 cm^3 sample was added 5cm^3 of distilled water and 2cm^3 olive oil; light green colouration with frothing was formed when shaken and allowed to settle.

2.3.1. Test for saponins

Two (2 cm^3) centimeter cube of test sample had 3 drop of $FeCl_3$ and 2 cm^3 of distilled water added, a greenish blue coloured solution was formed.

2.3.2.Test for phenol /Tannin

Two (2 cm^3) centimeter cube of test sample had 3 drop of $FeCl_3$ and 2 $cm^3$of distilled water added, a greenish blue coloured solution was formed.

2.3.3 .Test for steroid/glycoside

To 2cm^3 of the test sample was added 2 drop of acetic anhydride, boiled and cooled and 1 cm^3 H_2SO_4 added; there was no formation of deep red colour at the middle layer.

2.3.4. Test for alkaloid

About 2cm^3 of Wagner's reagent was added to 2cm^3 test sample with agitation, a dark red coloured precipitate was recovered.

2.3.5. Test for flavonoid

One molar (1M) sodium hydroxide solution was added to 2 $cm^3$of test sample. To this mixture was added 3 drop conc. HCl. A yellow colour developed which disappeared on standing.

2.4. Preparation of basic metabolite

About 2.50 g of the leaf extract was used to prepare the basic metabolite as stated by Ejele and Akujobi [23]. The extract was redissolved in 50 cm^3 of methanol and treated with 30 cm^3 of 10 % HCl and extracted with 2 x 30 cm^3 of chloroform using a separatory funnel. The organic layer ($CHCl_3$) was removed. The acid layer was treated with 10 % NaOH solution until the mixture became basic. The resulting solution was allowed to evaporate completely at room temperature to reveal a gel like compound which was dissolved in 95 % ethanol and filtered. The filtrate was

used for the phytochemical analysis and antibacterial tests without further purification. A similar experiment was performed for the seed extract.

2.5. Phytochemical Analyses of the Basic Metabolites

Approximately 0.750 g of each of the extracts was used for the analysis. These tests were carried out on the seed extract basic metabolite and leaf extract basic metabolite using standard methods with the view to identify the pharmacologically potent natural products present in them [24]. The summary of the results were presented in Tables 2 and 3.

2.6. Antimicrobial Sensitivity Test

The microbes were authenticated in the Department of Applied Microbiology, Ebonyi state University Abakaliki. Nutrient agar was used- this was weighed, dissolved in distilled water, autoclaved and poured into sterilized Petri dishes and allowed to solidify. The microbial samples were taken with sterile wire loop, streaked into the nutrient agar and incubated at 36^{o}C for 48 h. The agar well diffusion method was employed [25]. An inoculating loop was touched to three isolated colonies of each microbe placed on an agar plate and used to inoculate a tube of culture broth incubated at 36-37^{o}C until turbid and was subsequently diluted to match the standard turbidity. A sterile cotton swab dipped into the standardized microbial test suspension was used to inoculate the surface of the nutrient agar plate. This was allowed to settle for 5-7 min, then antimicrobial test disks were placed on the agar plate using a device and this was left to incubate at 35-38^{o}C for about 20 h, inhibition zone diameters were mapped out and measured to the nearest mm. The antimicrobial result of the crude seed sample appears in Table 4, that of the seed's basic metabolite in Table 5 and that of the basic metabolite from the leave sample in Table 6.

RESULTS

The preliminary qualitative phytochemical analysis of the crude seed sample, basic metabolites of the seed and leaf revealed the presence of alkaloids, steroids, terpenoids, phenols, saponins, tannins these are shown in tables 1, 2 and 3. Tables 4, 5 and 6 present inhibitory sensitivity of the various extracts.

Table 1 Phytochemical Analysis of Methanol Crude Seed Sample of *Datura stramonium*

phytochemical	Crude sample
saponin	++
phenol	++
steroid	-
alkaloid	+++
flavonoid	+
terpenoid	-
tannin	+++
glycoside	-
Carboxylic acid	+

+++ = strongly positive; ++ = positive; + = fair; - = not detected

Table 2 Phytochemical Analysis of the Basic Metabolite of the Seed Sample of *Datura stramonium*

Phytochemical	Basic Metabolite Crude Seed Sample
saponin	++
phenol	++
steroid	-
alkaloid	+++
flavonoid	+
terpenoid	-
tannin	+++
glycoside	-
Carboxylic acid	+

