Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v29i04.7007

COMPARATIVE EFFECTS OF ANTI MICROBIAL AND ANTIOXIDANT ACTIVITY OF NEEM, TURMERIC AND MANGO EXTRACTS AN INVITRO STUDY

Dr. Chetan Vinay Deshmukh[1*] Dr. Vibhuti Dilip Mistry[2,] Dr. Arun Suresh Dodamani[3,] Dr. Padma Vinay Deshmukh[4,] Dr. Swapnil Kamble[5,] Dr. Anand Maurya[6,] Mr. Shrikrishna Bhagat[7]

[1*]M.D.S; Assistant Professor & Ph.D. Scholar, Department of Public Health Dentistry, JMF's ACPM Dental College, Dhule, Maharashtra State.
[2]M.D.S; Assistant Professor, Department of Periodontology, JMF's ACPM Dental College, Dhule, Maharashtra State.
[3]M.D.S; Principal, Professor and Head, Department of Public Health Dentistry, JMF's ACPM Dental College, Dhule, Maharashtra State.
[4]Ph.D; Ex-Associate Professor & Head, Department of Microbiology, Smt. CHM College of Arts, Science,Commerce and Management, Ulhasnagar, Maharashtra State.
[5]Ph.D; Junior Scientific Assistant, Department of Microbiology, Smt. CHM College of Arts, Science,Commerce and Management, Ulhasnagar, Maharashtra State.
[6]Ph.D; Junior Scientific Assistant, Department of Microbiology, Smt. CHM College of Arts, Science,Commerce and Management, Ulhasnagar, Maharashtra State.
[7]M.Sc; M.Sc. (Research) Student, Department of Microbiology, Smt. CHM College of Arts, Science,Commerce and Management, Ulhasnagar, Maharashtra State.

***Corresponding Author:** Dr. Chetan Vinay Deshmukh
*(Assistant Professor & Ph.D. Research Scholar Department of Public Health Dentistry, Jawahar Medical Foundation's

ABSTRACT:

Introduction: Plants are the cheapest and safer alternative sources of antimicrobials and antioxidants. Neem extracts are known to have a strong antimicrobial effect and has several applications in herbal medicines. On the other hand, Turmeric extract is known to have a strong antioxidant effect and has been used as a part of remedies. Mango extract, which has been less explored so far, is a promising alternative with benefits of both.

Aim: The study aimed to compare the anti microbial and antioxidant activity of the alcoholic extracts of Neem leaves, Mango leaves and Turmeric rhizome.

Method: The antimicrobial activity of neem extract, turmeric extract and mango extract against *Staphylococcus aureus*, *Enterococcus fecalis* and *Streptococcus mutans*, was carried out using Agar Plate Method and the Minimum Inhibitory Concentration was determined. The antioxidant activity of neem, turmeric and mango extracts was carried out using the 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method.

Results: Antimicrobial activity was best shown by Neem extract followed by Mango and Turmeric Extracts. Antioxidant activity was best shown by Turmeric Extract followed by Mango and Neem

Extracts.The Mango extract was found to be almost equivalent to neem extract in anti- microbial activity and almost equivalent to turmeric extract in anti-oxidant activity.
KEYWORDS: Mango Extract; Neem Extract; Turmeric Extract; Antioxidant Activity; Antimicrobial Activity.

INTRODUCTION

Caries or periodontal diseases are infectious diseases caused by bacteria present in dental plaques and the removal of dental plaques is thus highly important for the health of oral cavities.

The mouth contains a wide variety of oral bacteria, few of which cause dental caries: *Streptococcus mutans* and Lactobacillus species among them. These organisms produce high levels of lactic acid due to fermentation of dietary sugars, and are resistant to the adverse effects of low pH, properties essential for cariogenic bacteria[1]. As the cementum of root surfaces is more easily demineralized than enamel surfaces, a wider variety of bacteria can cause root caries.

Enterococcus faecalis is a nonspore-forming, fermentative, facultative anaerobic Gram-positive coccus, recovered in high proportion from endodontic failure cases and in approximately one-third of root canal treated teeth with persistent lesions[2,3]. *E. faecalis* a very resistant species is able to invade dentinal tubules, and survive as a monoculture without the support of other bacteria and endure prolonged periods of nutritional deprivation. *E. faecalis* grows by forming biofilms which is an adaptive process[4]. Bacteria sequestrated in biofilms are protected and resist harder cidal action[5].

