INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DOI: https://dx.doi.org/ 10.33289/IJRPC.10.1.2020.10(3)

FORMULATION AND EVALUATION OF HERBAL COUGH SYRUP FROM SEEDS EXTRACT OF HEDGE MUSTARD

Vikash Sharma[1], Saurabh Singh[1], Arushi Dixit[2] and Alka Saxena[2]*

[1]G.C.R.G. Memorial Trust's Group of Institutions,
Faculty of Engineering, Lucknow, Uttar Pradesh, India.
[2]R&D Deapartment, Acube Life Sciences,
Lucknow, Uttar Pradesh, India.

ABSTRACT

In recent years there is a spurt in the interest regarding survival of Ayurvedic forms of medication. In the global perspective, there is a shift towards the use of medicine of herbal origin, as the dangers and the shortcoming of modern medicine have started getting more apparent, majority of Ayurvedic formulation are prepared from herbs. Syrup is very popular dosage form of cough and cold medications, ease of patient compliance. The objective of this study is to develop a herbal cough syrup and to check the anti bacterial activity of the extract of seeds of Hedge mustard used for the formulation of herbal cough syrup against different bacteria (*Staphylococcus aureus, E.coli, Salmonella sp., Pseudomonas aeruginosa , B.subtilis*), and to evaluate the physicochemical parameter of cough syrup as well. The cough syrup formulated with simple syrup 66.67% w/v as sugar base. Quality of final herbal syrup was evaluated with different parameters: physical appearance (colour, odour, taste), pH and turbidity. The formulated syrup under gone stability studies and microbial test for 72 hours, no turbidity was observed and no microbial growth was seen.

Keywords: Hedge mustard, Herbal cough syrup, Antibacterial activity, *Sisymbrium officinale.*

INTRODUCTION

Sisymbrium officinale (L.) Scop., synonym Erysimum officinale, commonly known as hedge mustard in English, erísimo in Spanish, erisimo or erba cornacchia in Italian, and velar in French, is a medicinal plant that belongs to the Brassicaceae family. This species could have potential for introduction into the leafy vegetable production for the minimally processed or fresh-cut industry. S. officinale is a terophyte scapose plant with a reddish-violet erect trunk, that present a lot of trichomes and many branches. Basal leaves are different from the upper ones with a dentate shape. Hedge mustard has a linear racemose inflorescence; each flower has four small (1–2 mm) yellow petals; the fruit is a tiny siliqua, close-fitting to the trunk. Flowering occurs in Spring–Summer, from May to July–August, depending on the climate. Siliqua pods usually are pubescent, once they reach maturity they release seeds. Seeds are very small, each siliqua can contain from 10 to 20 seeds. S. officinale is endemic in the Eurasian continent and widespread in all Italian regions from 0 to 1000 m. above sea level ,and rarely up to 2400 m above sea level.[1]

S. officinale is largely known as "singer's plant" and is used among singers, actors, and professionals who use the voice for working. The therapeutic activity of this plant is attributed to its sulfurated components. Dried flowering aerial parts contain: total glucosinolates (0.63–0.94%), mucilage (13.5–10.9%), total thiols (8.9–10.2%), and total flavonoids (0.50–0.56%). The main glucosinolate in S. officinale is glucoputranjivine.[2] historically, the sulphated compounds are reputed to stimulate the mucosal secretion in the upper respiratory

tract, so increasing expectoration. It is used mainly against the inflammations and catarrhs of the larynx, especially to combat hoarseness, as well as against cough, pulmonary catarrh, etc., and scurvy too. The fresh plant is preferably used. The pharmacological activity of Sisymbrium shows anti-inflammatory, analgesic, antitussive, myorelaxant and broad spectrum antimicrobial properties.[3]

It has also been found that the seeds contain small quantities of cardenolides. It is good for all diseases of the chest and lungs, hoarseness of voice. The juice made into a syrup with honey or sugar, is no less effectual for all other coughs, wheezing and shortness of breath, the seed is held to be a special remedy against poison and venom. It was formerly used for hoarseness, weak lungs and to help the voice. Herbalists use the juice and flowers for bronchitis and stomach ailments, among other uses, and as a revitalizer. In Tibetan medicine it is used to repress the symptoms of food poisoning.

