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A holistic approach for formulation and evaluation of poly herbal shampoos

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Abstract
The study aimed to formulate a pure herbal shampoo and to evaluate and compare its physico-chemical properties with the marketed synthetic and herbal shampoos. The herbal shampoo was formulated by addition of extracts in different concentrations of *Azadiracta indica*, *Annona squamosa*, *Citrus sinensis*, *Eucalyptus globulus*, *Sapindus mukorossi* in different proportions to a 10% aqueous gelatin solution. Small amount of methyl paraben was added as a preservative and pH was adjusted with citric acid. Several tests such as visual inspection, pH, wetting time, % of solid contents, foam volume and stability, detergency, dirt dispersion, skin and eye irritation tests and anti-head lice activity, etc. were performed to determine the physicochemical properties of both prepared and marketed shampoos. Polyherbal shampoo was clear and appearing. It showed good cleansing and detergency, small bubble size and marketed shampoos showed comparable results for 5 solid contents also. Compared to synthetic shampoos formulated polyherbal shampoos showed maximum paralysis or death time. When concentration increased, paralysis time decreased. The results indicated that the formulated shampoo is having excellent anti-head lice activity at par with commercially available shampoo. Death time of f3 formulation showed maximum effect on paralyse of human lice and cattle lice with in less duration in 18 minutes, 95% of lice killed. Saponin containing reeta used as a shampoo base and control death time period is 60 minutes. So, further research and development is required to improve its quality & safety.

Keywords: Poly herbal shampoo, formulation and evaluation

1. Introduction
Transilation of ethano-botanical information for isolation and identification of herbal constituents required for revealing the pharmacological status of the important photochemicals [1]. Now-a-days many synthetic, herbal, medicated, non-medicated shampoos are available in the market. Popularity of herbal shampoos among consumers is on rise because of their belief these products being of natural origin are safe and free from side effects [2]. Growth of head lice on the scalp of girl, cattle's, pets is a often problem. The problem can be overcome by using some natural extract which are available easy at cheaper coasts and more safety and effect than the synthetic shampoos [3].

Herbal shampoo is a cosmetic preparation in which uses plant secondary metabolites for washing of hair and scalp just like a regular shampoo. It is an alternative to the synthetic shampoo available in market. Herbalists today believe in helping people build their good health. Modern era all are very aware on herbal products than chemical products it is proved that herbs enhance health. In this formulation *Annona squamosa* seed oil extracted with petroleum ether, *Azadiracta indica* leaf extract, orange peel oil, eucalyptus globulus leaf oil andsoap nut extracts were used as an main ingredients for poly herbal lice shampoo. Custard apple plant is commonly found in India. It is properly known as sitafal in India, it is cultivated in all parts of India. The conical fruit with a green knobby skin in very sweet and eaten fresh or can be used for milk shakes, ice- creams and even sherbets [6]. The fruits contains large number of the plant used to treat diabetes, ulcer, wound, dysentery and ailment [5].

The grounded seeds can be applied a hair, to get rid of lice. The human head louse, *Pediculis human uscaptails*, a small insect causing a public health problem especially in poor sanitary condition [9]. Farmers in Vietnam use seed oil to control lice, leaf hoppers and plant hopper. In Thalianad, research has shown the ant head lice activity of *Annoa squamosal* [7]. *Azadiracta indica a.juss*, known as neem in vernacular belongs to family Meliaceae [8] is widely distributed in Asia, Africa and other tropical parts of the world [9]. Neem is a versatile medicinal plant almost every part of which is being used in folklore and traditional systems of medicine for the treatment of variety of human aliments. Neem contains secondary metabolites Azadirachatin, a major compound of neem has potent antifeedent, and growth had reproductive regulating properities. Nimbin is a liminoid form also known as a natural pesticidal prosperity. Neem tree

also considered as a natural insecticide plant. The quality of pesticide and pharmacological products depend upon the contents of Azadirachatin and Nimbin in the plant.
Rita or soap nut i.e *Sapindus mukorssi* pericarp extract is used as foaming agent has been used for hair cleansing property. Orange peel which is consider as a waste can be used for the extraction of essential oil has plenty applications ranging from flavoring agent in food, cosmetics its used as antidandruff agent, and insect repellants.

