International Journal of Scientific Research in Engineering and Management (IJSREM)
Volume: 05 Issue: 06 | June - 2021 ISSN: 2582-3930

FORMULATION OF PLANT BASED HERBAL SOAP AND ESTIMATION OF ITS ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY

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ABSTRACT – The present study was carried out to prepare herbal soaps with antimicrobial and anti-oxidant activities using different samples collected. The extracts were obtained by soxhlet extraction using petroleum ether solvent. The extracts were screened for antimicrobial (antibacterial) activity using the disk diffusion assay carried out against *Bacillus thuringiensis*, *Pseudomonas aeruginosa, Streptococcus pyogenes, E.coli* .Hydrogen Peroxide and DPPH radial scavenging assays were used to determine the anti-oxidant activity. Presence of phytochemical constituents was investigated. The extracts were used to prepare a solid soap. Petroleum Soxhlet extraction was the best bioactive extract exhibiting considerable antibacterial activity. Therefore this extract was used in the solid soap preparation. Thus the extracts can be successfully utilized to obtain herbal soap with improved antimicrobial and anti-oxidant activities.

KEY WORDS

Extract, Antibacterial activity, Anti-oxidant activity, Herbal soap

INTRODUCTION Soap is a well known common cleansing agent and are being used in our day to day life. WHO(World Health Organization) has reported a high prevalence with the percentage range to 21-87% of skin disorders in general population of developing countries of the world after reviewing 18 prevalence studies. Many skin beneficial medicinal herbs are available for treating almost all skin problems. Many authors have illustrated the definition of soap in many ways. In domestic purposes, soaps are used for washing, bathing and other types of housekeeping. In industrial purposes, soaps are used as lubricants and as thickeners etc., Potassium or Sodium salts are used to make insoluble soaps. One of the important characteristics of soap is hardness. Hardness refers to the hardness of the soap(how hard the soap is). For example, the soap which is made by Sodium salts shows little hardness compare to Potassium salts. The soap is produced by the process called "Saponification". The equation below represent the typical saponification reaction

Fat(Triglyceride) + NaOH → NaOOCR(Soap) + Glycerol

Where R represents the hydrocarbon chain or alkyl group.

■*Vigna radiata* commonly known as Green gram dal is a leguminous plant species belonging to the family Fabaceae family. Green gram is an annual plant. It has an efficient biological activity such as antibacterial and antioxidant. It is widely used for treating cancer, for weight loss, especially for skin glow and for acne. The cleansing properties of green gram dal face pack are highly effective in healing acne.

■*Cyperus rotundus*, commonly called as Nut grass is a perennial shrub. It contains various medicinal properties .It also have antifungal, antimicrobial, anti inflammatory properties. The paste of this is used in treating skin ailments and also in controlling acne.

■*Curcuma aromatica*, commonly known as kasthuri manjal or wild turmeric. It is mostly found in South Asia and is mostly limited to external purposes. It improves skin tone and reduces acne and its scars effectively. Slows down the growth of facial hair. It serves as an amazing treatment for pimples. And also for skin glow.

■Roots of vetiver tree is used. It have antimicrobial, antioxidant and anti inflammatory properties. It effectively reduces skin inflammation, treats skin infections and promotes overall skin health. Acne is a severe issue concerning many women. Vetiver has its own property to control acne.

■*Pterocarpus santalinus*, commonly called as red sandalwood which is a tree. It is best used for skin problems and have antimicrobial and antioxidant properties. And also used for acne treatment.

■*Senna auriculata*, commonly known as Aavaram poo(in Tamil) is a shrub. It has amazing health benefits, medicinal uses and skin care benefits. Aavaram poo face pack made with the dried flowers treats almost all skin problems like acne.

■*Rosa indica*, commonly known as paneer rose(in Tamil) belongs to a family of Rosaceae. It is cultivated as an ornamental plant. The dried flowers are used and were used in treating skin disorders. It exhibits antioxidant and antioxidant activity.

■ Babchi, the seeds in this plant have a wide range of coumarins. The derived babchi seed powder can be applied on the skin for curing several skin conditions. It also helps to improve health and skin colour.

