In vitro synergistic study on the effect of combined methanolic leaves extracts of *Eucalyptus camaldulensis* and *Senna occidentalis* against *Salmonella typhi.*

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Abstract

Nature has been a source of medicinal agents for thousands of years and since the beginning of man, however, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has led to the search of new antibacterial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the above disadvantages. The plants *Eucalyptus camaldulensis* (Myrtaceae) and *Senna occidentalis* (Fabaceae), are used traditionally for the treatment of wounds, boils, typhoid, malaria and other bacterial infections, this research was carried-out to evaluate the combined *in vitro* antibacterial activity of *S. occidentalis* and *E. camaldulensis* against *Salmonella typhi*. The antibacterial activity of the methanolic leaves extracts against the *S. typhi* was carried out using agar well diffusion and broth dilution methods. Five concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml were used and Ciprofloxacin (10 µg/ml) was used as control. The result obtained showed that methanolic leaves extracts of *S. occidentalis* and *E. camaldulensis* have antibacterial activity against *S. typhi*. The minimum inhibitory concentration of *S. occidentalis*, *E. camaldulensis* and their combination in ratio 1:1 were 6.25 mg/ml, 25 mg/ml and 25 mg/ml respectively. The minimum bactericidal concentration of *E. camaldulensis* and its combination with *S. occidentalis* are 50 mg/ml and 200 mg/ml respectively. The antibacterial activity increases as the concentration also increases. The extract when used alone yielded a significant activity than when combined together, this reduction in activity may be due to antagonistic interaction between some compounds present in the two extracts that inhibit the activity of one another.

Keywords: *Senna occidentalis*, *Eucalyptus camaldulensis*, *Salmonella typhi*, methanolic leaves extract, antibacterial activity.

INTRODUCTION:

Nature has been a source of medicinal agents for thousands of years and since the beginning of man. In Nigeria, almost all plants are medicinal and the application of medicinal plants especially in traditional medicine is currently well acknowledged and established as a viable profession (Kafaru, 1994). Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity.

It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity (Ebana *et al.,* 1991; Pamplona – Roger, 1999; Manna and Abalaka, 2000). However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Shariff, 2001). Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also associated with the emergence of widespread drug resistance (Shariff, 2001). During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has led to the search of new antibacterial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the above disadvantages. The plants *E. camaldulensis* (Myrtaceae) and *S. occidentalis* (Fabaceae), are used traditionally for the treatment of wounds, boils, typhoid, malaria and other bacterial infections (Bouamama *et al*., 2006). *S. occidentalis* (L.) a small shrub about 3 ft. high belong to Leguminosae family. It is native to the tropical regions of America and naturalized in Australia, eastern Africa, southern and eastern USA (Isah and Mujib, 2013). While *E. camaldulensis* originated mainly from Australia but now widely distributed around the globe, found in Pakistan, USA, South Africa and some tropical African Countries (Rejmanek and Richardson, 2011) and usually grows up to 20 m high, with 1-2 cm stem diameter and 7-10 cm long and greenish leaves.

Salmonella is a genus of rod-shape, Gram negative, non-spore forming, predominantly motile Enterobacteria with diameters around 0.7 to 1.5 µm, lengths from 2 to 5 µm and flagella that move in all directions. They are chemo organotrophs, obtaining their energy from oxidation and reduction reactions using organic sources and are facultative anaerobes. *Salmonella* is closely related to the *Escherichia* genus and are found worldwide in cold and warm blooded animals (including humans), and in typhoid fever, paratyphoid fever and food borne illness (Ryan *et al.,* 2004).

Salmonellosis is a water borne infection caused by a group of gram-negative motile rods belonging to the family of Enterobacteriaceae. The genus *Salmonella* which causes Salmonellosis comprises several species of which *S. typhi* and *S. paratyphi* are the main species pathogenic to man. The species *S. typhimurium* is the common cause of gastroenteritis, while *S. chloreraesus* is responsible for septicemia. Salmonellosis is associated with several symptoms including diarrhoea, vomiting, nausea, abdominal pain and fever. Untreated cases could become chronic or fatal. The portal of entry for Salmonellosis is through ingestion of contaminated food and/or water contaminated with animal or human faeces. The infection was commonly treated with antibiotics such as Ampicillin, Chloramphenicol, Trimethoprim/Sulpha-methaxazole, and Cephalosporin. However, resistance to these antibiotics have emerged. Salmonellosis can be controlled or prevented by adequate supervision of food and water, as well as improved personal hygiene (Ohalete *et al.,* 2011).

