



## Phytochemical analysis, antibacterial and antioxidant activities of *Entada africana* Guill. and perrott stem bark extracts

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### Abstract

*Entada africana* Guill and Perrot (*Fabaceae*) is predominantly a West African medicinal plant used in the treatment of several diseases. Multidrug resistance and the undesirable side effects of synthetic drugs underscore the need to search for alternatives from plants using bioassay guided technique. The hexane, dichloromethane, acetone and methanol stem bark extracts were screened for antibacterial activity. Relatively active acetone and methanol extracts were further analyzed for phytochemical contents and antioxidant property using standard methods. Results of the antibacterial screening indicates that the acetone extract had the best antibacterial property against *Enterococcus faecalis*, *Escherichia coli* and *Staphylococcus aureus* with inhibition zones ranging from 14.00±1.00 mm – 17.00±1.73 mm. Additionally, *E. faecalis* and *E. coli* had minimum inhibitory concentrations (MIC) of 0.39 mg/mL and 0.20 mg/mL respectively indicating moderate activity but weak on *Staphylococcus aureus* with MIC of 13.88 mg/mL. The quantitative phytochemical analysis of acetone and methanol extracts indicated 0.528±0.02 mg GAE/g and 0.650±0.85 mg GAE/g total polyphenol respectively. The acetone extract also yielded 0.500±0.017 mg RE/g of total flavonoids while the methanol extract gave 0.253±0.006 mg RE/g of total flavonoids. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) test for the crude extracts revealed similar antioxidant activity comparable to that of ascorbic acid standard and concentration dependent. The observed biological activities indicated the presence of bioactive phyto-constituents. The study therefore supports the therapeutic use of *Entada africana* as a herbal remedy in traditional medicine.

**Keywords:** *Entada africana*, Phytoconstituents, Bioactive, Antimicrobial, Antioxidant, Pharmacological.

### Introduction

For thousands of years, medicinal plants had been used in healthcare in various forms to alleviate or treat ailments in ethno-pharmacology globally. The healing powers of medicinal plants were not clear until the advent of modern methods of scientific analysis which demonstrated the existence of phyto-constituents such as alkaloids, terpenoids and phenolic compounds with a broad spectrum of biological and therapeutic activity such as antimicrobial, anticancer, anti-inflammatory, inhibition of cholesterol synthesis, antihypertensive, antiarrhythmias and antioxidants<sup>1,2</sup>. Studies had shown that there are variations in these classes of phytochemical substances in plants both within and across plant species. Genetic variations due to cross pollination and geographical locations do occur and had been suggested to account for observed differences. Consequently a given plant species in different parts of the globe may be used for different therapeutic purposes<sup>3</sup>. Furthermore, the fact that very scanty reports exists on the phytochemical analysis, antibacterial activity and antioxidant activity of *E. africana* underscores the need for phytochemical investigation of this medicinal plant using standard procedures as reported by several authors<sup>2,4-7</sup>. Previous assessments employed the use tannic acid as the standard while this report

uses gallic acid and rutin as standards for the determination of total polyphenols and total flavonoids contents respectively which are well defined phytocompounds relative to tannic acid, an oligomer. Information on the chemical constituents of medicinal plants does not only aid drug discovery but also reveals new sources of drug precursor materials<sup>4,5,8</sup>. Reports from Ougadougou, Togo and Cameroon indicates that the crude stem bark of *E. africana* possess antityphoidal, antimicrobial and antimalarial activities and inhibits the growth of resistant *E. coli*<sup>6,9-11</sup>.