In the oral tract, *S. aureus* has been associated with dento alveolar infections, and oral mucosal lesions. Moreover, staphylococcal colonization has been demonstrated from the tongue, saliva, mucosal surfaces, supra gingival tooth surfaces and the periodontal pocket[6,7] .

These bacteria collect around the teeth and gums in a sticky, creamy-coloured mass called plaque, which serves as a biofilm. Cariogenic bacteria are present in dental plaque, but are usually in low concentrations to cause problems unless there is a shift in the balance[8]. This is driven by local environmental change, such as frequent sugar, no biofilm removal (a lack of tooth brushing)[9]. If left untreated, the disease can lead to pain, tooth loss and infection[10].

Spread of anti-microbial resistance is one issue that has the potential of diminishing, if not nullifying, the benefits that mankind has reaped by utilising antimicrobial agents.

Resistance is not a new phenomenon that has posed various problems in treatment. Bacteria are known to build up resistance to a particular antimicrobial agent within a few years of the agent being cleared for application. What is new is the fact that the number of organisms that are posing challenge to anti-microbial agents and the occurrence of resistant infections are rising tremendously[11,12,13]. These resistant organisms are responsible for a number of increased community-acquired infections[14,15,16]. Unfortunately, the resistant micro bugs are no more the monopoly of healthcare institutes and hospitals. The factors responsible for the emergence and spread of anti-bacterial resistance have been worked upon and identified[17,18] . Amongst them, excess use and abuse of antibiotics constitutes the most essential factor.

Ayurveda uses herbs according to their energies or energetics. Herbs represent the most effective Ayurvedic approach to healing illness. Their action is strongest when they are fresh, but they may also be used as decoctions, infusions, teas, powders and pills. Interestingly, each herb appears to possess properties that work on multiple biochemical pathways capable of influencing several organ systems simultaneously. The ancient practice of combining and concentrating several plants by decoction (extracting together in boiling water) which exhibit a similar yet slightly different organ system focus, produces a finished product that treats the person in whole along with the presenting complaint.

The use of plants and herbs for dental care is a very common indigenous system of medicine. Chewing sticks (Neem) is most commonly used in the Middle East and Indian subcontinent. *Azadirachtaindica* (Neem)is commonly used as oral hygiene tool in different parts of the world, Several studies have demonstrated the anti-plaque anti carious and antibacterial effect of these sticks. A study was conducted to compare the effectiveness of antimicrobial activity of Neem and Arak

(*Salvadorapersica*, Miswak) chewing sticks' aqueous extracts. Data suggested that both chewing stick extracts were effective at 50% concentration on *Streptococcus mutans* and *Streptococcus faecalis*. Arak extract was more effective at lower concentrations for *Streptococcus faecalis*. The effect may be due to the difference in their chemical composition and variability in their pH[19].

Circuma longa(turmeric) is known to possess analgesic, antibacterial, anti-inflammatory, anti-tumor, anti-allergic, antioxidant, antiseptic, antispasmodic, appetizer, astringent, cardiovascular, carminative, cholagogue, digestive, and diuretic properties . There are many uses of turmeric in dentistry. The active constituent of turmeric, known as curcumin protects against free radical damage because it is a strong antioxidant[20]. and reduces inflammation by lowering histamine levels and possibly by increasing the production of natural cortisone by the adrenal glands[21].

Mangifera indica(Mango) is mentioned to be frequently used against diarrhoea and toothache.The plant also shows a number of other pharmacological actions .*Mangifera indica* leaves individually inhibited bacterial growth at very low concentrations**.** Barks, leaves and seeds of *Mangifera indica* contain tanins [22, 23, 24, 25] and this may also explain its effectivity against diarrhoea[26] **.** A number of workers have shown the anti-inflammatory properties of the plant[27, 28, 29]. The anti-inflammatory properties of *M. indica* may explain its use against toothache.

