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Susceptibility of Escherichia Coli Isolated from Oligospermia Patient to Gongronema Latifolium Leaves Extracts

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Abstract
Several studies has shown that the infection of the male genitourinary tract represent a significant healthcare problem and accounts for the major cases of oligospermia associated with microbial infections. The aim of this study was to evaluate the susceptibility of *Escherichia coli* isolated from oligospermia patient to *Gongronema latifolium* leaves extracts. A sample was collected from the hospital and subcultured on nutrient using streaking method, incubated at growth conditions. The phytochemical constituents of the leaves extracts of *G. latifolium* were determined quantitatively using spectrophotometric method. The antibacterial activity of the leaves extracts was carried out using agar well diffusion method. Tube dilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using double fold serial dilutions at concentrations 400 mg/ml to 25 mg/ml.The bacterial isolate were characterized and identified using colony description and biochemical tests.The phytochemical analysis of *G. latifolium* leaves extracts revealed the presence of alkaloids, saponins, flavonoids, steroids, cardiac glycosides, phenolics and tannins. The ethanolic extracts (17.00 mm) of *G. latifolium*showed more activity than aqueous extracts (6.00 mm) and their activities differed significantly ($P<0.05$) from Ciprofloxacin (20.33 mm). The MIC (200 mg/ml, 400 mg/ml and MBC (400 mg/ml, Nil) values revealed that the inhibitory and cidal activities of the ethanolic and aqueous extracts. This study has shown that *G. latifolium* leaves extracts showed pronounced activity against *E.coli* isolated from oligospermic patient and otherwise extract proved to be more effective.

INTRODUCTION

Oligospermia is an infection caused by *Escherichia coli*; a gram negative bacillus that belongs to the family of Enterobacteriaceae which is made up of species present in human and other animal intestine is the most common cause of microbial infertility. Oligospermia by microbial infection affects the semen in several ways, by damaging sperm, hampering their motility or by producing an inflammatory structure in the tract (Henkel and Schill, 1998). Oligospermia caused by *Escherichia coli* have been associated with 14.9% of oligospermia in microbial infertility.

According to Preethi *et al.* (2010), the use of traditional plants has been the oldest form of healthcare known to mankind. Over 50% of all modern clinical drugs are natural products origin and natural products play important roles in drug development in the pharmaceutical industry. A study by World Health Organisation (WHO) estimated that appropriately 80% of the world's inhabitants rely on traditional medicine for their primary healthcare (Akerele, 1993). Several studies indicated that medicinal plants contain compounds like peptides, aldehydes, essential oils, phenol, alkaloid and water or ethanol compounds. These compounds are significant in therapeutic application against animal pathogens.

Gongronema latifolium which belongs to the family Asclepiadaceae, contain about 21 species or more. The plant is a tropical rainforest plants primarily used as species and vegetable in traditional folk medicine (Ugochukwu and Babady, 2003). The leaf is commonly called "utazi" and "arokeke" in the south west and south eastern parts of Nigeria. Since the *Gongronema latifolium*, it has been used to treat cough, intestinal worms, dysentery and malaria. It is also taken as a tonic to treat loss of appetite and stomach ache. A common decoction of leaves is usually to treat diabetes and high blood pressure. Some properties have been exhibit by extracts of *Gongronema latifolium* such as hypoglycemic, hypolipidemic, antioxidative and anti-inflammatory properties (Ugochukwu and Babady, 2003). Despite the roles this of this *Gongronema latifolium*in healthcare, no work has been published on the role of this leaf in ameliorating the issue of oligospermia caused by *E. coli,* which is one of the problem couples are encounter nowadays. Thus, this work was taken to evaluate the susceptibility of *E. coli* isolated from oligospermia patient to *Gongronema latifolium* leaves extract

MATERIALS AND METHODS

Sample Collection: The fresh leaves of *Gongronema latifolium* were collected from Umuoma village, Uli Town, Ihiala Local Government Area, Anambra State.

Plate 1: *Gongronema latifolium* leaves

Preparation of Samples for Extraction: The leaves of *Gongronema latifolium* were plucked off and dried under shade at room temperature for 14 days. The dried samples were pulverized using electric grinder, weighed and kept ready for extraction of active indigents (Nwobu *et al.,* 2010).

Extraction Procedures: A 20 g portion f the sample was extracted y maceration in 200 ml of ethanol and water respectively for 3 days. The resulting extracts were subsequently filtered using Whatman No 1 filter paper. The extracts were evaporated to dryness at room temperature in a steady air current (Nwobu *et al.,* 2010).