In recent years, plant derived products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics and are available in health food shops and pharmacies over the counter as self-medication or also as drugs prescribed in the non-allopathic systems. Herbal medicines widely used in health-care in both developed and developing countries are complex chemical mixtures prepared from plants and are limited in their effectiveness because they are poorly absorbed when taken orally. Cough Syrup is liquid dosage form; the oral use of liquid pharmaceutical has generally been justified on the basis of ease of administration to those individuals who have difficulties in swallowing solid dosage forms. Syrup is a concentrated mixture of sugar and purified water. The high sugar content distinguishes syrups from other types of solutions. Syrups may or may not contain medication or added flavouring agents. Syrups without a medication, but with a flavouring agent, are called non-medicated or flavoured syrups. Flavoured syrups are often used as vehicles for unpleasant tasting medications: the result is medicated syrup. The high amount of sugar present in syrups predisposes them to bacterial contamination, so they often contain a preservative.[4] According to an estimate of the World Health Organization (WHO); about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs. Herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritics, liver diseases, cough remedies, memory enhancers and adoptogens.[5] As per WHO definition, there are three kinds of herbal medicines: raw plant material, processed plant material and medicinal herbal products. Herbal drugs are finished labelled products that contain active ingredients such as aerial or underground parts of plant or other plant material or combination thereof, whether in the crude state or as plant preparations.[6]

MATERIAL AND METHODS

Plant material

The seeds of Hedge mustard were collected from local market of Lucknow. It was authenticated by Acube Lifesciences, Lucknow.

Method of preparation of extract

The seeds were cleaned, shade dried and powdered mechanically and stored in air tight containers. The extraction was carried out by maceration. About 5 gm of powder was extracted with 80% methanol. The extract was kept for 48 – 72 hours and after that it was filtered. The extract was preserved in refrigerator at 4°C.

Antimicrobial activity

In vitro antibacterial activity of the methanolic extract was studied against gram +ve and -ve bacterial strains by the agar well diffusion method. Nutrient Agar was used as the bacteriological medium.The Nutrient agar media was melted and cooled to 48-50 ºC was then poured into sterile petridishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (50µl, 75 µl and 100 µl) was introduced in the well. The plates were incubated overnight at 37^{o}C. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotic ciprofloxacin.

Phytochemical screening

Qualitative phytochemical analysis for secondary metabolites was carried out for the crude extracts as per standard methods.

a) Saponin

5ml distilled water was added to 1ml plant extract and then shaken well, froth formation took place. Stability of froth confirms the presence of saponin in plant extract.

b) Tannin

1ml 5% $FeCl_3$ was added to 1ml plant extract. Appearance of dark blue, black or dark green confirms presence of tannin in plant extract.

c) Flavonoid
2ml 1% NaOH was added to 1ml plant extract, presence of yellow colour indicates the in plant extract.

d) Carbohydrate
1ml Fehling A and 1ml Fehling B was added to 2ml plant extract and then test tube was heated in water bath for 20 min. Appearance of red precipitate confirms the presence of carbohydrate in plant extract.

e) Protein
1ml of 1% $CuSo_4$ and 1ml of 1% NaOH was added to 2ml plant extract. Appearance of purple color confirms the presence of protein in plant extract.

f) Alkaloid
1ml iodine was added to 1ml plant extract. Appearance of reddish brown precipitate confirms the presence of alkaloid in plant extract.

g) Starch
1ml iodine was added to 1ml plant extract. Appearance of blue or black color confirms the presence of starch in plant extract.

h) Fat Test
1mL of distilled water and few drops of ethanol were added to 1mL of plant extract.
The white color precipitate formed showed the presence of fat in the plant extract.

i) Terpenoid Test
250µl chloroform was added to 500 µl plant extract then 625 µl Conc. H_2SO_4 was added to the solution. Reddish brown ppt, of the solution confirms presence of terpenoids.

Method of preparation of simple syrup (usp)
666.7 g of Sucrose was weighed and added to purified water and heated until it dissolved with occasional stirring. Sufficient boiling water was added to produce 1000 ml.[7]

Method of preparation of final herbal syrup
One part of decoction was mixed with five parts of simple syrup (1:5), peppermint oil (0.02%) and required quantity of Sodium benzoate (0.2%) was added to the above mixture (Sodium benzoate) act as a preservative to the above mixture. Solubility was checked by observing the clarity of solution visually. The final herbal syrup was then subjected for evaluation.

