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Antibacterial Potential of Novel Poly-Herbal Bio-Disinfectant

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ABSTRACT

Indigenous medicinal plants like *Azadirachta Indica, Mentha, Ocimum Tenuiflorum, Phyllanthus emblica and Aloe vera* is traditionally used for treating different ailments in India. It is considered as safe medicinal plants and modulates the numerous biological processes without any adverse effect. It is a vital principle in the prevention control, and reduction of any acquired infection. We aim to present the herbal preparation for human health hazard free bio disinfectant. Indigenous medicinal plants with reported effective results against *Escherichia coli, Staphylococcus aureus, Serratia marcescens, Bacillus, Pseudomonas aeruginosa, Salmonella typhi* along with least toxic impact on environment. The antimicrobial activities by agar well diffusion indicated by zone of inhibition were resulted against *Escherichia coli* (24 mm), *Staphylococcus aureus* (10 mm), *Serratia marcescens* (11 mm), *Bacillus* (20 mm), *Pseudomonas aeruginosa* (22mm), *Salmonella typhi* (15 mm). The highest potential was observed in methanolic extract as compared to aqueous extract with even concentration. The use of medicinal plants extracts in bio disinfectant spray is mainly due to the anti-allergic, antioxidant, antibacterial, antifungal, antiviral, and anti-inflammatory properties are present. The antioxidant activity was linearly proportional to the concentration and the result showed that the antioxidant activity of all the extracts was found to increase in concentration with increased absorbance of the reaction mixture indicates increase in antioxidant activity. The formulation was evaluated for its physical parameters. It is sure that these ingredients on combination behave as an effective bio-disinfectant product. It can be used as a gel, liquid, spray on medical device and it also plays an important role in the production of various types of disinfectant products. Natural herbal bio disinfectants are affordable, effective and environmentally friendly.

Keywords- Medicinal Plants, Phytochemicals, Antimicrobial agent, Antioxidant, Bio-disinfectant.

I. INTRODUCTION

Plants have many biologically active compounds which have latent for development as medicinal agents. Herbal medicines already formed the basis of beneficial use in the developing countries, but of recent, there has been rise in the use of herbal medicines in the developed world. Plants provide an alternate approach in search for new treatment. There is a plenty of plants reputed in traditional medicine to hold protective and therapeutic properties. It is likely the plants will continue to be a valued source of new molecules which may, after possible chemical manipulation, provide new and improved drugs. Bacterial resistance to antibiotics represents a serious problem for clinicians and the therapeutic industry and great efforts are being made to reverse this trend, one of them is the widespread screening of medicinal plants form the traditional system of medicine hoping to get some newer, safer, and more effective agents that can be used

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to fight infectious diseases [1]. Hands are primary mode of transmission of microbes and infections and hygiene is therefore the most important measure to avoid the transmission of harmful germs and prevent the infections. And hand hygiene is the single most important, simplest, and least expensive means of preventing nosocomial infections. Hand contact with ready-to-eat foods represents a very important mechanism by which pathogens may enter the food supply. Food handlers whose work involves touching unwrapped foods to be consumed raw or without further cooking or other forms of treatment have been identified a particular risk group. To protect the skin from harmful microorganisms and to prevent spreading of many contagious diseases, hand washing is absolutely an important precaution. Hand washing removes visible dirt from hands and reduce the number of harmful microorganisms such as E. coli, Salmonella can be carried by people, animal or equipment and transmitted to food [2]. Indigenous medicinal plants enriched with flavonoids, alkaloids, polyphenolic compound carry antiseptic, disinfectant and antiallergic activities. In this study we used *Azadirachta Indica*, *Mentha*, *Ocimum Tenuiflorum*, *Indian Gooseberry*, *Aloe vera* due their individual benefits [3]. All parts of Azadirachta Indica as a "Indian neem" is one of the oldest and important medicinal plant used in the many medicinal treatments like skin diseases, healthy hairs, improve liver function, detoxify the blood, anti-inflammatory, anti-diabetic, antiviral, anti-carcinogenic, immunomodulatory etc [4]. *Mentha (Mint)* are aromatic, almost exclusively perennial herbs. Botanists consider it as an astringent, antiseptic,antipyretic, antispasmodic, anti-catarrhal, antimicrobial, stimulant, and anti-aging properties [5]. *Ocimum Tenuiflorum* also called Tulsi. This plant is considered a sacred plant in Indian culture and used for holy purpose as well. Tulsi have properties like anti-ulcer, anti-oxidant, anti-inflammatory, anti-cancer, anti-diabetic, anti-stress, immunomodulatory, and neuroprotective activity. *Indian Gooseberry* (Amla) is a main herbal plant in Indian traditional medicine. That has antibacterial, antifungal, antiviral, and other pharmacological activities [6]. Aloe plant such as aloe vera is perennial succulents or xerophytes [7]. Their gel may be used as emollient and moisturizer in personal care products and inflammatory skin disorder like skin irritation. Because of these penetrating properties of all medicinal plants they can be used for preparing bio disinfectants. It is free from toxins and poisons that are hazardous to human health compared to other commercial bio disinfectant available today.

