Synergistic Effect of Eucalyptus (*Eucalyptus Camaldulensis*) and Guava (*Psidium Guajava)* Ethanolic Extracts on *Eschericia Coli* and *Staphylococcus Aureus*

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ABSTRACT

In-vitro antibacterial activities of the ethanolic leaf extracts of Eucalyptus camaldulensis and Psidium guajava and their combination were evaluated against Staphylococcus aureus, gram positive bacteria and Escherichia coli, gram negative bacteria using paper disc diffusion method. The extracts and their combination displayed broad spectrum activities against the test organisms with Eucalyptus camaldulensis leaf extract showing zone of inhibition of (18 mm) on E. coli and (20 mm) on Staphylococcus aureus at 1000 ug/disc, while Psidium guajava leaf extract showed inhibition zone of (17 mm) and (15 mm) at 1000 ug/disc on Escherichia coli and Staphylococcus aureus respectively. Similarly the combination (synergy) of the two extracts at 1:1 ratio presented higher activity than the individual extract with inhibition zone of (26 mm) and (22 mm) at 1000 ug/disc against Escherichia coli and Staphylococcus aureus respectively. Thus the results have proved the claim for the use of the two plants in the preparation of concoction for the treatment of gastro-intestinal diseases caused by these pathogens. This study also indicated that Eucalyptus camaldulensis and Psidium guajava leaves could be a good sources of antibacterial agents, however it is recommended that phytochemicals from these plants should be isolated and tested individually in order to find the bioactive compounds.

Keywords: Eucalyptus camaldulensis, Psidium guajava, Escherichia coli, Staphylococcus aureus, ethanolic extracts, phytochemicals and bioactive compounds

INTRODUCTION

Different plants genera and species were found to have antimicrobial potentials and this could lead to the discovery and development of new antimicrobials from natural source (Hammer, *et al.*, 1999; Sharififar *et al.*, 2009; Ilesanmi and Olawoye, 2011). The detection of the antimicrobial properties of a plant indicates that, such plant could be a candidate for the development of antimicrobial agent which is based on their phytochemical constituents (Maria *et al*., 2007). Concoction from the leaf of Eucalyptus and Guava had been used traditionally in the treatment of gastro-intestinal disorder such as food poisoning which is usually caused by *Staphylococcus aureus and Escherichia coli.*

Eucalyptus camaldulensis is perennial, single-stemmed, and medium sized to tall tree common and wide spread along water courses of Australian basin and belongs to the family Mytaceae (Bren and Gibbs, 1986, and Brooker *et al.*, 2000). Eucalyptus oil is officially in the Indian Pharmacopoeia as a counter-irritant and mild expectorant (IP, 1996), and official in the Chinese pharmacopeia as a skin irritant used in nerval pain (Tu, 1992). The present *Ayurvedic Pharmacopoeia* indicates its topical application for headache due to colds (Karnick, 1994). It is also used externally for cutaneous absorption in dosage forms, including the essential oil, liniment, and ointment (Leung and Foster, 1996; Wichtl and

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Bisset, 1994). The modern therapeutic applications for eucalyptus oil are supportable based on its history of use in well established systems of traditional medicine, phytochemicals investigations, as well as *in vitro* and *in vivo* studies in animals. Major compounds found in eucalyptus leaf extract include; tannins, flavonoids, steroid, alkaloid, glycoside, saponnins, terpenoids, reducing sugar and phenolics (Egwaikhide et al., 2009; Saxena et al., 2010).

Psidium guajava (guava), belonging to the family of *Myrtaceae*, is a native of tropical America and has long been naturalized in Southeast Asia. The positive effects of guava extracts on human ailments have been described and phytochemical studies have identified more than 20 compounds in guava extracts (Begum et al., 2002). Guava is rich in tannins, phenols, flavonoids, essential oils, lectins, vitamins, fatty acids and many other phytochemicals, and much of the guava's medicinal activity is related to its flavonoids content (Arima,2002).The major constituents of its leaves were identified to be tannins, *β*-sitosterol, maslinic acid, essential oils, triterpenoids and flavonoids (Arima and Danno, 2002; Begum et al., 2002; 2004). Decoction made from the leaves or infusion made from the flower of guava is used topically for wounds and skin sores, the leaves are also used in the treatment of Diarrhoea (Abdelrahim, 2002).