Evaluation of herbal syrup
Physicochemical parameters
The herbal syrup was evaluated for various physicochemical parameters such as physical appearance (colour, odour, taste), pH.
a) Color examination
Five ml final syrup was taken into watch glasses and placed against white back ground inwhite tube light. It was observed for itscolor by naked eye.

b) Odour examination
Two ml of final syrup was smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.

c) Taste examination
A pinch of final syrup was taken and examined for its taste on taste buds of the tongue.

d) Determination of pH
Placed an accurately measured amount 10 ml of the final syrup in a 100 ml volumetric flask and made up the volume up to 100 ml with distilled water. The solution was sonicated for about 10 minutes. pH was measured with the help of digital pH meter.

Stability testing
Stability testing of the prepared herbal syrup was performed on keeping the samples at accelerated temperature conditions. The final syrup was taken in culture tubes and were kept at accelerated temperature at 4°C, Room temperature and 47°C respectively. The samples were tested for all the physicochemical parameters, turbidity and homogeneity at the interval of 24 hr, 36hr and 72 hr to observe any change.

RESULTS AND DISCUSSION
The basic objective of this work was to develop herbal cough syrup from seeds of Hedge mustard. It has also been found that the seeds contain small quantities of cardenolides. The development of such herbal syrup will mark an important advancement in the area of Coughs, Use as a gargle or mouthwash, Chronic bronchitis, Urinary tract diseases, Swelling (inflammation) of the gallbladder and Other conditions.
The present investigation examines development and evaluation of herbal syrup.
Phytochemical screening investigations on the phytochemical screening of Hedge mustard extracts revealed the presence of carbohydrates, alkaloids, tannin, saponin, fat, terpenoid and flavonoids, which are known to be biologically active. These metabolites can exert antimicrobial activity through different

mechanisms. The antimicrobial activity of methanolic extract of hedge mustard seeds were tested against bacterial strains (*Staphylococcus aureus, E.coli, Salmonella sp., Pseudomonas aeruginosa , B.subtilis*), the extract showed zone of inhibition.

The formulated herbal syrup was found to be clear without particles and sweet in taste. The developed herbal syrup was evaluated for stability studies for 24, 36 and 72 hours with varying temperature of 4°C, room temperature and 47°C. There was no change observed in physical appearance (colour, odour, taste), pH of the formulated syrup. It did not produce turbidity at lower temperature of 4°C. It was clear homogenous liquid without turbidity at higher storage temperature of 47°C too. Thus it can be concluded that the herbal syrup was in suitable form which was developed.

CONCLUSION

In recent years there is a spurt in the interest regarding survival of Ayurvedic forms of medication. In the global perspective, there is a shift towards the use of medicine of herbal origin, as the dangers and the shortcoming of modern medicine have started getting more apparent, majority of Ayurvedic formulation are prepared from herbs. Syrup is very popular dosage form of cough and cold medications, ease of patient compliance.

Sisymbrium officinale, known as Hedge mustard, (formerly *Erysimum officinale*) is a plant in the family Brassicaceae. Hedge mustard contains an essential oil, rich in sweet-smelling sulphur compounds, consisting mainly of glucosinolates. It has also been found that the seeds contain small quantities of cardenolides.

The Phytochemical properties of the extract were evaluated. The methanolic extract showed the presence of various phytochemicals such as, Flavonoid, Alkaloid, Saponin, Protein, Carbohydrate, Starch, Fat, Terpenoid. The extract was tested for anti-microbial activity. Methanolic extract showed good activity at different concentration. The methanolic extract were formulated as a herbal cough syrup of one part of decoction was mixed with five parts of simple syrup (1:5), peppermint oil (0.02%) and required quantity of Sodium benzoate (0.2%)was added to the above mixture (Sodium benzoate) act as a preservative to the above mixture. Solubility was checked by observing the clarity of solution visually. The final herbal syrup was then subjected for evaluation.

ACKNOWLEDGEMENT

Authors are grateful to Acube Lifesciences, Lucknow for the support and providing facilities to carry out this work.

