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Comparative Study on Antibiogram and Phytochemical Analysis in Extraction of *Ocimum sanctum, Murraya koenigii, Mentha piperita* and *Coriandrum sativum,* against various Pathogens

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Abstract: **The Plants have the highest source of natural anti-microbial sources. The proposed study was passed out to estimate the antibacterial outcome of four important plants namely, *Ocimum sanctum, Murraya koenigii, Mentha piperita* and *Coriandrum sativum*. The crushed leaf materials of four selected plants were extracted with methanol and solvent extracts were evaporated for dryness with help of rotary evaporator. Remaining dry residue was dissolved in (M- Methanol, D- DMSO, H- distilled water, C- Control; 1:10 w/v) and different volume of tested leaf sample is applied for antibacterial action. The antibacterial screening of the four selected crude methanolic extracts were determined by the Kirby-Bauer's Disc diffusion technique on the following bacteria- *Staphylococcus aureus, Lactobacillus acidophilus, Escherichia coli* and *Streptococcus mutans*. The maximum zone of inhibitions were noted in the Curry leaf (*M. koenigii*) with solvent methanol and Distilled water against *S. aureus* 10 mm and 8 mm also with solvent DMSO against *Lactobacillus sps.* 07 mm. The Curry leaves shows the maximum zone of inhibition with solvent methanol and Distilled water against *S. aureus* 10 mm and 08 mm. Additional studies should be undertaken to explain the exact mechanism of action of anti-microbial effect to identify the active components which can be used in the drug development program.**

***Keywords*: Anti-biogram, DMSO, Methanol, Phytochemical and Water.**

I. INTRODUCTION

From the traditional health care system, we use plant as a good source of medicine/drugs. They have an essential part of traditional health care system as cure or food supplements in India and in maximum parts of the world. The herbal drugs as the main medicine in traditional system and also use of medicine practices since ancient times. Recently 20,000 plants are record in India as a medicinal plant, all most 800 medicinal plant species are used in curing of different diseases in 500 different communities (Kamboj, 2000). Herbal medicine is not only in Phyto product but it is also use in bee product and fungal.

An anti-microbial is a substance that kills and inhibits the growth of microbes like bacteria, fungi, protozoan etc. Anti-microbial treatments either kill microbes (micro-biocidal) or inhibit the growth of microorganisms (micro-biostatic). Several parts of plant beneficial in curing a large range of health-related issue. The secondary metabolites are synthesis by a plant that is important for medicine. Clinical value of various synthetic antibiotics is rise questioned nowadays of multidrug resistance o f pathogens because failures in chemotherapeutics and antibiotics shown by pathogenic microbial infection have led to the screening of numerous medicinal plants for effective microbial activity.

The plant has rich source of natural anti-microbial agent. Traditional healers claim that some medicinal plants have more effective treatment in infectious diseases in comparison to synthetic antibiotics (Mathur, *et al*, 2011). Plant origin of Biomolecules appear to be one of the most alternatives control in these antibiotic resistant human pathogens (Raghavendra, *et al*, 2006). Different extracts from traditional medicinal plants have been tested. Various reports showed the success of traditional herbs against microorganisms, as a result, plants are one of the

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foundations for modern medicine to achieve new principles (Evans, *et al*, 2002). Until natural products have been permitted as new antibacterial drugs, there is an urgent need to identify new substances active towards highly resistant pathogens (Recio, 1989; Cragg, *et al*, 1997). The medicinal plants are mostly used because of its easy availability and cost effectiveness. The active principles of various medicines found in plants are secondary metabolites. The anti-microbial actions of plant extracts may exist in a number of different components, including aldehyde and phenolic compounds (Lai and Roy, 2004). Again, Scalbert analysis exposed that tannins can be toxic to filamentous fungi, yeasts, and bacteria. Tannins determined to bind with cell walls of ruminal bacteria, that is control growth and protease activity (Scalbert, 1991). Alkaloid and its byproducts have actions against *Staphylococcus aureus* and methicillin-resistant S. aureus (Valsaraj, *et al*, 1997).

The Coriander leaves were used an anti-microbial analysis. Food preservative is from an ancient time & people have been using high salted, underground storage, smoking, under water, high molasses system acid, alcohol, and so on to increase shelf life of food. The industrial development chemical preservative is mostly used in the food processing industry. However, with the development of food industry along with great attention of peoples have higher demand of food safety, food processing, efficient preservation and food more secure. Coriander mostly studied & reported about its fruit in domestic as well as foreign. The essential oil is mostly extracted from its fruits and in few research reports about physiological roles of leaves and stems including antiseptic efficiency. Coriander used as a condiment and natural food preservative function also (Bashir, *et al*, 2011; Braide, *et al*, 2010).