An extract of *Mangifera indica* L showed a powerful scavenger activity of hydroxyl radicals and hypochlorous acid and acted as an iron chelator. The extract also showed a significant inhibitory effect on the peroxidation of rat-brain phospholipid and inhibited DNA damage by bleomycin or copper-phenanthroline systems [30] **.**

Studies have demonstrated the activity of crude extract *M. indica* leaves against both Gram positive and Gram negative bacteria. They have suggested it is an indication of broad spectrum of activity and thus can be used to source antibiotic substances for drug development that can be used in the control of these bacterial infections[31].

Significant in vitro activity has been shown by methanolic,ethyl acetate and chloroform extracts of *Mangifera indica* leaves against various pathogens including drug resistant *Staphylococcus aureus*[32]**.**

AIMS AND OBJECTIVES

The study was carried out to compare the antimicrobial activity of the alcoholic extracts of Neem leaves, Mango leaves and Turmeric rhizome against *Staphylococcus aureus* [MTCC 3160] [dento-alveolar infections], *Streptococcus mutans* [MTCC 890][DentalCaries] ,*Entrococcusfecalis* [MTCC 439] [Endodontic Failures]. Antioxidant activity of all three extracts was also determined.

MATERIALS AND METHODS

Plant Material

Collection

The plant material such as Turmeric Rhizome was obtained from authentic source while Neem leaves and Mango leaves were procured from the botanical garden at Smt. C.H.M. College, Ulhasnagar. The materials were identified and authenticated by the Department of Botany and voucher specimens of the same are maintained in the department. The parts were dried and powdered. (Figure 1)

Figure 1: The Three Extracts – Mango Leaves, Neem Leaves & Turmeric Rizome

Cold solvent extracts:

10gm of the powdered plant part was mixed with 100ml of ethanol and incubated for 24 hrs under shake flask condition. The mixture was then filtered and the filtrate was subjected to the process of evaporation. The residual extract was used for further studies[33] .

Test organisms.