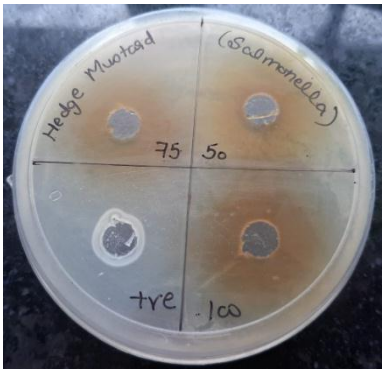


Fig. 1: MIC against *Salmonella sp.*

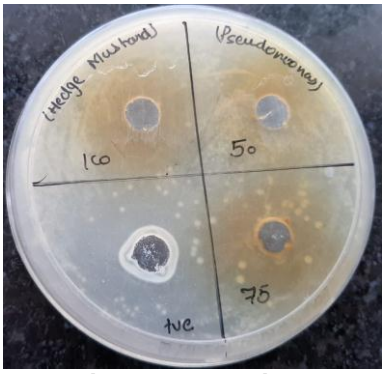


Fig. 2: MIC against *Pseudomonas aeruginosa*

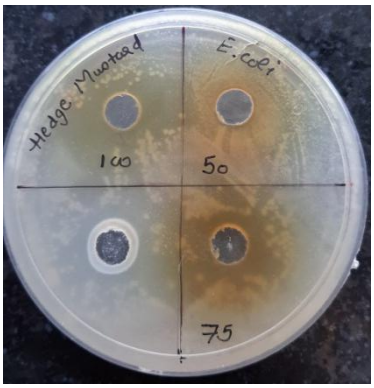


Fig. 3: MIC against *E.Coli*

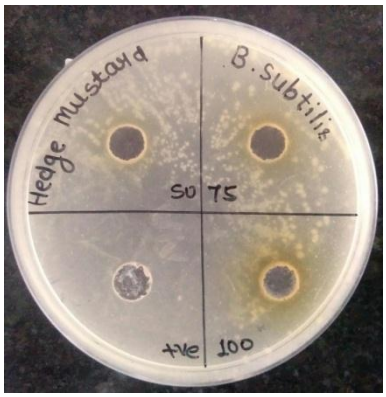


Fig. 4: MIC against *B.subtilis*

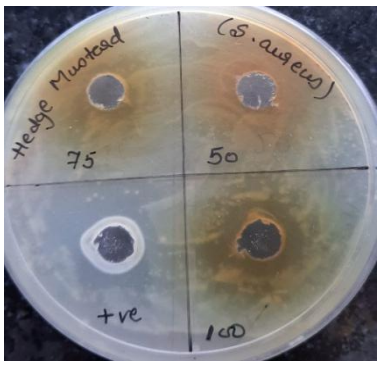


Fig. 5: MIC against *S.aureus*

Table 1: Result of Physicochemical parameters of developed herbal syrup

Sr. No.	Parameter	Result
1	Color	Yellow
2	Odor	Sweet aromatic
3	Taste	Sweet
4	pH	5.8

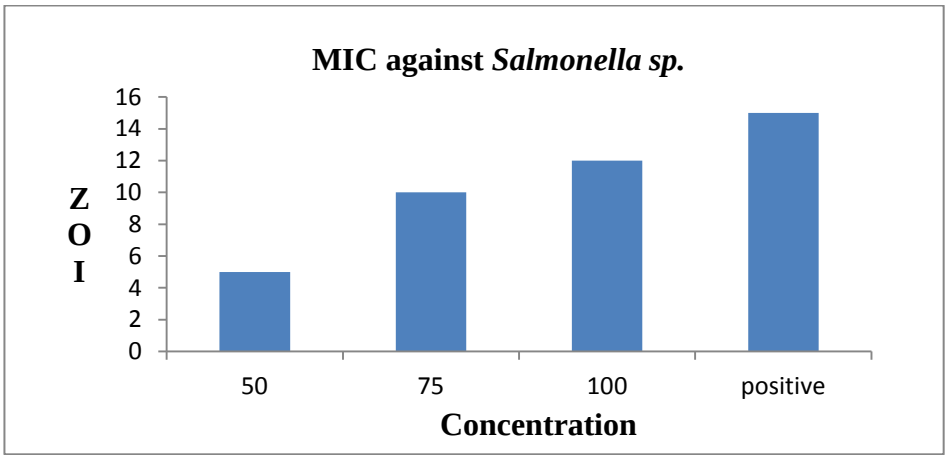
Table 2: Stability studies through Physicochemical parameters of developed herbal Syrup.

