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Novel formulation and evaluation herbal based lotion for the antimicrobial and antifungal properties

Shibanjan Paul Roy-Guide, Satyabrat Sarma and Shyam Prakash Rai

Abstract
By scientific increased evidence we know that plants are processed a vast and complex arsenal ingredients which have the ability to smooth the skin as well as restore very actively and protect and heal the skin. The present works deals with the development and analysis of new herbal lotion containing *Aniba rosaeodora*, *Ajania fruticulosa*, Meyer lemon, *Tithonia Diversifolia* and *Psidium guajava*. Formulations with different types of oil in water (O/W) herbal lotions were formulated by different concentrations of Lecithin and triethanolamine. Formulation of Triethanolamine and Lecithin was optimized 2.53:9.36. As we evaluated this prepared lotion has potent antimicrobial properties. This is the research which is guided by Guide-Mr. Shibanjan Paul Roy, Freelancer Scientist who lives in Race Course Para, Jalpaiguri. Researchers did research under his observation. The researchers are Mr. Satyabrat Sarma-Assistant Professor of Assam Downtown University and Mr. Shyam Prakash Rai completed B. Pharm from Assam Downtown University in 2019.

Keywords: Novel, evaluation, herbal, antimicrobial, antifungal

Introduction
Actually primarily added herbal extracts to cosmetic preparations due to several associated properties as antibacterial, anti-inflammatory and antioxidant properties. The lotion is a topical emulsion, suspensions and solutions are usually serve as vehicles for topically as emollients by applied drugs. They applied to the membranes and skin such as vaginal, nasal, rectal etc. By using other useful minerals herbal remedies enrich body with nutrients. The present invention can gives very effective protection to skin and free from toxicity when regularly used.

Materials and Methods
Plant Materials
These medicinal plants Aniba panurensis, *Ajania fruticulosa*, *Tithonia Diversifolia*, meyer lemon and *Psidium guajava* were collected by lab from Malbazar. The fruits of *Ajania fruticulosa*, leaves extract were sun dried for 7days.After drying the plants were crushed in powder. After for obtain juice of Meyer lemon was squeezed. The all plants were identified by our Guide-Mr.Shibanjan Paul Roy, Freelancer Scientist. The essential oils of *Aniba rosaeodora* and *Psidium guajava* were collected by lab.

Preparation of extracts
500gm of dried powder of parts of plants was extracted in ethanol with frequent agitation for 9days. Three times we did the process of extraction was carried out with the same sample. Then collected the filtrates and by water bath it will be evaporated. The Meyer lemon obtained by squeezing concentrated further in water bath.

Phytochemical Screening
All extracts are done by phytochemical screening out according to standard method (Trease & Evans 1989). After analyze the extracts for presence of tannins, phenols, flavonoids, glycosides, Tannins etc.

Antibacterial Property
Then evaluated antibacterial property by well diffusion method (Collins *et al*. 1995) [163]. The microorganisms used *Staphylococcus lugdunensis*, *Pseudomonas putida*, Fusobacterium nucleatum and *klebsiella oxytoca*. As in the sample total 20µl of test solutions of the sample and standard prepared of (2%, 4% and 6%) were then pipette by help of micropipette and

poured in prepared bore in swabbed of each nutrient agar plate with different bacteria with great care. Similarly, DMSO (10%) solution of 20 µl solution also poured in another bore as control. About 1hr the nutrient agar plates were allowed to stand till the test solution completely diffuses in the media. Then at the temperature 37 °C for 50hours. After 50hours, zone of inhibition was measured. MIC test then carried out by serial dilution method (Mhatre *et al*. 2014) [164]. The EO and extracts were serially diluted to obtain concentration from 225 mg/ml to 6.75 mg/ml. Full of microbial suspension which already prepared by a loop added to each test tube. Then incubated the test tubes at 37 °C for 25hours. The concentration of MIC of the highest dilution tube in which bacterial growth was totally absent.