Disinfectants are agents that disinfect by destroying or neutralizing the increase of disease carrying microorganisms. To disinfect is to purify in order to demolish or prevent the development of sickness conveying microorganisms. Disinfectants are products that are used on hard surfaces and objects to destroy or permanently inactivate fungi and bacteria [8]. With the entire world facing a pandemic of this proportion, we have taken a call back to our ways of hygiene passed down by our generations. It is important, now more than ever, to implement tried and tested methods to ensure that people around us maintain clean and healthy habits. But with every chemical product i.e. consumed over in the house, it is imperative to ensure safety standards. In the long term, harsh chemical disinfectants come with a range of side effects, including corrosion and skin irritation. Not to mention how hazardous they are to the environment. Polyherbal or Natural disinfectants are changing the method. That people use to disinfect their homes and offices, as they are designed to eliminate pathogens around us, without the unpleasant side-effects that the chemical disinfectants cause. We have an abundance of resources from nature that work wonders in keeping your home germ-free and spotless. In addition to being safe and friendly for the environment, our polyherbal or Natural disinfectants are safer for your home too. They are perfect for long term usage, as they have no adverse effect on your health [9]. The best natural disinfectants from polyherbal ensure. Children and pets are protected and wouldn't have to worry about toxic fumes that chemical products bring. Natural disinfectants are pocket-friendly and help you in adopting a better lifestyle i.e. both healthy and natural. While using regular home cleaning products and making it spick and span the way you know it, you actually end up eliminating all the good microbes that essentially help us our eco-system. We need good microbes to thrive, they help us develop immunity. Natural disinfectants, just like their chemical counterpart, kill the viruses and bacteria on various surfaces in your home and office. But the difference is that natural disinfectants don't harm you in any way. Not all bacteria are dangerous to our bodies; many help us stay healthy. Natural disinfectants don't kill the good bacteria, only the bad ones. They get rid of the disease-causing viruses and bacteria and keep you safe and healthy in this virus infected world. Ingredients of natural disinfectant come from medicinal plants that give us pleasant smell and a cleaner environment. Anything coming from plants can in no way hurt the environment. We have been working to bring safer and more environmentally sustainable natural disinfectants to market [10].

II. METHODOLOGY

Collection of samples:

The *Azadirachta Indica, Mentha, Ocimum Tenuiflorum, Indian Gooseberry, Aloe Vera* were collected from Dr. BAMU university of Marathwada, Aurangabad. All other reagent and chemicals used as analytical grade. Collected samples were dried naturally with the help of sun drying. After sun drying, the plant materials were made into powder with the help of grinder.

Extraction of samples:

Then 5 gm of each plant materials were weighed. About 20 gm of powdered plant materials to be extracted separately in 200ml of methanol/water using soxhelet

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extractor for 6hrs/sample or until the solvent became colourless. Temperature maintain till the boiling point of water and methanol i.e. 100°C & 64.7°C respectively.

Qualitative phytochemicals analysis:

A qualitative phytochemicals tests done for the presence of secondary metabolites; alkaloids, tannins, saponins, flavonoids, glycosides and phenols. And predominates over the others but also is helpful in searching for bioactive agents those can be used in the production of bio disinfectants [11, 12]. Preliminary qualitative phytochemicals screening was carried out following standard protocols.

Test for Alkaloids:

A. Mayer's reagent: 2 ml of plant extract was taken in a test tube and 2-3 drops of Mayer's reagent added on it. Presence of alkaloid was indicated by the appearance of green color precipitate in the solution. **B**. Wagner's reagent: When few drops of Wagner's reagent added in test tube containing 2 ml of extract, the appearance of brick color precipitate indicated the presence of alkaloids.

Test for Flavonoids:

A. Alkaline reagent test: 2 ml of plant extract was taken in a test tube and 2 ml of sodium hydroxide (2% w/v) solution was also added on it. An intense yellow color appeared in the test tube. On addition of few drop of dilute hydrochloric acid, it was colorless which indicated the presence of flavonoids.