2. Materials & Methods

Present study focused mainly safe, effective, effort table cost polyherbal shampoo designed, formulated and evaluate and compare its physicochemical properities with marketed synthetic shampoos for anti-head lice activity from waste products reused for society. *Azadiracta indica* seeds, A*nnona squamosa* seeds, *Citrus* sinensis peels, *Eucalyptus globules* leaves were collected from srikakulam forest in April 2016. The pericarp of *Sapindus mukorssi* commonly known as reeta, which produces rich lather when shaken with water due to their high content of saponins. They are collected from the Sri Venkateswara College of Pharmacy herbal garden, Srikakulam, Andhrapradesh, India. Plants were authenticated by a botanist of Andhra university prof. M. Venkaiah and the respective votcher specimen was deposited. Natural poly herbal shampoo compared with commercial available shampoos are Peritop-12%w/v, Moni Chem India, Med Lice, 10%w/v Medhel, India were purchased from the Siva chaitra sai medical &general stores from Vizianagaram, Andhra pradesh.

2.1 Preparation of Plant Extracts

500grams of custard apple seeds, 750grams of neem seeds, 500gms of orange peels, 650 grams of eucalyptus leaves were washed under water, and dried under room temperature. Custard apple seed powder subjected in soxhelet extractor for 6hrs with petroleum ether, neem seed oil collected from expression method, citrus peel powder subjected to steam distillation.
Aqueous extracts of reeta was collected by maceration process. All the extracts were extracted separately, filtered and concentrated vacuum evaporator, Custard apple oil obtained 20% v/w shampoo, neem 40% w/w, citrous oil 18% v/w, eucalyptus oil 16.88% v/w. Reeta aqueous extract 50% was obtained.

2.2 Formulation of poly herbal shampoo

All the herbal oils and extracts were mixed in different proportions to obtain a shampoo was shown in Table 1. Herbal extracts were added to 10% gelatin solution and were mixed with continuously stirring for 20 minutes.
The herbal shampoo was preferably using primary emulsion formula in the proportion of o: w: G as 4:2:1. Lemon juice 1ml, methyl paraben 0.05% added with stirring Finally the pH of solution was adjusted by adding optimum quantity of 1% citric acid solution few drops of rose essential oil also added for good aroma for anti-head lice shampoo and the final volume was made to 100ml of Reeta extract.

2.3. Anti-head lice shampoo evaluation tests

To evaluate the quality of commercial and formulated herbal shampoos, several quality control tests including organoleptic properties, physicochemical controls, eye irritation, stability tests were performed [12].

2.3.1 Physical appearance of poly herbal shampoo

The herbal formulation preparation was evaluated for the clarity, color, odour, and foam producing ability [13]. Texture was carried out by sensation, touch and vision respectively.

2.3.2 Determination of pH

The pH of F1, F2 and F3 in distilled water shampoos were measured by using pH meter (ELICO) at room temperature [14].

2.3.3 Determination of% solid contents

4 grams of each shampoo were placed in a previously clean, dry and weighed evaporating dish. The dishes and shampoos were weighed again to conform the exact weight of the shampoos. The liquid portion of the shampoo was evaporated by placing the evaporating dish on the hot plate, the weights and percent ±solid contents of shampoo left after complete drying were calculated [15].

Table 1: Composition of Formulated Herbal Shampoos

Ingredients	F1	F2	F3
Annona squamosa seed oil	5g	10g	15g
Azadiracta indica leaf extract	5g	10g	15g
Citrus sinensis peel oil	5g	10g	15g
Eucalyptus globulus leaf oil	5g	10g	15g
lemon juice	1ml	1ml	1ml
Reeta extract	20ml	20ml	20ml
Methyl paraben	0.05g	0.05g	0.05g
Acacia gum	2.5g	2.5g	2.5g
Gelatin solution	q.s.	q.s.	q.s.

2.3.4 Wash ability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually [16].

Fig 1: Dirt Dispersion

2.3.5 Foaming ability test

Foaming ability was determined by using cylinder shake method briefly, 50ml of the 1% commercial or formulated shampoo solution was placed in to a 250ml graduated cylinder. It was covered with one hand shaken 10 times. Total volume of the foam content after 1 min of shaking was recorded. Foam stability was evaluated by recording the foam volume after 1 min and 4 minutes of shake test. Foaming ability was determined by using cylinder shake method briefly, 50ml of the 1% commercial or formulated shampoo solution was placed in to a 250ml graduated cylinder. It was covered with one hand shaken 10 times. Total volume of the foam content after 1 min of shaking was recorded. Foam stability was evaluated by recording the foam volume after 1 min and 4 minutes of shake test [15-17].