Evaluation of Pharmaceutical parameters of Lotion

Basically Pharmaceutical evaluation of lotion formulations was carried out

PH: Lotion PH was measured with a digital PH meter. The solution was immersed in the PH meter before the 10% solution of lotion was prepared and the solution immersed in PH meter after the measurement recorded- (Namita & Nimisha 2013) [166].

Viscosity: The evaluation of viscosity done by Brookfield viscometer by using LV-64 spindle. By adjustment of rotation rate was 25RPM. Then into the spindle and the viscosity was measured for the formulated lotion.

Spread ability: By using the parallel plate method the spreaditibility of lotion determined. 20/20 cm were selected of Two glass slides. One of the slides placed about 1gm of the lotion formulation. Upon the top of the lotion the other slide was placed that lotion was sandwitched between the slides and 125gm of weight was placed upon the upper slide so, that lotion between two slide was pressed to form a thin layer uniformly. The weight was removed and the spread diameter was measured. (Garg *et al*. 2002) [167].

Stability Test: At different temperatures the formulated lotion was stored and humidity conditions 26±2 °C / 60±5% RH (at room temperature), 40±3 °C / 74±8% RH (accelerated temperature) for a period of 3.5 months then after studied for PH, viscosity and spreadability (Negi *et al*. 2012) [168].

Sensitivity Test: On the forearms of 6 volunteers a portion of lotion was applied and left for 25minutes. After 25minutes if anykind of irritation occurred then this was noted. (Draize *et al*. 1944) [165].

Washability Test: Over the skin a portion of lotion was applied and after under the forcing tap water for 10minutes must allowed to flow. The time was noted when the lotion completely removed.

Appearance: The odour, colour and homogeneity of lotion were visually determined.

Type of Emulsion Test: To determine the type of emulsion formed dye solubility and dilution test was conducted. (Tharwat 2013).

Results and Discussion

Phytochemical Screening

The phytochemical screening performed when the plants were undertaken as *Ajania fruticulosa* the phytochemical found as myrtenol, hexadecane, chrysanthenone, carvacrol, pinocarvone, myecene, eugenol, thymol, eucalyptol, terpinolene etc. as myrtenol used in orofacial pain and inflammation, carvacrol as antioxidant, anticancer properties, eucalyptol has high antifungal properties.

After Tithonia *Diversifolia* it has phenolics, flavonoids, tannins and saponins that was detected.

After we found that Meyer lemon has citronellol, alpha-terpineol, isopulegol, sabinene, limonene.

As we know that citronellol used in for ease of headache, reduce muscle spasms.

Sabinene has good anti-fungal and anti-inflammatory properties.

Limonene has potent antioxidant properties and it reduce the large amount of oxidative stress from body.

Aniba rosaeodora has linalool, alpha-terpinolene etc.

As linolool has anti-inflammatory properties and alpha-terpinolene has good antibacterial properties.

Psidium guajava has quercetin, apigenin, myricetin, gallic acid, hyperin etc.

We know quercetin used for allergies, cancer etc.Apigenin used as very potent antibacterial drug.

Antibacterial activity study

For antibacterial activity ethanol extract of plant were undertaken. Among these plants *Ajania fruticulosa* has very high antifungal and antimicrobial properties and other plants also has very good antibacterial and antifungal properties. But *Aniba rosadera* has less than others.