Time Duration (inhour)	Temperature (°C)	Physicochemical parameters				
		Color	Odor	Taste	pH	Turbidity/ Homogeneity
24	4°C	NC	NC	NC	5.8	No Turbidity
	Room temp	NC	NC	NC	5.8	X
	47°C	NC	NC	NC	5.8	No Turbidity
36	4°C	NC	NC	NC	5.8	No Turbidity
	Room temp	NC	NC	NC	5.8	X
	47°C	NC	NC	NC	5.8	No Turbidity
72	4°C	NC	NC	NC	5.8	No Turbidity
	Room temp	NC	NC	NC	5.8	X
	47°C	NC	NC	NC	5.8	No Turbidity

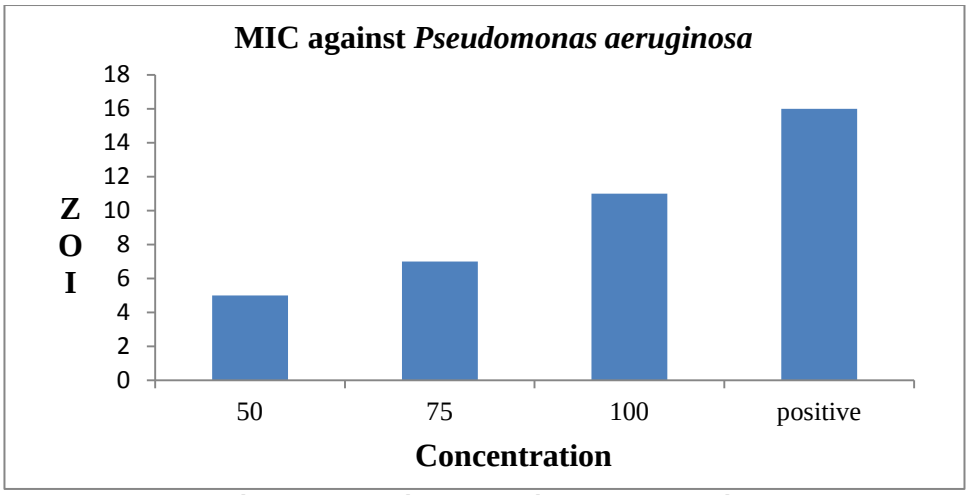
NC = No Change, X= Original condition

Table 3: The antimicrobial activity and MIC of the prepared extract

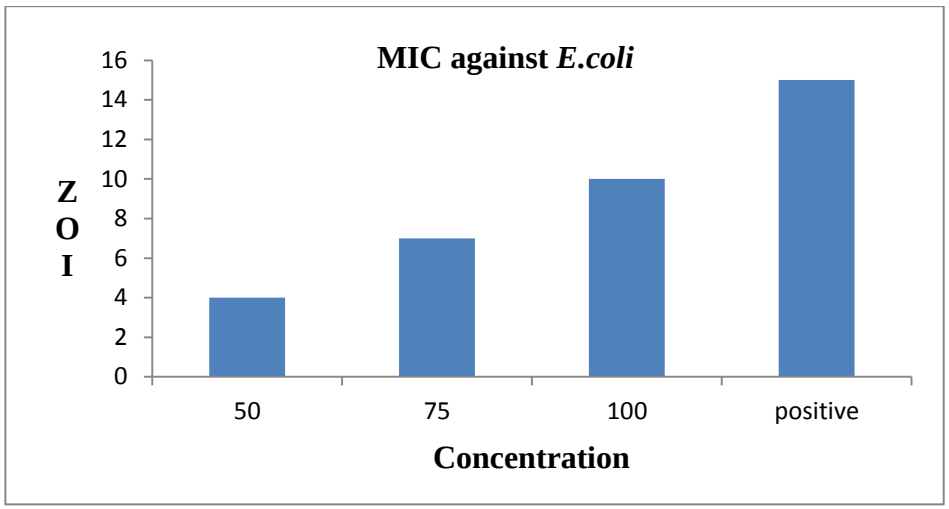
Test Bacteria	Zone of Inhibition (in mm)			
	50µl	75µl	100µl	positive
Staphylococcus aureus	3	5	8	13
E.coli	4	7	10	15
Salmonella sp.	5	10	12	15
Pseudomonas aeruginosa	5	7	11	17
B.subtilis	2	4	7	20