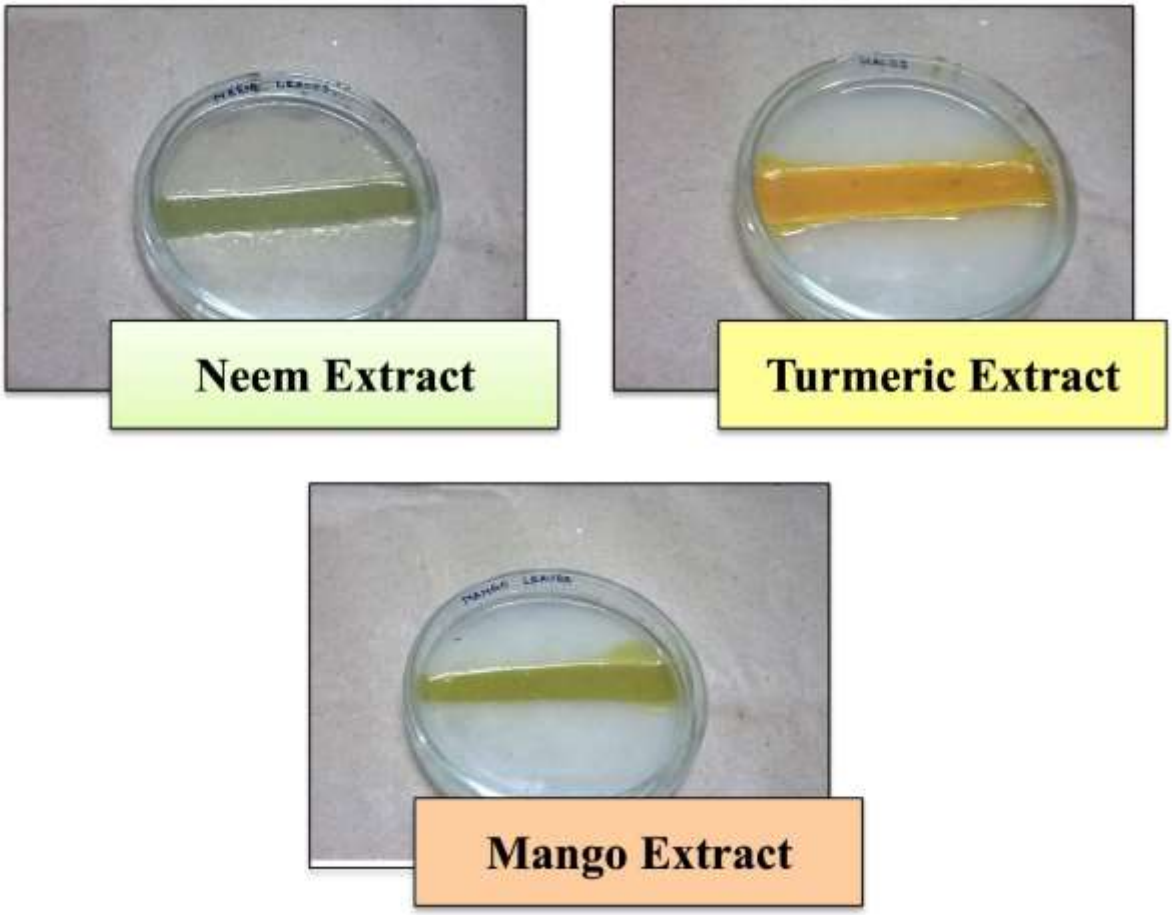
Standard strains of

Staphylococcus aureus **MTCC 3160**
Streptococcus mutans **MTCC 890**
Entrococcusfecalis **MTCC 439**
were used for the study

Agar ditch method[34]

The Agar Ditch Method was used to carry out the preliminary screening of the test compounds. This method allows a single compound to be evaluated against a number of test organisms simultaneously. (Figure 2)

Figure 2: Preliminary Antimicrobial testing done against the Micro organisms using the Agar Ditch Method using standardized 5% concentrations of each extract showed positive anti microbial activity against the three micro organisms.



Requirements

i] Test organisms:

The inoculum for the test was prepared using a 24 hours old culture broth whose density was adjusted to match with the turbidity of 0.5 McFarland standard[35]

PROCEDURE

A sterile agar plate was prepared using 20ml of sterile, molten Mueller-Hinton medium. A ditch of 8.0cm x 1.5cm was cut out aseptically in the medium.

The weighed quantity of the compound (powder or extract) was added to 10ml of sterile melted Agar medium to obtain the required concentration.

It was mixed thoroughly and aseptically poured into the ditch such that the surfaces of the agar in the plate and that in the ditch were even. The plates were kept in refrigerators for 10 minutes for proper setting of the agar. The plate was dried to remove any moisture on the agar surface.

The inocula prepared from the standard test strains were then streaked perpendicular to the ditch and parallel to each other. The plates with bacterial cultures were incubated at 37ºC for 24 hours. The growth was observed on the ditch and near to it, along the line of the streak of cultures. The Concentration used was 30mg/ml

Appropriate solvent controls were maintained and tested to eliminate the errors that may arise due to these solvents

Agar dilution method[36] **:**

Sterile plates of Mueller-Hinton agar with different concentrations of the antibacterial substance are inoculated with the test organisms and then incubated at suitable temperature. The lowest concentration of the substance which causes the complete inhibition of the organism with in a set time is taken as the inhibitory concentration of the test compound.

The growth of the organism on the surface was observed. The advantage of the test is that it allows the activity of different concentrations of the substance to be assessed against a wide range of organisms with the minimum use of the glass wares.

ii] Test compounds:

A range of concentrations from 1mg/ml to 30mg/ml was studied.

iii] Medium:

The medium used to perform the test was Mueller-Hinton Agar (Hi media)[37] for all the bacterial isolates.

Procedure

A series of the dilutions was prepared by adding different volumes of stock solution (3 %) to molten agar cooled to 45º C. These were then poured into sterile petriplates. Each plate was then spot inoculated with the test organisms.

After incubation for 24 hours, the plates were observed for growth and the minimum inhibitory concentration for different extracts was determined.

One plate was prepared without incorporating drug into it, which served as positive control and showed full growth of the organisms after incubation. This plate helped in comparing the growth of the organisms on the surface to determine the end point.

Appropriate controls of the solvents used to dissolve the extracts were also maintained and tested to eliminate possible errors.