MATERIALS AND METHODS

The fresh leaves of *S. occidentalis* and *E. camaldulensis* were collected from Gaskiya Layout, Zaria, Kaduna-Nigeria. The identity of the plants were confirmed by a plant taxonomist Mallam Namadi Ibrahim and the voucher specimen ABU0900218 and ABU02510 respectively lodged at Herbarium

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EXTRACTION OF *SENNA OCCIDENTALIS* AND *EUCALYPTUS CAMALDULENSIS* LEAVES

The leaves were washed thoroughly with tap water and rinsed finally with distilled water. The sample was then air dried at room temperature for 7 days. After drying, the sample was ground with a mortar and pestle to obtain a relatively coarse powder. The dried powder was kept in an air-tight container.

The 100 g of the dried powder was dissolved into 1 L of methanol. The mixture was shaken then left to soak for 3 days in the laboratory. The preparation was first filtered using a clean cloth to remove debris then re-filtered using Whatmann's filter paper. The resultant filtrate was concentrated to dryness at 40 ˚C under reduced pressure. The percentage recovery was calculated and the dried extract was stored in an air-tight container until use. This method was employed for both plants.

PHYTOCHEMICAL SCREENING

The methanolic extracts of leaves of *S. occidentalis* and *E. camaldulensis* were subjected to qualitative test for detection of the presence of bioactive components that include Molisch's test for detection of Carbohydrates, Dragenduff test for detection of Alkaloids, Ferric chloride test for detection of Tannins, Keller- Killiani's test for detection of Cardiac Glycosides, Frothing test for detection of Saponins, Libermann-Burchard's test for detection of Steroids and Triterpenoids, Free anthraquionone test for the detection of Anthraquinones **(**Sofowora, 1982; Trease and Evans, 2002; Adegoke *et al.,* 2010).

SCREENING OF *SENNA OCCIDENTALIS* AND *EUCALYPTUS CAMALDULENSIS* LEAVES EXTRACT FOR ANTIBACTERIAL ACTIVITY

Screening of *S. occidentalis* methanolic leaves extract for antibacterial activity was done by agar well diffusion method using standardized microorganism cell suspension at 1:5000 dilutions. Each labelled medium plate was uniformly seeded with a test organism by means of flooding with 2 ml of the standardized inoculum discarded after thoroughly covering the surface of the medium. Wells of 10 mm in diameter and 2 cm apart were punched with a sterile cork-borer of 10 mm on the dried inoculated culture medium. The extract was poured into four wells in a graded concentration. Ciprofloxacin antibiotic suspension was used to fill the fifth well along with the test extract as reference controls. Approximately, 100 µl of the various concentrations (25, 50, 100, and 200 mg/ml) of methanol extract of leaves of *S. occidentalis* and 10 µg/ml of Ciprofloxacin were used. The plates were left for 30 min. at room temperature as pre-diffusion time and incubated for 24 h. The resultant zones of inhibition which served as an indication of antibacterial activity were measured in millimeters (Ibekwe *et al*., 2001). This process was carried out in duplicate. The same process was applied for the screening of *E. camaldulensis* for antibacterial activity.

SCREENING FOR COMBINED EFFECT OF *SENNA OCCIDENTALIS* AND *EUCALYPTUS CAMALDULENSIS* FOR ANTIBACTERIAL ACTIVITY

Screening of combined effect of *S. occidentalis and E. camaldulensis* methanolic leaves extracts for antibacterial activity was done by agar well diffusion method using standardized microorganism cell suspension at 1:5000 dilutions. Each