A dichloromethane/methanol (1:1v/v) extract column fraction of *E. africana* was found to be a more potent anti-inflammatory agent than a standard anti-inflammatory agent *Baicalin* due to significant levels of polyphenols content<sup>12</sup>. A strong correlation was also found to exist between polyphenol content and antioxidant property of chemical substances with Pearson correlation coefficient,  $R^2 = 0.994$  in ferric thiocyanate,  $\text{Fe}(\text{SCN})_3$ , assay and  $R^2 = 0.914$  in DPPH solution test<sup>13,14</sup>. Antioxidants are considered as phytochemical compounds that neutralize and prevent free radicals from inflicting harm to human body physiology. Free radicals which are produced during cellular respiration, infections and by solar radiations are known to be the underlying causes for many human ailments

such as cardiovascular diseases, diabetes, cancer, Alzheimer's disease and neurological disorders. Vitamins A, C, E and polyphenols (tannins, phenolic acids and flavonoids) are useful in the neutralization of these free radicals. The activities of these phytochemical substances are further complimented by those of superoxide dismutase, glutathione peroxidase and catalase enzyme systems of the human body. In a healthy body, a balance is always maintained between the rate of production and removal of free radicals<sup>5,15</sup>.

*Entada africana* (*E.africana*) is a perennial plant that grows to heights of 4 – 10 m and 90 cm in girth. It is predominantly a West African medicinal plant that is quite valuable as a herbal remedy in the treatment of many diseases that include but not limited to fever, diabetes mellitus, arrow poison, stomach upset and diarrhea<sup>5,16</sup>. The root decoction extracts serve as a tonic and a stimulant while extracts of the stem bark possess abortifacient properties. The leaves are used for wound dressing among Nigerians and the Ghanians. Literature studies had shown the existence of several classes of bioactive phytoconstituents such as alkaloids, flavonoids, cardiac glycosides, steroids, saponins, terpenoids, coumarins and polyphenols in the methanol extract of *E .africana* stem bark. Despite the numerous traditional medicinal uses of *E. africana*, only few reports are found in literature<sup>5,17,18</sup>. Consequently, the absence any report on *E. africana* stem bark acetone extract vis-à-vis its methanol extract informed the need for this study. It is also quite important to carry out the phytochemical investigation of *E. africana* of Gombe State Nigeria, given geographical, regional and seasonal variations in phytoconstituents and their therapeutic efficacy even for the same plant species<sup>3</sup>. The evaluation of phytochemical constituents, the antibacterial activity and antioxidant property of *E. africana* is necessary to determine some of its specific medicinal values for possible commercial exploitation and hence the needs for this study.

## Materials and methods

**Sample collection, identification and handling:** The *E. africana* stem bark sample was collected by a herbalist in Gombe in the month of November, 2014 from Akko Local Government area of Gombe State, Nigeria. It was later identified by Dr. K. P. Yoriyo of Biological Sciences Department, Gombe State University of Nigeria with Voucher No. FHJ-227. The stem bark was shredded into pieces and shade dried on cleaned ceramic slab surface. This was later pulverized to powder and stored properly until required for use.

**Extraction:** Successive extraction was carried out on 2.68 Kg stem bark sample of *E. africana* with hexane, dichloromethane and acetone. Pulverized plant samples were soaked in the different solvents for periods of five to seven days per solvent so as to achieve maximum yield. These were filtered and concentrated on a rotary evaporator at 45°C. Similarly, another portion of the pulverized stem bark sample of *E. africana* (1.0 Kg) was defatted with hexane and then extracted with 70% methanol to obtain methanol extract<sup>19</sup>.

**Quantitative Phytochemical Analysis:** Polyphenols and flavonoids are phytochemical compounds of great importance with a broad spectrum of biological and therapeutic properties such as anticancer, antiinfective, antioxidant, anti-inflammation, antidiabetic, cardiovascular and hepatoprotective properties<sup>20</sup>. These are naturally polar substances that are better extracted with polar solvents such as acetone and methanol. Consequently, the phytochemical analysis of polyphenols and flavonoids was carried on the acetone and methanol stem bark extracts<sup>21,22</sup>.