Antioxidant Activity: The antioxidant activity of neem, turmeric and mango extracts was carried out using the 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. (Figure 3)

Figure 3: Antioxidant Activity of the three extracts

Antioxidant activity of the Neem extracts

Antioxidant activity of the Mango leaves extracts

Antioxidant activity of the Turmeric leaves extracts

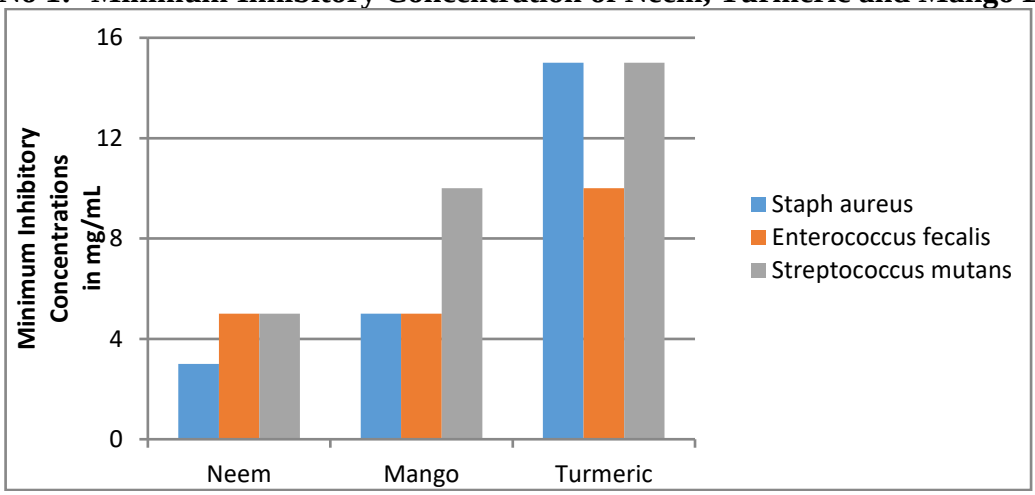
RESULTS

Results obtained from the preliminary screening using Agar Ditch method indicated that all the three standard strains were inhibited by the cold ethanolic extracts of Neem, Haldi and Mango at a concentration of 30mg/ml. (Table 1; Graph 1)

Table No 1: Minimum Inhibitory Concentration of Neem, Turmeric and Mango Extracts

Extracts	***Minimum Inhibitory Concentration level [mg/ml]***		
	S. aureus **MTCC 3160**	***E. fecalis*** **MTCC 890**	***Str. mutans*** **MTCC 439**
Neem Extract	**3**	**5**	**5**
Mango Extract	**5**	**5**	**10**
Turmeric Extract	**15**	**10**	**15**

Graph No 1: Minimum Inhibitory Concentration of Neem, Turmeric and Mango Extracts



The MIC of the Neem extract was determined by Plate Dilution method to be 2mg/ml for *Staphylococcus aureus* and 4mg/ml for both *Enterococcus feacali*s and *Streptococcus mutans*.
In case of Turmeric, the MIC values were found to be higher. *Staphylococcus aureus* and *Streptococcus mutans* were inhibited at a concentration of 14mg/ml while *Enterococcus feacalis* showed inhibition at9mg/ml. (Table No 2 & 3)

Table No.2: Comparison of antibacterial activity of three groups against test micro-organisms

Extract	**Minimum Inhibitory Concentration [mg/mL]**		
	***S. aureus* MTCC 3160**	***E. fecalis* MTCC 890**	***Str. Mutans* MTCC 439**
Neem Extract	3.40 ± 0.894	5.00 ± 0.707	5.00 ± 0.707
Mango Extract	5.40 ± 1.140	5.40 ± 0.548	10.40 ± 0.894
Turmeric Extract	15.00 ± 0.707	10.20 ± 0.837	15.20 ± 0.837
F value	221.846	83.733	195.300
P value	0.001*	0.001*	0.001*

One way ANOVA; *indicates significance at p≤0.05

Table No 3: Pairwise comparison of antibacterial activity among three groups

Dependent Variable	**Extracts (I)**	**Extracts (J)**	**Mean Difference (I-J)**	**p value**
S. aureus	Neem	Mango	-2.000*	0.005*
		Turmeric	-11.600*	0.001*
	Mango	Turmeric	-9.600*	0.001*

E. fecalis	Neem	Mango	-0.400	0.389
		Turmeric	-5.200*	0.001*
	Mango	Turmeric	-4.800*	0.001*
S. mutans	Neem	Mango	-5.400*	0.001*
		Turmeric	-10.200*	0.001*
	Mango	Turmeric	-4.800*	0.001*

