

Evaluation of antibacterial activity of herbal hand sanitizer gel from leaves extract of *Eclipta alba linn.*, plants

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ABSTRACT

Objective: The objectives of this study were to determine the minimum inhibitory concentration (MIC) of *Eclipta alba linn.*, leaves extract against Gram positive and Gram negative bacteria like *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus*. The hand sanitizer gels containing *Eclipta alba linn.*, leaves extract was formulated and evaluated to determine the effectiveness of the gels against bacteria.

Methods: The gel base was optimized by preparing ten formulations containing carbopol 940 and *Eclipta alba linn.*, leaves extract at different concentrations ranging from 100mg to 1000mg. The physical stability of hand sanitizer gels containing *Eclipta alba linn.*, leaves extract was measured at $4\pm 2^{\circ}\text{C}$, $25\pm 2^{\circ}\text{C}$, and $30\pm 2^{\circ}\text{C}$ for 12 weeks.

Results: The prepared poly herbal formulations exhibited good antimicrobial effect. The best hand sanitizer gel base containing carbopol-940 and glycerine was maintained at a pH of 6.50. The hand sanitizer gel formulations 6 and 8 had good stability for 12 weeks. Formulation 8 was found to decrease the number of bacteria at a good level, as a result formulation 8 was preferred to other formulations in the ethical studies.

Conclusion: Formulation 8 gel with 800mg concentration *Eclipta alba linn.*, leaves extract was more effective than the other formulation.

Keywords: Antibacterial, Hand sanitizer gel, Minimum inhibitory concentration, Physical stability, *Eclipta alba linn.*, leaves extract.

Introduction

The world was facing the outbreak of communicable diseases such as H1N1 Swine Flu, Bird Flu, Small pox, Measles, Ebola virus, Marburg, Hantaviruses, and the recent one Covid 19 corona virus. In such pandemic situation it is very necessary for everyone to utilize hand sanitizers to maintain health and hygiene and to overcome these diseases. Hence, the necessity of the hand hygiene products such as liquid hand wash and hand sanitizers emerged in the modern world. In global market there are so many hygienic products available like liquid hand wash, sanitizer spray etc. In global market Hand sanitizer sales has been expected to grow from \$919 million in 2016 to \$ 1,755millions in 2023. The products available in the market were classified into gel, foam, sprays and others. Gel hand sanitizers accounted maximum share in the market for the year 2016(Hand sanitiser market, size, share &industry analysis 2022)

The liquid hand wash removes the dirt and micro organisms from the hands, thus performing the dual action of cleaning and sanitizing. The sanitizers are available in both herbal and non- herbal formulations with varying colors and flavors and were majorly used by the people of institutional and residential segments. In preparation of Hand sanitizer, people can use alcoholic or non alcoholic based formulations (Kampf, G et al., 2004). Common name of *Eclipta alba linn.*, is Bhringraj. It is an annual herbaceous plant, commonly known as king of hairs. It is an erect plant, branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile and lanceolate belonging to family Asteracea (Prasad KV et al., 2012).The leaves has chemical constituent Wedelolactone which acts as an therapeutic agents in treatment of diseases like Antihepatotoxic (Wanger H et al., 1986), Antibacterial(Manoj Kumar Pandey et al., 2011) Jaundice etc(Upadhyay RK et al., 2001). Dried aerial parts of *Eclipta alba linn.*, were collected from Sir C R R College of pharmaceutical sciences medicinal garden and authenticated by Dr Prasad (Department of botany, Sir C R Reddy autonomous College, Eluru) where the voucher specimens were deposited.

The treatment of particular area in skin disorders can be enhanced by topical route, as it enhances the drug delivery systemically. In topical administration importance is given to single phase gel systems which are absorbed by passive diffusion, ionic or electro chemical diffusion methods. In the present study, we evaluated the preliminary phytochemical potential of herbal sanitizer gel formulations of different aqueous extract concentrations of *Eclipta alba linn.*, The antimicrobial activity was tested in microbiology lab of Sir C R Reddy College of pharmaceutical sciences, Andhra Pradesh, India under aseptic conditions for treatment of manifestation caused by several pathogenic bacteria. It was essential to find out new sources of antimicrobial drugs which can be procured easily without having any side effects and which have high economic potential.

MATERIALS AND METHODS

2.1 Collection and authentication of Plant Materials

The plant specimen for the proposed study *Eclipta alba linn.*, was collected from the Sir C R REDDY College of pharmaceutical sciences and other irrigated fields in and around west godavari District, andhra pradesh, India. The herbarium of these plants was identified and authenticated by Dr. prasad, Professor, Department of Botany, Sir C R REDDY autonomous College, Eluru, Andhra Pradesh.