Foam Index-F1

T1	T2	T3	T3	T5	T6	T7	T8	T9	T10
1mm	0.9	0.8	0.8	0.9	0.7	1	1	0.8	0.9

Foaming index=1000/8=125

Foam Index- F2

T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
1	0.8	0.7	1	0.4	0.8	0.9	0.9	0.8	0.6

F2=1000/8=125

Foam Index-F3

T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
1	0.9	0.9	0.8	0.9	0.9	0.9	0.9	0.7	0.9

F3=1000/1=111.1

Foam Index-F4

T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
0.4	0.7	1	0.4	0.6	0.8	0.7	0.8	0.5	0.5

F4=1000/1=111.1

Foam Index-F5

T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
0.5	0.6	0.4	0.7	0.5	0.6	0.7	0.6	0.6	0.9

Foaming index of F5=1000/10=100

F1	F2	F3	F4 Peri TOP	F5 Medlice	Mean
125	125	111.1	111.1	100	114

Fig 2: Foaming ability test

2.3.6 Wetting time test: A convas paper was cut into 1 inch diameter disc having an average weight of 0.44g. The smooth surface of disc was placed on the surface of 1%v/v shampoo solution and the stop watch started the time required for the disc to begin to skin was noted down as the wetting time [17]

Fig 3: Disc Diffusion Test

2.3.7 Eye irritation tests

Animals (Albino rats) were collected from animal house, about 1% shampoo was dipped into the eyes of rats (n=3). Albino rats with their eyes open with clips at the lid, the irritation was recorded at specific intervals over on 2 mins for a period of 5 secs. Observed the reactions to the irritants can include swelling of eyelid inflammation of the iris, ulceration, haemorrhages and bleeding. The polyherbal shampoo showed no harmful effect on skin and eye revealed by the testing of irritation tests. This happens due to the avoid of synthetic surfactants. So many synthetic surfactants showed inflammation of the eyelid and corneal irritation, skin ulceration but in this polyherbal shampoo, all the ingredients are obtained from natural source so that it does not produce any adverse effect on eyes.

2.3.8 Evaluation of conditioning performance

A hair flees of a Srikakulam women were obtained from a local salon. It was cut in to four swatches of the tresses with approximately the length of 10cm and weight of 5g. A swatch without washing served as the control. Other three tresses were washed with the commercial and formulated shampoos in an identical manner. For each cycle, each tress was shaken with the mixture of 10g of a sample and 15g of water in a conical flask for 2 min and then rinsed with 50ml water. the girl students were blind folded and asked to touch and rate the 4 tresses for conditioning performance from score 1 to 4 (1 = poor, 2 = satisfactory 3 = good, 4 = all excellent

2.2.9 Skin irritation tests

Skin irritation test is carried out by using open patch method. A small amount of each polyherbal shampoo was applied for each group (n=3) on the backside of rat after shaving an area 1 cm^2. Observed any signs of irritations like itchness, pain, breakout on skin, redness or rash, etc. for a period of 15-20 min after application. There was no significant irritation observed in F1 and F2 whereas slight erthmea was observed in F3 and F4. So F1 and F2 are found to be safe to use as polyherbal shampoo.

3. Stastical Analysis

Data were analyzed using ANOVA. All tests were performed in triplicate and data are expressed as means ± standard deviation. ANOVAs single factor was used for determining significance p values <0.05 were considered as significant.

4. Results & Discussion

4.1 Formulation of Herbal Shampoo

A pure herbal shampoo was formulated by mixing petroleum ether extract of custard apple seed oil, ethanolic extract of neem oil, citrous oil, aqueous extract of soap nut in definite amounts shown in table 1. These plant materials contain photochemical like triglycerides, limonene, 1,8 cineole, azadiractin, soap nut contains saponins which are natural surfactants possessing good detergency and foaming properties. An ideal shampoo must possess adequate viscosity to facilitate removal from the bottle. So many natural materials available we select 10% gelatin solution for this purpose as it shows pseudo plastic behavior and forms clear solutions. Lemon juice 1ml was also added as natural oxidant, chelating agent and anti-dandruff agent.