The objective of this study was to analyses the anti-microbial function of *Ocimum sanctum, Mentha piperita*, *Murraya koenigii*, and *Coriandrum sativum.* The further work to know the minimum concentration and phytochemical analysis to check the presence of secondary metabolites.

II. MATERIALS AND METHODS

A. Sample Collection

The sample was collected from the Chirondi, Ranchi. The full-grown plant of Tulsi, Coriander, Mint and Curry leaves (*Ocimum sanctum*, *Coriandrum sativum*, *Mentha piperita* and *Murraya koenigii*,). Only healthy leaves of Tulsi, Coriander, Mint and Curry leaves was collected.

B. Solvents Used

The plant extracts are prepared by using the organic solvent. The common structure of organic solvents (at least 1 carbon and 1 hydrogen atom), low molecular weight, volatility and lipophilicity and they occur in liquid firm at room temperature. Secondary metabolites were needed for plants, that is organic in nature and organic solvents is used to dissolve secondary metabolites. On the time of extraction solvents diffuse in to the solid plant material and solubilize compound with similar polarity. The following five solvents was used for plant extraction, first three are organic solvents-Methanol, Chloroform and Hot Water (Agarry, *et al*, 2005).

C. Preparation of Plant Extract

Leave samples (Tulsi, Coriander, Mint and Curry leaves) were collected from Chirondi area. The leaves are separated from plant and washed properly. The leave samples were kept in room temperature for drying after that dry sample was grinded through mortar-pestle to make powder form. The grind samples individually mixed with different solvents in 1:10 ratio. After mixing, keep the solution in dark place for 48 hrs. After 48 hrs. the sample was filtered through filter paper in a clean and air-dried beaker. (Note-The weight was taken of the empty beaker for calculate the difference after collecting filtrate). The obtained filtrate was kept in room temperature for complete dry. The weight of beaker having the solid filtrate was then measured in order to calculate the difference. After that the DMSO (Dimethyl sulphoxide) were added and obtained filtrate. Note-Add with double amount of DMSO to filterate will give the conc. of 500 mg/ml and so on. Lastly, the solution obtained were the plant extract (Suleyman, *et al*, 2009).

D. Pathogens Used

The analysis of Antibacterial activity of Coriander, Mint, Tulsi and Curry leaves was performed against four bacterial pathogens which are given below- *Streptococcus mutans, Escher ichia coli, Staphylococcus aureus* and *Lactobacillus acidophilus.*

E. Agar Disc Diffusion method

The agar disc diffusion is best methods to analyze the anti-microbial against numerous microorganisms. The zone of inhibition measures the diameter in mm (Bauer, *et al*, 1966).

Procedure:

The Nutrient media are prepared for bacteria. The culture media and petri dish were sterilizing in autoclaved. After autoclaving, they were place in Laminar air flow. In LAF, the media were carefully poured into the Petri dish and allowed to get solid form. After that paper disc was prepared and sterilize. The leave extract was loaded into the disc and the plates was incubated at 37^0C Temperature for 24 hrs. After that analyzed the result in growth plate and measure the diameter in mm of zone of inhibition.

Antibiotic optimization test is optimizing the different antibiotic against pathogens by disc diffusion technique to get the best result of antibiotic among the tested antibiotics.

Procedure:

Nutrient media was prepared and sterilized in autoclaved. The sterilized culture media was poured into Petri dish in Laminar air

flow. After the media get solid state, the pathogenic culture was spreaded (20 µl) over the media and plates was marked with bacterial culture name. After spreading disc are made in tetrad form. After prepared disc of antibiotics are Erythromycin (E- 10 mg), Vancomycin (VA- 30 mg), Neomycin (N- 30 mg), Gentamicin (GEN- 10 mg), Tetracycline (TE- 30 mg), Amoxycillin (AMX- 10 mg), Ampicillin (AMP- 10 mg), Penicillin (10 units) placed in petri dish. After that plates are keep into the incubator for 24 hrs. The zone of inhibition is observed and measure, the maximum activity of antibiotics against pathogens. The maximum zone of inhibition marked as a good antibiotic.