B. Shinoda Test: 2 ml of plant extract was taken in a test tube. 5 drops of Hydrochloric acid and 0.5 gm of magnesium powder was added on it. Pink color was observed in the solution containing flavonoids.

Test for Saponins: Foam test: The extract solution was diluted with distilled water and taken in a test tube. There was a suspension formed for minutes. 2 cm layer of foam indicated the presence of saponins.

Test for Terpenoids:

Crude extract was dissolved in 2ml of chloroform and was evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added; a reddish-brown coloration at the interface indicates the presence of terpenoids.

Test for Glycosides:

A. Salkowski's test: Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown color indicates the presence of glycosides. **B.** Keller-Kilani test: Crude extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl3. The mixture was poured into another test tube containing 2 ml of Concentrated H_2SO_4. A brown ring at the interface indicates the presence of glycosides.

Test for Polyphenols and Tannins:

Crude extract was mixed with 2 ml of 2% solution of FeCl3. A blue green or blue-black coloration indicated the presence of polyphenols and tannins.

Test for Steroids: A) Crude extract was mixed with 2 ml of chloroform and concentrated H2SO4 was added sidewise. A red color produced in the lower chloroform layer indicates the presence of steroids. **B)** Crude extract was mixed with 2 ml of chloroform. Then 2 ml of each of concentrated H2SO4 and acetic acid was poured into the mixture. A greenish coloration indicates the presence of steroids.

Test for carbohydrates:

A. Fehling's Test: Equal amount of Fehling A and Fehling B reagent were mixed and 1ml of crude extract added on it. Gently tube was placed in boiling water bath for 10 minutes. Appearance of brick red precipitate indicated the presence of reducing sugars.

B. Benedict's test: 1ml of crude extract mixed with 1ml of Benedict's reagent and placed in boiling water bath for 10 minutes, a reddish brown precipitate indicated the presence of carbohydrates.

C. Molisch's Test: 1ml of Molisch's reagent was added to 1ml of crude extract and the mixture was shaken properly. After that 1ml of conc. H2SO4 was poured carefully alongside of the test tube. Appearance of a violet ring at the interface indicated the presence of carbohydrate.

D. Iodine Test: 1ml of Iodine solution was mixed with 1ml of crude extract. A dark blue or purple coloration indicated the presence of carbohydrate.

Test for Proteins: Biuret's Test: 1ml of Biuret reagent was added to 1ml of crude extract. The mixture was shaken well and incubated at 37°C for 10 minutes. Appearance of red or violet color indicated the presence of proteins.

Million's Test: 1 ml of crude extract was mixed with 1ml of Million's reagent, if precipitate appeared which turned red on gentle heating confirmed the presence of proteins.

Ninhydrin Test: 1 ml of crude extract was mixed with 1ml of 0.2 % solution of Ninhydrin and boiled for few second, if violet color appeared indicating the presence of amino acids and proteins.

Antimicrobial Activity of plants extracts (Well diffusion method):

The screening of antimicrobial activity of plant extract against pathogens was performed by using well diffusion method. The test organisms were inoculated in nutrient broth and incubated overnight at 37°C to adjust the turbidity. Sterile petri plates were taken for testing the antimicrobial activity of plant extracts against test microorganisms i.e., *Escherichia coli, Staphylococcus aureus, Serratia marcescens, Bacillus, Pseudomonas aeruginosa, Salmonella typhi,* etc. The sterile nutrient agar media plates were prepared and allowed to solidify. After solidification 100µl of overnight incubated test organisms were spread on plates by using glass spreader. Wells were prepared in the seeded agar plates. About 20µl of plant extracts were introduced in the wells and allow it to diffuse in it. Then the plates were incubated overnight at 37°C. The antimicrobial agents diffuse in the agar medium and inhibit the growth of the microbial strain tested therefore clear zone or zone of inhibition were observed [13], The antimicrobial spectrums of the extracts were determined for the bacterial species in terms of zone sizes around each well. The antimicrobial activity

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was measured by measuring diameter (mm) of the resulting zone of inhibition against the tested organisms. The antimicrobial activity was carried out by both individual and mixture of the plants extract.

Antioxidant Assay (Reducing power assay):

This assay is often used to protect cells form damage caused by free radicals (unstable molecules made by the process of oxidation during normal metabolism.) and used to measure antioxidant capacity of plant sample and nutritional supplements containing polyphenols. The ferric reducing powder assay is based on the ability of the antioxidant to reduce fe3+ to fe2+ in the presence of potassium ferricyanide and ferric chloride [14].