+++ = strongly positive; ++ = positive; + = fair; - = not detected

Table 3 Phytochemical Analysis of the Basic Metabolite of the Leaf of *Datura stramonium*

Phytochemical	Basic Metabolite
saponin	+
phenol	+
steroid	+++
alkaloid	+++
flavonoid	-
terpenoid	+
tannin	++
glycoside	++

+++ = strongly positive; ++ = positive; + = fair; - = not detected

Table 4 Antimicrobial Sensitivity of the Crude Seed Extract against the Four Pathogens/ Control Drug

Test organism	Basic metabolite 50mg/10ml (IZD mm)	Control drug Ampiclox 50mg/10ml(IZD mm)
Escherichia coli	-	8
Staphylococcus aureus	-	8
Klebsiella pneumonia	-	8
Salmonella typhi	-	8

Table5 Antimicrobial Sensitivity of the Seed Basic Extract against the Four Pathogens/ Control Drug

Test organism	Basic metabolite 50mg/10ml (izd mm)	Control drug Ampiclox 50mg/10ml(IZD mm)
Escherichia coli	10	20
Staphylococcus aureus	-	20
Klebsiella pneumonia	-	20
Salmonella typhi	20	20

- *No Inhibition*

Table 6Antimicrobial Sensitivity of the Basic Metabolite of Leaf Extract against the Four Pathogens

Test organism	Basic metabolite 50mg/10ml (IZD mm)	Control drug Ampiclox 50mg/10ml(IZD mm)
Escherichia coli	-	18
Staphylococcus aureus	8	18
Klebsiella pneumonia	8	18
Salmonella typhi	28	18

- No Inhibition

DISCUSSION

The methanol crude sample of *Datura stramonium* contained saponin, phenol, alkaloid flavonoid tannin and carboxylic acid but there was no indication of steroid, terpenoid and glycoside [9]. The alkaloid precipitated out from the test solution indicating a very strong presence of this phytochemical. This supported the report of the presence of tropane alkaloids from the aerial part of this plant species [17, 26]. Most saponins are biologically active compounds of medicinal plants and play a variety of physiological functions [27]. Alkaloids exhibit useful antimicrobial activity, likewise flavonoids exhibit various bioactivities which include, antibacterial, antivirus, anti-inflammatory, analgesic, antitumour and hepatoprotective actions [28-29]. The presence of saponin and alkaloid was in order since some alkaloids are easily bound with some saponins in natural products; the basic metabolite showed the same trend. The partitioning of the seed extract into basic metabolite did not change the qualitative presence of the phytochemicals. The basic metabolite from the leaf extract showed a remarkable shift in the trend. There were moderate detection of saponin, phenol and terpenoid, a remarkable presence of tannin, glycoside and strong presence of steroid and alkaloid.

The crude seed sample showed no inhibitory effects against *Escherichia coli*, this was in agreement with the report of Banso and Adeyemi in 2006 that the methanol extracts of *Datura stramonium* and *Datura inoxia* had little or no activity against *Escherichia coli* and *pseudomonas aeruginosa* [30]. As could be seen from Table 4, the crude seed extract did not inhibit the growth of the four microorganisms at the concentrations used.

The basic crude seed extract inhibited the growth of *Escherichia coli* and *Salmonella typhi* with IZD values of 10 mm and 20 mm respectively even higher than the control drug Table 5. The inhibitory sensitivity was also not in line with the report of Banso and Adeyemi, 2006; it inhibited the growth of *Salmonella typhi* strongly. This showed that some synergic effects presented by the phytochemicals in the crude sample were removed by partitioning the extract into various components especially the basic component. The basic metabolite of the leaf extract Table 6 inhibited the growth of three out of the four pathogens: 8 mm for *Staphylococcus aureus* and *Klebsiella pneumonia* respectively and 28 mm for *Salmonella typhi*. This plant extract showed more inhibitory effect compared to the orthodox control drug ampiclox at the same concentration.

The encouraging results – the strong inhibitory efficiency against *Salmonella typhi* and other micro organisms suggested the possibility of using this compound for infections caused by bacteria and principally typhoid fever which is endemic in most developing countries of the world. This would reduce cost as the plant could easily be sourced and an infusion in local gin or alcoholic drink for some hours would release the phytochemicals; this would also go a long way in reducing the incidences of side effects and bacterial resistance associated with ampiclox and other orthodox antibiotics [31]. The isolation and characterization of the pure isolates would be the next stage; to

ascertain the structures and antimicrobial sensitivity of each phytochemical present in the basic metabolite of the seed and leaf extracts of this plant species.

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