Post hoc LSD; * indicates significance at p<0.05

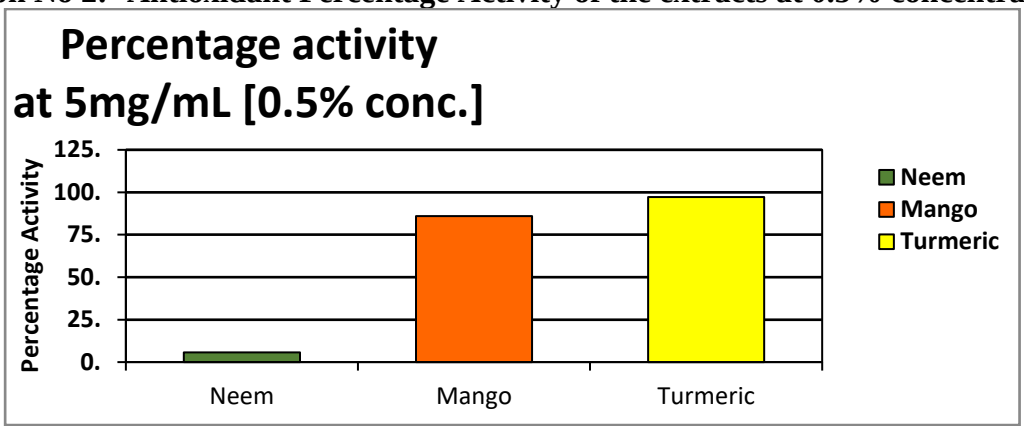
Mango extract showed uniform activity that is comparable with Neem, where it showed inhibition against all the three test organisms at a concentration of 4mg/ml.

Antioxidant activity of the extracts screened was found to be comparable. Neem leaves extract showed maximum activity(5.71%) at a concentration of 5mg/ml while Turmeric showed 97.2% activity at 3.5mg/ml. It has been reported in earlier studies that Curcumin (present in Turmeric) shows strong antioxidant, activity. Present study with cold ethanolic extract of the Turmeric rhizome alsoindicated that the extract showed significant activity. The cold ethanolicextractof mango leaves showed an antioxidant activity of 85.98% at 3mg/ml which is comparable with that of Turmeric .(Table No. 4; Graph No. 2)

Table No 4: Anti Oxidant Activity of Neem, Turmeric and Mango Extracts

Extract Concentration		**Percentage Activity**		
Mg/mL	**%**	**Neem**	**Mango**	**Turmeric**
0.5	0.05	0	17.76	27.1
1	0.1	0	36.44	56.07
1.5	0.15	0	56.07	85.98
2	0.2	0	71.02	95.33
2.5	0.25	0	83.18	96.26
3	0.3	0	**85.98**	96.26
3.5	0.35	0	85.98	**97.20**
4	0.4	0	85.98	97.20
4.5	0.45	0	85.98	97.20
5	0.5	**5.71**	85.98	97.20

Graph No 2: Antioxidant Percentage Activity of the extracts at 0.5% concentration]



DISCUSSION

Since prehistoric times humans have used indigenous plants to treat infectious diseases [38.]While a number of synthetic and natural antibacterial agents are available for controlling bacterial infections, increased resistance calls for new antibacterial drugs, one source of which are traditional medicinal plants. [39] Many medicinal plants around the world contain many compounds with antibacterial activity.[40] Moreover, to many communities in the developing counries, antibacterial pharmaceuticals are not accessible to the majority of the people who need them. The use of botanical medicines is generally on the rise in many parts of the world.[41, 42] Ethnobotanical surveys carried out in Uganda have reported a number of plants that are used in the treatment of infectious diseases, including *Mangifera indica* (L.)[43, 44, 45] *Mangifera indica* is a large evergreen tree, with a heavy, dome-shaped crown. It belongs to the family Anacardia- ceae. It is found all over the tropical regions of the world where it is used as a horticultural and medicinal plant. Fruits contain protein, fat, carbohydrate, minerals, vita- mins A, B and C and amino acids. The fruits also yield a resin that is said to contain mangiferene, mangiferic acid, resinol and maniferol and others.[46, 47] The leaves contain the glucoside mangiferine. The bark of the mango tree contains 16–20% tannin.[46, 47] The leaves have been reported to contain saponins, glycosides, unsaturated sterols, poly- phenols, euxanthin acid, mangiferine, mangin, gallic tan- nins, etc. The ashes of the leaves are used to treat burns, scalds, sores, cough and diarrhoea in South America and other parts of the world.[47, 48] The use of leaf extracts as antiseptics in the treatment of burns, scalds, sores, wounds, abscesses and other infections in humans and animals has been reported in a number of ethnobotanical surveys. [49, 50, 44]