2.2 Preparation of plant extracts

The fresh whole plant of *Eclipta alba linn.*, leaves were washed with distilled water to removed unwanted foreign materials like soil and dusts. After, washed plant material was dried under shade at room temperature without direct exposure of sunrays. It was then coarsely grounded by using mechanical device. The powdered plant material was passed through sieve no 40 and stored in an airtight container for further use.

The coarsely powdered plant materials of *Eclipta alba linn.*, (2000 g) were extracted separately to exhaustion in a soxhlet apparatus for 72 hours by using water and methanol (95%) solvent (Merk & Spectrum Chemicals, India) systems. All the extracts were filtered through a cotton plug followed by whatman filter paper (No.1) and then concentrated by using a rotary evaporator at low temperature (45 -

preserved in airtight containers and kept at 6°C until further use.

2.3. Chemicals and Reagents

Carbopol 940 is collected from Himedia laboratories Pvt. Ltd. Secunderabad, sodium lauryl sulphate, methyl paraben and glycerin were taken from SDFCL Laboratories, Mumbai. All other reagents / chemicals used were analytical grade.

2.4 Microbial strains used

Antibacterial effect of *Eclipta alba linn.*, was determined against 2 gram negative bacteria viz., *Proteus vulgaris* NCIM 2813, and *Pseudomonas aeruginosa* NCIM 2200 and 2 gram positive bacteria viz., *Bacillus cereus* NCIM 2458, and *Staphylococcus aureus* NCIM 2602.

Methods

Phyto-chemical analysis

The crude aqueous extract of leaf of *Eclipta alba linn.*, plants was subjected to preliminary qualitative phytochemical investigation. The various test such as alkaloids (Dragendorff's reagent), proteins (Biuret test), carbohydrates (Molisch's test), flavonoids (Lead acetate test), glycosides, amino acids (Ninhydrin test), phenolic compounds (FeCl₃ solution), saponins (Foam test) were conducted to determine the presence or absence of the phyto constituents on the basis of colour changes (Tanu Sharma et al., 2017, Sabri et al., 2012) (Table no. 1).

Standardization of inoculums

The inoculums prepared from the stock cultures, were maintained on nutrient agar at 4°C and sub cultured onto Nutrient broth using a sterile wire loop (Majumdar S.H et al., 2015).

Antimicrobial Studies of herbal extract and herbal extract hand sanitiser

The screening of anti-microbial efficacy of the herbal extract hand sanitiser was performed on various micro organisms by using dip well method as per standard procedure. 5 petri plates were taken for testing the antimicrobial activity of herbal extract and 10 petri plates were taken for testing the antimicrobial activity of herbal extract hand sanitiser against four different microorganisms i.e. *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus*. The plates were filled with

Muller Hinton agar solution 25ml and allowed for solidification. After solidification the microorganisms from the subculture were inoculated into the nutrient agar media and 15 discs were inoculated with different concentrations of herbal extracts and herbal extract hand sanitiser formulation respectively (Ningsih, D et al., 2014). The plates were incubated at 37°C for overnight. After 24 hours of incubation, the plates were observed for the zone of inhibition. From the zone of inhibition the anti microbial activity of formulation is estimated as shown in the Table no. 4, 5, below.

Determination of Minimum Inhibitory Concentration (MIC) of the extracts

The MIC is defined as the lowest concentration that completely inhibits the growth of microorganisms for 24 hrs incubation. Determination of minimum inhibitory concentration of extracts was determined by preparing different concentrations of extracts 200µg, 250µg, 300µg, 500µg and 700µg were added respectively to the nutrient broth (Table 4). A 50µl volume of each dilution was added aseptically into the wells of Mueller Hinton agar plates that were already seeded with the standardized inoculums of the test bacteria. All experiments were performed in triplicate (Suhad Faisal Hatem, et al, 2015). The agar plates were incubated at 37°C for 24 hours. The lowest concentration of extracts showing a clear zone of inhibition was considered as the MIC.

Statistical analysis

Values are given in the mean \pm SD, and the differences between values were determined by the student's t-test. Values of $P < 0.01$ were considered significant.