■*Curcuma zedoaria* is also known as kichili kizhangu(in Tamil).It is a herb and used for their medicinal properties. It manages the symptoms of skin problems due to its antioxidant and antibacterial properties.

These medicinal herbs were collected and shade dried for some days and extract was obtained using Soxhlet method of extraction Research has shown that the extract obtained has been used to formulate soap.

The aim of the present study is to formulate a plant based herbal soap for treatment against pimple and to estimate its antimicrobial and antioxidant activities.

2. MATERIALS AND METHODS:

*Curcuma aromatica, Vigna radiata, Vetiveria zizanioides, Senna auriculata, Rosa indica, Psoralea corylifola, Curcuma zedoaria, Cyperus rotundus, Pterocarpus santalinus,*Beakers,Conicalflasks**,**
Pipette,Test tubes**,** Petri plates. Caustic soda, petroleum ether, DMSO, beef extract, acid hydrolysates of casein, starch, agar, 0.2Mm DPPH, ethanol, ascorbic acid, 0.1M phosphate buffer, 4mM hydrogen peroxide in phosphate buffer, benedict's reagent, biuret reagent, chloroform, hydrochloric acid, sulfuric acid, dragendroff's reagent, acetic anhydride. Soxhlet apparatus, Calorimeter.

METHOD

Herbs collection and processing

The collected herb were shade dried for 2 days and ground into coarse powder.

Petroleum ether extraction by Soxhlet apparatus

Soxhlet apparatus was used to obtain herbs extract. The ground powder was loaded into Soxhlet apparatus along with 300ml of organic solvent (petroleum ether). Extraction procedure was carried for 4 hours and extract obtained was stored in a bottle.

Soap preparation

100ml of water was poured into a plastic jar and 30 grams of NaOH was weighed and added into the container containing water. After mixing it was kept aside for sometime to cool. Meanwhile, 150ml of coconut oil was measured and poured into another jar. 20ml of medicinal herbs extract was added into container containing coconut oil. After NaOH got cooled, oil and herb extract mixture was added into NaOH solution and mixed it. Then mixture was poured into mould. It was kept aside for 48 hrs.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Benedict's Test: To a 0.5ml of filtrate, 0.5ml of benetict's reagent was added. The mixture was heated on a boiling water bath for 2 min. A Characteristics colored precipitate indicates the presence of sugar

Test for Protein: To confirm the presence of protein biuret test was done. The extraction boiling with 2ml of biuret reagent, violet color appears indicating the presence of proteins.

Test for saponins: Addition of 5ml of distilled water to the extract. Formation of 1cm foam layer showed the presence of saponins.

Test for steroids: 1ml of extract was dissolved in 10ml of chloroform and added an equal volume of concentrated sulfuric acid from sides of the test tube. The upper layer turns into red.

Test for alkaloids: To 5ml of extract 2ml of hydrochloric acid was added. 1ml of dragendroff's reagent was added to the acidic medium. An orange or red precipitation was immediately produced which indicates the presence of alkaloids.

Test for terpinoides: To the extract 1ml of chloroform and 1ml of acetic anhydride was added following the addition of 1ml of concentrated H_2SO_4. Formation of reddish

violet color indicates the presence of triterpinoides.

Salkowski's test for glycosides: 2ml of chloroform was added to the extract followed by gentle addition of concentrated H_2SO_4 will give reddish brown color indicating the presence of glycosides.

AGAR WELL DIFFUSION ASSAY

The antimicrobial assay was performed by agar well diffusion method. The molten Mueller hinton agar was inoculated with 100microliter of the inoculum and poured into the petri plate. The wells were punched in the plates using cork-borer(0.85cm).100microliter of test sample was introduced into each well. The plates were incubated for overnight at 37 C for bacteria. Microbial growth was determined by measuring the diameter of zone of inhibition. The respective controls were maintained for each bacteria. The result was obtained by measuring the zone of inhibition.

DPPH RADICAL SCAVENGING ACTIVITY

The free radical activity of the herb extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH solution of DPPH in ethanol(0.1mM) was prepared and 1.0ml of this solution was added to 2.0ml of herb extract at different concentrations (100-500microliter/ml). After 30 mins, the adsorbance was measured at 517 nm. Ascorbic acid was used as the positive control.

