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Comparative Analysis of Antibacterial Activity between *Annona Senegalensis* and *Cassia Singueana Root* Extracts Against *Proteus Mirabilis*

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ABSTRACT

Medicinal plants are traditionally used in the treatment of human infections. The present study was carried out to compare the potential of *Annona senegalensis* and *Cassia singueana* root extracts against *Proteus mirabilis* related infections. The crude methanol root extracts were subjected to disc diffusion method to determine the activity of the crude methanol extracts. The methanol extracts of the two medicinal plants were further fractionated using solvents of different polarity (n-Hexane, ethyl acetate and n-butanol). The fractions were further subjected to antibacterial activity using same disc diffusion method in which *Cassia singueana*e thylacetate fraction prove to be the most active fraction, and no activity was observed in n- hexane fraction of the two extracts.

Keywords; *Annona senegalensis, Cassia singueana, proteus mirabilis, nosocomial infection*, antiproteus activity.

1.0 INTRODUCTION

Resistance in gram-negative bacteria has been increasing over the years; this is mainly due to the spread of strains producing extended spectrum ß-lactamase (Sui 2002). Although *P. mirabilis* remains susceptible to nearly all antimicrobial with the exception of few (egtetracyclines), However, drug resistance has been increasingly reported for this specie, and the diffusion of resistance to extended-spectrum cephalosporins due to the production of extended-spectrum β-lactamases (ESBLs) has become of great concern (Stürenburg, and Mack 2003), as it is one of the commonest gram-negative pathogens encountered in clinical specimens and can cause a variety of community or hospital-acquired illnesses, including urinary tract, Wound, and Bloodstream infections (O'Hara *et al.*, 2000).

Extended-spectrum β-lactamases (ESBL) production has been reported among *P. mirabilis* in several epidemiological settings, with a prevalence that can exceed 20% in some areas **(***Winokur, et al*., 2001**).** This trend is a matter of major concern, since *P. mirabilis* is a common cause of human infections and accounts for a higher percentage of Nosocomial infections (Jacobsen *et al.*, 2017), while ESBL-producing *P. mirabilis* strains are usually resistant to several antimicrobial agents and can result in difficult-to-treat infections **(**Luzzaro *et al.*, 2000**).**

Other barriers towards orthodox treatment of *P. mirabilis* include the misconception that most diseases caused by this bacterium are not serious, embarrassment about a physical examination especially when associated with urinary tract infection as the stigma associated with UTI remains a pervasive barrier to testing and management.

There is abundant justification for the use of herbs by the various traditional healers identified, and modern-day technology, innovations and education, have, however, made a lot of impact on the herbalist and on the practice of traditional medicine in Nigeria (Adesina, 2005), and natural products have historically been of crucial importance in the identification and development of antibacterial agents (Moloney, 2016), A total of 34 plant species belonging to 18 different families, selecbased on n the basis of folklore medicinal reports practiced by the tribal people of Western Ghats, India, were assayed for antibacterial activity

against some Gram-negative bacteria using the disc diffusion method out Of these 16 plants showed activity (Sen *et al.*,1998).

Cassia singueana is a medicinal plant reputed to be of beneficial effect in the traditional system of medicine. The plant is used for various ailments that include: sterility in women, abortive, urinary schistosomiases, hernia, abdominal pain, fever, anticonvulsant, constipation, heartburn and snake bite. Previous studies on the plant showed weak antimicrobial activity, and phytochemicals constituents that include alkaloids, anthraquinones, quinones, steroid and triterpenes. (Adzu and Gamaniel, 2003).

Scientific Research (Mordi and Momoh2009), *clearly indicates that Proteus species are the most commonly isolated organism from wounds, and account for 26.79% of the 560 specimens, among which P.mirabilisoccupying the largest share of about 65% of all the Proteus species isolated.*
Bloodstream infection (BSI) due to *Proteus mirabilis* strains is a relatively uncommon clinical entity, and its significance has received little attention(Andrea *et al.*,2005), However, drug resistance has been increasingly reported for this specie, and the diffusion of resistance to extended-spectrum Cephalosporins due to the production of extended-spectrum β-lactamases (ESBLs) has become of great concern (Stürenburg, and Mack 2003).