**Evaluation of total polyphenol:** Folin-ciocalteu reagent (1.5 mL, 10%) was added to the plant extract (100 µL, 1 mg/mL) solution. After 2 mins, Na<sub>2</sub>CO<sub>3</sub> (1.2 mL, 20%) was added to the mixture. This reaction mixture was allowed to stand for further 30 mins. Absorbance values were read at a wavelength of 765 nm on a Spectrophotometer with model No. 6405 UV/Vis. From the absorbance values of a two-fold serial dilution solution of gallic acid (100µL, 0.5 mg/mL), a standard curve was obtained by plotting absorbance values against solution concentrations. The total phenolic content of extract was evaluated using the gallic acid calibration curve<sup>8</sup>.

**Evaluation of total flavonoids:** A solution of AlCl<sub>3</sub> (100 µL, 0.2 M) was mixed with the plant extract (100 µL, 10 mg/mL) solution. A drop of acetic acid was used to acidify the mixture and then made up to 5 mL with methanol. The mixture was kept standing for 40 mins before absorbance values were taken at a wavelength of 415 nm. The blank consisted of the plant extract (100 µL, 10 mg/mL), a drop of acetic acid and diluted to 5 mL with methanol without AlCl<sub>3</sub> solution. Rutin solution (100 µL, 0.5 mg/mL) was used to obtain the calibration curve under the same conditions as test samples. All determinations were performed in triplicate<sup>23</sup>.

**Antibacterial Screening:** The agar disc diffusion method was adopted for the antibacterial susceptibility test with slight modification<sup>11</sup>. Briefly, Clinical isolates of *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus* were incubated overnight in Mueller Hinton broth prepared according to manufacturer's standard. The cultures were standardized using 0.5 McFarland turbidity standards. About 25mL of sterilized Mueller Hinton agar was poured into 100 mm petridish and allowed to solidify. 100µL inoculums size of microbes containing approximately 1 x 10<sup>6</sup> cfu/mL was smeared unto agar surfaces. Punched sterile filter paper discs impregnated with extracts at 100 mg/mL were placed on the agar surface together with standard commercially manufactured discs of gentamicin at 10 µg/mL. The plates were incubated in incubators overnight at 37°C for bacteria. After 24 hrs plates were examined for inhibition zones. All determinations were carried out in triplicate.

**Antioxidant Activity Procedure: DPPH free-radical Assay:** Different sample solution concentrations were prepared by

dissolving 0.49, 0.98, 2.0 and 3.0 mg in minimum amount of methanol and made up to 2 mL to produce approximate concentrations of 0.25, 0.5, 1.0 and 1.5 mg/mL respectively. Similarly 100 mL solution of 39.4 mg DPPH was prepared in methanol to give a 1 mM solution<sup>24</sup>.

The solutions were kept for 10 mins after which absorbances were read at wavelength of 517 nm. Two (2 mL) of DPPH solution was added to 0.5 mL of each plant sample solution. The mixture was shaken and kept for 10 mins and the absorbance read at 517 nm for solutions, control and the standard. The percentage inhibition was calculated according to equation 1. The same method was applied to butylatedhydroxyanisole (BHA), which was used as the standard prepared in distilled water<sup>25</sup>.

$$\text{Inhibition (\%)} = \left( \frac{A_{\text{blank}} - A_{\text{test}}}{A_{\text{blank}}} \right) \times 100 \quad (1)$$

Where:  $A_{\text{blank}}$  = Absorbance of blank and  $A_{\text{test}}$  = Absorbance of sample or control.

## Results and discussion

**Table-1:** Percentage yield of Extracts.

Solvents	Extract yield (gram)	Percentage yield (%)
Hexane	46.25	1.73
Dichloromethane	7.12	0.27
Acetone	134.00	5.00
Methanol	119.00	11.90

**Table-2:** Total Polyphenol and Total Flavonoid contents.

Extract	Polyphenol (mgGAE/g)	Flavonoid (mgRE/g)
Acetone	0.528±0.02	0.500±0.017
Methanol	0.650±0.09	0.253±0.006

