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ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACT OF GREWIA MOLLIS SMITH (MALVACEAE) ON CLINICAL ISOLATES OF ESCHERICHIA COLI FROM CASES OF DIARRHEA.

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ABSTRACT

Background: *Escherichia coli* is one of the leading causes of primary intestinal infections, particularly diarrhoea and other types of opportunistic infections of humans. The antimicrobial, phytochemical contents and minimum inhibitory concentration of *Grewia mollis Smith* (Malvaceae) were determined in a quest to evaluate their potentials as sources of alternative medicine for the treatment of diarrhoea.

Methods: The bacteriological investigation was carried out on 25 clinical isolates of *Escherichia coli* from cases of diarrhoea which includes; sub-culturing onto eosin methylene blue agar and incubated at 37^0C for 24 hours. Gram staining, lactose fermentation, indole formation from tryptophan, gelatin liquefaction and Voges - Proskauer test were carried out on the isolates. The antimicrobial, phytochemical analysis and minimum inhibitory concentration (MIC) of *Grewia mollis* extract were determined using Evans and Trease method.

Results: *Grewia mollis* leave methanol, ethyl acetate and N- hexane extracts gave extraction yield 8.40, 3.86 and 2.55 respectively. Tannins, saponins, flavonoids and alkaloids were detected as bioactive compounds from the leave of *Grewia mollis* investigated. The minimum inhibitory and minimum bactericidal concentration of the extract elicited remarkable antimicrobial activity in correlation to the polarity of each solvent.

Conclusion: Therefore, *Grewia mollis* leave extracts hold the potential for clinical application.

Keywords: Antimicrobial activity, *Grewia mollis*, *Escherichia coli*, Diarrhea.

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INTRODUCTION

Grewia mollis Smith (Malvaceae) known as Oora Igbo in Yoruba land is a shrub or small tree, up to 20 ft. high, it is made up of small leaves with pale greenish-white beneath the yellow flowers. It has variable or broadly rounded or obliquely truncate appearances, apex acute to slightly acuminate, margin toothed, 3-veined from the base, glabrous to sparsely minutely stellate-pubescent above, densely greyish to brownish-white pubescent beneath.

Grewia mollis is a useful multipurpose plant, providing a wide range of products, including timber for local use, food, fibre, fodder and traditional medicines (Youssef et.al.,2012). *Grewia mollis* is widely distributed in northern Nigeria and some African countries. Approximate estimation of 100g dry matter of *Grewia mollis* bark in Nigeria contains protein 3.7 g, fat 1.1 g, carbohydrate 45.3 g, fibre 45.3 g, Ca 3474 mg, Mg 743 mg, P 79 mg, Fe 10.4 mg and Zn 0.5 mg. In Togo, a decoction of the stem bark is drunk to treat diarrhoea. Several ethnomedicinal uses of the infusion, decoction, maceration, or mucilage from the leaves, roots, or stem bark of Grewia. mollis have been documented. In Nigeria, the fruit is used as a febrifuge. Grewia mollis is frequently used in traditional rituals in Sudan and Ethiopia (Goyal,2012).

Medicinal plants have been found useful in the cure of a number of diseases including bacterial disease and they are a rich source of antimicrobial agents. Although medicinal plants produce slow recovery, the therapeutic use of medicinal plants is becoming popular because of their lesser side effects and low resistance to microorganisms. Millions of people in developing countries will always depend on herbal medicines for their health care because they believe that the medicinal herbs are always available locally, cheap, affordable and are prescribed by traditional practitioners of medicine who are part of the community(Neuwinger et. al.,2000). About 12.5% of the 422,000 plant species documented worldwide are reported to have medicinal values.

Diarrhoea is the condition of having at least three loose, liquid, or watery bowel movements each day. It often lasts for a few days and can result in dehydration due to fluid loss. It is usually a symptom of an infection in the intestinal tract, which can be caused by a variety of bacterial, viral, and parasitic organisms. Signs of dehydration often begin with loss of the normal stretchiness of the skin and irritable behaviour. This can progress to decreased urination, loss of skin colour, a fast heart rate, and a decrease in responsiveness as it becomes more severe. About 1.7 to 5 billion cases of diarrhoea occur per year. It is most common in developing countries, where young children get diarrhoea on average three times a year (Albert et.al.,1995).