Determination of Extract Value: The concentration of the extracts were determined by evaporating 1.0 ml of the extracts in evaporating dish of known weight in an oven to dryness and weighed. The weight of the residue was obtained by subtracting the weight of the empty dish from the weight of dish and residue. The above process was repeated in duplicat.

Preparation of Test Sample: In this study, concentrations of 500 mg/ml of the extracts were used to screen for the antimicrobial activity. This was done by using the modified method of NCCLS (2000). Here, 2.5 g of the extract was dissolved in each of the extracting solvents.

Preparations of Media for Isolation: The media used for this study included: Nutrient agar (oxoid), Mac Conkey agar (oxoid), mannitol salt agar (oxoid), Muller Hiton agar (oxoid) and Nutrient broth (oxoid). The media were prepared according to the manufacturer's instruction and sterilized by autoclaving at 121ºC, 15psi for 15 minutes.

Collection and Identification of Test Organism: The test organism used in this work was collected from the already cultured plate of semen sample. The organism obtained were aseptically subcultured on nutrient agar plate and incubated at 37ºC for 24 h. the pure culture of the test organism was identified using gram staining, colonical description and biochemical reactions.

Maintenance of Test Organism: The isolated test organisms were used for the antibacterial sensitivity testing. Prior to the test, the organism was subcultured on nutrient agar plate at 37ºC for 24 h. then the 24 h cultures were transferred into nutrient broth and incubated at 37ºC for 24 h.

Sensitivity Testing Using Agar-well Diffusion Method: Each labeled plate was uniformly inoculated with the test organism using pour plating method. A sterile cork borer of 5 mm diameter was used to make wells on the medium. One tenth milliter (0.1 ml) of various concentrations of the extracts were dropped into each labeled wells and then incubated at 37ºC for 24 h. antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation. Ciprofloxacin (500 mg/ml) was used as control (Iheukwumere and Umedum, 2013).

Determination of Minimum Inhibitory Concentration (MIC): Here, various concentrations of the test extracts were obtained using double-fold serial dilution. Each dilution was assayed against the test organism using tube dilution method. One milliter of the organism was added into each dilution and incubated at 37ºC for 24 h. the MIC was defined as the lowest concentration able to inhibit any visible bacteria growth. This was

determined and recorded (Iheukwumere and Umedum, 2013).

Determination of Minimum Bactericidal Concentration (MIC): Here, equal volumes of various concentrations of those tubes that did not produce any visible growth from MIC was plated on fresh sterile pour plate and incubate at 37 ºC for 24 h. the lowest concentration of the extracts that killed the test organism was taken as the MBC (Iheukwumere and Umedum, 2013).

RESULTS

The characteristics and identities of the test organism are presented in Table 1. The isolated organism was *Escherichia coli*. This organism was characterized and identified using their colonial description, Gram staining reaction and biochemical tests. The quantitative phytochemical analysis of the leaves of *Gongronema latifolium* was shown in Table 2. The result revealed the presence of alkaloids, saponins, flavonoids, phenolics, tannins and glycosides. The phytochemical constituents may be responsible for the activity of the leaf extracts of *Gongronema latifolium.* The diameter zones of inhibition of *Gongronema latifolium* (Utazi) leaves extracts against *E. coli* was showed in Table 3. The ethanolic extract inhibited the test organism more than the aqueous extract and their inhibitory activities differed significantly ($P<0.05$) from that of ciprofloxacin (control). The absolute ethanol (0.1 ml) and distilled water (0.1 ml) used in this study as extracting solvents had no effect on the tested organism.The result of minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the ethanol and aqueous extracts of *Gongronema latifolium* are shown in Table 4. The result of the analysis showed that the ethanol extract exhibited more pronounced activity than the aqueous extract. The ethanolic and aqueous leaf extracts of *G. latifolium* exhibited almost similar MICs compared to ciprofloxacin (control) which had more pronounced activity. The ethanolic leaf extract of *G. latifolium* and ciprofloxacin (control) showed similar bactericidal activity compared to aqueous leaf of *G. latifolium* which had no bactericidal activity against *E. coli.*

Table 1: Characteristics and identity of the test organism

Parameter	*E. coli*
Appearance on agar plate	Red
Margin	Entire
Shape	Rod
Coagulase	-
Indole	+
MR test	+
VP test	-
Glucose	A/G
Maltose	A/G
Mannitol	A

