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# In Vitro Antimicrobial Activity of *Sapindus mukorossi* and *Emblica officinalis* Against Dental Caries Pathogens

**Kamal Rai Aneja, Radhika Joshi* and Chetan Sharma**

Department of Microbiology, Kurukshetra University, Kurukshetra -136119. India
Corresponding author Email: joshi_radhika31282@yahoo.com

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## Abstract

The *in vitro* antimicrobial activity of *Sapindus mukorossi* and *Emblica officinalis* fruit extracts were studied against *Streptococcus mutans, Staphylococcus aureus, Lactobacillus acidophilus, Candida albicans* and *Saccharomyces cerevisiae*. The acetone, ethanol, methanol, hot water and cold water extracts of *S.mukorossi* exhibited antimicrobial activity against one of the tested microorganisms i.e. *S.cerevisiae*. All the five extracts of *E.officinalis* showed inhibitory activity against *S.mutans* while the acetonic, hot and cold aqueous extracts showed inhibitory activity against *S.aureus* also. The largest zone of inhibition was obtained with the acetonic extract of *S.mukorossi* against *S.cerevisiae* (29.65mm) and hot water extract of *E.officinalis* against *S.aureus* (40.32mm). Minimum inhibitory concentrations (MIC) of the extracts were also determined against the selected microorganisms showing zones of inhibition ≥8mm. This study depicts that the fruits of *Sapindus mukorossi* and *Emblica officinalis* possess very good antifungal and antibacterial activities respectively and can be used as a potential source of novel antimicrobial agents used to cure dental caries.

**Key words :** Dental caries, *Sapindus mukorossi*, *Emblica officinalis*, antimicrobial activity, zone of inhibition, minimum inhibitory concentration.

## Introduction

Dental caries is a very common problem that affects all age groups. It is a process in which the enamel and the dentine are demineralised by acids produced by bacterial fermentation of carbohydrates (de Soet and de Graff, 1998). In real life, it is the most common infectious disease affecting human beings (Balakrishnan *et al.,* 2000). Medicinal plants since ancient times have been employed for prophylactic and curative purposes (Joshi and Joshi, 2005; Amadi *et al.,* 2007). The present study reports the antimicrobial activity of five different solvent extracts of *Sapindus mukorossi* and *Emblica officinalis* on dental caries causing microorganisms.

*Sapindus mukorossi* Gaertn., a member of the family *Sapindaceae*, is commonly known by several names such as soapnut, soapberry, washnut, ritha, reetha, aritha, dodan and doadni. It is a deciduous tree widely grown

in upper reaches of Indo-Gangetic plains, Shivaliks and sub Himalayan tracts at altitudes from 200m to 1500m. It is one of the most important trees of tropical and sub-tropical regions of Asia, usually with straight trunk, nearly 4-5m in height, pinnately compound leaves and lanceolate leaflets. Flowers are small, terminal and polygamous. The fruit is valued for the saponins (10.1 %) present in the pericarp and constitutes up to 56.5 per cent of the drupe known for inhibiting tumor cell growth. The fruits are expectorant, emetic, contraceptive, for treatment of excessive salivation, epilepsy, to treat chlorosis, head lice, migranes, eczema, psoriasis and for removing freckles (Kirtikar and Basu, 1991). The powdered seeds are employed in the treatment of dental caries, arthritis, common cold, constipation and nausea (Dhar *et al.*, 1989). It cleanses the skin of oily secretion and is even used as a cleanser for washing hair and a hair tonic, and forms a rich, natural lather.

*Emblica officinalis* Gaertn., a member of the family *Euphorbiaceae*, is commonly called by several names such as amalaka, aavalaa, amla, amlaki and Indian gooseberry. It is found throughout India, Pakistan, Bangladesh, China, Sri Lanka and the Malayan Peninsula. It is a deciduous tree, 8-15 m tall, with alternate, subsessile leaves and greenish to creamy-yellow, unisexual, actinomorphic, trimerous flowers (Warrier *et al.*, 1995). The fruit is a very rich source of Vitamin C (600mg/100g). The leaves, bark, fruit and root bark are used to cure many diseases (Dey, 1980). The fruit has anti-viral, antibacterial, anti-cancer, anti-allergy, and anti-mutagenic properties. It is rich in phenols, tannins, polyphenols, flavonoids, kaempferol, ellagic acid and gallic acid (Nair and Chanda, 2007).

**Materials and Methods**

*Sapindus mukorossi* and *Emblica officinalis* fruits were obtained from the local market of Delhi, India. Dr. B.D.Vashistha, Botany Department, Kurukshetra University, Kurukshetra confirmed the identification of the samples.

*Extraction of plant material*

The samples were carefully washed under running tap water followed by sterile distilled water. These were air dried at room temperature ($40^{O}$C) for five days and pulverized to a fine powder using a sterilized mixer grinder and stored in air-tight bottles. Four different solvents namely ethanol, methanol, acetone and aqueous (hot and cold) were used for extraction. A 10g amount of pulverized fruits was separately soaked in 100ml of acetone, ethanol, methanol (100% each) and cold sterile distilled water for 24h. Also the same amount (i.e. 10g) of pulverized fruits was immersed in 100ml of hot sterile distilled water and allowed to stand for 30min on a waterbath with occasional shaking and kept undisturbed for 24h. Each preparation was filtered through a sterilized Whatman No.1 filter paper (Ogundiya *et al.*, 2006) and the filtered extract was concentrated under vacuum below 40ºC using Heidolph, VE-11 rotaevaporator (Bag *et al.*, 2009). The dried extract thus obtained was exposed to UV rays for 24hrs and checked for sterility on nutrient agar plates and stored in labelled sterile bottles in a freezer at 4ºC until further use (Nkere and Iroegbu, 2005).

*Test Microorganisms*

Three dental caries causing bacteria *Streptococcus mutans* (MTCC*497), *Staphylococcus aureus* (MTCC 740), *Lactobacillus acidophilus* (MTCC *447) and two yeasts *Candida albicans* (MTCC 227) and *Saccharomyces cerevisiae* (MTCC 170) were procured from Microbial Type Culture Collection, IMTECH, Chandigarh. The microorganisms were subcultured on the specific media recommended for different microorganisms such as Brain heart infusion agar (*S.mutans*), Nutrient agar (*S.aureus*), Lactobacillus MRS agar (*L.acidophilus*), Malt yeast agar (*C.albicans* and *S.cerevisiae*) and incubated aerobically at $37^{O}$C. The media were procured from HiMedia Laboratory Pvt. Ltd., Bombay, India. Identification of all the strains was confirmed by standard biochemical and staining methods (Aneja, 2003; Benson, 2004; Cappuccino and Sherman, 1995).

*Antimicrobial Assay*

The acetone, methanol, ethanol, cold and hot water extracts were used for the screening. Antimicrobial activity of various extracts was determined by the agar well diffusion method (Okeke *et al.,* 2001). In this method, pure isolate of each microbe was subcultured on the recommended specific media for each microorganism at $37^{O}$C for 24hrs. A plate of each microorganism was taken and a minimum of four colonies were touched with a sterile loop and transferred into normal saline (0.85%) under aseptic conditions. Density of each microbial suspension was adjusted equal to that of $10^{6}$ cfu/ml (standardized by 0.5McFarland standard) and used as the inoculum for performing agar well diffusion assay (Aneja *et al.,* 2010). One hundred microlitre (100µl) of inoculum of each test organism was spread onto the specific media plates so as to achieve a confluent growth. The agar plates were allowed to dry and wells or cups of 8mm were made with a sterile borer in the inoculated agar plates and the lower portion of each well was sealed with a little specific molten agar medium. The extracts were reconstituted in 20% DMSO for the bioassay analysis (Rajasekaran *et al.,* 2008). A 100µl volume of each extract was propelled directly into the wells (in triplicates) of the inoculated specific media agar plates for each test organism. The plates were allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at $37^{O}$C for 24h (Aneja *et al.,* 2009; Khokra *et al.,* 2008; Rios *et al.,* 1980). Sterile DMSO (20%) served as the negative control and ciprofloxacin (for bacteria) and amphotericin-B (for fungi) served as the positive control. The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone of inhibition was greater than 8mm (Hammer *et al.,* 1999). The experiments were performed in triplicates and the mean values of the diameter of inhibition zones with ± standard deviation were calculated (Aneja and Joshi, 2009a, b).

*Determination of Minimum Inhibitory Concentration (MIC)*

MIC is defined as the lowest concentration of a compound/extract/drug that completely inhibits the growth of the microorganism in 24h (Thongson *et al.,* 2004). The MIC was determined by following the modified agar well diffusion method (Rajasekaran *et al.,* 2008). A twofold serial dilution of each extract was prepared by first reconstituting the powder (100:1mg/ml) in 20% dimethylsulphoxide (DMSO) followed by dilution in sterile distilled water (1:1) to achieve a decreasing concentration range of 50mg/ml to 0.39mg/ml. A 100 µl volume of each dilution was introduced into wells (triplicate) in the specific media agar plates already seeded with 100µl of

microbial inoculum adjusted with sterile distilled water equal to that of $10^6$ cfu/ml (standardized by 0.5McFarland standard) of the test microbial strain. All test plates were incubated aerobically at 37ºC for 24 hrs and observed for the inhibition zones. The lowest concentration of each extract showing a clear zone of inhibition (>8mm), considered as the MIC, was recorded for each test organism (Nkere and Iroegbu, 2005; Aneja *et al.,* 2009).

**Results and Discussion**

The results of antimicrobial activity of the five extracts of *S.mukorossi* and *E.officinalis* by agar well diffusion method have been shown in Table 1 and Table 2 respectively. From the data presented in the table 1, it is evident that all the five extracts of *S.mukorossi* i.e. hot aqueous, cold aqueous, acetonic, methanolic and ethanolic showed antimicrobial inhibitory activity against only one of the five tested dental caries causing microorganism *S.cerevisiae*, with the mean diameter of the highest zone of inhibition being 29.65mm. S. cerevisiae survived upto 12.5mg/ml in the acetonic extract, thus having a MIC of 25mg/ml (Table 3). Although no inhibitory activity of *S.mukorossi* was shown against *S.mutans, S.aureus, L.acidophilus* and *C.albicans*, the antifungal activity was much higher than the control (amphotericin-B, with the mean diameter being 11.94mm), thus *S.mukorossi* possesses very good antifungal properties against *S.cerevisiae*. The major active ingredient responsible for antifungal activity of *S.mukorossi* might be saponin. Saponins are generally known as non–volatile, surface–active compounds that are widely distributed in nature, occurring primarily in the plant kingdom (Oleszek, 2002). Saponins have a diverse range of properties, including foaming and emulsifying (Price *et al.,* 1987), pharmacological and medicinal properties (Attele *et al.,* 1999), haemolytic properties (Sparg *et al.,* 2004), as well as antimicrobial, insecticidal, spermicidal and molluscicidal activities (Saxena *et al.,* 2004; Shiau *et al.,* 2009).

All the five tested extracts of *E.officinalis* as presented in table 2 showed antimicrobial activity against *S.mutans* with the mean diameter of the highest zone of inhibition being 18.96mm and an MIC of 50mg/ml (table 4), as it survived upto 25mg/ml, while the acetonic, hot and cold aqueous extracts of *E.officinalis* showed excellent inhibitory activity against *S.aureus* with the mean diameter of highest zone of inhibition being 40.32mm produced by hot aqueous extract (much higher than the positive control ciprofloxacin 34.66mm) and an MIC of 12.5mg/ml, as it survived upto 6.25mg/ml. *L.acidophilus, C.albicans* and *S.cerevisiae* did not show any inhibitory activity when assayed against *E.officinalis* fruit extracts. Thus *E.officinalis* possesses good antibacterial activity but no antifungal activity. The excellent activity of *E.officinalis* against *S.aureus* shows a very good potential of *E. officinalis* to treat the diseases caused by *S.aureus*. The possible reason for the antibacterial activity of *E. officinalis* might be due to the tannins present in its fruits. The fruits have 28% of the total tannins distributed in the whole plant. The fruit contains two hydrolysable tannins Emblicanin A and B, which has antioxidant properties, one on hydrolysis gives gallic acid, ellagic acid and glucose wherein the other gives ellagic acid and glucose. The fruit also contains Phyllemblin (Wealth of Asia, 1998; Ghosal, 1996; Dictionary of Indian Medicinal Plants, 1988).