**Table-3:** *E. africana* Crude Extract at 100 mg/mL; Inhibition Zones (mm).

Organisms	Hexane	Dichloromethane	Acetone	Gentamicin (10 µg/mL)
<i>Enterococcus faecalis</i>	9.00±1.73	8.00±0.75	17.00±1.73	30.00±2.31
<i>Escherichia coli</i>	10.33±1.53	10.67±0.58	14.67±1.53	14.33±0.58
<i>Klebsiella pneumoniae</i>	8.33±0.58	9.33±1.15	0.00	18.00±0.00
<i>Pseudomonas aeruginosa</i>	12.67±2.31	9.67±1.53	0.00	14.67±1.53
<i>Salmonella typhi</i>	0.00	10.67±0.58	14.00±1.00	18.00±1.63
<i>Staphylococcus aureus</i>	0.00	0.00	14.67±1.53	20.67±1.15

**Table-4:** MIC and MBC of Bacterial Strains.

Organisms	MIC (mg/mL)	MBC (mg/mL)
<i>Enterococcus faecalis</i>	0.39	12.50
<i>Escherichia coli</i>	0.20	25.00
<i>Staphylococcus aureus</i>	13.88	91.00

**Table-5:** Susceptibility test, MIC and MBC of Methanol Extract of *E. africana* Stem bark.

Organisms	Methanol Extract (100 mg/mL)			Gentamicin (10 µg/mL)		
	Inhibition Zones (mm)	MIC	MBC	Inhibition zones (mm)	MIC	MBC
<i>E. coli</i>	9.7±1.3	12.50	50.00	21.0±1.0	5	10
<i>S. aureus</i>	11.3±0.6	6.25	25.00	23.7±0.5	2.5	5
<i>Klebsiella pneumoniae</i>	10.0±0.0	12.5	≤ 100	22.7±1.5	5	≤ 10
<i>S. typhi</i>	10.67±0.58	12.5	≤ 100	21.3±1.3	5	10

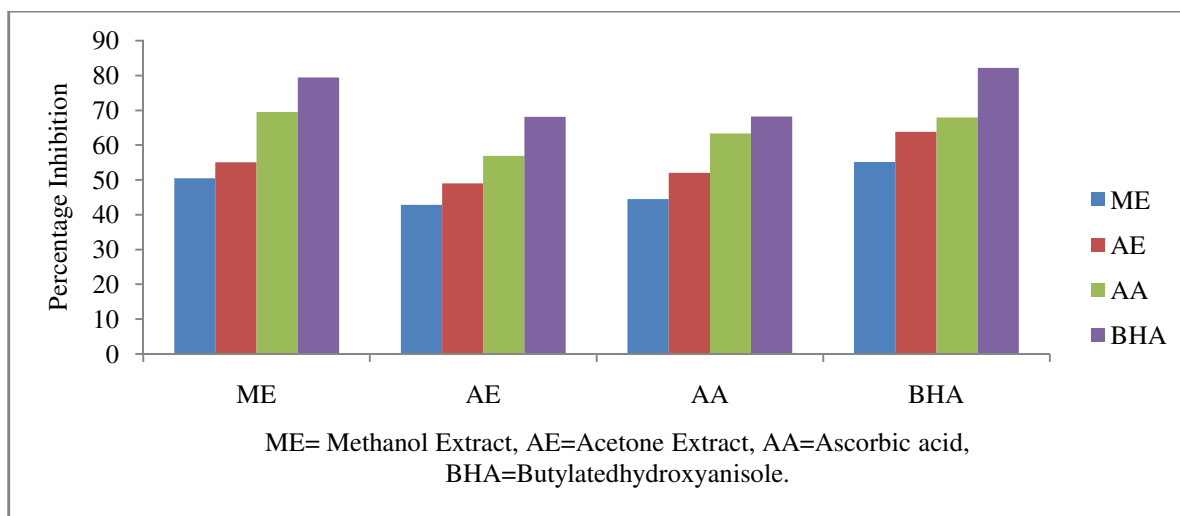


Figure-1: *E. africana* stem bark crude extract Antioxidant Activities.

