OBAFEMI AWOLOWO UNIVERSITY

ANTIMICROBIAL ACTIVITY OF THE ETHANOLIC ROOT EXTRACT OF *MEZONEURON BENTHAMIANUM* BAILL (CAESALPINIACEAE)

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Antimicrobial Activity of the Ethanolic Root Extract of *Mezoneuron benthamianum* Baill (Caesalpiniaceae)

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DEDICATION

To God Almighty, the owner and the source of my life who gave me the privilege, strength and understanding to complete this work. I owe Him everything.

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CERTIFICATION

This is to certify that this study was carried out by OLADEJI, Oladokun Layiwola of the Drug Research and Production Unit (DRPU), Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

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ABSTRACT

This study investigated the activities of the ethanolic root extract of *Mezoneuron benthamianum* Baill (Caesalpiniaceae) and its partitioned fractions on some microbial isolates from the oral cavity and determined the killing rate of the most active fraction. This was with a view to providing scientific basis for the use of the root in the treatment of oral infections.

The root of *M. benthamianum* was air-dried, ground to powder and macerated using 70 % ethanol with constant shaking with a mechanical shaker for 72 hours. The extract was concentrated *in-vacuo* at 50 ^{o}C using a rotary evaporator and freeze dried. The *in-vitro* susceptibility test was done with the ethanolic root extract and its partitioned fractions (petroleum spirit, chloroform, ethyl acetate and aqueous) on *Staphylococcus aureus* (NCIB 8588) and clinical isolates of *Streptococcus mutans*, *Streptococcus pyogenes*, *Streptococcus salivarius*, *Staphylococcus aureus* and *Candida albicans* isolated from human oral cavity using the agar well diffusion method. The minimum inhibitory concentration of the fractions was determined using the two fold agar dilution method at a range of 0.39 to 25 mg/ml for each fraction. The time-kill-assay for the most active fraction i.e. the ethyl acetate fraction was carried out on all the organisms. Bio-autographic assay was also carried out to find out the component that is responsible for its antimicrobial activities. The values obtained were subjected to inferential statistical analysis.

The result showed that the ethanolic root extract had appreciable activity against all the test organisms at varying concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml with increasing zones of inhibition as the concentration increased. The rank order for the activity of the partitioned fractions at the test concentration of 25 mg/ml was observed as ethyl acetate (20.6 mm-23.7 mm) > chloroform (14.7 mm-18.7 mm) > aqueous (13.7 mm-18.3 mm) and > petroleum spirit (11.3 mm-13.3 mm). The minimum inhibitory concentration gave a rank order of ethyl acetate (0.039 mg/ml) < chloroform (0.78 mg/ml-6.25 mg/ml) < aqueous (3.13 mg/ml-12.5 mg/ml) < petroleum spirit (6.25 mg/ml- 25 mg/ml). It was also observed that the activity of the ethyl acetate fraction was higher than the activity of the ethanolic extract at the same concentration of 25 mg/ml. The killing rate experiment showed that the ethyl acetate fraction had a rapid rate of action on the organisms at the test concentrations of 0.39 mg/ml (MIC concentration) and 0.78 mg/ml (MICx2). At concentration of 1.17 mg/ml (MICx3) the time of kill of all the organisms by the ethyl acetate fraction was ≤ 5 minutes, which was similar to the standard antibiotics used at their MIC values e.g. 0.039 mg/ml of Ampicillin and Tetracycline for the bacteria; and 0.078 mg/ml of Amphotericin B for the fungus used. The chromatographic result of the ethyl acetate fraction showed two distinct bands and the bio-autographic assay performed on the plate revealed that only one component (the upper band) proved effective in inhibiting all the test organisms.

The study concluded that *M. benthamianum* ethanolic root extract was highly effective against oral isolates with its ethyl acetate fraction being the most active.

CHAPTER ONE

1.1 Introduction

Plants have served as a source of new pharmaceutical products and inexpensive starting materials for the synthesis of some known drugs. Components with medicinal properties from plants play an important role in conventional Western medicine. In 1984, at least 25% of the Western medicine issued in the US and Canada were derived from or modeled after plant natural products and 119 secondary metabolites were used globally as drugs. It has been estimated that 14-28% of higher plant species are used medically. Only 15% of all angiosperms have been investigated chemically and 74% of pharmaceutically active plant derived components were discovered after following up on ethnomedicinal use of the plant (Farnsworth, 1994).

The traditions of collecting, processing and applying plant and plant-based medications have been handed down from generation to generation. In many African countries, traditional medicines, with medicinal plants as their most important components, are sold in marketplaces or prescribed by traditional healers (without accurate dose value) in their homes (Herdberg and Staugard, 1989). Because of this strong dependence on plants as medicines, it is important to study their safety and efficacy (Farnsworth, 1994). The value of ethno-medicine and traditional pharmacology is nowadays gaining increasing recognition in modern medicine because the search for new potential medicinal plants is frequently based on an ethno-medicinal basis. In the ethno-pharmacological approach, local knowledge about the potential uses of the plants is very useful as compared to the random approach where indigenous knowledge is not

taken into consideration. Numerous plants are used in Africa as chewing sticks for the cleansing of the mouth and for the treatment and prevention of oral infections. These plants include *Azadiracta indica, Salvadora persica, Albizia coriara, Salix subserrata, Boscia coriacea* to mention a few (Kassu *et al.,* 1999).

Medicinal plants which form the backbone of traditional medicine have in the past few decades been the subject for very intense scientific studies and this has been as a result of the acknowledgement of the value of medicinal plants as a potential source of new compounds of therapeutic value. Plant based traditional knowledge has become a recognized tool in the search for sources of drugs. Compounds inhibiting microorganisms, such as benzoin and emetine have been isolated from plants. It is possible that anti-microbial compounds from plants may inhibit micro-organisms by a different mechanism than the presently used antibiotics and may have clinical value in the treatment of resistant microbial strains. For this reason, it is therefore important to investigate plants as alternative sources of anti-microbial compounds (Cox, 1994).

Continuous effort is still being carried out in the area of medicinal plants research for the discovery and possible isolation of active principles from plants that have being reported traditionally to be used in the treatment of infectious diseases. One of such plants is *Mezoneuron benthamianum* whose root is reported to be used in Ibadan area, Nigeria as chewing stick for the treatment of pain resulting from oral infections. Its leaves are used in folk medicine for the treatment of dermal infections and wounds in Ghana. The leaves are considered in Senegal to be antiseptic and used in cleaning, healing of refractory sore (Verger, 1997).

The oral cavity supports a vast community of microorganisms some of which are infectious in nature and have been implicated in the etiology of oral infectious diseases. It is therefore important to keep these odontopathogens in check before they damage the oral cavity. Oral infectious diseases of these organisms are very common and are increasingly getting difficult to treat due to widespread resistance to existing drugs. Given the high importance of infection as regards to health, it is not surprising that anti-infective agents are high on the list for drug development and a number of species used traditionally have undergone screening (Bhandari, 1990; Said *et al.,* 1996; Babu *et al.,* 1996; Holmstrup *et al.,* 2003).

The aim of this work is to investigate the antimicrobial activity of the ethanolic root extract of *Mezoneuron benthamianum* against some odontopathogens commonly found in the human oral cavity that have been implicated in the etiology of oral infectious diseases.

1.2 Literature Review

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases. About 25 to 50% of current pharmaceuticals are plant derived. Traditional healers have long used plants to prevent or cure infectious conditions; Western medicine is trying to duplicate their successes. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids