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EVALUATION OF PHYTOCHEMICAL, ANTIOXDATIVE AND ANTIMICROBIAL EFFECT OF *MAGNIFERA INDICA*, LEAF EXTRACTS ON ORAL DENTAL PATHOGENS

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Magnifera indica leaves, Phytochemical, Antioxidant, Antimicrobial activity, Oral dental pathogen.

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ABSTRACT: A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources. Chemical substances used for prevention of dental caries are known to have many side-effects. Thus, natural products should be explored for their anticaries action. Present study evaluate the antimicrobial effect of alcoholic and aqueous extracts of *Magnifera indica* leaves against oral dental pathogens *Streptococcus mutans*, *Staphylococcus aureus*. Ethanolic extract was found to be more effective and dose dependent against both pathogens. Phytochemical and antioxidative study was also performed using various methods of testing on the aqueous and ethanolic extracts for the presence of phenols, alkaloids, saponins, terpenoids, flavonoids, tannins and reducing sugar. Both leaf extracts showed higher anti oxidative capacity and phytochemicals content like alkaloids and steroids which are active antimicrobial components in plants. Present investigation provides preliminary information for using mango leaf extracts in the prevention of oral diseases such as dental caries.

INTRODUCTION: Bio- active compounds from plants are significant and important source of new drugs that are likely to lead to new and better treatments for dental caries. Phenolic and flavanoid contents provide antioxidant activities that may underlie the anti-inflammatory and anticaries potential**.** Dental caries is an infectious microbial disease that results in localized dissolution and destruction of the calcified tissues of the teeth. Oral microorganisms play a vital role in initiation and progression of dental caries.

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It can be prevented by mechanical plaque removal or by the use of chemical agents [1]**.** The early stage of dental caries is characterized by a destruction of superficial dental structures caused by acids which are by-products of carbohydrates metabolism by *Streptococcus mutans*, a cariogenic bacterium [2] Artificial drugs have unpleasant side effect, on the other hand, the number of drug resistant microorganisms is increasing, so researches are trying to pay more attention to herbal drugs. *Mangifera indica* is a species of mango in the Anacardiaceae family. It is found in the wild and cultivated in India.

The present study was intended to determine the phytochemical constituents, antioxidant capacity and antimicrobial activity of ethanolic and aqueous extracts from leaves of *Magnifera indica* on oral dental pathogen.

MATERIALS AND METHODS:

Collection of Plant material and Extract Preparation: The fresh and tender guava leaves were collected from a local garden in Kalyan. The authentication of plant leaves was done at the Blatter Herbarium, (Herbarium specimen no NYL 1) St. Xavier college, Mumbai. The leaves were thoroughly washed, shade dried and then crushed by electric grinder. 30 gm of mango leaves powder subjected to soxhlet extraction. The extracted solutions were concentrated in a rotary evaporator and stored at 4 °C for further use.

Procurement of the microorganisms: Freeze-dried forms of the microorganisms *Streptococcus mutans* (MTCC 890) was obtained from Microbial Type Culture Collection MTCC, Chandigarh, and a glycerol stock of *Staphylococcus aureus* was obtained from School of Biotechnology and Bioinformatics, D. Y. Patil University.

Antimicrobial Activity tests: Antibacterial effect of medicinal plant extracts were checked by Well-diffusion method. The ampoules containing freeze-dried forms of the micro-organisms were opened and revived in Brain heart infusion Broth (hi-media) for *Streptococcus mutans*, and Nutrient broth (hi-media) for *S aureus.* 20, 40 & 60 micro litres of the ethanolic and aqueous extracts of Mango leaves were incorporated in the 8 mm wells which were bored onto the Mueller hinton agar plates which have been inoculated separately with *S. mutans*, and *staphylococcus aureus* using the spread plate technique (1 x 10^8 cfu/ ml). Streptomycin for *S. aureus*, Vancomycin for *Streptococcus mutans*, was used as control Antibiotics. After 24 hrs at 37 °C incubation, the plates were observed for the results and zone of inhibitions measured. The experiment was done in triplicate and values are presented as Mean $\pm$SD.

Estimation of phytochemical constituents:

Estimation of total phenol content (TPC): The total phenol content was determined by Folin-Ciocalteu reagent method [3] and expressed in terms of gallic acid equivalent (mg/g) [4].

Estimation of total flavonoids (TF): The total flavonoid content was determined by aluminium chloride method [5] and expressed in terms of quercetin equivalent (mg/g).

Estimation of sugars: Estimation of sugars in the extract was done by DNSA method [6]. Maltose is a reducing sugar which will reduce 3,5 – dinitro salicylic acid (DNSA) to 3 – amino – 5 – nitro salicylic acid in alkaline medium that is orange coloured and absorbance was measured at 525 nm. Sugar content was expressed in terms of maltose equivalent (mg/g).

α, α-diphenyl- β-picryl-hydrazyl (DPPH) radical scavenging assay: The free radical scavenging activity was measured using 2, 2-diphenyl-1-picryl-hydrazyl or 1, 1- diphenyl-2-picryl-hydrazyl by the method of McCune and Johns [7]. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen–donating antioxidant due to the formation of the non radical form DPPH-H. The absorbance was measured at 517nm [4]. DPPH scavenging activity was expressed in terms ascorbic acid equivalent (mg/g).

Nitric oxide (NO) radical scavenging assay: Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interact with oxygen to produce nitrite ions, which were measured using the Griess reagent at 540 nm [8, 4]. NO radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g).

Ferric reducing antioxidant power (FRAP) assay: FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri(2-pyridyl)- s-triazine (TPTZ). Decrease in the absorbance (593 nm) is proportional to the antioxidant content [4]. The antioxidant capacity was expressed in terms of ascorbic acid equivalent (mg/g).

Estimation of reducing power (RP): The reducing power was determined by the method of Athukorala *et al.* 2006 [9]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid per-oxidation processes, so that they can act as primary and secondary antioxidants. Absorbance was measured at 700 nm [4]. RP was expressed in terms of ascorbic acid equivalent (mg/g).

Superoxide anion (SO) radical scavenging assay: The superoxide anion scavenging activity

was measured as described by Robak and Gryglewski (1998) [10]. In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen from PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture [4]. SO anion scavenging activity was expressed in terms of Gallic acid equivalent (mg/g).

Hydrogen peroxide (H_2O_2) radical scavenging assay: The ability of plant extracts to scavenge hydrogen peroxide is determined according to the method of Ruch *et al.* (1989) [11]. H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (OH^-). Decrease in absorbance at 230 nm was determined [4]. H_2O_2 radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g).

Statistical Analysis: Analysis of the antibacterial action of the extracts of mango leaves was carried out at different concentrations, by comparing the mean diameter of the inhibition haloes as a variable. For anti-oxidative activity Mean ± SD for samples in triplicate was used for comparisons.

RESULTS AND DISCUSSION: Essential oils and extracts of plants, have been used for thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens [12]. Present work showed that the mango extracts inhibited bacterial growth but their effectiveness varied. Ethanolic extract was found to be very effective in inhibiting the growth of *S. mutans* as compare to *S. aureus*. Aqueous extracts are effective at higher concentration against *S. mutans* and *S. aureus*.

All the gram positive microorganism's viz., *S. aureus*, *S. mutans* were more susceptible bacteria to all plant extracts. This may be due to differences in cell wall structure between Gram-positive and Gram-negative bacteria, with Gram-negative outer membrane acting as a barrier to many environmental substances including antibiotics [13]. Effective antimicrobial activity of aqueous extract may be because of active compounds which are flavonoids with different levels of antibacterial activity **(Fig. 1, 2** and **3)**.

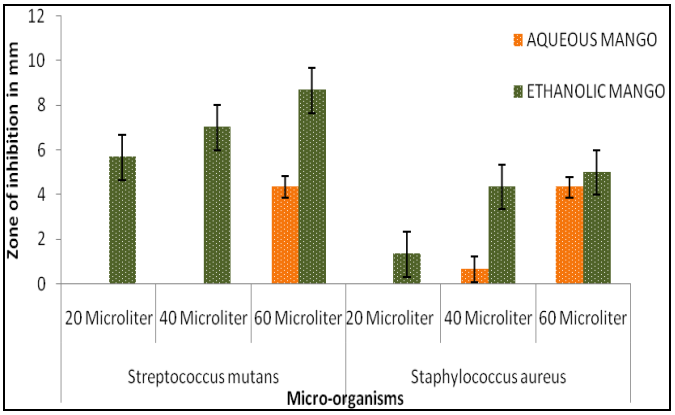

FIG. 1: EFFECT OF VARIOUS CONCENTRATIONS OF *MAGNIFERA INDICA*, LEAF EXTRACTS ON *STREPTOCOCCUS MUTANS*, *STAPHYLOCOCCUS AUREUS*

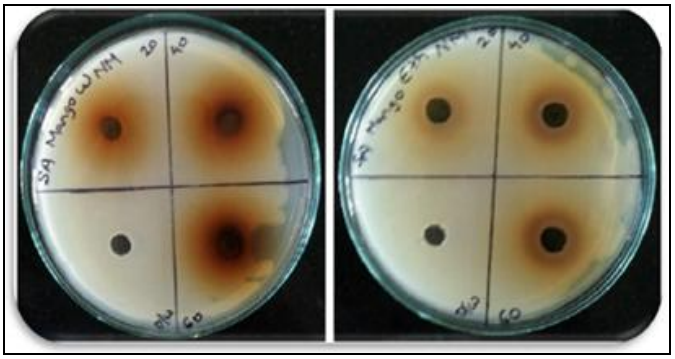

FIG. 2: ZONE OF INHIBITIONS EXHIBITED BY MANGO EXTRACTS IN *STAPHYLOCOCCUS AUREUS*

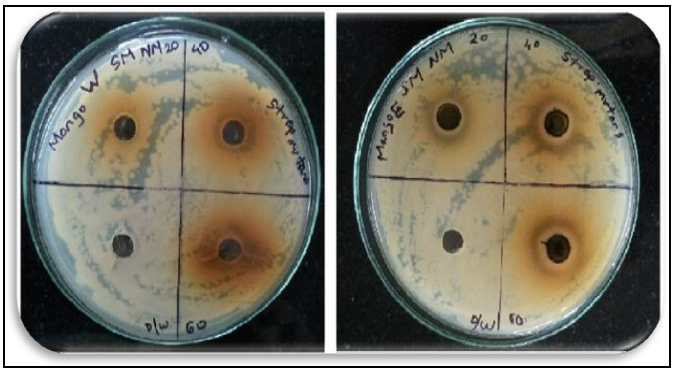

FIG. 3: ZONE OF INHIBITIONS EXHIBITED BY MANGO EXTRACT IN *STREPTOCOCCUS MUTANS*

The structure elucidation study reveals that five flavonoid compounds are quercetin, quercetin-3-O-α-L-arabinofuranoside, quercetin -3 –O –β -D-arabinopyranoside, quercetin-3-O-β-D glucoside and quercetin-3-O-β-D-galactoside [14] similar trends

was also observed in our results that the total phenolic content is higher in aqueous extracts of mango leaves than the ethanolic extracts **(Table 1)**.

TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF *MAGNIFERA INDICA*, LEAF EXTRACTS

Tests	Standard equivalent in Aqueous extract (µg/g)	Standard equivalent in Ethanolic extract (µg/g)
Total phenol content	242.92 ± 15.67	148.17 ±19.19
Total flavonoids	681.92 ± 29.84	936.66 ±29.84
Sugar content	3914 ± 58.69	5611.3 ±104.28

Reducing power of plant extract was reported to be directly correlated with its antioxidant and is based on the presence of reductants like Quercetin-3, 5-diglucoside and cyanidin-3-sophoroside-5-glucoside which exert antioxidant activity by breaking the free radical chain and donating a hydrogen atom [1, 12]. Reducing power is highest in ethanolic extract and this can also be linked with higher content of reducing sugar in same extract **(Table 2)**.

TABLE 2: A**NTIOXIDANT ACTIVITY OF *MAGNIFERA INDICA*, LEAF EXTRACTS**

Tests	Standard equivalent in Aqueous extract (µg/g)	Standard equivalent in Ethanolic extract (µg/g)
DPPH scavenging assay	1514.88 ± 18.68	947.26 ± 50
No radical scavenging assay	180.66 ± 6.11	1238 ± 46.28
Reducing power assay	551.33 ± 33.04	1301.04 ± 10.03
SO radical scavenging assay	369.59 ± 11.58	146.55 ± 24.16
H_2O_2 Radical scavenging assay	2214.33 ± 39.64	2131.7 ± 29.7

DPPH scavenging activity in mango leaves was estimated to be higher in the aqueous extract. This can be associated to the presence of total phenols in the extract [16]. Aqueous extract shows high SO scavenging activity which is associated to the concurrent higher total phenolics and flavanoids in the same extract [17]. Both extract shows higher hydrogen peroxide scavenging activity. Two major valuable compounds, namely ethyl gallate and penta-O-galloyl-glucoside, have been isolated from mango peels that have potent radical scavenging ability [18, 19]. Current data shows that are *Mangifera indica* leaves are potential source of antioxidant and phytochemicals which can be used effectively as dental therapeutic agents.

CONCLUSION: Recent research studies indicate that, the natural products derived from plants continue to be used in prophylaxis and treatment of dental caries and diseases. Our study indicates that mango leaves have potent pharmacological components possessing antioxidant and anti microbial activity with low or no side effects. It can be used effectively in herbal oral preparations as dental therapeutic agents. With further research in this field, we can get much safer alternatives for caries prevention in children.

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CONFLICT OF INTERST: The authors declare that there is no conflict of interests regarding the publication of this paper.

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