Total deaths from diarrhoea are estimated at 1.26 million in 2013—down from 2.58 million in 1990. In 2012, it was the second most common cause of death in children younger than five (0.76 million or 11%). Frequent episodes of diarrhoea are also a common cause of malnutrition and the most common cause in those younger than five years of age. Other long term problems that can result include stunted growth and poor intellectual development. In sub-Saharan Africa, primary caregivers display poor perceptions about the signs of dehydration, dysentery, and management of diarrhoea. Diarrhoea remains an important public health problem particularly in developing countries (Nataro et.al.,1997). Escherichia coli, a prominent member of the indigenous flora of the human tract and also a major etiologic agent of gastroenteritis belong to the family Enterobacteriaceae. Gastroenteritis caused by *Esch. coli* ranges from simple diarrhoea to a more severe form with a debilitating loss of fluids and electrolytes. Gastroenteritis is most serious and sometimes fatal in nutritionally deprived infants and elderly debilitated adults. Escherichia coli causes diarrhoea by invading the intestinal epithelial cells and eliciting an

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inflammatory response. The exact cause of diarrhoea is unknown, but it may be due to the cytotoxic effects of lipopolysaccharide (Knuttonet.al.,1987).

This study was therefore designed to examine the antimicrobial activity of leaves extracts of *Grewia mollis* on isolates of Escherichia coli from cases of diarrhoea in solvents of varied polarity.

Materials and Methods Sample Collection Clinical Samples Clinical isolates of Escherichia coli from cases of diarrhoea were collected from the medical microbiology laboratory unit of the Oluyole Primary Health Care clinic in Ibadan and were confirmed by sub-culturing on to Eosin Methylene Blue, examined macroscopically for morphological characteristics, Gram staining and other conventional bacteriological tests which include lactose fermentation, indole formation from tryptophan, gelatin liquefaction and Voges-Proskauer test was carried out on the isolates.

Plant Samples

The leaves of *Grewia mollis* were collected from the forest along Benin- Ore road in southwest Nigeria and it was authenticated at the Department of Pharmacognosy Herbarium Laboratory of the Olabisi Onabanjo University with voucher number GM3345 and the specimens were deposited at the location.

Extraction method

A quantity of 200 grams each of powdered leave was placed in a screw-capped bottle containing 2000mL of distilled methanol, ethyl acetate and N-hexane. It was soaked for 5 days after which it was filtered using Whatman No.1 filter paper. It was then concentrated using a rotary evaporator and kept in the refrigerator at 40C for further use.

Phytochemical Screening

Phytochemical screening was performed to identify some bioactive compounds in the methanol extracts of *Grewia mollis*. The phytochemicals were detected by colour tests.

Test for tannins: About 0.5 g of the extracts were boiled in 10mL of distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black colouration. A Blue-black colour was observed.

Test for alkaloids: 0.5g of extracts were diluted to 10mLwith acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2mL of dilute ammonia. To 5mL of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10mL of acetic acid. It was divided into two portions. Mayer‘s reagent was added to one portion and Draggendorff’s reagent to the other. The formation of cream/reddish brown precipitates was observed as positive for alkaloids.

Test for flavonoids: A volume of 5mL of 10% dilute ammonia solution was added to a portion of the aqueous filtrate of the extract. Concentrated H2SO4.(1mL) was added. A yellow colouration that disappears on standing indicates the presence of flavonoids.

Test for saponins: 0.5 g of the extracts was added to 5mL of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for anthraquinone: 0.5g of the extracts were boiled with 10mL of sulphuric acid (H2S04) and filtered while hot. The filtrate was shaken with 5mL of chloroform. The chloroform layer was pipetted into another test tube and 1mL of dilute ammonia was added. The resulting solution was observed for colour changes.

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Antimicrobial Assay

The antimicrobial activity of the Grewia mollis was carried out using the agar well diffusion technique. The surface of the agar medium in a culture plate was inoculated with 0.2ml of 1:100 of an overnight culture of each isolate of *Escherichia coli.* Using a sterile corn borer, 8mm wells were bored into the agar medium. Then varied concentrations of the plant with positive and negative controls (10 µg/mL gentamicin and 50% methanol) were introduced to the wells and plates were allowed to stand on the bench for 1 hour for pre-diffusion before incubation at 370C for 24 hours. The zones of growth inhibition (mm) were taken as a measure of antimicrobial activity.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentrations of the extracts was determined by dissolving 4mg of extract into 10mL of (50%) methanol to attain a stock concentration of 400µg/mL. Furthermore, 5mL of the mixture was diluted serially with 5mL of the 50%v/v methanol resulting in concentrations of 200µg/mL, 100µg/mL, 50µg/mL and 25µg/mL respectively. The same procedure was repeated for each extract with ethyl acetate and N-hexane solvents. Two millilitres (2mL) of the extract from each dilution and stock was mixed with 18mL of molten sensitivity test agar medium resulting in concentrations; 200µg/mL, 100µg/mL, 50µg/ml, 25µg/mL, 12.5µg/mL and poured into sterile Petri- plate allowing the agar to set The surface of the agar was allowed to dry before streaking with 1:100 dilution of an overnight culture of the test organisms. The plates were incubated accordingly after which the lowest concentration preventing visible growth was taken as Minimum Inhibitory Concentration (MIC) of the extract.

