

# Fractionation of Methanolic Crude Extract of Root of *Senna siamea* Decreases Antimicrobial Activity

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## Abstract:

The effect of different fractions of methanolic crude extract of root of *Senna siamea* on *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* was investigated. Phytochemical analysis of root of *Senna siamea* revealed the presence of tannins, saponins, flavonoids, steroids, cardiac glycosides, and anthraquinone. The methanolic crude extract of the root of the plant exhibited highest activity on *Escherichia coli* and *Salmonella typhi* both with zone of inhibitions of 30.00 mm and least activity on *Staphylococcus aureus* with a value of 23.00 mm. The extract was further separated chromatographically using different solvents, four fractions were collected and designated: I, II, III and IV which were eluted using methanol/water (1:1), ethylacetate/methanol (19:1), benzene/methanol (9:1) and acetic acid/methanol (1:1). Among the four fractions, fraction IV had least effect on the test organisms ranging from 0.00 mm to 20.00 mm while fraction I had highest effect on the test organisms ranging from 13.00 mm to 20.00 mm. This study revealed that fractionation of the methanolic crude extract of the root of *Senna siamea* resulted in the decrease of activity on the test organisms.

## Keywords:

*Escherichia coli*; Fractionation; Phytochemical analysis; *Salmonella typhi*; *Senna siamea*; *Staphylococcus aureus*

## 1. INTRODUCTION

Medicinal plant is any plant used for extraction of pure substances either for direct medicinal use or hemi-synthesis of medicinal compounds which can be used for therapeutic purpose or as precursor for the synthesis of useful drugs [1]. Medicinal plants have been in use for the past fifty centuries which until the last two and a half centuries was the main source of treatment to man and his domestic animals [2].

*Senna siamea* is a non-nitrogen fixing leguminous tree in the sub-family *Caesalpinioideae* of the family *Leguminosae*. It has been widely planted in many south-east Asian countries for erosion control, wind breaks, fuel wood and pole wood. It is commonly called *Thailand shower*, *Yellow cassia*, *Minjin* or *kissod* and has many regional names [3]. The plant has many medicinal values for instance it acts on

the lower bowel and is useful in alleviating constipation. It increases the peristaltic movement of the colon. It is most often prepared as infusion [4]. Macerated juice of the fresh leaves is recognized by traditional healers as a remedy for parasitic diseases such as enteriobiasis or any skin related disorders like dermatoyositis [5].

A crude plant is a complex mixture and its evaluation for the large array of compounds in the complex mixture may interact antagonistically or synergistically interfering with or masking the activity of one another. Secondly, the vast majority of active compounds in crude extract is present at a very low concentration and therefore may not show high specific activity [6].

This study was undertaken to fractionate the methanolic crude extract of root of *Senna Siamea* using column chromatography and evaluate the effect of the fractions on *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* so as to pave way in elucidating the active component responsible for the observed activity if any.

## 2. MATERIALS AND METHODS

### 2.1 COLLECTION OF PLANT MATERIAL

The root of *Senna siameae* was collected around Yolde Pate Ward in Yola South Local Government Adamawa State, identified and authenticated at the Department of Plant Sciences, Modibbo Adama University of Technology, Yola, Adamawa State.

### 2.2 MICROORGANISMS

The microorganisms *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* were collected from microbiology laboratory of Federal Medical Centre Yola and the department of Microbiology Modibbo Adama University of Technology, Yola, Adamawa State. All the microorganisms are clinical isolates identified in the mentioned laboratories.

### 2.3 EXTRACT PREPARATION

The root of *Senna Siamea* was removed from the plant, washed and air-dried for 5-days at room temperature. The root of the plant was pounded using pestle and mortar, after which the powdered form were macerated in methanol and left overnight. The mixture was filtered and evaporated using rotary evaporator.

### 2.4 PHYOCHEMICAL ANALYSIS

Chemical test was conducted on the crude extract using standard procedures to identify the constituents as described by Trease and Evans[7], Sofowora[1].