F. Incubation & determination of Zone of Inhibition (ZOI)

Loading the sample, after that the plates was incubated in straight position for 24 hrs. at 37^0C for bacterial growth. After that plate are observed for measuring the zone of inhibition and calculate the value.

G. Phytochemical analysis

Phytochemical analysis used for chemical assay and find out the various phytochemicals are presence in the plant extract. Mostly phytochemical are classified as secondary metabolites of plant and that is responsible for the anti-microbial activity of the plant (Manisha *et al*, 2009; Rishikesh, *et al*, 2013).

1) Test for deoxy sugars

A volume of the 5 ml plant extract is mix with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. After that add 1 ml conc. sulphuric acid in solution. A brown ring is interface that is indicate the presence of deoxy sugar appearances of cardio glycosides.

2) Test for reducing sugars

The plant extract was treated with 5 ml of Fehling solution and place in water bath. The formation of yellow or red Colour precipitate shows the presence of reducing sugars.

3) Test for saponin

2 gm of crushed leave sample was boiled in 20 ml distilled water in water bath than filtered. The 10 ml of filterate was mixed with 5 ml distilled water and shaken strongly, an appropriate determined froth formed. In frothing 3 drops of olive oil mixed and shake it. The formation of emulsion was then observed.

4) Test for oil & fats

A small amount of plant extract was taken between 2 filter papers and press it. Oil observe by filter paper that shows the presence of oil.

5) Test for terpenoids

5 ml of plant leave extract was mixed with 2 ml chloroform and conc. Sulphuric acid was added that form a layer. A reddish brown colour of the interface was formed to indicate the presence of terpenoides.

6) Test for tannins

In test tube 0.5 gm dried leave sample was taken and add 20 ml of water and boiled after that filtered. A few drops of ferric chloride were added and observed for blue-black or brownish green color.

7) Test for phenols

A few drops of ferric chloride solution and alcohol mixed with the plant extract. A red colour or blue green shows the presence of phenols.

8) Test for amino acid & proteins

Take 1 ml plant extract, and add 2 drops of freshly prepared 0.2% ninhydrin reagent and heated. Blue colour appears that indicate the presence of proteins.

9) Test for quinines

The plant extract was taken and add few drops of sodium hydroxide mixed and shaken vigorously. A red or blue green colour appear that shows the presence of quinones.

III. RESULTS AND DISCUSSIONS

Samples collection of Plant leaves- The leave sample were collected from Chirondi, Ranchi, the full-grown plant of Tulsi, Coriander, Mint and Curry leaves (*Ocimum sanctum, Coriandrum sativum, Mentha piperita* and *Murraya koenigii*,). After select the healthy leaves of Tulsi, Coriander, Mint and Curry leaves were collected.

A. Mint Leaves B. Coriander Leaves

C. Curry Leaves D. Tulsi Leaves

Figure 1: Leaves samples A, B, C and D dry in room temperature.

Table 1: Phytochemical study of *Coriandrum sativum* (Coriander)-

S. No.	Phytochemical Test	Colour Indication	Result
1	Deoxy Sugar	Brown ring	Positive
2	Terpenoids	Reddish brown	Positive
3	Tannins	Blue black	Positive
4	Quinines	Blue green	Positive
5	Flavanoids	Yellow Colour	Positive
6	Phlobatannins	Red precipitation	Positive

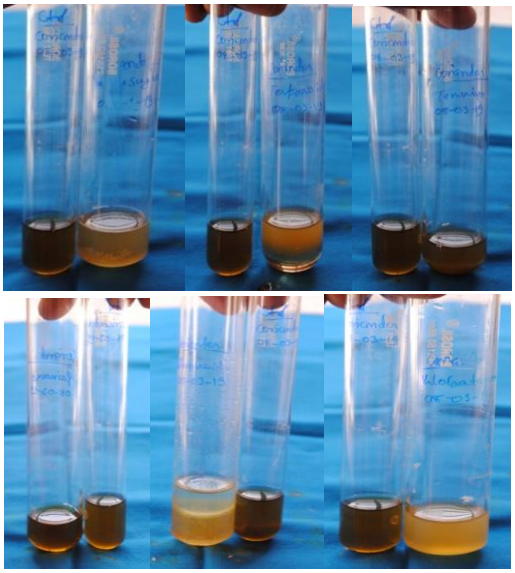

Figure 2: Phytochemical Test Result of Deoxy Sugar, Terpenoids, Tannins, Quinines, Flavanoids and Phlobatannins.