Inhibition(%) = [1-(abs sample/abs control)]*100

HYDROGEN PEROXIDE SCAVENGING ACTIVITY

The Hydrogen Peroxide scavenging was determined according to the method of ruch et al. a solution of hydrogen peroxide was prepared in phosphate buffer and their concentration was determined specrometically from the absorption at 230 nm. Various concentrations pf extract were added to hydrogen peroxide and incubated for 10 min. The absorbance at 230 nm was determined against blank containing phosphate buffer without hydrogen peroxide.The percentage scavenging of hygrogen peroxide and ascorbic acid was calculated using the formula

Inhibition(%)=[1-(abs sample/abs control)]*100

Where, Abs sample =absorbance of herb extract

Abs control = ascorbic acid

ACTIVITY OF HERB EXTRACT AGAINST PIMPLE SAMPLE

Agar well diffusion method was adopted for evaluation of anti-microbial activity. A well 10 mm in diameter bored on the surface of the agar medium using sterile cork borer. Herbs extracts were introduced into each well at different concentrations (25, 50, 75 microlitre). The plate was incubated at 36 c for 4 days. At the end of the incubation, the plate was observed for growth and inhibition

RESULT

Herbal soap preparation: Soap was prepared using extract and coconut oil. Picture at bottom was the prepared soap.

a.soap prepared with powder extract

b.soap from herbs

Preliminary phytochemical analysis of petroleum ether extract of herbs:

The results of preliminary phytochemical analysis of petroleum ether extract of medicinal herbs are given in the Table 4.1. The results showed that the phytochemical constituent like saponin was present but carbohydrates, proteins, glycosides, flavonoids, alkaloids, triterpenoids were absent.

Antibacterial activity (agar well diffusion assay) of petroleum ether extract of herbs

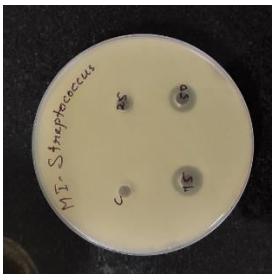
For *Bacillus thuringenisis* at the concentrations (25,50,75 microlitre) the diameter of zone of inhibition was 5mm, 7mm, 10mm.

For *Streptococcus pyogenes* the concentrations (25,50,75 microlitre) the diameter of zone of inhibition was 4mm, 5mm, 7mm.

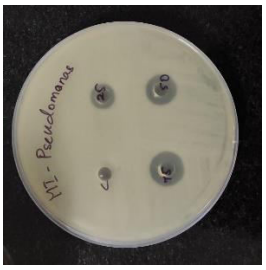
For *Pseudomonas aeruginose* at concentrations (25, 50, 75 microlitre), the zone of inhibition was 6mm, 12mm, 12mm.

Among these results *Pseudomonas aeruginose* has highest diameter of 12mm zone of inhibition at both concentrations of 50, 75.

a.*E.coli* b.*Bacillus thuringenesis*



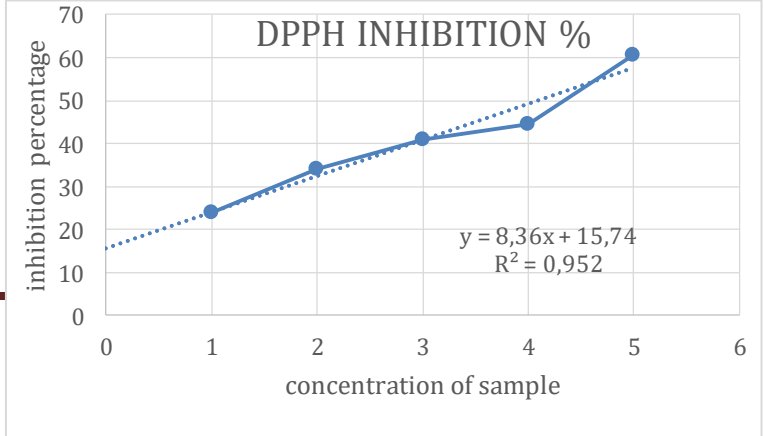
c.*Sterptococcus pyogenes*



d. *Pseudomonas aeruginose*

DPPH RADICAL SCAVENGING ACTIVITY

From the DPPH radical scavenging activity result showed that the highest DPPH antioxidant activity of the herb extract was found to be 60.7 % at 600microlitre and IC_{50} was 5.37mg/ml