Potassium Ferric Ferricyanide + Chloride Antioxidant Potassium+ Ferrous ferrocyanide Chloride

Protocol:

Various concentration of Ascorbic acid were prepared and add in 1ml in each test tubes and labelled them as 0.1, 0.2, 0.3, up to 1 ml in one test tube stand and it referred as standard. In another test tube stand different volume of plant extracts were added and labelled them as 0.1, 0.2, 0.3, up to 1ml and mark them as Test. Blank was prepared by using 1ml of distilled water for both stand. 2.5 ml of Phosphate buffer (pH-6.6) and 2.5 ml Potassium ferricyanide (1%) were added in each test tube and boiled for 20 minute at 50 ^{0}C. To that mixture 2.5ml of TCA was added and centrifuged for 10 minute at 2000 rpm. The supernatant was collected and 1 ml of distilled water and 0.5 ml of freshly prepared ferric chloride (0.1%) solution were added. The absorbance of solution was then measured at 700 nm. The reducing power activity was indicated by the increase in the optical density [15].

Method of preparation of Bio-Disinfectants:

Carbapol 940 was added to deionized water with constant stirring. After uniform mixing 0.1% NaOH solution was added drop wise to avoid the formation of possible air bubble in the gel, kept aside for 24hrs. The quantity of denatured alcohol and deionized water were separated in five equal quantities. In each part of denatured alcohol, Plant extract, Sorbitol, methyl paraben, Tween 80 and glycerine were uniformly mixed. To this five-alcoholic mixture, five equally separated quantities of deionized water were added and mix until the mixture was uniform. With continuous stirring, then all the solutions mixed together uniformly. After ensuring the solutions are mixed properly to one another perfume was added.

The method of preparation of bio-disinfectants were carried out for both mixture of methanolic and aqueous extracts, and also for the individual extract of medicinal plants. After preparation of bio disinfectants were filled in dispenser bottles and labelled it. Preparations of powder hand wash: The 0.8 gm sodium lauryl sulphate (SLS) was weighed and soaked in plant extract and allowed to dry it. After drying 1 gm of sorbitol and 0.1 ml of perfume were added in it. (To prepare hand wash mix the powder in 100 ml water and shake then use after proper mixing.)

III. RESULTS

The phytochemicals characteristic of both the mixture of methanolic and aqueous extract were tested. From the table, phytochemicals characteristics mixture of both the extract it could be seen that, proteins, carbohydrates, phenols and tannins, flavonoids and saponins were present in all the plants. Flavonoids were absent only aqueous extract of mixture of medicinal plants show in Table 2. The result obtained in this study that suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial.

Table 2: Qualitative Phytochemicals screening of Methanolic & Aqueous extract

Sr.No.	TEST	OBSERVATION	
		Methanolic extract	Aqueous extract
1	Alkaloids	+++	+++
2	Flavonoids	++	-
3	Saponins	++	++
4	Terpenoids	+++	+++
5	Glycosides	+++	++
6	Polyphenols & Tannins	+	+
7	Steroids	++	++
8	Carbohydrate	+	+++
9	Protein	++	++

Note: (+) = Trace amount, (++) = Moderate amount, (+++) = High amount, (-) = Absence

The screening of antimicrobial activity of both plant extract against organism was performed by using well diffusion method. (Table 3). The Antimicrobial activity of mixture of Methanolic and Aqueous extract against various organism were shown in the Table No 3. However, they vary in zone of inhibition against the tested organisms. Both the extract produced the widest zone of inhibition against Escherichia coli, Staphylococcus aureus, Salmonella typhi and Pseudomonas aeruginosa by using well diffusion method.

Antimicrobial activity of Mixture of Methanolic & Aqueous extract against Escherichia coli, Staphylococcus aureus, Salmonella typhi by using well diffusion method. The review table is not, only limited to antimicrobial activity, it contains phytocompounds isolated from methanolic and aqueous extract of plants which might be active constituent responsible for microbes inhibition.