**Table 1. PHYTOCHEMICAL ANALYSIS OF ROOT OF *SENNA SIAMEA*.**

Phytochemicals	Result
Tanins	+
Saponins	++
Flavonoids	+
Steroids	+
Alkaloids	-
Terpenoids	-
Cardiac glycosides	++
Anthraquinones	+
Phenols	-

++ = Highly present    + = present    - = absent

## 2.5 SEPARATION OF METHANOLIC CRUDE EXTRACT OF ROOT OF *SENNA SIAMEA*.

Slurry was prepared by dissolving 30g silica gel in 100ml methanol: water (1:1) and packed in a column (1.5 × 30cm). The column was loaded with 15ml of the crude extract and sequentially eluted with benzene/methanol (9:1), and acetic acid/methanol (1:1) ethylacetate/methanol (19:1). The fractions were collected separately, concentrated under pressure using rotary evaporator [8].

## 3. ANTIMICROBIAL ACTIVITY TESTING

The effect of the methnolic crude extract of root of *Senna siameae* was tested according to the method described by Emeruwa [9]. Wells were made on the surface of 19ml nutrient agar plates which was seeded with 0.1ml of  $10^{-6}$  test organisms. Exactly 0.5ml of crude extract was aseptically introduced into the wells made. The plates were allowed to stand on the working bench for 30 minutes after which the plates were incubated for 24 hours at 37°C in an incubator. The presence of zone of inhibition was regarded as positive and was expressed in terms of average diameter of the zone of inhibition.

### 3.1 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

MIC of the most active fraction (I) was determined by dilution of the crude extract to various concentrations of 10, 5, 2.5, 1.25 and 0.65 mg/ml respectively. Equal volume of crude extract and nutrient broth were mixed in a test tube. Specification, 0.1ml of standardized inoculum ( $1.0 \times 10^{-4}$  cfu/ml) was added in each tube. The tubes were incubated aerobically at 37°C for 18-24. Tubes containing extract and growth media without inoculum was used as control. The lowest concentration of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tube was regarded as MIC [10].

## 4. RESULTS

## 5. DISCUSSION

Phytochemical compounds have been reported to be of great medicinal value. The phytochemical compounds found in the methanolic crude extract of root of *Senna Siamea* such as tanins, saponins,

**Table 2.** EFFECT OF METHANOLIC CRUDE EXTRACT OF ROOT OF *SENNA SIAMEA* ON THE TEST ORGANISMS

TEST ORGANISMS	ZONE OF INHIBITION(mm)
<i>Escherichia coli</i>	30.00
<i>Salmonella typhi</i>	30.00
<i>Staphilococcus aureus</i>	23.00

**Table 3.** EFFECT OF DIFFERENT FRACTIONS ON THE TEST ORGANISMS

TEST ORGANISMS	FRACTION I (mm)	FRACTION II (mm)	FRACTION III (mm)	FRACTION IV (mm)
<i>Escherichia coli</i>	13.00	10.00	9.00	-
<i>Salmonella typhi</i>	20.00	20.00	18.00	20.00
<i>Staphilococcus aureus</i>	15.00	13.00	15.00	12.00

FRACTION I=Methanol/water, FRACTION II= Acetic acid/methanol, FRACTION III= Benzene/methanol FRACTION IV= Ethylacetate/methanol

flavonoids and cardiac glycosides have been associated with antimicrobial activity by other workers [11, 12]. However, since there is a family of these compounds, there may be a need to determine which specific compound(s) among them, exhibit the effect against the test organisms.

Different mechanisms of action of these phytochemicals have been suggested, such as interference with the phospholipids bilayer of the cell membrane which has as a consequence a permeability increase and loss of cellular constituents, damage of the enzymes involved in the production of cellular energy and synthesis of structural components, and destruction or inactivation of genetic material. In general mechanism of action is considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport and coagulation of cell contents [13]. So, it is possible that, the bioactive compounds in the root of this plant exerted its effect via some of the mentioned mechanisms.