labelled medium plate was uniformly seeded with a test organism by means of flooding with 2 ml of the standardized inoculum discarded after thoroughly covering the surface of the medium. Wells of 5 mm in diameter and 2 cm apart were punched with a sterile cork-borer of 10 mm on the dried inoculated culture medium. The combined extract in the ratio 1:1 was poured into four wells in a graded concentration. Ciprofloxacin antibiotic suspension was used to fill the fifth well along with the test extract as reference controls. Approximately, 100 µl of the various concentrations (25, 50, 100, and 200 mg/ml) of methanolic leaves extracts (*E. camaldulensis* and *S. occidentalis*) and 10 µg/ml of Ciprofloxacin were used. The plates were left for 30 min. at room temperature as pre-diffusion time and then incubated for 24 h. The resultant zones of inhibition which served as an indication of antibacterial activity were measured in millimeters (Ibekwe *et al*., 2001). This process was carried out in duplicate.

MEASUREMENT OF MINIMUM INHIBITORY CONCENTRATION (MIC)

The susceptibility of *S. typhi* to methanolic leaves extracts of *E. camaldulensis* and *S. occidentalis* was assessed using the macro-dilution method. 3 sets of 10 test tubes containing sterile nutrient broth were prepared. Different concentrations of methanolic leaves extract of *E. camaldulensis* (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.26, 0.78, 0.39 mg/ml), *S. occidentalis* (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.26, 0.78, 0.39 mg/ml) and the combination of the methanolic leaves extracts of *E. camaldulensis* and *S. occidentalis* (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.26, 0.78, 0.39 mg/ml) were dispensed into each set of 10 test tubes containing sterile nutrient broth. 100 µl of standardized bacterial suspension of *S. typhi* was added to each tube and incubated for 24 h at 37 ºC (Ibekwe *et al*., 2001). The tubes were observed for bacterial growth and the MIC for *E. camaldulensis* and *S. occidentalis were* identified to be 25 and 6.25 mg/ml respectively. The MIC of the combined methanolic leaves extracts (1:1) was found to be 25 mg/ml.

MEASUREMENT OF MINIMUM BACTERICIDAL CONCENTRATION (MBC*)*

MBC was described as the highest dilution (the lowest concentration) with no growth on the plate. On nutrient agar plate, 100 µl of cultures from the tubes showed no growth were cultured using spread plate method, and all the plates were incubated for 24 h at 37 ºC. The concentration that showed no growth on the nutrient agar plate is regarded as the minimum bactericidal concentration (MBC) (Hugo and Rusell, 2011).

RESULTS

Table 1: Phytochemical composition of methanolic leaves extracts of *E. camaldulensis* and *S. occidentalis*.

PHYTOCHEMICAL	**INFERENCE**	
	E. camaldulensis	*S. occidentalis*
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Alkaloids	+	+
Cardiac Glycoside	+	+
Anthraquinone	+	+
Steroidal glycosides	+	+

Key: + = Presence

Table 2: Zones of inhibition of methanolic leaves extracts of *E. camaldulensis, S. occidentalis,* and combination of the two extracts (1:1) against *S. typhi*

Extract/Concentration	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml
***S. occidentalis* (mm)**	20	15.5	11	11
***E. camaldulensis* (mm)**	21	18	15	12
Combined extracts (mm)	27.5	26	24	20

Table 3: Minimum inhibitory concentration of the methanolic leaves extract of *E. camaldulensis, S. occidentalis and* combination of the two extracts (1:1).

Extract/Concentration	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.5625**	**0.7812**	**0.3906**
S. occidentalis	-	-	-	-	-	-	+	+	+	+
E. camaldulensis	-	-	-	-	+	+	+	+	+	+
Combined extracts	-	-	-	-	+	+	+	+	+	+

Key: + = concentration of extract(s) that shows no activity, - = concentration of extract(s) that shows activity

Table 4: Minimum bactericidal concentration of the methanolic leaves extracts of *E. camaldulensis, S. occidentalis* and combination of the two extracts (1:1)

Extract/Concentration (mg/ml)	200	100	50	25	12.5	6.25
***S. occidentalis* (mm)**	+	+	+	+	+	+
***E. camaldulensis* (mm)**	-	-	-	+	+	+
Combined extracts (mm)	-	+	+	+	+	+

Key: + = concentration of extract(s) that shows no activity, - = concentration of extract(s) that shows activity