Table 1: Zone of inhibition of plant extracts against different bacteria

Extract	Zone of inhibition (mm)				
	Concentration (%)	*Staphylococcus lugdunensis*	*Pseudomonas putida*	*Fusobacterium nucleatum*	*Klebsiella oxytoca*
Aniba rosaeodora	2	6.91±2.77	8.91±2.83	6.91±1.83	4.58±1.83
	4	9.91±2.77	10.58±2.4	9.25±2.25	8.58±2.77
	6	10.91±2.77	12.25±2.25	11.91±1.83	11.25±2.25
Ajania fruticulosa	2	16.25±2.25	15.91±1.83	16.58±3.77	13.25±2.25
	4	18.58±1.83	18.58±2.4	17.25±2.25	16.58±2.77
	6	21.88±1.83	20.25±2.25	20.25±2.25	19.91±2.83
meyer lemon	2	15.91±2.77	18.25±3.25	18.58±2.4	19.58±2.77
	4	20.25±2.25	21.25±2.25	21.25±2.25	20.58±2.77
Tithonia diversifolia	6	22.25±2.25	23.5±2.25	22.25±2.25	22.58±2.77
	2	14.91±2.77	14.25±2.25	14.91±2.4	13.25±2.25
	4	18.58±2.4	20.58±2.77	15.91±2.77	15.91±2.83

	6	22.58±2.77	23.25±2.25	19.25±2.25	20.58±2.77
Psidium guajava	2	16.58±3.33	17.25±2.25	14.91±2.4	14.91±2.77
	4	19.91±1.83	18.58±1.58	18.25±2.25	18.58±2.83
	6	23.58±3.33	23.58±2.77	21.25±2.25	20.91±2.4
Standard cipro	6	25.58±2.4	26.91±2.77	26.25±2.25	25.58±2.83
Standard Amox	6	25.58±1.83	25.25±2.25	25.91±2.4	26.25±2.25

Among the five different combination of extracts, ratio 2 (R2) showed good antibacterial property as compared to remaining others. Since R2combination shows highest antibacterial activity, same was used for lotion preparation.

Table 2: Zone of inhibition of ratios of extracts against different bacteria

Microorganisms	R1	R2	R3	R4	R5	Std. Cipro	Std. Amox
Staphylococcus	19.25±2.25	21.91±	18.58±	16.58±	16.91±	26.25±2.25	27.25±2.25
lugdunensis		2.4	2.4	1.83	1.83		
Pseudomonas	17.58±	21.91±	16.91±	17.58±	13.91±	26.25±2.25	27.25±2.25
putida	1.83	1.83	1.83	1.83	1.83		
Fusobacterium Nucleatum	15.58±	19.91±	17.25±2.25	14.25±2.25	16.25±2.25	26.91±2.4	26.91±3.33
	1.83	2.78					
Klebsiella	15.25±2.25	17.58±	15.91±	13.25±2.25	13.58±	25.91±2.4	27.91±2.4
oxytoca		2.4	2.78		1.83		

R1= 1:1:1:1; R2= 1:1:1:2; R3=2:1:1:1; R4=1:2:1:1; R5=1:1:2:1 (*Ajania fruticulosa*: Meyer lemon: *Tithonia Diversifolia*: *Psidium guajava*)

Table 3: Antibacterial activity of final formulation compared to the marketed product Zone of Inhibition (mm) of final formulation (F2)

Microorganisms	Formulation	Std.
Staphylococcus lugdunensis	21.25±2.25	24.58±1.82
Pseudomonas putida	20.58±2.78	23.58±3.33
Fusobacterium Nucleatum	18.58±1.83	23.91±2.4
Klebsiella oxytoca	17.58±2.77	23.91±1.83

Minimum Inhibitory Concentration: It was shown that *Ajania fruticulosa*, *Tithonia Diversifolia* and *Psidium guajava* against *Staphylococcus lugdunensis* MIC of 25 mg/ml. MIC of 25 mg/ml was shown by Meyer lemon against Pseudomonas putida. Against Fusobacterium nucleatum the best MIC (50 mg/ml) was shown by *Psidium guajava* as compared to other extracts and essential oil. Against *Klebsiella oxytoca* all of extracts and essential oils showed MIC of 100 mg/ml which is not very much effective.