Table 2: Phytochemical analysis of *Murraya koenigii* (Curry)-

S. No.	Phytochemical Test	Colour Indication	Result
1	Deoxy Sugar	Brown ring	Positive
2	Terpenoids	Reddish brown	Positive
3	Tannins	Blue black	Positive
4	Quinines	Blue green	Positive
5	Flavanoids	Yellow Colour	Positive
6	Phlobatannins	Red precipitation	Positive

Table 3: Phytochemical analysis of *Ocimum sanctum* (Tulsi)-

S. No.	Phytochemical Test	Colour Indication	Result
1	Deoxy Sugar	Brown ring	Positive
2	Terpenoids	Reddish brown	Positive
3	Tannins	Blue black	Positive
4	Quinines	Blue green	Positive
5	Flavanoids	Yellow Colour	Positive
6	Phlobatannins	Red precipitation	Positive

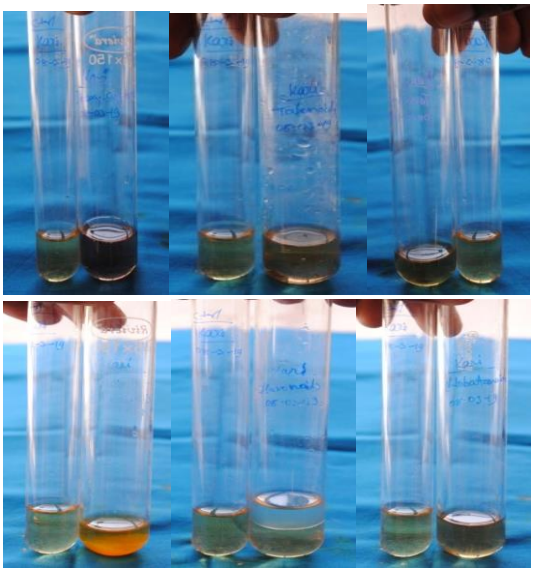

Figure 3: Phytochemical Test Result of Deoxy Sugar, Terpenoids, Tannins, Quinines, Flavanoids and Phlobatannins.

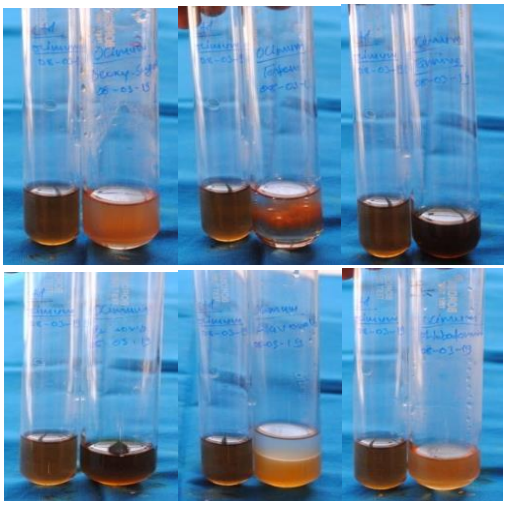

Figure 4: Phytochemical Test Result of Deoxy Sugar, Terpenoids, Tannins, Quinines, Flavanoids and Phlobatannins.

Table 4: Phytochemical analysis of *Mentha piperita* (Mint)-

S. No.	Phytochemical Test	Colour Indication	Result
1	Deoxy Sugar	Brown ring	Positive
2	Terpenoids	Reddish brown	Positive
3	Tannins	Blue black	Positive
4	Quinines	Blue green	Positive
5	Flavanoids	Yellow Colour	Positive
6	Phlobatannins	Red precipitation	Positive

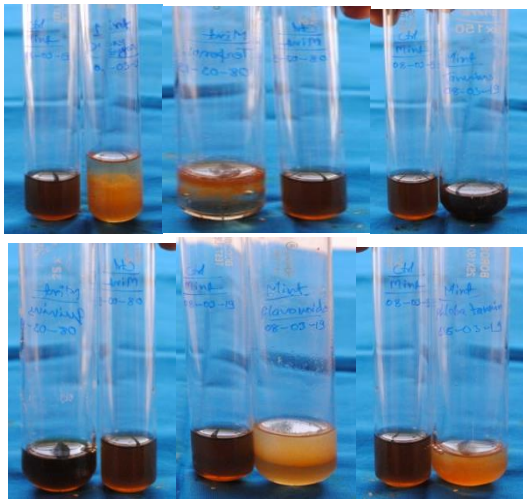

Figure 5: Phytochemical Test Result of Deoxy Sugar, Terpenoids, Tannins, Quinines, Flavanoids and Phlobatannins.

