DETERMINATION OF ANTIBACTERIAL POTENTIAL OF SWEET FLAG EXTRACT

Mamta Chopde[a], Dhanshree Longadge[b]
[ab]Department of Cosmetic Technology, Nikalas Mahila Mahavidyalaya, Khamla, Nagpur-440015,India

A B S T R A C T

To defend the skin from microorganism and to avoid spreading of numerous contagious diseases. Hand wash is extremely important precaution. Synthetic antimicrobial agents like triclosan, cresol, and phenol are normally used and they are irritating to skin at high concentration and many people are allergic to them. Asarone is the active constituent in sweet flag which possess antimicrobial activity. Minimum Inhibitory Concentration of Sweet Flag extract was studied to determine antimicrobial activity against skin pathogens which was found to be at 0.25%. The 0.25% of extract was incorporated in liquid soap formulation and was tested for its antimicrobial activity.

Keywords: Antibacterial, Sweet Flag, Asarone, Liquid Soap, Natural Cosmetics

1. Introduction

Natural cosmetics are the emerging trend in the field of cosmetic. The usage of herbal of extracts has been increasing in personal care system and there is a great demand for herbal cosmetics. (Sofia.et al. 2015)

Skin especially hands are needed to protect from bacterial pathogens as they are the most exposed part of our body. Proper hand hygiene is the single most important, simplest and least expensive means of preventing health care associated infection. (Londe, et al. 2015)

Many of the chemical antibacterial agents are available in the market as alcohol based liquid soap and chlorohexidine product. Their product helps to reduce health care associated transmission of contagious diseases more effectively. They have some shortcoming or adverse

effects. Their frequent use can lead to skin irritation and also resistance among pathogens.

Plants are rich in a wide variety of secondary metabolites like phenolic compound, tannins, terpenoids, alkaloids and flavonoid which have been found to give excellent antimicrobial activity.

Therefore, research has been done towards making natural products with improved quality yet less expensive and with minimum side effect over chemical products (Pal, et al.2015)

Hence the aim of the present study was to prepare liquid soap using sweet flag extract which is considered to possess the antimicrobial property.

2. Experimental

The rhizomes and small plant of *Acorus calamus* were procured from the local market of Nagpur district of Maharashtra, India and authenticated at Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University

2.1. Extraction of Sweet Flag: -

Extraction of sweet flag was carried out by maceration process. (Kaur, et al. 2014) Dried rhizomes of sweet flag were crushed into powder Form. 100 gm of powder was immersed in 800 ml ethanol in macerating bottle. It was kept aside at non disturbing place for period of seven days, with frequent stirring. After seven days the macerate was filtered through filter paper. The obtained filtrate was sun dried and extract was obtained.

2.2. Phytochemical screening of sweet flag extract: -

The extract of sweet flag individually is analyzed for the various classes of phytochemicals such as flavonoids, alkaloids, tannins, proteins, carbohydrates glycosides & saponin by using standard phytochemical methods (Kokate, et al. 2003)

Table 1 Result of phytochemical investigation

Sr. No.	Phytochemicals	Name of test	Result
1.	Alkaloids	a) Mayer's test	+
		b) Wagner's test	+
2.	Carbohydrates	a) Molisch's test	+
3.	Glycosides	a) Modified Born Tragers test	+
4.	Saponins	a) Froth test	+
		b) Foam test	+
5.	Phytosterols	a) Salowsk's test	+
6.	Phenols	a) Ferric Chloride test	+
7.	Tannins	a) Gelatin test	+
8.	Flavonoids	a) Alkaline reagent test	+
		b) Lead acetate test	+
9.	Proteins	a) Xantoproteic test	+
10.	Diterpenes	a) Copper acetate test	+

2.3. Determination of Anti-microbial property of sweet flag extract: -

Test organisms were procured from Rajiv Gandhi Biotechnology Institute, Nagpur. The (MIC) minimum inhibitory concentration test determines antimicrobial activity of material against specific bacteria by Cup plate method (Luangtongkum, et al. 2017).

Procedure: -

- Escherichia. Coli was sub cultured in nutrient broth.
- Petri plates, pipettes, nutrient agar were sterilized.
- Different concentration of sweet flag extract, tetracycline and gentamicin were prepared.
- 1 ml (106 cfu) of culture from nutrient broth was poured in sterile petri plates.
- After some time moderate hot agar was poured in sterile Petri plates.
- It was allowed to solidify for half hour.
- 1 ml diluted cultures from nutrient broth were poured in nutrient agar.
- Cups were made with the help of sterile stainless steel borer.
- Comparison was done between sweet flag extract, gentamicin and tetracyclin.

Table 2 Comparison of sweet flag extract with gentamicin and tetracyclin

Organism	Sweet flag extract dilution (Zone of inhibition in mm)				Gentamicin concentration (zone of inhibition in mm)			Tetracyclin concentration (Zone of inhibition in mm)			Minimum Inhibitory Concentration
	1:2 dil	1:3 dil	1:4 dil	1:5 dil	0.5mg/ml	0.1 mg/ml	0.01 mg/ml	0.5 mg/ml	0.1 mg/ml	0.01 mg/m	
E. coli *(*10^6 cfu)	6.5	5	2	0	4	2	2	10	6	3	1:4 dilutions

(a) (b)

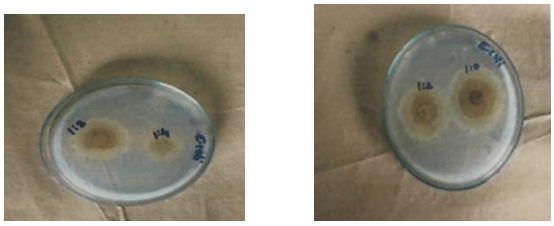

Fig.1 (a) (b) Sweet Flag extract showing activity against E. coli

2.4. Formulation and development of liquid soap: -

The base for liquid soap was selected and after selection of base (Wilkinson, etal.1982) for product sweet flag extract was incorporated in base in 0.25% in concentration as per the result of minimum inhibitory concentration.