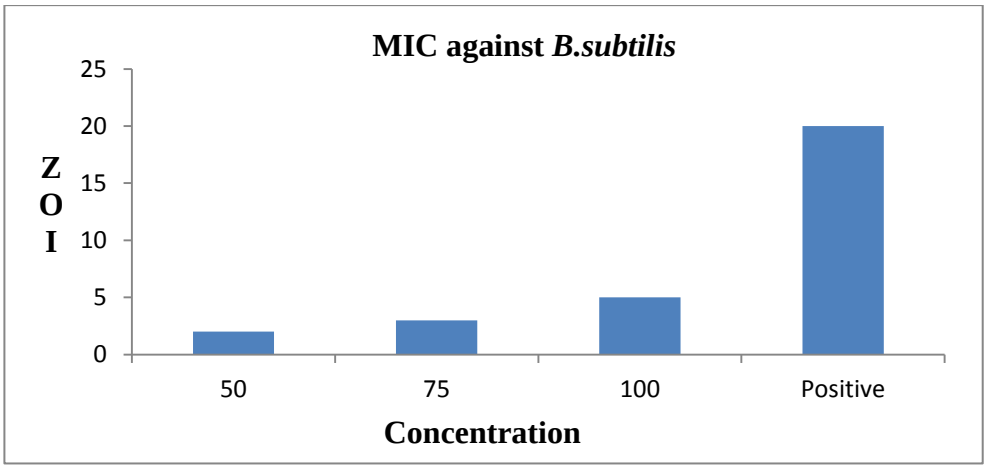
Graph 1: MIC against *Salmonella sp*



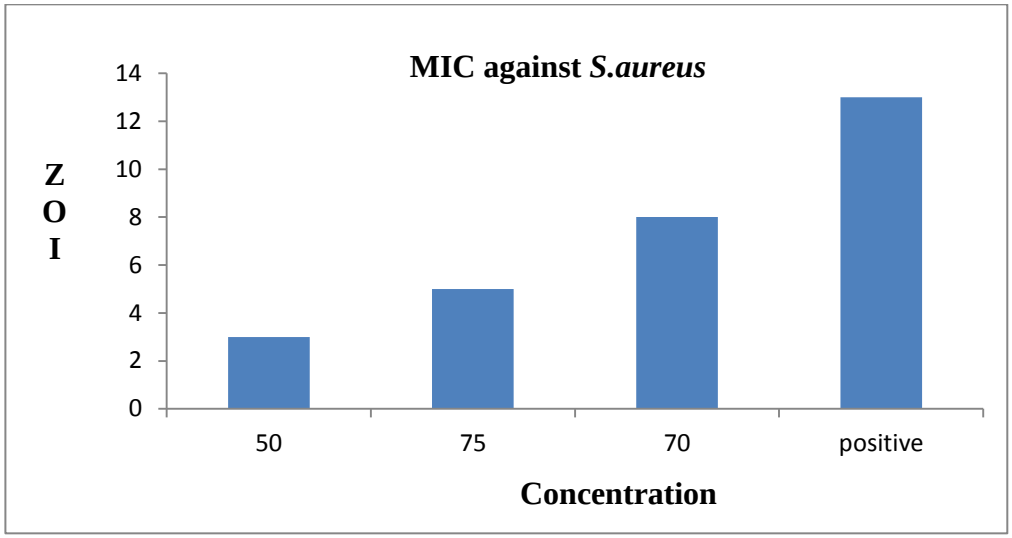
Graph 2: MIC against *Pseudomonas aeruginosa*



