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RESEARCH ARTICLE

IN VITRO ASSESSMENT OF THE ANTIPLAQUE PROPERTIES OF CRUDE M.ALBA LEAF EXTRACT

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ABSTRACT

Traditionally, mulberry (*Morusalba*) is chewed in toothache to avoid further destruction or cavitation of the tooth. *Morusalba* has gathered great attention for its antioxidative and antidiabetic effects and is an important ingredient of herbal tea. In spite of being known as nature's tonic, very limited information is available regarding the anticariogenic potential of the crude extract of the leaves of the mulberry. However, a flavonoid, kuwanon-G, which had been isolated from the root bark of *Morusalba*, shows antibacterial activity against *Streptococcus mutans*only. The present study intends to study the antiplaque potential of the hot methanol extract of *Morusalba* leaves.

The minimal inhibition concentration (MIC) of hot methanol extract of *Morusalba*(2.5mg/ 10ml) was used to study its effect on the virulence factors of biofilm forming *Streptococcus mutans*. The strategies for effective treatment against the dental caries includes the elimination of mutans streptococci, inhibition of the colonization, reduction of the glucosyltransferase activity, inhibition of glucanproduction. The hot methanol extract of leaves of *Morus alba* (10mg/ 10ml) has shown more efficiency minimal bacterial concentration (MBC) which means it more efficient in complete inhibition of *Streptococcus mutans* bacteria. The minimal inhibition concentration (MIC) of hot methanol extract of *Morusalba*(2.5mg/ 10ml) was shown in complete inhibition glucosyltransferase production, reduction of the glucosyltransferase activity in terms of glucan production, reduction in the bacterial density of the biofilm and the decrease in thickness of the preformed biofilm of *Streptococcus mutans*. An extension of this work in future will be to investigate the components present in the hot alcoholic extract of *Morusalba* leaves.

INTRODUCTION

Dental plaque (biofilm) formation is a naturally occurring process, resulting from bacterial interactions with the acquired salivary pellicle formed over the surface of the tooth shortly after brushing the tooth. Although the newly formed plaque lacks any pathogenic potential due to an insufficient number of microorganisms present, the persistence of dental plaque allows for multiple bacterial interactions, resulting in various pathologies such as gingivitis, caries, periodontitis, and peri-implantitis (Marsh 2006; Bordone and Bortolaia 2003). This can be cured by distinct mouthwashes with antiplaque agents such as chlorhexidine, fluoride, and cetylpyridinium chloride are recommended for use in conjunction with tooth brushing because rinsing with mouthwashes in addition to tooth brushing has been found to impart superior plaque control compared to tooth brushing alone (Feres *et al.* 2009).

These agents have bactericidal or bacteriostatic action against gram- positive microorganism than gram- negative microorganism (De Freitas *et al.* 2003). Several studies, both *in vitro* and *in vivo*, have evaluated the efficacy of the antiplaque agents mentioned above (Pizzo *et al*. 2008; Featherstone 2000). But, there are some adverse effects of these antiplaque agents on human health. Despite the widespread use of different sources of fluoride, dental caries continues to be the single most prevalent and costly oral infectious disease worldwide (National Institutes of Health 2001; Marsh 2003; Dye *et al.* 2007). Virulent biofilms that are tightly adherent to oral surfaces are a primary cause of infectious diseases in the mouth, including dental caries (Bowen and Koo 2011).

Therefore, now scientists found natural compounds which work against plaque. Historically all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex form of crude extracts, mixtures, etc. Today a substantial number of drugs are developed from plants (Fabricant and Farnsworth 2001) which are active against a number of diseases. The majority of these involve the isolation of the active ingredient (chemical compound) found in a particular medicinal plant and its subsequent modification. In the developed countries 25 percent of the medical drugs are

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based on plants and their derivatives (Principe, 2005) and the use of medicinal plants is well known among the indigenous people in rural areas of many developing countries. In the past our ancestors made new discoveries of the healing power of plants through trial and error. Although some of the therapeutic propertiesattributed to plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and thousands of years (Gurib-Fakim, 2006).

Traditionally, mulberry (*Morusalba*) is chewed in toothache to avoid further destruction or cavitation of the tooth. *Morusalba*has garnered great attention for its antioxidative and antidiabetic effects and is animportant ingredient of herbal tea (Wang *et al.* 2009).In spite of being known as nature's tonic, very limited information is available regarding the anticariogenic potential of the crude extract of the leaves of the mulberry. However, a flavonoid, kuwanon-G, which had been isolated from the root bark of *Morusalba*, shows antibacterial activity against *Streptococcus mutans*only. The antibiofilm activity of *Morusalba*leaf extract is not studied (Lokegaonkar and Nabar 2011). The present study intends to study the antiplaque potential of the hot methanol extract of *Morusalba* leaves.

MATERIALS AND METHODS

This study was conducted in Institute of Biotechnology and Allied Sciences (IBAS), Sikar&Jhalawar Medical College & Hospital, Jhalawar, Raj.

The plaque sample was collected by patients coming in the regular OPD in Department of Dentistry, Jhalawar Medical college, Jhalawar Raj.Leaves of *Morusalba*was shade dried at room temperature and then ground in an electrical grinder. The ground material was passed through sieve of mesh size 60 to obtain a fine powder which was used to prepare the extract. Cold extractions as well as hot extraction procedures were followed to procured crude and partially purified fractions respectively.