DISCUSSION

The percentage yield from both samples upon extraction with 70 % Methanol (*S. occidentalis* and *E. camaldulensis*) were estimated to be 23.25 % and 20.25 % respectively. The antibacterial activities of the methanolic leaves extracts of *S. occidentalis* and *E. camaldulensis* were investigated against *S. typhi*. The series of trials and evaluation techniques to which the methanolic leaves extracts of *S. occidentalis* and *E. camaldulensis* were subjected has proven antibacterial potential of the extract at 25, 50, 100, 200 and 400 mg/ml (Table 2). An increase in concentration of the extracts yielded increase in the zone of inhibition. This was supported by Tsado *et al.,* (2016) where he concluded that the zone of inhibition demonstrated by the leaves extract of *N. laevis* and *C. adansonii* against some selected pathogenic bacteria, including *S. typhi* increase with increase concentration. The ability of the methanolic leaves extracts of *S. occidentalis* and *E. camaldulensis* to inhibit the growth of *S. typhi*, a typhoid causing bacteria explained why it is used in folk medicine to treat typhoid in Nigeria and other African countries. The antibacterial activity demonstrated by leaves extracts of these plants could be linked to phytochemical constituents especially saponins, tannins and flavonoid which were reported to exert antibacterial activity as mentioned in several studies (Akiyama *et al.*, 2001 and Dzoyem *et al.*, 2013). The combined antibacterial effect of the methanolic leaves extracts of *S. occidentalis* and *E. camaldulensis* from zone of inhibition showed a little increase compared to either of the methanolic extract. Assay showing zone of inhibition cannot distinguish between bacteriostatic and bactericidal activity and that differences in zone of inhibition cannot be used to compare drug potencies, zone of inhibition only provides information to which the bacterial pathogens is susceptible (Furuno *et al.*, 2014).

The minimum inhibitory concentration of *S. occidentalis* and *E. camaldulensis* was found to be 6.25 mg/ml and 25 mg/ml respectively (Table 3). Upon combination of the two extracts (*S. occidentalis* and *E. camaldulensis*), the minimum inhibitory concentration was found to be 25 mg/ml (Table 3). This shows that lesser methanolic leaves extract of *S. occidentalis* is required to inhibits the growth of *S. typhi* compared to the methanolic leaves extract of *E. camaldulensis*. Therefore, *S. occidentalis* is more potent than *E. camaldulensis* according to this study.

S. occidentalis was found to be bacteriostatic, this is because the minimum bactericidal concentration of methanolic leaves extract of *S. occidentalis* (Table 4) showed bacterial growth indicating that the methanolic leaves extract of *S. occidentalis* has no bactericidal activity, this is supported by the work of Adamu *et al.*, (2018), which states that *S. occidentalis* is bacteriostatic against *S. typhi*.

Minimum bactericidal concentration of methanolic leaves extract of *E. camaldulensis* (Table 4) showed minimum bactericidal concentration of 50 mg/ml. This is similar to a study conducted by Adamu and Yusha'u (2018), which reported the bactericidal effect of *E. camaldulensis* against *Salmonella* sp. The minimum bactericidal concentration of the combination of methanolic leaves extracts of *S. occidentalis* and *E. camaldulensis* (Table 4) was found to be 200 mg/ml. This increase in

the minimum bactericidal concentration value explained the reduced activity of the combined methanolic leaves extracts compared to the methanolic leaves extracts of *E. camaldulensis* when used alone. Therefore, the combined effect of methanolic leaves extract of *S. occidentalis* and *E. camaldulensis*, since it is found in the study that *S. occidentalis* is bacteriostatic action.

CONCLUSION

Phytochemical analysis of the methanolic leaves extracts was carried out, methanolic leaves extracts of *S. occidentalis* and *E. camaldulensis* have antibacterial activity against *S. typhi*. Antibacterial activity increases as the concentration also increases. The combination of the methanolic leaves extracts of *S. occidentalis* and *E. camaldulensis* showed an interaction. The interaction pattern is not synergistic, the antagonistic interaction observed with the extracts may be due to some compounds present which inhibit one another from expressing their full potential to act against *S. typhi.*

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