This study reports that the leaves of M. indica contain alkaloids, anthracenosides, coumarins, flavonones, sugars, tannins, steroids and saponins. These, together with chlorophyll, are responsible for the extract colours observed. The fruity or sweet smell of the extracts is due to the presence of esters and essential oils in the plant extracts. [40, 48] Some of these compounds have been reported to possess antimicrobial activity.[47]

This study reported that the neem leaf extract was found to have the best antimicrobial activity with significant difference when compared to the antimicrobial activity of the mango and turmeric extracts. These results are in concurrence with the study carried out by Hegde Vibha et al (2013)[51] where antimicrobial activity of neem extract was more than the other agents for both microorganisms, the difference being statistically significant. The antimicrobial activity of sodium hypochlorite solution was only less than neem extract for both microorganisms. Turmeric solution showed good activity only against *C.albicans*. The other 2 solutions showed weak antimicrobial activity.

The study reported that the Neem extract was effective against E. *fecalis.* This finding is in concurrence with the study conducted by Bohara Aarti et al (2010)[52]. The results obtained in this in vitro study showed that neem leaf extract is a viable medicament against C. albicans, E. faecalis and even mixed state. Thus Neem has proven to be a viable antimicrobial agent and thus is confirmed by

this study as well.

Comparison shows significant differences in anti-bacterial activity of neem compared with mango (p=0.005) and turmeric (p=0.001) against *S. aureus*. Difference in anti-bacterial activity of mango and turmeric against *S. aureus* was also found to be significant (p=0.001). Thus Neem was found to be most effective as an antimicrobial agent followed by Mango and Turmeric against S. *aureus.*

Difference in anti-bacterial activity of neem and mango against *E. fecalis* was found to be non-significant (p=0.389). However differences in anti-bacterial activity of neem vs turmeric and mango vs turmeric were found to be significant (p=0.001). Thus Neem was found to be most effective as an antimicrobial agent with Mango equivalent to Neem and followed by Turmeric against *E. fecalis.*

Anti-bacterial activity of neem compared with mango and turmeric against *S. mutans showed significant differences* (p=0.001). Difference in anti-bacterial activity of mango and turmeric against *S. mutans* was also found to be significant (p=0.001) Thus again, Neem was found to be most effective as an antimicrobial agent followed by Mango and Turmeric against *S. mutans.*

The antioxidant properties observed shows that at 0.35% concentration the turmeric extract showed the highest antioxidant percentage activity at 97.2% which was followed by Mango Extract – at 0.3% concentration, the Mango extract showed 85.98% of antioxidant percentage activity and Neem proved to be a very weak antioxidant agent showing no antioxidant percentage till 0.5% concentration and at 0.5% Neem showed a weak antioxidant percentage activity of 5.71%. These findings were in accordance with the study conducted by Jayprakasha GK et al (2002)[53] where it was found that turmeric oil exhibited a markedly antimutagenicity but fraction III (containing aromatic turmerone (44.5%), curlone (19.22%) and turmerone (10.88%) as major compounds) was the most effective. The antioxidant effects of turmeric oil and its fractions may provide an explanation for their antimutagenic action.

CONCLUSIONS

Thus it can be safely deduced that antimicrobial activity was best shown by Neem extract followed by Mango and Turmeric Extracts. Antioxidant activity was best shown by Turmeric Extract followed by Mango and Neem Extracts whereas Mango extract was found to be almost equivalent to neem extract in anti-microbial activity and almost equivalent to turmeric extract in anti-oxidant activity.

The present study which was conducted to compare the antibacterial and antioxidant activities of the three plant parts ,namelyethanolic extracts of Neem leaves, Turmeric Rhizome and Mango leaves indicate that Mango leaves exhibit significant antibacterial and antioxidant activities as compared to the extracts of Turmeric and Neem which are good either as anti oxidant or antibacterial agents.

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