Separation of parent methanolic crude extract of the root of *Senna siamea* using column chromatography gave four fractions designated: I,II,III ,and IV. Of all the fractions, fraction IV showed the least effect against the test organisms. Those that showed better effect, the range and type of organisms showing susceptibility varied with fraction, which indicates that there were several types of compounds with antimicrobial effect among the phytochemical constituents of the root of *Senna siamea* plant. Thus, fraction I probably contained the highest proportion of these antimicrobial compounds followed by fraction II and III in that order. It is not surprising, therefore, fraction I showed a wider spectrum of activity than other fractions; but whether this superiority of activity means that it contains more potent antimicrobial principles or it contains compound acting together synergistically or additively needs to be

**Table 4.** MINIMUM INHIBITORY CONCENTRATION OF THE MOST ACTIVE FRACTION (I) ON THE TEST ORGANISMS.

TEST ORGANISMS	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.65 mg/ml
<i>Escherichia coli</i>	-	+	+	+	+
<i>Salmonella typhi</i>	-	-	-	+	+
<i>Staphilococcus aureus</i>	-	-	+	+	+

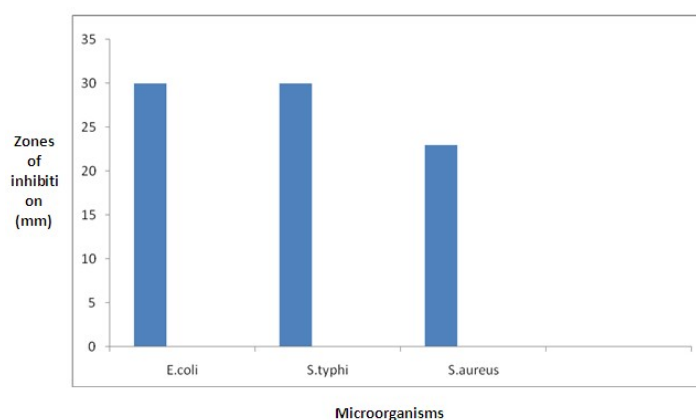
-=No Growth, +=There is Growth

ascertained. On the other hand, the superior effect of fraction I could be due to the presence of higher concentration of the bioactive components in it.

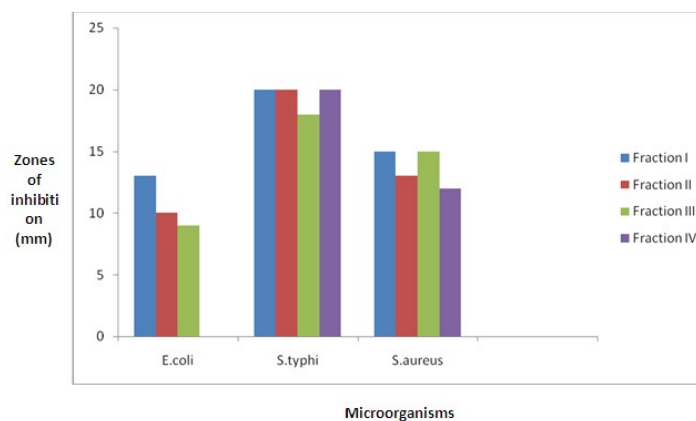
Fractionation of the crude extract resulted in the decrease effect against the test organisms. This observation is in agreement with the view of Nwodo, [6] who stated that fractionation of crude extract could produce loss or gain of activity depending on the nature of interaction between the constituent compounds of the extract.

In conclusion, this study which evaluated the effect of different fractions of methanolic crude extract of root of *Senna siamea* may enhance the effective use of the root of the plant on the diseases caused by the test pathogens.

## APPENDICES



**Figure 1.** Effect of methanolic crude extract of root of *Senna siamea* on the Test organisms



**Figure 2.** Effect of Different Fractions of methanolic crude extract of root of *Senna siamea* on the Test organisms

## ACKNOWLEDGMENTS

The authors are very grateful to the department of Biochemistry, Modibbo Adama University of Technology, Yola, Adamawa State, for giving them the privilege to use their laboratory during study. The authors are also indebted to the chief Laboratory Technologist of the department for his assistance.

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