Determination of Minimum Bactericidal Concentration (MBC)

Selected plates with no discernible growth from the MIC were streaked on a Nutrient Agar medium and incubated at 37oC for 24 hours. The lowest concentration of the plant extract which shows no growth of the test organisms on recovery nutrient agar plates were taken as minimum bactericidal concentration.

RESULTS

Table 1: % extraction yield of leaf Grewia mollis from selected solvents

Solvents	Weight of the extract		% yield
	Initial weight(g)	Extract weight(g)	
Methanol	200	16.8	8.4
Ethyl-acetate	200	7.62	3.86
N-hexane	200	5.1	2.55

Table 2: Qualitative Phytochemical profile of Grewia mollis leaf extract

Phytochemical	Methanol extract	Ethyl acetate extract	N-hexane extract
Alkaloid	+	-	-
Saponins	+	+	+
Flavonoids	+	+	+
Tannins	-	-	+
Anthraquinone	-	-	+

Key: + = Present - = Absent

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Table 3: Antimicrobial Susceptibility Test [AST]

Test Organism	Zone of Growth Inhibition (mm)											
S/N	200 µg/mL			100 µg/mL			50 µg/mL			25 µg/mL		
	M	EA	H	M	EA	H	M	EA	H	M	EA	H
Esch. coli 1	32	18	14	22	16	12	16	14	10	12	10	0
Esch. coli 2	28	20	14	20	14	10	15	10	10	12	0	0
Esch. coli 3	28	16	24	20	14	12	15	14	12	12	10	10
Esch. coli 4	27	20	16	17	18	12	14	14	10	10	10	0
Esch. coli 5	23	16	24	18	12	20	12	10	16	10	0	10
Esch. coli 6	25	16	24	18	14	12	12	10	10	10	0	0
Esch. coli 7	25	18	16	18	14	14	12	14	12	10	10	10
Esch. coli 8	25	16	14	18	12	10	12	10	0	10	0	0
Esch. coli 9	28	24	20	21	20	18	16	14	14	12	10	10
Esch. coli 10	30	28	16	22	24	12	16	16	10	12	14	0
Esch. coli 11	26	24	20	18	18	16	14	20	12	10	10	10
Esch. coli 12	26	18	24	21	16	20	16	14	16	12	12	10
Esch. coli 13	26	16	20	21	14	18	14	12	14	10	10	0
Esch. coli 14	30	20	28	22	18	26	14	18	20	10	14	14
Esch. coli 15	0	0	0	0	0	0	0	0	0	0	0	0
Esch. coli 16	16	10	14	14	10	0	14	10	0	10	0	0
Esch. coli 17	20	10	12	14	10	0	12	10	0	10	0	0
Esch. coli 18	28	24	18	18	18	14	12	14	12	10	10	10
Esch. coli 19	28	20	24	20	16	18	16	14	16	12	14	12
Esch. coli 20	28	12	14	20	10	10	14	0	0	10	0	0
Esch. coli 21	23	16	18	18	14	18	12	10	14	10	10	10
Esch. coli 22	21	18	20	16	16	18	12	16	14	10	12	14
Esch. coli 23	22	18	16	16	14	16	12	14	12	10	0	0
Esch. coli 24	22	12	16	18	10	14	12	12	0	10	0	0

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Table 4: Determination of Minimum Inhibitory Concentration of Grewia mollis