On the other hand, *Proteus mirabilis* is one of the most common gram-negative pathogens encountered in clinical specimens and can cause a variety of community- or hospital-acquired illnesses, including urinary tract, Wound, and Bloodstream infections (O'Hara *et al., 2000)* although it is not the most common cause of UTI in the normal host (Fluit*et al.*,2000), even in patients with recurrent UTI, the incidence of infections by this organism is only a few percentage points higher(Fluit*et al*,2000), but the need for the intensive studies on the organism lies in the fact that this organism infects much higher proportions (up to 44%) of patients with complicated urinary tracts; making it the most common nosocomial infection. While infecting the urinary tract, *P. mirabilis* has a predilection for the kidney, likewise, the bacterium causes cystitis and acute pyelonephritis, but the production of urinary stones, and importantly, being the organism the most commonly isolated among proteus specie in wounds (Mordi and Momoh, 2009).

The present work, compare the activity of the two medicinal plants that are traditionally used in northern part of Nigeria to treat wounds, blood stream and urinary tract infections, caused by *Proteus mirabilis...*

2.0 MATERIALS AND METHODS

To ensure reproducible result, the following materials and methods were employed in the research.

2.1 Materials

2.2 Collection and Preparation of Sample

The roots of the plants obtained within Sokoto and Zamfara States were identified and authenticated at the Herbarium, Botany Unit, Department of Biological Sciences, UsmanuDanfodiyo University, Sokoto. The collected plants roots were then washed with clean water and air-dried under shade, thereafter, they were pulverized to small pieces using pestle and mortar. (Harborne, 1983).

2.3 Methods

2.3.1Preparation of Plant Extracts

One hundred gram (100g) of each of the plant materials was weighed and soaked in n-hexane 24 hours to remove fat from the plant materials prior to methanol extraction as described by Wagner *et al*., 1984, and the percentage yield of the extracts was calculated as follows

$$\%yield = \frac{weight\ of\ extract}{initial\ weight\ of\ sample} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The residues were prepared and used for the screening of antibacterial activity. The methanol residue of the two plants were further dissolved with distilled water and fractionated with 3 solvents of different polarity, namely: n-hexane, ethylacetate and n-Butanol and the fractions were screened for the antibacterial activity.

2.3.2Determination of Antibacterial Activity

Clinical isolates of *Proteus mirabilis* was obtained from Microbiology Laboratory,

Usmanu Danfodiyo University Teaching Hospital, Sokoto. (UDUTH).

2.3.3Extract preparation

One gram of each plant sample was dissolved in 20ml distilled water to prepare stock solution (50mg/ml), followed by serial dilution of the stock solution to prepared working solution of 5, 10, 20, 30, 40 and 50 mg/ml concentrations using the dilution formula in equation 2.

$$C_1V_1 = C_2V_2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

2.3.4 Media Preparation (nutrient agar 250cm^3)

Seven (7) gram of the nutrient's agar powder (purchased from stock) was dissolved in 250ml distilled water in a conical flask, the mouth of the conical flask was stoppered with cotton wool, and then the conical flask was wrapped with aluminum foil and then autoclave for 15 minutes at 121^{o}C. (Jahangirian*et al*., 2013).

The Media was poured on to Petri dishes on the laboratory bench, dispensed aseptically, then flamed and allowed to solidify.

2.3.5 Antibacterial Activity

The prepared media were inoculated with the test organism, the bacterium was spread on the plate using sterile bent glass rod, thereafter, various holes were made with cork-borer (6mm) on the plate, and then 5, 10, 20, 30, 40 and 50mg/ml concentration of the various plant extracts, positive control (Gentamycin 5mg/ml) and negative control (Distilled water) were transferred into various holes made on the media.