Table 3- Base formula for Liquid Soap

Sr. No.	Ingredients	Base with Active
1.	Xanthum gum	0.25%
2.	Glycerin	10%
3.	Reetha extract	5%
4.	Cocodiethanolamide	8%
5.	EDTA	1%
6.	Methyl paraben	0.15%
7.	Citric acid	0.2%
8.	Water	Up to 100 ml
9.	Sweet flag extract	0.25%

2.5. Evaluation of liquid soap with sweet flag extract: -

2.5.1. Accelerated stability study of final product.

The final product was evaluated on different parameters. The colour, odor, pH and
Viscosity were checked and the product was found to be stable

2.5.2. Determination of Foaming Power: -

General: - This method determines the ability of the liquid soap to produce lather

Procedure: -

About 5 gm of the liquid soap was weighted accurately. In a 100 ml glass beaker 10 ml of water was added, cover the beaker with watch-glass and allow to stand for 30 minutes. This operation was carried out to disperse the liquid soap.

The content of the beaker was stirred with glass rod and the slurry was transferred to 250 ml graduated cylinder, ensuring that no foam (more than 2 ml) should produce.

As the temperature of the content of the cylinder reached 30^{o}c. The cylinder was stopped and 12 complete shake were given, upside-down and vice-versa. After that the cylinder was allowed to stand still for 5 minutes and the volumes of (a) foam plus water (v 1 ml) and (b) water only (v 2 ml) was noted (IS 7669-1990).

2.5.3 Determination of matter insoluble in alcohol: -

General: -This method determines the amount of non-volatile soluble matter in liquid soap.

Procedure: -

About1gram of sample was weighted and transferred to a beaker.100 ml of ethanol was added to it. Stirred well and filtered through the filter paper. The residue was washed again with small amount of ethanol. Ethanol was allowed to evaporate from filter paper and the residue was completely transferred to the previously dried and weighted petri plate and the residue was kept at the temperature of 105^{0} C to the constant mass (IS 4199-2001)

3. Result and Discussion

Sweet flag extract was procured from the local market of Nagpur, district Maharashtra and authenticated from Department of Botany, RTM Nagpur University with authentication number 9963.Extraction of the active was done by the maceration process. Dark colored extract was obtained.

Evaluation of active was carried out which included phytochemical screening test and MIC (Minimum Inhibitory Concentration)

Phytochemical screening was done for the detection of alkaloids, carbohydrates, glycoside,

Saponins, phytosterols tannins, and flavonoids as shown in table 1.

MIC (Minimum Inhibitory Concentration) of sweet flag extract against E. coli was determined. Zone of inhibition was found to be 2 mm at the dilution ratio of 1:4. As shown in Fig 1.

1:4 = 0.25% sweet flag extract

After the evaluation of active, base of liquid soap was formulated on the basis of trial and error. After selection of base 0.25% extract was incorporated in the product. The antimicrobial evaluation showed that sweet flag extract gives antimicrobial activity against E coli at 0.25%.

The product showed all the desired properties with respect to colour, odor and pH as per BIS specification. The liquid soap was also found to possess satisfactory properties like viscosity and foaming power. Foaming power was found to be 120ml. Matter insoluble was observed to be 0.103%. It was also observed that the active used in the product was compatible with other raw material of the product.

4. Conclusion: -

The present research work deals with formulation and evaluation of liquid soap using sweet flag extract. The developed formulation was evaluated for its antibacterial activity against *E. coli* and the extract showed antimicrobial activity at a concentration of0.25% hence it can be concluded that liquid soap with sweet flag extract gives antimicrobial property and has additional benefit of cleansing and foaming ability. It also has good antioxidant and anti-inflammatory property.

Acknowledgement-

Authors want to thank Department of Botany RTM Nagpur University for gift sample of microorganism.

REFERENCES

1. Riberio,S., Estanqueiro,M.,Oliveria, M.B & Lobo.S.(2015).Main benefits & application of plant extact in skin care products. *Cosmetics* (2), 48-65
2. Londe, J.,Jagtap,S., Doshi,C.,& Jagade,D.(2015).Formulation of herbal hand wash with potential antibacterial activity.*Intrnational jouranal of research in advent technology (13-14),*11
3. Pal,P.K.,Mandal,P., Kumar,R., Sannd,R.,Singh,H., &Kumar,B.K.(2015). Formulation & evaluation of hand wash. *World jouranalof pharmacy & pharmaceutical science, (4),*4188
4. Deep,K., Sharma,A.k., S.Dash., & Bal,N.(2014).Vacha(acuros calamus linn) A valuable medicinal plant.*International jouranal of ayurveda & pharma research,*3
5. Kokate,C.K.,Purohit,A.P.,&Gokhale,S.B .(2003).Phytochemical investigation.*Pharmacognosy,*(pp105-109)*(24).*
6. Luangtonkum, T.,Morishtia, T.Y.,Ison, A.J.,Zhang,Q.(2017) Comparision of antimicrobial susceptibility testing of camphylobacter spp by the agar dilution & diffusion methods.*Jouranal of clinical microbiology* 591-593.
7. Wilkinson, J.B., & Moore, R.J.(1982).*Harry'scosmeticology*,7th eidition (pp 654) George &Godwin Longman house
8. IS 7669;1990. Specification of shampoo, soap based, Bureau of Indian Standards,(First Rivision)
9. IS 4199;2001. Specification of toilet soap ,Bureau of Indian Standards.