Base on the information above this study is designed to determine the in-vitro synergistic effect of Eucalyptus and Guava ethanolic leaf extracts against *Escherichia coli* and *Staphylococcus aureus*, so as to prove the claims of traditional herbalist in the use of concoction made from the combination of eucalyptus and guava leaf for the treatment of stomach disorder caused by these two pathogens.

MATERIAL AND METHODE

Collection And Processing Of Plants Materials

The leaves of the *Eucalyptus camaldulensis* and *Psidium guajava* plants were collected from the Biological garden of Science Laboratory Technology Department, School of Technology Kano State Polytechnic. The leaves were washed to remove the dust and then dried in the laboratory for about two weeks. After drying the leaves were grinded separately using mortar and pestle to get their powdered form.

Extraction of The Plant Materials

100 g of each powdered material was extracted with 250 ml of petroleum ether using soxhlet apparatus so as to remove the oil from the leaves. The defatted plant materials were then extracted with 90% ethanol separately and the extracts were allowed to dry at room temperature in order to remove the solvent. The extracts were kept in sterile bottles under refrigeration conditions until use (Betoni *et al*.2006).

Preparation of Extract Impregnated Paper Disc

Extract impregnated paper disc were prepared according to the method of Idris and Yusha'u (2012), with modifications. Briefly, discs of 6mm diameter were punched out from Whatman No.1 filter paper and placed into bijou bottles in batches of 50 discs; the discs were then sterilized by autoclaving at 121^{o}C for 15 minutes and then allowed to cool. Three different concentrations (1000 µg/disc, 500 µg/disc and 250 µg/disc) were made from the *E. camaldulensis, P. guajava* ethanolic extract and their combination (synergy) respectively.

One hundred milligram (100 mg) of each of the extracts was dissolved in 1ml of DMSO to get 100,000 µg/ml stock solutions. To two bijou bottles containing 50 sterile paper discs each, 0.5 ml of the eucalyptus leaf extract was added in the first one and guava leaf extract in the second one from the stock solutions to arrived at 1000 µg/disc each of the extract after

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even distribution. Similarly, 0.5 ml each of the stock solution was serially double diluted to get 50,000 µg/ml and 25,000 µg/ml in a separate bijou bottle. These were use to prepare 500 µg/disc and 250 µg/disc. Synergy was prepared by combining the stock solution of eucalyptus and guava extract in the ratio (1:1) which was then use to prepare 1000 µg/disc, 500 µg/disc and 250 µg/disc.

Test Organisms

Two human pathogenic organisms: *Escherichia coli*, gram-negative bacteria and *Staphylococcus aureus*, gram positive bacteria were used as test organisms to evaluate the synergistic activity of Eucalyptus and Guava ethanolic leaf extracts. These organisms were provided by the Post Graduate Laboratory, Department of Biological Sciences, Faculty of Science, Bayero University Kano, Nigeria.

Inoculum Preparation

A loop full of isolated colony from 24 hrs culture of the test organism was taken and transferred in to 2mls of sterile normal saline and the turbidity of the suspension was adjusted so as to obtain turbidity visually comparable to that of 0.5 McFarland standards as described by Cheesbrough (2006) and adopted by Yusha'u *et al*., (2009).

Bioassay Procedure

For the sensitivity test, agar disc diffusion method was employed as described by Kirby and Bauer (1996) and adopted by Yusha'u *et al*., (2009) and Bashir *et al*., (2011) with mordification. In the test, nutrient agar plates were prepared and the surface of the agar plates dried in a hot air oven at 35^{o}C. Using sterile swab stick, nutrient agar plates were aseptically seeded with the previously standardized test organism. Discs containing different concentrations (1000 µg/disc, 500 µg/disc and 250 µg/disc) each of eucalyptus and guava extracts, and the combination (synergy) of the two extracts were placed firmly and sufficiently spaced on to the surface of inoculated plates, and disc with only DMSO and gentamicin sulphate GEN served as negative and positive controls respectively . The plates were allowed for pre-diffusion time of 15 minutes, and then incubated at 37^{o}C for 24 hours. The diameters of the zones of inhibition formed were measured with the aid of a standard ruler to determine the sensitivity of the extracts and the synergy on the test organisms.