4.2 Evaluation Parameters

4.2.1Physical Appearance

A shampoo like any other cosmetic preparation should have good appealing physical appearance. The formulated herbal shampoo and marketed herbal shampoos were evaluated for physical characteristics such as color, odor, and transparency in Table-2. Our shampoo shows F1, F2, F3 light brown, transparent and good odor. With marketed shampoos (F4 & F5) there was no significant difference observed in transparency and foaming characteristics between synthetic shampoos and formulated shampoo except for odor and color.

4.2.2 pH

Most shampoos are formulated as either neutral or slightly

alkaline to minimize the damage to hair the pH of shampoo also helps him minimizing irritation to eyes enhance the quality of hair and maintain the ecological balance to scalp [12]. The pH of tested synthetic shampoo as well as marketed shampoo (peritop-F4, med lice-F5) were found within the preferred range between 5 and 7 [14] and are presented in Table 2, but the pH of formulated shampoo was found to be nearly neutral.

4.2.3% of solid content

Good shampoos usually have 20% to 30% is solid content it is easy to applied and rinse daily out from hair if it does not have enough solid it will be too watery and was away quickly similarity too many solids will be hard to work into the hair or very hard to wash out the present solid content of all the tested shampoo was found within Rangers of 20 to 25 percentage and are expected to wash out easily [18].

4.2.4 Dirt Dispersion

Dirt dispersion is very vital tool for evolution of cleansing action of shampoo that causes the ink to concentrate in the foam are considered of poor quality because ink or dirt that stays in foam is difficult to rinse away and gets redeposit on the hair the dirt should stay in the water portion for achieving better cleaning action all shampoos concentrated the ink in the water portion ensures their satisfactory cleaning ability and actual effectiveness [19].

4.2.5 Surface tension

The term indicates the amount of surfactant present in the shampoo to reduce the surface tension. Lesser the surface tension, stronger is the cleaning ability of a shampoo. A shampoo is considered of good quality if it decreases the surface tension of pure water from 72.28 dyn/cm to about 40 dyn/cm. So all the tested shampoos showed similar reduction in the surface tension ranging from 35.78 dyn/cm to 39.94 dyn/cm. The reduction in surface tension is an indication of a good detergent action. The formulated shampoo reduce surface tension to 39.94 dyn/com which is comparable to synthetic shampoo 38.78 dyn/cm. However among all the shampoos, peritop has showed lowest surface tension of 35.78 dyn/cm indicating that it has a strongest cleaning ability. The commercial synthetic shampoos may contain excessive deteregents which can strip the hair upto 80% of the oil and thus damage the air. Using the mild detergent in our shampoo we have ensured that our formulated shampoos have good cleansing activity.

4.2.6 Foaming ability and foaming stability

Foaming or gathering is very important to the consumer and therefore it is considered as an important parameter in evaluation of shampoo herbal essences and formulated shampoo produced the foam volume above 100 ml (F1, F2, F3, F4 and F5) the foam generated by formulated shampoo were small compact uniform denser and stable similar to commercial samples all tested shampoo had the same form volume for 5 minutes showing that their foam has good stability the higher forming property of formulated shampoo maybe due to soap nut [20].

4.2.7 Wetting time

The wetting ability of surfactant independent on its concentration and is constantly used to test its efficacy the convert disc method quick efficient and reliable test to evaluate the wetting time of 3 shampoo was found in order F1 less than F2 less than F3 is peritop (pyrithium). It can be concluded that contains the maximum concentration of detergent because it has the least wetting time by contract over formulated shampoo inhibited maximum waiting time so it contains maximum concentration of detergent because it has the least waiting time by contest over formulated shampoo exhibited maximum wetting time so it contains minimum concentration of detergent because it had the least wetting time by contrast over formulated shampoo exhibited maximum wetting time. So it contains maximum concentration of detergent.

4.2.8 Eye irritation tests

Animals (Albino rats) were collected from animal house, about 1% shampoo was dipped into the eyes of rats (n=3). Albino rats with their eyes open with clips at the lid, the irritation was recorded at specific intervals over on 2 mins for a period of 5 secs. Observed the reactions to the irritants can include swelling of eyelid inflammation of the iris, ulceration, haemorrhages and bleeding. The polyherbal shampoo showed no harmful effect on skin and eye revealed by the testing of irritation tests. This happens due to the avoid of synthetic surfactants. So many synthetic surfactants showed inflammation of the eyelid and corneal irritation, skin ulceration but in this polyherbal shampoo, all the ingredients are obtained from natural source so that it does not produce any adverse effect on eyes.