Test Organism	M.E µg/mL	E. A µg/mL	N.H µg/mL
Esch. coli 1	25	100	200
Esch. coli 2	50	100	200
Esch. coli 3	25	50	100
Esch. coli 4	50	100	100
Esch. coli 5	25	100	200
Esch. coli 6	50	100	100
Esch. coli 7	50	200	200
Esch. coli 8	50	100	100
Esch. coli 9	100	100	100
Esch. coli 10	100	200	200
Esch. coli 11	100	100	200
Esch. coli 12	100	50	50
Esch. coli 13	50	100	50
Esch. coli 14	25	100	50
Esch. coli 15	ND	ND	ND
Esch. coli 16	100	200	200
Esch. coli 17	50	200	200
Esch. coli 18	50	100	200
Esch. coli 19	50	100	100
Esch. coli 20	50	200	200
Esch. coli 21	100	200	200
Esch. coli 22	100	100	50
Esch. coli 23	100	200	200
Esch. coli 24	100	100	100
Esch. coli 25	50	200	200

DISCUSSION

Results obtained from this study shows the antimicrobial potency of *Grewia mollis*. *Grewia mollis* was prominent among the medicinal plants discovered during an ethnobotanical survey of local plants used to treat diarrhoea in Southwestern Nigeria. Ethnomedicinal approach, rather than taxonomic criteria was used in selecting *Grewia mollis* for this study. The susceptibility of these Escherichia coli to *Grewia mollis* in this study indicated the therapeutic potentials of this plant against etiologic agents of diarrhoea. The percentage yield of methanol extract was higher (8.40%) than ethyl acetate(3.86%) and N-hexane extract (2.55%) which could be attributed to the selective reaction of the composition of the extract to chemicals of different polarity. Most polar constituents were recorded in methanol, which could be due to its intermediate polarity which makes it an excellent extractant. The phytochemical analysis showed the presence of saponins, alkaloids, tannins, flavonoids in varied proportions while anthraquinones were absent which agrees with the study of Kubmarawa(2007) on preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. The presence of secondary metabolites of therapeutic values could be a source of antimicrobial activity of the leave extract against *Escherichia coli.* The pharmacological activity of most medicinal plants have been found to be directly related to the types of a secondary metabolite that they contain, therefore the presence of tannin, alkaloids, saponins and other secondary metabolites may be responsible for the observed antimicrobial activity(Shangalet.al.,2012).

Methanol extract of Grewia mollis leave has antimicrobial activity at 200, 100. 50 and 25µg/ml than ethyl acetate and N-hexane extract. Ninety-six per cent (96%) of the isolates were

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susceptible to methanol extract at 25µg/ml, while 52% and 36% of the test isolates were susceptible to ethyl acetate and N-hexane respectively. Test isolates 15, 16, 17, 20 and 25 were resistant to N-hexane at 50 µg/ml and 3 of the isolates were also resistant to hexane at 100 µg/ml. Hundred per cent of the isolates were susceptible 200 µg/ml and most at 100 µg/ml as shown in Table 3 in this study which agrees with Al-Youssef (2012) on the biological evaluation of constituent of *Grewia mollis*. (Al-Youssef, 2012)

Sixteen per cent of the test isolates were susceptible at MIC 25 µg/mL while 52% and 32% of the test isolates were susceptible at MIC of 50 and 100 µg/mL ethanol extract respectively. While the MIC of ethyl acetate and N-hexane against the test isolates ranged between 50 and 200 µg/ml as shown in table 4 which corroborates the study of Shagal et.al.,(2012) on antimicrobial activity of root, stem and bark extract of *Grewia mollis*.

The minimum inhibitory concentration (MIC) values for methanol, ethyl acetate and n-hexane on *Escherichia coli* is remarkable in this study which is the suggestive therapeutic potential of Grewia mollis (Ashiku et.al.,2012). The minimum bactericidal concentration of the isolates elicited remarkable antimicrobial activity, 44% of the isolated were susceptible at 50µg/ml methanol while the remaining 66% at 100 µg/ml were also susceptibly eliminated. Eighteen(18) of twenty-five (25) isolates were susceptible to ethyl acetate extract at 200 µg/ml while the remaining seven isolates were also eliminated at 100 µg/ml. the isolates were also susceptible to N-hexane, between a range of 50 µg/ml – 200 µg/ml while only isolates 15 were not determine sequel to its resistance to extract solvents varied fractions which corroborated the findings of Kubmarawa (2007) on antimicrobial screening of 50 medicinal plants from Nigeria.

CONCLUSION

Although in vitro activity cannot be directly translated into effective use of the plant in herbal medicine, observation of bioactivity do give some indication of possible curative effects. However, additional studies are recommended to explore further other compounds of therapeutic values in *Grewia mollis* and its medicinal application for the treatment of diarrhoea

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