RESULT

The in-vitro antibacterial activity of eucalyptus and guava ethanolic leaf extracts and their combination (synergy) were evaluated on *Staphylococcus aureus* and *Escherichia coli*. The result indicated that both plants have inhibitory effect on the tested organisms as shown in table 1 and 2. The result shows that *Staphylococcus aureus* was slightly more sensitive to eucalyptus leaf extract at all concentrations than guava leaf extracts compared to the sensitivity of *Escherichia coli* as shown in table 1. However with guava leaf extract, the reverse was the case where *Escherichia coli* showed wider zones of inhibition than *Staphylococcus aureus* at all concentrations as shown in table 2. Similarly the combination of the two plants extract showed wider zone of inhibition than the individual extracts as shown in table 3. The combination of the two plants (synergy) showed more effect on *Escherichia coli* at all tested concentrations than *Staphylococcus aureus*. It was also observed that dimethyl sulphur oxide (DMSO) which served as negative control presented no activity on the test organisms, where as GEN (positive control) produced inhibition zones of 23 mm and 25 mm on *Escherichia coli* and *Staphylococcus aureus* respectively. And GEN (positive control) presented higher zones of inhibition on the test organisms at all tested concentrations

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than the individual extracts and their combination except for *E. coli at* 1000 µg/disc which produced inhibition zone of 26 mm and is wider than the positive control (23 mm).

Table 1. Antibacterial effect of *Eucalyptus camaldulensis* ethanolic extract

Isolates	concentration/zone of inhibition (mm)				
	1000 µg/disc	500 µg/disc	250 µg/disc	DMSO	GEN 30 µg/disc
E. coli	18	14	10	0.0	23
Staph. aureus	20	16	11	0.0	25

GEN = Gentamycin

DMSO = Dimethyl sulphur oxide

Table 2. Antibacterial effect of *Psidium guajava* ethanolic extract

Isolates	concentration/zone of inhibition (mm)				
	1000 µg/disc	500 µg/disc	250 µg/disc	DMSO	GEN 10 µg/disc
E. coli	17	13	10	0.0	23
Staph. aureus	15	12	8	0.0	25

GEN = Gentamycin

DMSO = Dimethyl sulphur oxide

Table 3. Antibacterial effect of Combined *Eucalyptus camaldulensis* and *P. guajava* ethanolic extracts (synergy)

Isolates	concentration/zone of inhibition (mm)				
	1000 µg/disc	500 µg/disc	250 µg/disc	DMSO	GEN 10 µg/disc
E. coli	26	22	12	0.0	23
Staph. aureus	22	16	11	0.0	25

GEN = Gentamycin

DMSO= Dimethyl sulphur oxide

DISCUSSION

The results of this study show that ethanolic leaf extracts of Eucalyptus and guava demonstrated inhibitory activity against the tested organisms at the concentration used. The activities produced by these plants extracts vary between the tested organisms which may be due to the cell wall differences of the tested organisms (Nester *et al*., 1998; Karou *et al*. ,2006) and difference in phytochemical composition of the plants(Abdelrahim, 2002). Eucalyptus and guava leaf extract showed inhibitory effect on the tested organisms, this is in line with the findings of other authors which show that eucalyptus and guava leaf extracts have inhibitory effects on *E. coli* and *Staph. aureus* (Akin-Osanaiye *et al.*, 2007), while Sherry *et al*. (2001) revealed that topical application of eucalyptus oil clears methicillin resistance *Staphylococcus aureus* infection. The methanol extract of *E. camaldulensis* has

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been found to be effective against *Staphylococcus aureus* (Babayi *et al.*, 2004). In other work, agar well diffusion method was used to test the antibacterial activity of *Psidium guajava* extract. And the result shows that methanolic extracts of *Psidium guajava* leaf inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* at concentration as low as 100 µg /ml (Mohamed *et al.*, 2012). It was observed that positive control (GEN 30 µg/disc) presented wider zone of inhibition compared to the individual extracts on the tested organisms. This may be due to the fact that the positive control GEN is a purified form of the antimicrobial agent where as the leave extracts are crude extracts in their un purified forms (Yusha'u, 2011).

The synergy of the extracts showed wider zone of inhibition than the individual extracts on tested organisms. This may be due to the combined effect of the extracts which is described as additive synergistic effect (Ochei and Kolhatkar, 2000). This means that the combination of the extracts also have more effect on *E. coli* than *Staph. aureus* as observed in table 3.

CONCLUSION

In summary, the ethanolic leaf extracts of *Eucalyptus camaldulensis* and *Psidium guajava* have inhibited the growth of *Staph. aureus* and *E. coli*. The combination of the two extracts (synergy) has higher antimicrobial activity against *E. coli* compared with that of *Staph. aureus.* And the synergy of the extracts showed wider zone of inhibition than the individual extracts. However it is recommended that phytochemicals of the plants should be separated and tested individual in order to find the bioactive compounds present.

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