**Table 1:** Antimicrobial activity of fruit extracts of *Sapindus mukorossi* on dental caries causing microorganisms determined by agar well diffusion method on specific media for each test microorganism.

| *Sapindus mukorossi* extracts (mg/ml) | Diameter of inhibition zones (mm) | | | | |
|---|---|---|---|---|---|
| | *Sapindus mukorossi* | | | | |
| | *S.m.* | *S.a.* | *L.a.* | ***C.a.*** | ***S.c.*** |
| Acetone | - | - | - | - | 29.65±0.57 |
| Methanol | - | - | - | - | 20.96±1 |
| Ethanol | - | - | - | - | 24.97±1 |
| Hot aqueous | - | - | - | - | 19.65±0.57 |
| Cold aqueous | - | - | - | - | 20.32±0.57 |
| ciprofloxacin (5µg/ml) | 27.32±0.57 | 34.66±0.57 | 25.65±0.57 | nt | nt |
| amphotericin B (100units/ml) | Nt | Nt | Nt | 13±0 | 11.94±1 |
| DMSO | - | - | - | - | - |

**(-) = no activity, nt = not tested, *S.m. = Streptococcus mutans, S.a.= Staphylococcus aureus, L.a.= Lactobacillus acidophilus, C.a.= Candida albicans, S.c.= Saccharomyces cerevisiae,* * Values, including diameter of the well (8 mm), are means of three replicates, † ± Standard deviation.**

**Table 2:** Antimicrobial activity of fruit extracts of *Emblica officinalis* on dental caries causing microorganisms determined by agar well diffusion method on specific media for each test microorganism.

| *Emblica officinalis* extracts (mg/ml) | Diameter of inhibition zones (mm) | | | | |
|---|---|---|---|---|---|
| | *Emblica officinalis* | | | | |
| | *S.m.* | *S.a.* | *L.a.* | *C.a.* | *S.c.* |
| Acetone | 18.96 * ±1 † | 36.98±1 | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| Methanol | 12.31±0.57 | - | - | - | - |
| Ethanol | 15.31±0.57 | - | - | - | - |
| Hot aqueous | 11.64±0.57 | 40.32±0.57 | - | - | - |
| Cold aqueous | 10.31±0.57 | 33.98±1 | - | - | - |
| ciprofloxacin (5µg/ml) | 27.32±0.57 | 34.66±0.57 | 25.65±0.57 | nt | Nt |
| amphotericin B (100units/ml) | Nt | Nt | Nt | 13±0 | 11.94±1 |
| DMSO | - | - | - | - | - |

(-) = no activity, nt = not tested, *S.m. = Streptococcus mutans, S.a.= Staphylococcus aureus, L.a.= Lactobacillus acidophilus, C.a.= Candida albicans, S.c.= Saccharomyces cerevisiae,* * Values, including diameter of the well (8 mm), are means of three replicates, † ± Standard deviation.

**Table 3:** MIC of fruit extracts of *Sapindus mukorossi* against dental caries causing microorganisms on specific media for each microorganism, determined by modified agar well diffusion method.

| *Sapindus mukorossi fruit extracts* | MIC (mg/ml) | | | | |
|---|---|---|---|---|---|
| | Acetone | Methanol | Ethanol | Hot water | Cold water |
| *Saccharomyces cerevisiae* | 25 | 25 | 25 | 25 | 25 |

**Table 4:** MIC of fruit extracts of *Emblica officinalis* against dental caries causing microorganisms on specific media for each microorganism, determined by modified agar well diffusion method.

| *Emblica officinalis fruit extracts* | MIC (mg/ml) | | | | |
|---|---|---|---|---|---|
| | Acetone | Methanol | Ethanol | Hot water | Cold water |

| | | | | | |
|---|---|---|---|---|---|
| *Streptococcus mutans* | 50 | 50 | 50 | 50 | 50 |
| *Staphylococcus aureus* | 12.5 | - | - | 12.5 | 12.5 |

## Conclusion

Since the acetonic and hot water extracts of fruits of *Sapindus mukorossi* and *Emblica officinalis* were most effective against the tested dental caries causing microorganisms, purification and toxicological studies of the plants and *in vivo* trials should be carried out so that it can be used as a potential source for the development of a phytomedicine to act against dental caries causing microbes. The antimicrobial activities can be enhanced if the phytoactive components are purified and adequate dosage determined for proper administration.

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