Table 4: Chemical Composition of formulation in percentage

Formulation No.	Lecithin	Cetostearyl alcohol	Orange wax	Mineral Oil	TEA	Propylene Glycol	Water
F1	9.99	1.22	9.74	12.20	2.99	1.22	QS to 100 ml
F2	9.99	1.22	9.74	12.20	2.99	1.22	QS to 100 ml
F3	4.99	1.22	9.74	12.20	3.99	1.22	QS to 100 ml
F4	17.06	1.22	9.74	12.20	2.99	1.22	QS to 100 ml
F5	14.99	1.22	9.74	12.20	1.99	1.22	QS to 100 ml
F6	14.9	1.22	9.74	12.20	3.99	1.22	QS to 100 ml
F7	9.99	1.22	9.74	12.20	4.40	1.22	QS to 100 ml
F8	9.99	1.22	9.74	12.20	2.99	1.22	QS to 100 ml
F9	2.82	1.22	9.74	12.20	2.99	1.22	QS to 100 ml
F10	9.99	1.22	9.74	12.20	2.99	1.22	QS to 100 ml
F11	9.99	1.22	9.74	12.20	1.57	1.22	QS to 100 ml
F12	9.99	1.22	9.74	12.20	2.99	1.22	QS to 100 ml
F13	4.99	1.22	9.74	12.20	1.99	1.22	QS to 100 ml

Active plant extracts and essential oils combination was 5% in the formulation composition

Effect of independent variables on lotions

Effect on Viscosity of Lotion

It was shown that triethanolamine has negative effect on the viscosity whereas Lecithin has a positive effect on viscosity. Triethanolamine decreases viscosity by the increase in the concentration and increase in concentration of lecithin increase the viscosity.

Effect on pH of Lotion

The positive effect of triethanolamine that represents by the positive magnitude of the coefficient of Triethanolamine. The increase of pH of the formulation by the increase in concentration of Triethanolamine. Similarly, the increase in concentration of lecithin causes the decrease of PH of the formulation.

Effect on Spreadability of Lotion

Triethanolamine increases the spreadability of lotion when triethanolamine has positive effect. In contrast the Lecithin increases the spreadability of the lotion decreases. Triethanolamine indicated the positive coefficient value of the increase in spreadability as the concentration of Trimethanolamine increases.

Evaluation of Pharmaceutical parameters of lotions

The pH of the lotion maintained within the limit by which we known that lotion is safe to use for skin and it is stable. The pH of lotion shows it's stability. As the viscosity of the medium increase by with the stability of lotion increases. Among 13 different formulations the value of viscosity differed from 4909 cps to 10188 cps. As the spreadability

range for lotion was from 7 to 13 cm. We calculated the measured spread diameter was minimum of the lotion was 8.3 cm and maximum 10.4 cm.

The lotion was water removable and not irritating the skin. The lotion was found to be yellowish white in colour by the final appearance. During the formulation emulsion formed of lotion was evaluated for the type of emulsion. With the sample oil dye miscible dye (Sudan III) was mixed and observed under microscope. Surrounded by water phase small red droplets of oil can be observed. This helps to evaluate that lotion was oil-in water emulsion. The formed of the lotion was oil in water type.

Optimization of formulation

The pH suitable range for hand lotion was 4-7 and spreadability the desired range was 7 to 13cm. The viscosity should such that the lotion can be easilyspread over the skin surface. Then for the response parameters the optimized formulation was evaluated and the value optimized 5.7, value of viscosity was 5562 cps and the value of spreadability 9.8. All the parameters were suitable for application over skin and were within the limit. The desired results showed (2.53:9.36) ratio of Triethanolamine and Lecithin as optimum. So, on the basic ratio obtained from the software final optimized formulation was developed.