The inoculated plates were incubated at 37^{o}C for 24 hours. After 24 hours of incubation, diameters of zones of inhibition of various extract against the test organisms were measured in millimeters. (Hugo and Russel, 1983). Values greater than 6mm (the diameter of the hole) indicates some activity.

3.0 RESULT AND DISCUSSION

3.1 Results

Results obtained are presented in Tables 3.1 – 3.6.

3.1.1Result of Percentage Yield of the Extract

The Percentage Yield of Methanol Extract of the selected Plants is in Table 3.1

3.1.2 Antibacterial Activity of the Plant Extracts

The results of Antibacterial Activity of the two (2) selected Plants Extracts are in Table 3.2

3.1.3 Phytochemical Screening of the *Cassia Singueana*

The result for the phytochemical analysis of the crude Methanol Extract (CME), n-Hexane Fraction (NHF), Ethylacetate Fraction (EAF) and n-Butanol fraction (NBF) of *Cassia singueana*root Extracts are in Table 3.3 below

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3.1.4 Phytochemical Screening of the *Annona senegalensis*

The result for thephytochemical analysis of the crude Methanol Extract (CME), n-Hexane Fraction (NHF), Ethylacetate Fraction (EAF) and n-Butanol fraction (NBF) of *Annona senegalensis* root Extracts are in Table 3.4 below.

3.1.5 The Antibacterial Activity of the Fractions

The Results for the Antibacterial Activity of the Methanol Extract Fractions of *Annona senegalensis* and *Cassia singueana* are in Table 3.5.

Table 3.1 Percentage Yield of the Methanol Extracts of the selected Plants used

S/N	Plant	Percentage Yield (%)
1	*Annona senegalensis*	4.22
2	*Cassia singueana*	18.44

Table 3.2. The Antibacterial Activity of some Plant Extracts on *Proteus mirabilis*

Bacterium	***Proteus mirabilis***					
Extract concentration (mg/ml)	5	10	20	30	40	50
Plant extract	zone of inhibition (mm)					
Annona senegalensis	6.00	6.00	9.00	12.00	12.50	14.00
Cassia singueana	16.00	18.00	19.20	22.00	23.50	25.00
Gentamycin (+ control)	23.00	25.00	28.00	30.00	32.00	34.00
Distilled water (-control)	6.00	6.00	6.00	6.00	6.00	6.00

Table 3.3 Phytochemical Constituents of the crude methanol extract (CME), n-Hexane fraction (NHF), ethyl acetate fraction (EAF) and n-Butanol fraction of *Cassiasingueana* root extracts

S/N	**TEST**	**CCME**	**CNHF**	**CEAF**	**CNBF**
1	Carbohydrate				
	I- molishs test (general test)	+	+	+	+
	II- fehlings test (reducing sugar test)	+	+	+	+
2	Anthraquinones				
	I- bornstragers test	-	-	-	-
3	Cardiac glycosides				
	I- Salkwoskis test	+	-	+	+
	II- Kellakiliani test	+	+	+	+
4	Saponins				
	I- Frothing test	+	-	+	+
5	Steroids/ triterpenes				
	I- Lieberman- Buchard's test	+	+	+	+
6	Flavonoids				
	I- Sodium hydroxide test	+	+	+	+
	II- Iron (III) chloride test	+	+	+	+
	III- Shinoda's test	+	+	+	+
7	Tannins				
	I- Iron (III) chloride test	+	+	+	+
	II- Strong lead sub acetate test	+	+	+	+
8	Alkaloids				
	I- Dragendorff's reagent	+	+	+	+
	II- Wagner's reagent	+	+	+	+
	III- Mayers test	+	+	+	+

+Present - Absent

Table 3.4 Phytochemical Constituents of the crude methanol extract (CME), n-Hexane fraction (NHF), ethyl acetate fraction (EAF) and n-Butanol fraction of *Annona senegalensis* root extracts