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Table 3: The Antimicrobial activity by well diffusion method

Sr. No	Pathogenic Microorganisms	Observation	
		Methanolic extract	Aqueous extract
1	*Escherichia coli*	24mm	16mm
2	*Staphylococcus aureus*	10mm	12mm
3	*Serratia marcescens*	11mm	14mm
4	*Bacillus*	20mm	11mm
5	*Pseudomonas aeruginosa*	22mm	18mm
6	*Salmonella typhi*	15mm	17mm

The reducing power activity was indicated by the increase in the optical density. The Antioxidant assay of standard Ascorbic acid and ferric reducing ability of Ascorbic acid at various concentrations was shown in Fig.2.

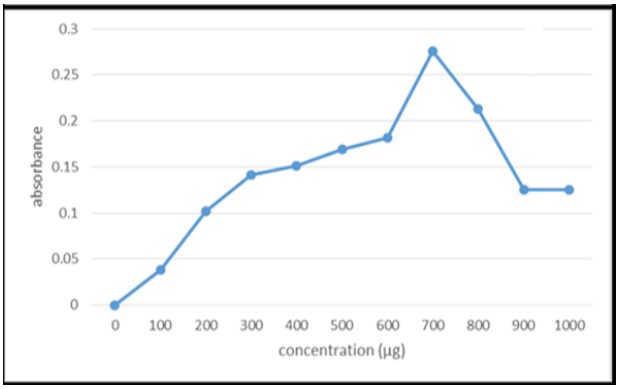

Fig.1. Antioxidant assay: Ferric reducing ability of Ascorbic acid at various concentrations

The reducing power activity was indicated by the increase in the optical density. The Antioxidant assay of mixture of Methanolic and aqueous extract were performed (Fig.2) and measuring the optical density at 700nm. The ferric reducing ability of both the extracts at various concentrations was shown in Tables 4 and 5.

Table 4: Antioxidant assay of Methanolic extract

Sr. No	Different volume of mixture of Methanolic extract (mL)	Phosphate Buffer and Potassium Ferric cyanide	Boiling water bath for 20 min at 50°C	Trichloro acetic acid	Centrifuge at 3000 rpm for 10 min	Upper layer	Distilled water	Ferric chloride	Optical density at 700nm
1	Blank	2.5 mL in each test tubes		2.5 mL in each test tubes		2.5 mL in each test tubes	2.5 mL in each test tubes	0.5 mL in each test tubes	0
2	0.1								0.2132
3	0.2								0.3877
4	0.3								0.6745
5	0.4								1.0149
6	0.5								1.2256
7	0.6								1.4055
8	0.7								1.7806
9	0.8								2.1743
10	0.9								2.1356
11	1								2.0606

Table 5: Antioxidant assay of aqueous extract

Sr. No.	Different volume of mixture of Aqueous	Phosphate Buffer and Potassium Ferric cyanide	Boiling water bath for 20 min at 50°C	Trichloro acetic acid	Centrifuge at 3000rpm for 10 min	Upper layer	Distilled water	Ferric chloride	Optical density at 700 nm

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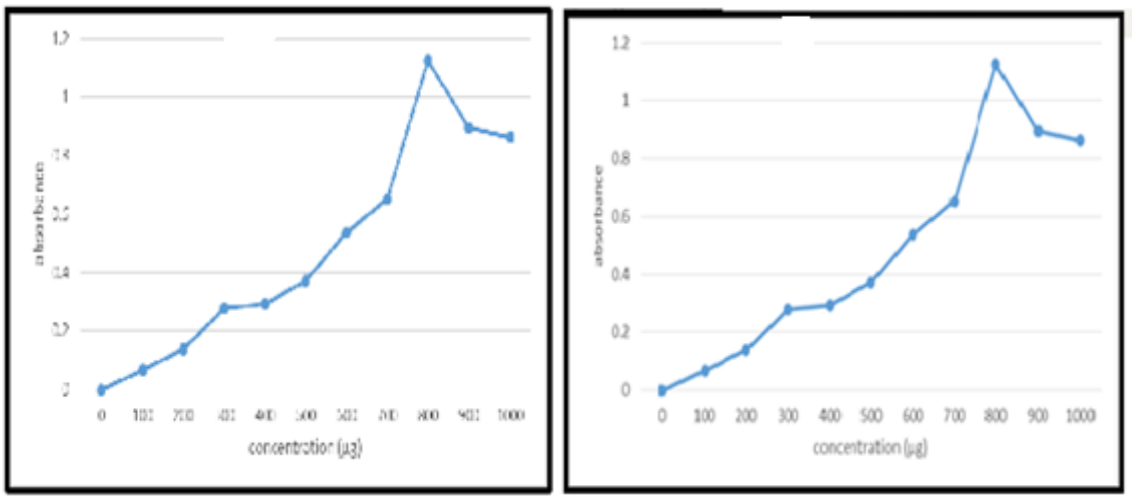
	extract (mL)								
1	Blank	2.5 mL in each test tubes		2.5 mL in each test tubes		2.5 mL in each test tubes	2.5 mL in each test tubes	0.5 mL in each test tubes	0
2	0.1								0.0671
3	0.2								0.1396
4	0.3								0.2776
5	0.4								0.2926
6	0.5								0.3716
7	0.6								0.5356
8	0.7								0.6523
9	0.8								1.1251
10	0.9								0.8936
11	1								0.8637