Table 5: Value of Viscosity, pH and spreadability obtained for 13 different formulations

Formulation	pH (4-7)	Viscosity (<10000) cps	Spreadibility (7-13) cm
F1	5.58	5963	9.4
F2	5.75	5682	9.8
F3	7.03	5014	10.3
F4	5.42	10188	8.3
F5	5.14	9237	8.5
F6	6.20	7573	8.6
F7	6.79	6926	8.9
F8	5.6	5830	9.6
F9	5.97	4909	10.4
F10	6.02	5440	9.9
F11	5.12	6179	9.1
F12	6.06	5373	10
F13	5.26	5215	10.1

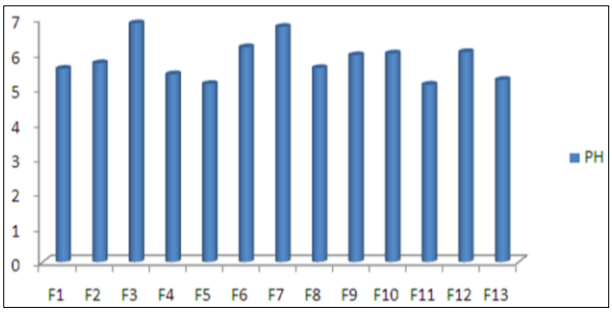

Fig 1: pH

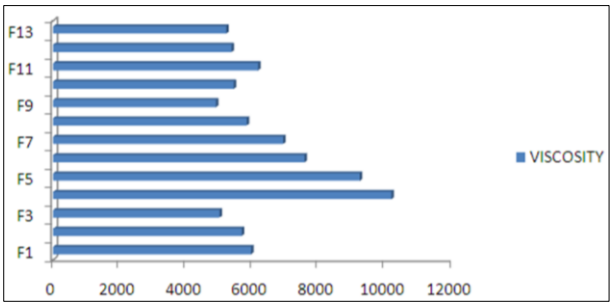

Fig 2: Viscosity

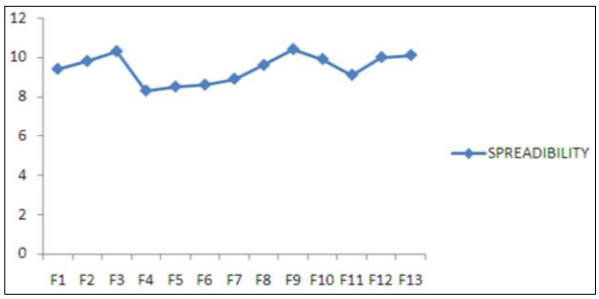

Fig 3: Spread ability

Stability Test

The final optimization of lotion of stability test was carried out and it was found that the lotion was stable in room temperature and for at least 3months the temperature was accelerated. The value of pH, viscosity and spreadibility compared as initial value

Table 6: Stability test at room temperature and accelerated temperature (of optimized formulation)

Parameters	Initial value	Room temperature (25±2) ºC			Accelerated temperature (40±2) ºC/75% RH		
		1 month	2 month	3 month	1 month	2 month	3 month
Viscosity	5562 cps	5555 cps	5566 cps	5560 cps	5551	5565	5572
pH	5.7	5.8	6	5.9	5.9	6	6.2
Spreadability	9.9 cm	9.7 cm	10 cm	9.8 cm	9.7 cm	10 cm	10.1 cm

Conclusion

Oil in water type lotion was properly formulated by using formulation of *Ajania fruticulosa: meyer lemon: Tithonia Diversifolia: Psidium guajava*at the ratio of 1:1:1:2. This is possible as optimum different plant extracts as well as synergize the cosmetic properties of prepared products differentiate to individual extracts. From this present invention it has been revealed that lotion was stable in room temperature and accelerated temperature for at least three months. In this research there is a problem that's for memorize I named the F1-Muzzpiydeemon as like others F2 to F13.

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Bokaro. In this research total works done under the guidance of Guide-Mr. Shibanjan Paul Roy. Mr. Satyabrat Sarma and Mr. Shyam Prakash Rai performed for the plants extraction and others work and note the reading.

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