S/N	**TEST**	**CCME**	**CNHF**	**CEAF**	**CNBF**
1	Carbohydrate				
	I- molishs test (general test)	+	+	+	+
	II- fehlings test (reducing sugar test)	+	+	+	+
2	Anthraquinones				
	I- bornstragers test	-	+	-	-
	Cardiac glycosides				
	III- Salkwoskis test	+	-	-	+

S/N	TEST		CCME	CNHF	CEAF	CNBF
	IV-	Kellakiliani test	+	+	+	+
4	Saponins					
	II-	Frothing test	+	+	+	+
5	Steroids/ triterpenes					
	II-	Lieberman- Buchard's test	+	+	+	+
6	Flavonoids					
	IV-	Sodium hydroxide test	+	+	+	+
	V-	Iron (III) chloride test	+	+	+	+
	VI-	Shinoda's test	+	+	+	+
7	Tannins					
	III-	Iron (III) chloride test	+	+	+	+
	IV-	Strong lead sub acetate test	+	+	+	+
8	Alkaloids					
	IV-	Dragendorff's reagent	+	+	+	+
	V-	Wagner's reagent	+	+	+	+
	VI-	Mayers test	+	+	+	+

+Present - Absent

Table 3.5 Antibacterial Activity of the Methanol Fractions of *Cassia singueana*

Bacterium	***Proteus mirabilis***				
Extract concentration (mg/ml)	5	10	20	30	40
Plant extract	zone of inhibition (mm)				
n- Hexane	6.00	6.00	6.00	6.00	6.00
Ethylacetate	8.00	9.00	11.60	13.50	14.20
Butanol	6.00	6.00	7.20	8.00	9.00
Gentamycin (+ control)	22.00	24.00	28.00	31.00	33.00
Distilled water (-control)	6.00	6.00	6.00	6.00	6.00

Table 3.6 Antibacterial Activity of the Methanol Fractions of *Annona senegalensis*

Bacterium	***Proteus mirabilis***				
Extract concentration (mg/ml)	5	10	20	30	40
Plant extract	zone of inhibition (mm)				
n- Hexane	6.00	6.00	6.00	6.00	6.00
Ethylacetate	6.00	6.00	6.60	7.10	7.50
Butanol	6.00	6.00	6.20	6.20	6.50
Gentamycin (+ control)	22.00	24.00	28.00	31.00	33.00
Distilled water (-control)	6.00	6.00	6.00	6.00	6.00

3.2 Discussion

In this work, three medicinal plants were selected based on their traditional role in the treatment of infectious diseases, including those diseases caused by *P. mirabilis*.

The two plants were subjected to methanol extraction (after de-fatting) in order to mimic the traditional method of extraction with water alongside other compounds.

De-fatting, eliminate low polarity constituents, therefore make easy the fractionation procedure.

In the methanol extraction, Maceration method was employed, and the result obtained for the percentage yield of the two selected plant root shows that *C. singueana* has a higher percentage yield of 18.44%, almost 32% higher than the percentage yield of the leave methanol extract as reported by Ode and Asuzu 2011, while, on the other hand *A. senagalensis* has percentage yield of 4.2%.

The crude methanol extracts were then tested for their antibacterial activity against the bacterium (*P. mirabilis*) using disc diffusion method, and *C. singueana* crude methanol extract shows remarkable antibacterial activity across all the concentrations used against the test organism (*P. mirabilis)* than the *A. senegalensis* methanol extract, this high activity (25.00mm at 50mg/ml) demonstrated by *Cassia singueana*crude methanol extracts may be as a result of the

presence of phytochemicals such as Tannins, Saponins and Alkaloids (Table 3.3) that are known to have antibacterial activity (Halilu*et al.,* 2013), even though *A. senegalensis* was also reported to contain the phytochemicals, their less or inactivity may be as a result of antagonizing effect exert by the bioactive component from other components in the plants extract, or as a result of the absence of the active compound responsible for the anti-*Proteus* effect.