Formulation of Herbal Hand Sanitizer Gel

Formulation of Herbal Hand wash Gel Form literature review, Carbopol 940 was found to be excellent gelling agent for the preparation of hand wash gels. Procedure Various hand wash gel formulations were prepared (table no: 1) using carbopol - 940 as gelling agents. The desired concentration of gelling agent, sodium lauryl sulphate, glycerin were measured accurately and dispersed in purified water with moderate stirring (Mounika A et al., 2017). The required quantity of methyl paraben was dissolved in remaining quantity of purified water by gentle heating. Desire quantity of eclipta alba linn., plant leaf extract added. Triethanolamine was used to adjust the pH. The formulated hand wash gel was filled in collapsible tubes and stored at cool and dry place until further evaluation.

EVALUATION OF HERBAL HANDSANITISER

Physical Evaluation

Physical evaluation (color, dour) was done by sensory and visual inspection(Abhishek Shound et al., 2016)

Grittiness

1ml of gel was taken on finger tips and rubbed between two fingertips then the formulation was evaluated(Palash Mandal, et al., 2017)

pH

One gram of sample of poly herbal hand wash gel was taken and dissolved it into 100ml distilled water. The pH of solution was measured by previously standardized digital pH meter(Rathi P. B et al., 2015).

Viscosity

The viscosity of hand wash gel was determined by using digital Brook filed viscometer(S -62, MODEL DV2TLVTJO) at 30⁰C with a spindle speed of the viscometer rotated at 60 , 80 and 100 rpm. The assembly was connected to a thermostatically controlled circulating water bath maintained at 30⁰C. Measured quantity of hand wash gel was taken into a beaker and the tip of viscometer was immersed into the hand wash gel and viscosity was measured in triplicate(Singh Deep Hussan, et al., 2012)

Spread ability

A sample of 0.5 g of each formula was pressed between two slides and left for about 5 minutes where no more spreading was expected Diameters of spreaded circles were measured in cm and were taken as comparative values for spreadability. The results obtained are average of three determinations.

Foam Height

One gram of sample of hand sanitiser gel was taken and dispersed in 50ml distilled water. Dispersion was transferred to 500ml measuring cylinder. Volume was made up to 100ml with water. 25 strokes were given and kept it aside. The foam height above the aqueous volume was noted (Tarun Kumar Guleri, L. et al., 2022)

al., 2013).

Foam Retention

25ml of the 1% hand sanitiser gel was taken into 100ml graduated cylinder. The cylinder was covered with hand and shaken 10 times. The volume of foam at 1 minute interval was recorded for 4 minutes.

Determination of percentage free alkali:

About 5 gm of sample was taken in a conical flask and added to it into 50 ml of neutralized alcohol. It was boiled under reflux on a water bath for 30 minutes, cooled and 1 ml of phenolphthalein solution was added. It was then titrated immediately with 0.1N HCL.

Alcohol Insoluble Matter:

5 gm of sample was taken in a conical flask. Added it to 50 ml of warm ethanol and shaken vigorously to dissolved The solution was filtered through a tarred filter paper with 20 ml warm ethanol and dried it at 105°C for 1 hour. The weight of dried paper was taken(Zeeshan Asfisar et al., 2016)

Formula

$$\% \text{ alcohol insoluble matter} = \text{Wt. of residue} \times 100 / \text{Wt. of sample}$$

Temperature Stability:

The stability studies were carried out for all the gel formulation by freeze - thaw cycling. Here, by subjecting the product to a temperature of 4°C for 1st the month, the at 25°C for 2nd month and then at 40°C for 3rd month and studied for appearance, pH, viscosity and spreadability(Dalia abd el-rhman et al., 2012)

RESULTS AND DISCUSSIONS

Antimicrobial activity was done for leaves aqueous extract of *Eclipta alba linn.*, due to presence of phenols by doing different chemical tests. During antimicrobial study the leaves aqueous extract showed concentration dependent increase in zone of inhibition against Gram positive, Gram negative bacteria by

cup plate method. The results of antibacterial are shown in Table 4. The antibacterial activity of the aqueous extract was found to be good ($35 \pm 1.2\text{mm}$). Among the gram negative bacteria tested, *Proteus vulgaris* showed highest zone of inhibition ($35 \pm 1.2\text{ mm}$) in the aqueous extract at the dose of $700\text{ }\mu\text{g}$ per disc respectively whereas *Pseudomonas aeruginosa* showed the lowest zone of inhibition ($26 \pm 1.8\text{ mm}$) at the dose of $300\text{ }\mu\text{g}$ per disc respectively. Among the 2 tested gram positive bacteria, *Staphylococcus aureus* exhibited the highest zone of inhibition ($32 \pm 1.6\text{mm}$) which was followed by *Bacillus cereus* ($15 \pm 1.5\text{mm}$). With reference to the chemical constituents of *Eclipta alba linn.*, such as alkaloids, Flavanoids, phenols, terpenoids and the results of the present evaluation, it suggests that antimicrobial potential may be due to phenols, flavanoids and alkaloids. The present results are in accordance with the findings of the researcher who found phenols, alkaloid and flavanoid crude extracts of *Eclipta alba linn.*, was found active against *Proteus vulgaris* and *Staphylococcus aureus*.