F1 (5% Conc.) F2 (10% Conc.) F3 (15% Conc.) F4 (12% Conc. Peritop) F5 (10% Medlice)

Fig 4: Eye irritation test

4.2.9 Skin irritation tests

Skin irritation test is carried out by using open patch method. A small amount of each polyherbal shampoo was applied for each group (n=3) on the backside of rat after shaving an area 1 cm^2. Observed any signs of irritations like itches, pain, breakout on skin, redness or rash, etc. for a period of 15-20 min after application. There was no significant irritation observed in F1 and F2 whereas slight erthmea was observed in F3 and F4. So F1 and F2 are found to be safe to use as polyherbal shampoo.

F1 (5% Conc.) F2 (10% Conc.) F3 (15% Conc.) F4 (12% Conc. Peritop) F5 (10% Medlice)

Fig 5: Skin irritation test

Table 2: Physicochemical evaluation of formulated and Marketed shampoo

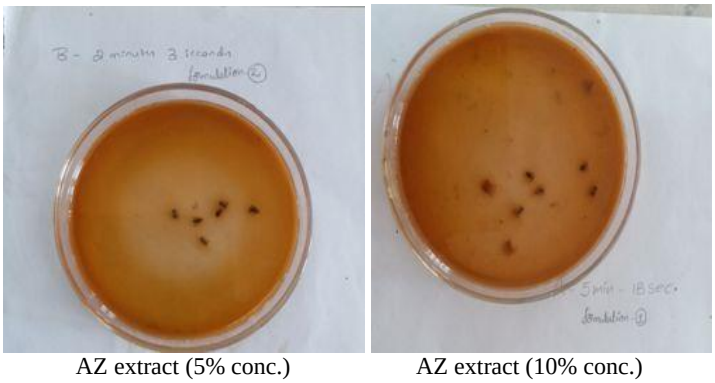
	F1 (5%w/w)	F2 (10%w/w)	F3 (15%w/w)	F4 (12%w/w Peritop)	F5 (10%w/w Medilice)
pH	6.00	6.012	6.15	6.09	7.00
% Solid content	23.75	25.32	24.24	22.37	23.45
Foam volume	65	65	72	58	60
Foam type	Small dense	Small dense	Small dense	big	big
Surface tension dyn/cm	35.78	35.34	39.94	38.78	37.52
Wetting time	184+8	187+4	240+2	240+2	308+6
Skin irritation	No irritation	No irritation	No irritation	No irritation	No irritation
Eye irritation	No irritation	No irritation	Slight Erythmea	No irritation	Slight Erythmea

4.2.10 Anti-headline activity of formulated shampoo

F1, F2, F3 formulated shampoos were tested for antiheadlice activity by comparing with marketed formulations like peritop (F4) and medlice (F5). 0.05 mL of each shampoo was spreaded on a petridish in a thin layer over a 2 cm^2 area. Equal sized head lice (n=6) were collected from college girls and cattle placed on petri dishes containing the above mentioned shampoos. Paralysed head lice which were determined as deadlice or paralysed lice were counted every 5 min unless all the lice were dead. Reeta extract (Shampoo base) was also tested for antiheadlice activity and it is considered as positive control. The same procedure has been conducted for crude extracts like *Sapindus murkosi* extract, *Azadiracta indica* extract, *Annona squamosa*, *Citrus sinensis* peel oil and *Eucalyptus globulus* leaf oil which showed significantly less death time for lice when compared to marketed formulations. The below data explains that plant materials contain insecticidal property due to the presence of Azadirachatin, 1, 8-cineole, triglycerides, oleic acid and lemonine. So all the formulated shampoos showed synergistic effect when concentration increases, paralysis time decreased. All the tested headlice killed within 18.35±5.51 minutes for F3 formulation whereas the marketed formulation Peritop has showed 25.25±4.05 min and Medlice has showed 29.15±0.03 min as death time. *Sapindus murkosi* extract which is a shampoo base killed all the headlice within 60 min. All the results are represented pictorially in Figures 7 and 8. All the formulated shampoos show maximum killing effect on headlice with safety using minimum concentration and less time.