The Antioxidant assay of poly herbal compounds revealed the presence of anti-oxidant potential. The reducing ability was linearly proportional to the concentration and the result showed that the antioxidant activity of all the extracts was found to increase in concentration with increased absorbance of the reaction mixture indicates increase in antioxidant activity.

The ferric reducing ability of both the extracts increased with increase in concentration and obtained results were compared with standard ascorbic acid. The Antioxidant assay of Methanolic and aqueous extract at various concentrations were shown in Fig.2.



a b

Fig.2. Antioxidant assay of mixture sample, a. Ferric reducing ability of Methanolic extract at different concentrations. b. Ferric reducing ability of aqueous extract at different concentrations

a b c d e

Fig.3. Formulated Bio disinfectant products: a – Methanolic extract Hand sanitizer; b – Aqueous extract Hand sanitizer; c –Hand sanitizer in liquid and gel; d – Formulated hand wash; e – Formulated Powder hand wash

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The formulated Bio disinfectants like A) Methanolic extract Hand sanitizer, B) Aqueous extract Hand sanitizer C) Hand sanitizer in liquid and gel, D) Formulated hand wash E) Formulated Powder hand wash (Fig.3). The physiochemical parameter of these formulated Bio disinfectants is determining such as color, odour, pH, appearance were tested. The formulation exhibited good appearance characteristics as well as the pH was found in the range of 6 -6.4 which is the desired pH. It is safe, effective, beneficial, affordable and overall chemical free way of stopping transmission of diseases. The methanolic and aqueous based Bio disinfectant containing Azadirachta Indica, Mentha, Ocimum Tenuiflorum, Indian Gooseberry and Aloe vera which shows various antibacterial, antifungal, and antiseptic activities which helps in treating bacterial diseases and remove pathogens to matintain sanitization, the results are tabulated in (Table 6).

Table 6: Physicochemical parameters of bio disinfectants

Sr. No	Parameters	Methanolic Extract		Aqueous Extract		Individual Extracts	
		Gel	Liquid	Gel	Liquid	Gel	Liquid
1	Color	Light green	Light green	Golden yellow`	light yellow	Yellow	Pale Yellow
2	Odour	characteristics	Aromatic	Characteristics	Aromatic	Characteristic	Aromatic
3	pH	6.4	6.3	6.2	6.2	6	6

IV. DISCUSSION

The prepared plants extracts are tested for its quality and efficacy. Hence, evaluation tests like physicochemical properties were carried out. The plants extract showed excellent effect against various microorganisms. The formulations of bio disinfectants are good in appearance, homogeneity and smooth texture in both liquid and gel consistency. *Aloe Vera* and *Mentha* used in the respective formulations shows better results. Whereas, *Azadirachta Indica, Ocimum Tenuiflorum and Indian Gooseberry* used in bio disinfectants shows excellent germicidal activity against various microorganisms. The herbals used in formulations are widely used for medicinal purpose. These herbals are also rich in various compounds. The main idea behind combining two or more herbals to get additive effects of a active constituents of different plants [16]. Hand hygiene is a simple and least expensive means of preventing hospital acquired infections specially derived from environmental surfaces [17]. The people use to disinfect their homes and offices as they are designed to eliminate to pathogens around us, without unpleasant side effects that the chemical disinfectants cause. Natural remedies are more acceptable in the belief as they are safer with fewer side effects than the synthetic ones. Herbal formulations have emergent demand in the global market.

V. CONCLUSION

The gel/liquid formulation is good in appearance and homogeneity. Thus the treatment is simple, cost effective, eco-friendly, reachable for all and the components present in the bio disinfectants have no side effect to human compared to chemical treatment. It is concluded that the bio disinfectants have a significant antimicrobial effect on the specified microorganisms. Thus, there is a immense potential in establishing the use of antimicrobial herbal products as a measure to control the multidrug resistance microbes as well as check their spread though hands form one geographical region to another.

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