Preparation and evaluation of physicochemical parameters of formulations

The physicochemical parameters of the prepared hand sanitizer were determined. Parameters such as color, odour, appearance, pH were tested. The formulations exhibited good appearance characteristics as well as the pH was found in the range of 6.51 to 6.81 which is the desired pH. Other parameters such as percentage free alkalie, foam height, foam retention, alcohol insoluble matter and high temperature stability were determined; The results are tabulated in Table 3.

Antimicrobial screening of the prepared formulations

The extracts of different concentrations that exhibited maximum antimicrobial activity were leaves aqueous extracts of *Eclipta alba linn.*, with different cultures of gram positive and gram negative zones of inhibition ranging from $35 \pm 1.2\text{mm}$ to $15 \pm 1.5\text{mm}$. Hence by using these extracts, different concentrations of hand sanitiser formulations was done in table no. 1. The antibacterial activity of the hand sanitiser F_4 , F_6 , F_8 and F_{10} was found to be good. Among the gram negative bacteria tested, *Proteus vulgaris* showed zone of inhibition $39 \pm 2.0\text{mm}$ in the F_8 , $36 \pm 2.0\text{mm}$ in the F_{10} , $31 \pm 2.0\text{mm}$ in the F_4 whereas *Pseudomonas aeruginosa* showed zone of inhibition $37 \pm 1.9\text{mm}$ in the F_9 , $36 \pm 2.0\text{mm}$ in the F_7 , $21 \pm 1.8\text{mm}$ in the F_1 and Among the gram positive bacteria, *Bacillus cereus* showed the zone of inhibition $38 \pm 1.9\text{mm}$ in the F_8 , $36 \pm 2.09\text{mm}$ in the F_6 , $19 \pm 1.2\text{mm}$ in the F_1 , *Staphylococcus aureus*

exhibited the highest zone of inhibition 32 ± 1.6 mm in the F₈, 30 ± 2.0 mm in the F₃, 23 ± 1.2 mm in the F₁. The ten different concentrations of formulations exhibited good zones of inhibition ranging from 39 ± 2.0 to 19 ± 1.2 mm. The results are tabulated in **Table 5**.

Conclusions

The plant leaves of *Eclipta alba linn.*, were extracted using water as solvent were subjected to antimicrobial screening. Results revealed that most of the highest concentration extracts exhibited good antimicrobial effect. This is in accordance with the antimicrobial activities of this plant listed in the literature. Furthermore these extracts with different concentration exhibiting maximum activity were selected and were included in our prepared hand sanitizer formulations. The prepared formulations when tested for antimicrobial activity exhibited zones of inhibition. Furthermore the prepared hand sanitizer formulations were standardized by evaluating various physico chemical properties such as pH, spreadability, appearance, extrudability, high temperature stability, in which they exhibited satisfactory characters. Thus, it is good in this pandemic situation of covid found to provide safe and healthier living through germ-free hands. Although the removal is not 100% but a major number can be reduced.

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Table No: 1 Phytochemical analysis of an aqueous extract of *Eclipta alba*

S.no.	Phytochemicals	Test	Result
1.	Carbohydrate	Fehling's test	-
2.	Phenols	Fecl3 test	+
3.	Flavonoids	NH3 test	+
4.	Alkaloids	Wagner's test	-
5.	Steroids	Salkowski's test	-
6.	Tannins	Lead acetate test	+
7.	Saponins	Frothing test	-
8.	Glycosides	Nitroprusside test	-
9.	Quinones	-	+
10.	Amino acids	Ninhydrin test	+
11.	Coumarin	UV light test	+

Table No: 2 Formulation of herbal hand wash gel with carbopol - 940 as gelling agent

Composition (%w/v)	Formulation code									
	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	F ₇	F ₈	F ₉	F ₁₀
Eclipta alba linn.,Aq.extract	100mg	200mg	300mg	400mg	500mg	600mg	700mg	800mg	900mg	1000mg
Carbopol-940	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Glycerin	1	1	1	1	1	1	1	1	1	1
Sodium lauryl sulphate	1	1	1	1	1	1	1	1	1	1
Methyl Paraben	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Purified water up to(in ml)	100	100	100	100	100	100	100	100	100	100