+ = Positive, A = Acid, - = Negative, G = Gas

Table 2: Phytochemical constituents of *Gongronema latifolium* leave extracts

Parameter	Amount (g/100g)
Alkalods	10.32
Flavonoids	0.14
Tannins	7.28
Saponins	3.14
Phenolics	0.87
Cardiac glycosides	0.14
Steriods	0.02

Table 3: Diameter Zone (mm) of Inhibition of *Gongronema latifolium* against *Escherichia coli* using 5 mm cork borer

Extract (400 mg/ml)	Zone of inhibition (mm)
EEG	17.00 ± 0.81
AEG	6.00 ± 1.63
CPX	20.33 ± 1.57
Absolute Ethanol (0.1 ml)	-
Distilled water (0.1 ml)	-

EEG = Ethanolic extract of *Gongronema latifolium,* AEG = Aqueous extract of *Gongronema latifolium,* CPX = Ciprofloxacin

Table 4: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the leaf extracts

Inhibitory substance	MIC (mg/ml)	MBC (mg/ml)
EEG	200	400
AEG	400	-
CPX	100	200

EEG = Ethanolic extract of *Gongronema latifolium,* AEG = Aqueous extract of *Gongronema latifolium,* CPX = Ciprofloxacin

DISCUSSION

Infection of the semen by *Escherichia coli* is a powerful mechanism that can lead to sperm damage, deformity and eventually Oligospermia (Askienazy-Elbhar 2005). Reduced motility of spermatozoa has found n seminal fluid sample which contain high concentration of bacteria (Pellati *et al.,* 2008). Oligospermia dangers about 10-15% of the couples all over the world (Carlsen, 1992). Although some therapeutic agents are used in treatment of this infection, serve problem encountered worldwide is development of drug resistant organism due to it overuse and self-medication (Slama *et al.,* 2005). This present was carried out to evaluate the susceptibility of *Escherichia coli* isolated from oligospermia patient to *Gongronema latifolium.*

The present study revealed that the leaves extracts of *Gongronema latifolium* showed pronounced activity against *Escherichia coli,* which was a predominant bacterium isolated from oligospermia patients. The presence of alkaloids, phenolics, tannins, saponins, flavonoids, steroids and cardiac glycosides in the studied plant extracts corroborated with the works of many researchers (Akah and Okafor, 1992; Ali *et al*., 2001; Morebise et al., 2002; Iheukwumere and Umedum, 2013). The phytochemicals may be responsible for the activity of *Gongronema latifolium* leaves extracts on *Escherichia coli* isolated from oligospemia patient as described by many researchers (Morebise *et al.*,2002; Nwokeke, 2008; Nwobu *et al*., 2010; Iheukwumere and Umedum, 2013)).

The pronounced activity of the extracts against tested organisms corroborated with the works of Iheukwumere and Umedum (2013). The activity of ethanolic extract against aqueous extract indicates that the active phytochemical constituents of the plant parts have more ability to dissolve in ethanol solvent than aqueous solvent used this study. Similar conclusions were drawn by Nwobu *et al.* (2010) and Iheukwumere and Umedum (2013). Though aqueous extract produced higher amount of extract but exhibited relatively lower activity than the ethanolic extract which obtained in lower quantity. This indicates that the amount of yield did not always influence the inhibition of microbial growth but the active ingredients found in the extract play the major role. The study highlighted that ethanol was able to extract more of the phytochemical constituents because ethanol is an organic polar solvent, and most of the phytochemical constituents are organic in nature. This observation suggested that the organic solvent extraction is suitable to verify the antibacterial properties of medicinal plants (Ali *et al.,* 2001; Iheukwumere and Umedum, 2013)

The results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration of the leaf *G. latifolium* showed that ethanolic and aqueous leaf extracts of *G. latifolium* exhibit reasonable activity against *E. coli* as well as ciprofloxacin (control) which had more pronounced activity. This means that oligospermia infection caused by *E. coli* could be managed effectively by *G. latifolium* leaves extracts. Also, further research involving *in vivo* assays will be needed to establish the relationship between the MICs and MBCs obtained in this study and effective dosage that should be administered in ethnomedical practice.

CONCLUSION

The study has supported the folklore usage of the leaves of *Gongronema latifolium* and suggested that the leaves could be more economical and safe in handling cases oligospermia associated with *Escherichia coli.*

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