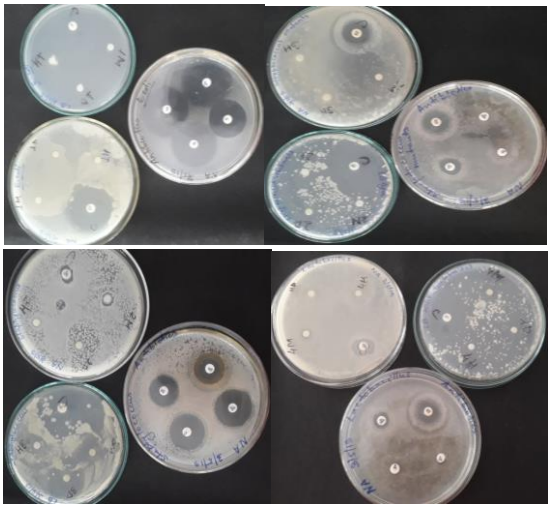

Figure 6: Show the ZOI of Antibiotics and solvents test results

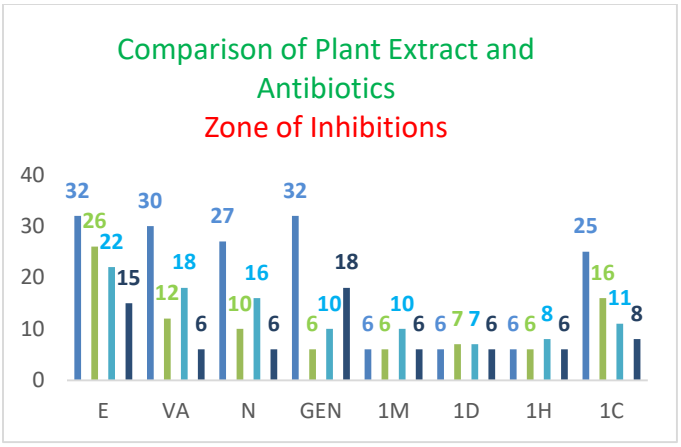

Figure 7: Graph shows comparison of Plant Extract and Antibiotics of Zone of Inhibitions

Table 5: The table showing for antibiotic sensitivity test of the zone of inhibition against pathogen bacterial strains-

S. No.	Pathogens	Zone of Inhibition in mm of Antibiotics							
		ANTIBIOTICS				LEAVES EXTRACT IN SOLVENTS (*C. sativum*-1, *M. koenigii*-3, *M. piperita*-2, *O. sanctum*-4)			
1	*E. coli*	E	VA	N	GEN	1M	1D	1H	1C
		32	30	27	32	6	6	6	25
2	*Lactobacillus acidophilus*	VA	TE	AMX	AMP	4M	4D	4H	4C
		26	12	10	6	6	7	6	16
3	*S. aureus*	VA	TE	AMX	AMP	2M	2D	2H	2C
		22	18	16	10	10	7	8	11
4	*S. mutans*	AMX	P	AMP	TE	3M	3D	3H	3C
		15	6	6	18	6	6	6	8

List of Antibiotics- Erythromycin (E- 10mg), Vancomycin (VA- 30mg), Neomycin (N- 30mg), Gentamicin (GEN- 10mg), Tetracycline (TE- 30mg), Amoxycillin (AMX- 10mg), Ampicillin (AMP- 10mg), Penicillin (10 units).

M- Methanol, D- DMSO, H- distilled water, C- Control;
1-*C. sativum*, 2- *M. piperita*, 3-*M. koenigii*, 4-*O. sanctum*

An anti-microbial agent that kills or inhibits the growth of microorganism like bacteria, fungi, protozoan's etc. The leaves of Curry, Tulsi, Mint and Coriander have anti-microbial properties. The current study was observing the anti-microbial activity of Curry leaves, Tulsi, Mint and coriander than identify the minimum inhibitory concentration and presence of secondary metabolite compounds by the phytochemical test (Cock, 2008; Zhi –Xin, *et al*, 2012; Chang, *et al*, 2011).