Test Bacterial isolate

Culture of plaque forming bacteria *Streptococcus mutans* was procured from Microbial Type Culture Collection, Chandigarh. Collected *Streptococcus mutans* culture was checked for purity. Pure culture was maintained on Agar slant at 4^0 C.

Cold Extraction

Cold extraction was done in water, 50% hydro alcohol as well as absolute alcohol. 10 gm dried and powdered plant material was suspended in 50 ml of solvent (alcohol/water and 50% hydro alcohol) for 48 h. The suspension was filtered through Whatman filter paper no.1 then vacuum dried with the help of rotary vacuum evaporator. The dried residue was used as extract and solvent was recycled.

Hot Extraction

Reflux method of solvent extraction was used for successive separation of different partially purified organic constituents present in dried plant material. Solvent series used for successive separation was as follows:

Pet. ether → Benzene → Acetone → Alcohol →Methanol

This method involves continuous extraction of powdered dried plant material in soxhlet apparatus with a series of organic solvents. Each time before extracting with next solvent the plant material was air dried at room temperature. 20 gm dry plant powder was kept in soxhlet extraction unit and extracted with 140 ml petroleum ether till all petrol soluble fractions was extracted. Residue was dried and used for extraction with next solvent. Same procedure was repeated with each solvent and finally residue was macerated with chloroform water to obtain aqueous fraction.

Assay of Antibacterial Activity of Plant Extract

Antibacterial activity of *Morusalba* leaf extract was done by agar well diffusion method (Collee *et al.*, 1996). For this purpose, 50% methanol and 100% acetone were used as solvent to prepare 10mg/10 ml concentration of extract. The agar plates were seeded with 0.1 ml of *Streptococcus mutans* culture (0.5 Mcfarland) by spread plate method. Subsequently, 9 mm wide wells were bored within these agar plates using a sterile cork borer 150 µl stock solution of respective extract was filled into the wells and the plates were incubated at 37^o C. Acetone and 50% methanol controls were also maintained along with test samples. The antimicrobial activity was checked as clear zones surrounding the wells.

Estimation of Minimum Inhibitory Concentration (MIC) of Selected Plant Part

MIC of extract or fraction showing best activity was determined by dilution method using MRS broth. For this purpose 20mg/10 ml was used as a stock solution. Stock was added to MRS media to prepare 10 mg/10ml, 05 mg/10ml, 2.5 mg/10ml, 1.25 mg/10ml, 0.625 mg/10ml, 0.312 mg/10ml and 0.156 mg/10ml respectively.

These tubes were then respectively inoculated with pure culture of *Streptococcus mutans* (10 µl of 0.5 Mcfarland) and incubated at 37^0C for 24h.

RESULT AND DISCUSSION

Extracts of *Morusalba* leaves provides antimicrobial potential against harmful microorganisms. The use *M.alba*in the treatment of Atherosclerosis (Enkhmaa *et al.* 2005; Katsube *et al.* 2006), Diabetes mellitus (Tierney *et al.* 2002), Immunonutrition and cancer (Katsube *et al.* 2006; Martin-Moreno *et al.* 2008). Neuroprotective functions (Kumada *et al.*2008; Ikuta *et al.* 1985; Seeram *et al.* 2001), Skin tone (Lee *et al.* 2002) was found to effective. But, till now, very limited information is available regarding the anticariogenic potential of the crude extract of the leaves of the mulberry. The antiplaque properties of *Morusalba* are not studied.

The strategies for effective treatment against the dental caries includes the elimination of mutans streptococci, inhibition of the colonization, reduction of the glucosyltransferase activity, inhibition of glucan production (Lokegaonkar and Nabar 2011). In present study, we tested the hot methanol extract of *Morusalba*against such biofilm properties. The minimal inhibition concentration (MIC) of hot methanol extract of *Morusalba*(2.5mg/ 10ml) was used to study its effect on the virulence factors of biofilm forming *Streptococcus mutans*. The strategies for effective treatment against the dental caries includes the elimination of mutans streptococci, inhibition of the colonization, reduction of the glucosyltransferase activity, inhibition of glucan production. In present study, we tested the hot methanol extract of *Moursalba*against such antiplaque properties.

The hot methanol extract of leaves of *Moursalba* (10mg/ 10ml) has shown more efficiency minimal bacterial concentration (MBC) which means it more efficient in complete inhibition of *Streptococcus mutans* bacteria. The hot methanol extract of *Mours alba* (2.5mg/10ml) has shown more The minimal inhibition concentration (MIC) of hot methanol extract of *Morus alba*(2.5mg/ 10ml) was shown in complete inhibition glucosyltransferase production, reduction of the glucosyltransferase activity in terms of glucan production, reduction in the bacterial density of the biofilm and the decrease in thickness of the preformed biofilm of *Streptococcus mutans*. An extension of this work in future will be to investigate the components present in the hot alcoholic extract of *Morusalba* leaves.

Table no. 1 Serial order of MIC and MBC stock tubes

Test Tube S.No.	Stock (mg/10ml)
1	10mg/ 10ml
2	05mg/ 10ml
3	2.5mg/10ml
4	1.25mg/10ml
5	0.625mg/10ml
6	0.156mg/10ml

Fig no. 1 First tube show the MBC and Third tube show MIC

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