S.no	Volume of extract sample microlitre	Concentration of extract(mg/ml)	Od at 517 nm	Inhibition %	IC_{50} mg/ml
1	Same		0.627	0	-
2	200	1	0.476	24.1	1
3	microl	2	0.418	34	2.18
4	itre	3	0.371	40.9	3.00
5	Contr	4	0.349	44.4	3.42
6	ol	5	0.247	60.7	5.37

S.no | Volume of extract sample microlitre | Concentration of extract(mg/ml) | Od at 517 nm | Inhibition % | IC_{50} mg/ml
1 | Same | | | | 0.627 | 0 | -
2 | 200 | 1 | | | 0.476 | 24.1 | 1
3 | microl | 2 | | | 0.418 | 34 | 2.18
4 | itre | 3 | | | 0.371 | 40.9 | 3.00
5 | Contr | 4 | | | 0.349 | 44.4 | 3.42
6 | ol | 5 | | | 0.247 | 60.7 | 5.37

Graph 1 DPPH Inhibition Percentage

TABLE 1 DPPH SCAVENGING ASSAY

S.no	Volume of extract sample microlitre	Concentration of extract(mg/ml)	Od at 517 nm	Inhibition %	IC_{50} mg/ml
1	Control	0	0.846	0	
2	200	1	0.576	32	0.98
3	400	2	0.478	43.5	1.91
4	600	3	0.346	59.2	3.19
5	800	4	0.247	70.9	4.14
6	1000	5	0.175	80	4.88

HYDROGEN PEROXIDE SCAVENGING ACTIVITY

From the H_2O_2 radical scavenging activity result showed that the highest H_2O_2 antioxidant ativity of the herb extract was found to be 80% at 1000 microlitre and IC_{50} was 4.88 mg/ml.

TABLE 2 H_2O_2 SCAVENGING ASSAY

ACTIVITY OF EXTRACT AGAINST PIMPLE SAMPLE

The antibacterial activity of extract was evaluated according to zone of inhibition formed. Zone of inhibition was observed against pimple sample.zone of inhibition was checked against pimple sample at different extract concentration (25, 50,75 microlitre). The diameters of zone of inhibition formed were 13mm, 14mm, 16mm. among these results 16mm was highest zone of inhibition formed at 75microlitre concentration.

DISCUSSION

Applicability of the plant extracts we have collected in preparation of herbal soap products have not been extensively investigated so far. According to this research, it was found that the petroleum ether Soxhlet extract was effective towards herbal soap products. The samples collected itself possess biological properties such as antibacterial, antioxidant activities. It also have been tested for its activity against pimple. Thus by increasing the biological efficiency proper solution for treating certain skin diseases due to bacterial organisms can be obtained. The samples are *Vigna radiata,Curcuma aromatica, Senna auriculata etc.,* these samples are known for its well known medicinal activity. The extract was prepared by using Soxhlet method of extraction. The extract was obtained by using petroleum ether as a solvent. Petroleum ether was chosen as solvent because it has the ability to extract maximum phytochemicals or bioactive components from the sample collected. Phytochemical tests were carried out. The phytochemical constituent like saponin was present but carbohydrates, proteins, glycosides, flavonoids, alkaloids, triterpenoids were absent. The antibacterial activity was tested using four different bacterial strains viz., *Bacillus thuringiensis, E.coli, Pseudomonas aeruginosa* and *Sterptcoccus pyogenes*. The antibacterial activity was observed after 24 hours of incubation. The formation of zone of inhibition was observed in the petroleum ether extract of medicinal herb. The diameter was checked for four bacterial strains.

CONCLUTION

Though there are many herbal soaps available in the market few of the herbal soap still has some chemicals in it.

But the soap we prepared has no chemicals and also the soap showed good antioxidant and antibacterial property.

So, this soap would be better alternative to chemical soaps and it also helps in protecting the skin from skin infections.

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