**Discussion:** The percentage recovery of extracts for the various solvents used for the extraction of *E. africana* stem bark are shown above (Table-1). Low recoveries were observed in each case. This is to be expected due to the fibrous nature of the *E. africana* stem bark. The determination of the total polyphenol and total flavonoids contents were carried out according to established methods<sup>8,23</sup>. From a gallic acid calibration curve ( $y = 2.443x + 0.035$ ,  $R^2 = 0.999$ ), the total polyphenol contents of both acetone and methanol were found to be  $0.528 \pm 0.020$  mg GAE/g and  $0.650 \pm 0.09$  mg GAE/g respectively. Rutin calibration curve,  $y = 0.170x + 0.001$ ,  $R^2 = 0.972$ , was used to evaluate the total flavonoids of the acetone and methanol extracts. Results indicate  $0.500 \pm 0.017$  mg RE/g for acetone extract and  $0.253 \pm 0.006$  mg RE/g for methanol extract as their respective total flavonoid contents (Table-2). From these results, the polyphenol content of methanol extract is greater than that of acetone extract. This observed difference may be due to methanol being more polar than acetone and may extract more polar phytochemicals such as tannins. Such findings are consistent with the physicochemical properties of the solvents<sup>5</sup>. On the other hand, the flavonoid content of methanol was lower than that of acetone extract. The acetone molecules can interact with the non-polar portions of organic molecules more than the methanol molecules and therefore could potentially extract more lipophilic flavonoids<sup>19</sup>.

The antibacterial screening of *E. africana* stem bark extracts revealed moderate activity for acetone extracts with inhibition zones range of 14 mm – 17 mm and a weak activity for both hexane and dichloromethane extracts (Table-3). This may be explained partly due to the existence of more flavonoids and polyphenols in the acetone extract relative to the hexane and dichloromethane which might contain little or none of them at all. The well known antibacterial property of flavonoids is due to their ability to achieve complex formation with extracellular soluble proteins and the cell wall of bacteria. Some lipid soluble flavonoids can penetrate bacterial cell walls and cause the

disruption of cell membranes<sup>26</sup>. The moderately active acetone extract was assessed for minimum inhibitory and minimum bactericidal concentrations on *Enterococcus faecalis*, *Escherichia coli* and *Staphylococcus aureus* due being sensitive to the extract (Table-4). Results confirm the presence of moderate activity on *Enterococcus faecalis* and *Escherichia coli* but weak on *Staphylococcus aureus*. The observed high MBC values confirm the bacterial isolates to be resistant species<sup>27</sup>. Similarly, Table-5 shows the results of the antibacterial screening of *E. africana* stem bark methanol extract. The result shows slightly weaker growth inhibition relative to that of acetone extract. The relatively high flavonoids content of acetone extract compared to methanol extract may partly explain such the difference in antibacterial activity.

The antioxidant activity of both the acetone and methanol stem bark extracts of *E. africana* were assessed using DPPH stable free radical (Figure-1). Results showed that their antioxidant activity is concentration dependent for both acetone and methanol stem bark extracts. The fifty percent inhibitory concentrations ( $IC_{50}$ ) were evaluated using SPSS 16.0 software. The methanol extract exhibited a relatively higher antioxidant property ( $IC_{50} = 0.261$  mg/mL) compared to acetone extract ( $IC_{50} = 0.595$  mg/mL) and ascorbic acid standard ( $IC_{50} = 0.443$  mg/mL). Butylatedhydroxyanisole, a synthetic antioxidant under the same conditions had a much higher antioxidant activity ( $IC_{50} = 1$   $\mu$ g/mL). However the antioxidant activities of both extracts are quite significant relative to that of ascorbic acid and hence may partly explain the use of *E. africana* in traditional medicine cancer management and other related diseases.

## Conclusion

The observed antibacterial and antioxidant properties of the acetone and methanol stem bark extracts of *E. africana* correlates with the presence of bioactive principles whose

activities may be improved if purified. Consequently, the purification and subsequent determination of the biological properties of isolates is recommended. It is also important to characterize the isolates so as to evaluate the structure activity relationship of the compound(s) obtained. The effort may yield quite a substantial evidence for the use of *E. africana* in traditional medicine.

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