Graph 3: MIC against *E.Coli*



Graph 4: MIC against *B.subtilis*



Graph 5:MIC against *S.aureus*

Table 1: Result of Phytochemical Tests

Tests	Extracts of Tamarind fruit	Extracts of Bay leaves	Extracts of Mustard leaves
Alkaloids	Positive	Positive	Positive
Saponin	Positive	Positive	Positive
Tannin	Positive	Positive	Positive
Flavonoid	Positive	Positive	Positive
Starch	Negative	Negative	Positive
Carohydrate	Negative	Positive	Positive
Protein	Positive	Negative	Negative
Fat	Positive	Positive	Positive

Table 2: Result of Antibacterial Tests

Sample	Organisms	ZOI (in mm)
Positive control (Ciprofloxacin)	*S. aureus*	13
	E. coli	10
	B. subtilis	7
	Palm bacteria	22
Tamarind	*S. aureus*	15
	E. coli	12
	B. subtilis	09
	Palm bacteria	15
Mustard	*S. aureus*	12
	E. coli	09
	B. subtilis	05
	Palm bacteria	12
Bay Leaf	*S. aureus*	2
	E. coli	2
	B. subtilis	12
	Palm bacteria	10

Table 3: Result of Chromatography

Samples	Solute	Solvent	Retention Factor
Leaves of Bay	2.8	4.8	0.5
Leaves of Mustard	2.5	5.8	0.4
Fruit of Tamarind	2.8	4.8	0.5

Table 4: Formulation of hand sanitizer

Excipients	Quantity(in gm)
Extracts of Leaves of Mustard	1.5
Extracts of Leaves of Bay	1.5
Extracts of Fruit of Tamarind	1.5
Camphor	1.5
Glycerin	0.75
Water	3ml
Alcohol	6ml
Polysorbate	0.15
SLS (Sodium lauryl sulphate)	0.15

Table 5: Evaluation

Chemical/Physical parameter	Result
pH	4
Colour	Light brown
Odour	Aromatic
Texture	Fine
Appearance	Translucent

Table 6: MIC against Palm bacteria, *S aureus, B. Subtilis* and *E. coli*

Concentration	Zone of Inhibition(in mm)			
	Palm Bacteria	***S. aureus***	***B. subtilis***	***E. coli***
Positive Control (Ciprofloxacin) (10µl)	10 mm	20 mm	15 mm	12mm
Formulated Hand Sanitizer (100 µl) (Undiluted)	9 mm	4 mm	8 mm	5 mm
Formulated Hand Sanitizer (100 µl) (1:2)	8 mm	0.5mm	2 mm	3 mm
Formulated Hand Sanitizer (100 µl) (1:4)	5 mm	0 mm	0 mm	2 mm
Formulated Hand Sanitizer (100 µl) (1:8)	0 mm	-	-	0 mm

Table 7: Comparison of Different Hand Sanitizer

Sample	Volume (µl)	ZOI (mm)
Dettol	100	2
Lifebuoy	100	4
Himalaya	100	5
Herbal	100	15

REFERENCES

1. Guarise M, Borgonovo G, Bassoli A and Ferrante A. Evaluation of Two Wild Populations of Hedge Mustard (Sisymbrium officinale (L.) Scop.) as a Potential Leafy Vegetable. Horticulturae. 2019;5:13.
2. Carnat A, Fraisse D, Carnat AP, Groubert A and Lamaison JL. Normalization of hedge mustard, Sisymbrium officinale L. Annales Pharmaceutiques Françaises. 1998;56: 36–39.
3. Calcinoni O Sisymbrium. Singers' Plant Efficacy in Reducing Perceived Vocal Tract Disability. Journal of Otolaryngology-ENT Research. 2017;8(2):00243.
4. Kaushik A, Chauhan V and Dr Sudha. Formulation And Evaluation Of Herbal Cough Syrup. European Journal of Pharmaceutical And Medical Research. 2016;3(5):517-522.
5. Michael M. Herbal Formulas For Clinic and Home. Bisbee, AZ 85603. 1995;1-34.
6. Choudhary N and Sekhon BS. An overview of advances in the standardization of herbal drugs. J Pharm Educ Res. 2011;2(2):55-70.
7. Goswami PK and Srivastava RS. Development and evaluation of herbal syrup from root extract of nothosaerva brachiata and gomphrena celosiodies. International journal of research in pharmacy and chemistry. 2016;6(3):473-475.