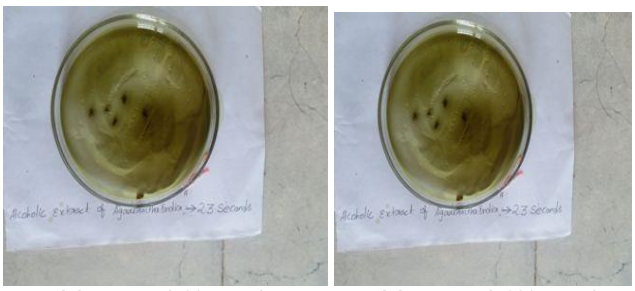
Table 3: Anti head lice activity of formulated and marketed shampoo

S. NO.	Formulations	Paralyse Time Or Death Time (Min)
1.	Formulation 1 -5%	30.76 ±5.05
2.	Formulation 2-10%	20.22±3.15
3.	Formulation 3-15%	18.35±5.51
4.	Peritop 12%w/w	25.25±4.05
5.	Medlice 12%w/w	29.15±0.03
6.	*Sapindus murkosi* extract (SM)	60 Minutes
7.	*Azadiracta indica leaf* extract	6 ±0.6
8.	*Annona squamosa* seed oil	5±15
9.	*Citrus sinensis* peel oil	5±75
10.	*Eucalyptus globulus* leaf oil	7±25



AZ extract (5% conc.) AZ extract (10% conc.)

F1 (5% conc.) F2 (10% conc.) F3 (15% conc.)



AS extract (5% conc.) AS extract (10% conc.)

Fig 6: Anti head lice activity of crude extracts at different concentrations

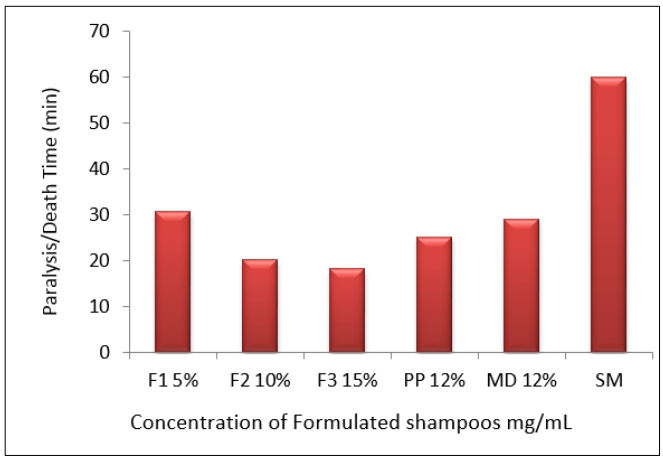


Fig 7: Anti head lice activity of Formulated shampoos at different concentrations

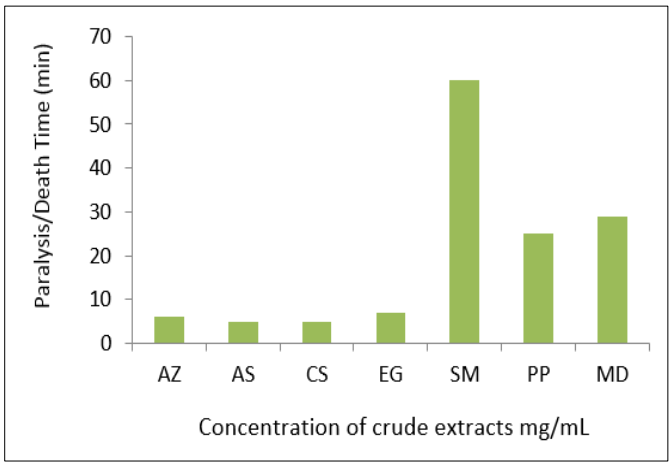


Fig 8: Anti head lice activity of crude extracts at different concentrations

5. Conclusion

The aim of this study was to formulate a completely polyherbal shampoo which is at poor with the synthetic shampoo available in the market. We formulated Herbal shampoo by using plant extracts which are constantly used traditionally launched for the hair cleaning, anti-dandruff, anti-head lice activity action across Asia. All the ingredients used to formulated shampoo are safer than silicone and poly quaternion synthetic conditioning agents which can greatly reduce the hair protein loss during combing instant of using cationic conditioners. We have used *Azadiracta indica*, Custard apple, citrus oil. Soap nut provides antidandruff ant head lice activity. Several tests were performed to evaluate and compare results with that of marketed shampoo showed comparable result with that of marketed shampoo for quality control test but further research and development is require to improve its overall quality.

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