The phytochemical screening of the crude methanol extract of the two plants and their fractions (*Cassia singueana)* and its ethyl acetate fraction (Table 3.3 and 3.4), reveal the presence Alkaloids, Tannins Saponins, Cardiac glycocides, Steroids and Flavanoids, the classes of secondary metabolites that are known to be responsible for the antibacterial activities demonstrated by plant extracts (Halilu*et al.,* 2013). But Anthraquinones were not detected in all the extracts and fractions although it was earlier detected in *C. singueana extract*. (Azdu and Gamaniel, 2003).

The crude methanol extract of *Annona senegalensis*shows an increasing trend of activity against the test organism.

Observing antibacterial activity in *C. singueana*methanol extract is a clear indication that the compound with the antibacterial activity may be present in that extract. But the crude extract contain mixture of so many compounds alongside with the targeted compound, thisprompted us to the fractionation of the methanol extracts to further separate other non-active compounds using liquid-liquid extraction.

In liquid-liquid extraction, also known as solvent – solvent extraction, it's inevitable fact that like dissolves like, ie polar compounds will prefer polar solvent, and non-polar will prefer non polar solvent, with regard to this, we use solvents of different polarity (n- Hexane, ethyl acetate and n-butanol), in order to separate compounds based on their relative solubility in two different immiscible liquids, three solvents of different polarity were used to achieve proper distribution in suitable solvents, this include n-hexane, ethyl acetate and n-butanol.

The three fractions of the two methanol extracts were further subjected to antibacterial activity using the same disc diffusion method, and *C. singueana* ethyl acetate fraction (CEAF) was found to show the highest activity (8.20mm at 40mg/ml) against the test organism among the six (6) solvents fractions tested, even though some activity was observed in *C. singueana*n-Butanol fraction (CNBF) (3.0mm at 40mg/ml), the ethyl acetate fraction demonstrated the highest activity (8.20mm) against the test organism at that same concentration (Table 3.4), but *C. singueana*n-hexane fraction (CNHF), *A. senegalensis*n- hexane fraction(ANHF) show no activity against the test organism across the concentrations.

However, ethyl acetate and n-butanol fractions of *A. senegalansis, (ie.,* AEAF and ANBF) shows little activity at higher concentration (7.50 and 6.50 mm at 40 mg/ml respectively).

From the antibacterial activity of the methanol extract (Table 3.2) and that of the solvent fractions (Table 3.4); it can be observed that the antibacterial activity drastically decreases from 17. 50mm (most potent plant) at the concentration of 40mg/ml to 8.20mm (most potent fraction) at the same

concentration, this may be as result of synergistic effect exert by the compound from other compound in the methanol extract which were remove during fractionation process. But the result for the antibacterial activity of the isolated compound (Table 3.6) is in concordant with the result obtained for the ethyl acetate fraction (Table 3.4).

The antibacterial activity of the fractions clearly indicates that *C. singueana*ethyl acetate fraction is the most potent fraction; hence it may contain the active compound at a higher concentration compared to other Fractions.

4.0 CONCLUSION AND RECOMMENDATION

4.1 Conclusion

Out of the two plants selected for the study, *Cassia singueana* prove to be the most potent against the target organism (*P.mirabilis).* It showed an inhibition zone of 19mm where the Gentamycin gave 28mm. That is, it had 68% activity compared to the Gentamycin. The ethylacetate fraction of the plant extract showed inhibition zone of 8mm against 27mm for Gentamycin; that is about 30% of Gentamycin activity.

Thus, it can be concluded that the plant actually has some level of activity against the test organism although it is much weaker than the existing drug (Gentamycin).

Therefore, the study justified the ethnomedical claim of the use of the plant in treatment of wounds, bloodstream and urinary tract infections.

4.2 Recommendations

Further research needs to be carried out to include;

a. Isolating the active compound responsible for the observed activity.
b. Testing the activity of the isolated compound on other pathogenic bacteria.
c. Evaluation of the chronic and acute toxicity of the compound.
d. Confirmation of possible synthetic route for the compound.

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