Staphylococcus aureus susceptibility to Gentamycin in current study relates favorably with reports issued in the Southwestern part of Nigeria (Uwazuoke JC, Aririatu, 2004). In the earlier years, CAZ has basically been prescribed for treating Staphylococcal infections because of reliable sensitivity results it obtainable in vitro. It was reported by (Ndip, *et al*, 1997) with 78% and (Amadi *et al*, 2008) who noted sensitivity of 85.4%. Meanwhile, the susceptibility of *Staphylococcus aureus* to Cloxacillin (CXC) an analyzed the study is in variance with the

recent trend. But there is no correlation with the reports of other researchers (Obiazi, *et al*, 2007) however, all observed low susceptibility response to Cloxacillin. The resistance in high-level should be associated with previous exposure of these antibiotics to bacteria which may have create resistance in other side antibiotic abuse is relatively high in this area of research. This can arise from self-medication and inadequate prescription of drugs (Akharaiyi, *et al*, 2017). Complete resistance of the *Staphylococcus aureus* isolates to CAZ was observed. This should be due to frequently use of this drug by persons and such can develop resistance to this drug overtime. However, this outcome is contrasting to the remark of (Dang-Thic *et al*, 2014) who described total susceptibility with CAZ. (Dang-Thic *et al*, 2014) stated that the present market has numerous products c ontaining ceftiofur (a third-generation cephalosporin) as such ph armacologists could be worried about the create of resistance to this antibiotic in the near future.

The leave samples were collected from Chirondi, Ranchi area than performing the antibiogram test, MIC test and Phytochemical test the overall result show the increased activity of all plants. The plant samples are used with solvents that is Methanol, DMSO and Distilled water for preparing their extracts in solvents. The highest zone of inhibitions were recorded in the case of Curry leaves (*M. koenigii*) with solvent methanol and Distilled water against *Staphylococcus aureus* 10 mm and 8 mm also with solvent DMSO against *Lactobacillus sp*. 07 mm. The Curry leaves shows the maximum zone of inhibition with solvent methanol and Distilled water against *Staphylococcus aureus* 10 mm and 08 mm (Table 5). The minimum concentration of the extract observed as MIC. This result supports the previous claim of (Deshwal and Vig, 2012). In case of *S. typhi* showed no sensitivity for all tested with plant extracts, however Gram-negative bacteria showed some degree of susceptible to other plants extracts and *E. coli* showed best susceptibility against *R. serpentine*. *P. aeruginosa* showed susceptibility against *O. tenuiflorum.* Although, it is resistant against the other plant extracts. This implied that the gram-positive bacteria were high susceptible to the plant extract than the gram-negative bacteria. It is Possible because of the presence of outer membrane that serves as an effective barrier in gram negative bacteria (Nikaido, 1999; Adesokan, *et al*, 2007).

The tested plant sample with different solvents, the minimum MIC value find and the range is from 3.47-0.57 mg/ml. The lowest MIC value show in coriander with chloroform plant extract against *S. aureus* was 0.02 at conc. 0.57 mg/ml (Gholamali J, *et al*, 2007; Chaudhry NM, *et al*, 2006).

The phytochemical test shows the presence of secondary metabolite compounds in Tulsi, Curry, Mint and Coriander leave is Deoxy sugar, Terpenoids, Tannins, Quinines, Phlobatannins and Flavanoids. In the Tulsi, Curry, Mint and Coriander leaves presence of these secondary metabolites were analyzed.

CONCLUSION

There is a long history of use of the Medicinal plants to progress health and promote oral hygiene. Plant contains phytochemicals like deoxy sugar, tannins, terpenoids, quinines, Flavanoids and Phlobatannins which have marked defensive and curative action. Only one herb can show a variety of effects like antibacterial, anti-inflammatory, antifungal activity and many more. Herbs in medical practice will prove to be a valuable help in health treatment. But there is less information about quality, safety and greater efficiency of these products for use in drugs field.

After doing all the work it was concluded that all plants *Murraya koenigii, Ocimum sanctum, Mentha piperita* and *Coriandrum sativum* have anti-microbial properties. Initially phytochemicals test reveals the presence of secondary metabolites which is responsible for its anti-microbial activity. The molecular characterization of the genes responsible for many anti-microbial activities and by screening identifying and purifying several active molecules the desired drug can be designed after process of research and evaluation. One important advantage of using *Murraya koenigii, Ocimum sanctum, Mentha piperita* and *Coriandrum sativum* for medicinal purpose is that it is mostly available and thus future drug agent will be economy. Being an herbal plant, drug found will have no side effects.

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