Formulation Code Color	Appearance and homogeneity	Grittiness	Colour	pH	Foam Height (in ml)	Foam Retention (in ml)	Spread ability (cm)	Viscosity			% free alkali	Alcohol insoluble matter
								60 (RPM)	80 (RPM)	100 (RPM)		
F ₁	Translucent	Non gritty	Light brown	6.56	180	15	10	39.5	49.8	90	0.12	4.6
F ₂	Translucent	Non gritty	Light yellow	6.78	175	17	11	41.6	55.3	100	0.15	4.8
F ₃	Translucent	Non gritty	Light yellow	6.61	182	11	10.5	150	160	180	0.18	4.4
F ₄	Translucent	Non gritty	Light yellow	6.79	179	19	12	190	225	250	0.12	4.5
F ₅	Translucent	Non gritty	Light yellow	6.73	184	23	11.5	237	269	292	0.16	4.2
F ₆	Translucent	Non gritty	Light brown	6.81	196	26	9	255	289	311	0.18	4.3
F ₇	Translucent	Non gritty	Light brown	6.54	193	22	8.56	300	335	368	0.14	4.6
F ₈	Translucent	Non gritty	Light brown	6.75	200	27	8.34	368	375	385	0.17	4.1
F ₉	Translucent	Non gritty	Light brown	6.51	201	21	8.25	378	382	390	0.16	5.2
F ₁₀	Translucent	Non gritty	Light brown	6.53	196	28	7.9	385	400	402	0.13	5.4

From above table F₁₀ formulation was optimized and subjected for stability studies for 3 months

Evaluation of stability studies

Evaluation parameters for Carbopol-940 as gelling agent hand wash gel

Months	Formulation Code Color	colour	Appearance and homogeneity	Grittiness	pH	Foam Retention (in ml)	Foam Height (in ml)	Spread ability (cm)	Viscosity			% free alkali	Alcohol insoluble matter
									60 (RPM)	80 (RPM)	100 (RPM)		
1 st month	F ₈	Light brown	Translucent	Non gritty	6.82	12	186	9.5	345	355	380	0.18	5.2
2 nd month	F ₈	Light brown	Translucent	Non gritty	6.76	18	195	11.2	365	375	390	0.13	5.6
3 rd month	F ₈	Light brown	Translucent	Non gritty	6.78	22	172	12.4	380	397	401	0.19	5.8

Table 4: Zones of inhibition (mm) of leaves extracts of *eclipta alba linn.*

Extract Concn of Drug ($\mu\text{g/ml}$) / Organisms	Zone of inhibition (diameter mm)			
	<i>Proteus vulgaris</i>	<i>P.aeruginosa</i>	<i>Bacillus cereus</i>	<i>Staphylococcus</i>
200	32 ± 2.0	28 ± 1.8	25 ± 1.2	24 ± 1.8
250	30 ± 1.2	27 ± 2.0	16 ± 1.7	22 ± 1.6
300	29 ± 1.7	26 ± 1.8	15 ± 1.5	23 ± 1.5
500	31 ± 2.2	27 ± 1.1	22 ± 1.7	28 ± 1.4
700	35 ± 1.2	29 ± 1.8	26 ± 1.2	32 ± 1.6
control	0	0	0	0

Table 5: Antimicrobial screening of the prepared formulations (mean \pm SD) (n=3)

Sl no	Formulation	Zones of inhibition in mm			
		<i>Proteus vulgaris</i>	<i>P.aeruginosa</i>	<i>Bacillus cereus</i>	<i>Staphylococcus</i>
01	F ₁	28 ± 2.0	21 ± 1.8	19 ± 1.2	25 ± 1.5
02	F ₂	27 ± 1.3	30 ± 2.0	26 ± 13	28 ± 1.9
03	F ₃	29 ± 1.5	28 ± 1.9	25 ± 1.7	30 ± 2.0
04	F ₄	31 ± 2.0	30 ± 2.0	27 ± 1.2	32 ± 1.2
05	F ₅	25 ± 1.6	34 ± 2.3	30 ± 1.6	28 ± 1.7
06	F ₆	30 ± 2.0	32 ± 1.2	36 ± 2.0	25 ± 1.5
07	F ₇	28 ± 1.7	$36 \pm 2.0^*$	29 ± 1.5	32 ± 1.2
08	F ₈	$39 \pm 2.0^*$	30 ± 2.0	$39 \pm 1.9^*$	$41 \pm 2.0^*$
09	F ₉	32 ± 1.2	37 ± 1.9	32 ± 1.2	32 ± 1.5
10	F ₁₀	36 ± 2.0	29 ± 1.5	30 ± 2.0	30 ± 2.0