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Original Research Article

'Psoriasis warning' from Anti-Psoriatic AYUSH Drugs

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Abstract

Rancidity of several anti-psoriatic drugs of AYUSH (Ayurveda & Siddha) were studied and the ill effects of acid value was established by free radical scavenging assay, nitric oxide inhibition, anti-glycation, anti-angiogenesis and fibroblast toxicity. Our findings clearly show that rancidity not only produced severe side effects but also modified the therapeutic constituents from herbal source thus making the Ayush preparations more harmful to the psoriatic patients than as remedial. Several anti-psoriatic oils were compared with Dr. JRK's 777 oil and Psorolin oil. Findings clearly show that the Dr. JRK's 777 oil and Psorolin oil is the best, safe and effective drug for psoriasis than many other Ayush oils available in the market. Details are presented in the paper.

Keywords: 777 oil, *Wrightia tinctoria*, Herbal treatment for Psoriasis, CAM assay, Rancidity.

INTRODUCTION

Psoriasis is an incurable auto-immune disorder of the skin that manifest as hyper proliferation of the epidermal cells with impaired skin barrier effect. The inflammatory changes do occur at different time intervals. Although several predisposing factors are hypothesized but the disease remain incurable and at best psoriasis can be managed and the remission phase can be prolonged [1, 2].

In modern medicine several drugs such as anti-mitotic preparations, topical/oral immune suppressive agents etc., are used however such therapy has definite side effects as well. UV therapy is also used and similarly monoclonal antibody therapy is also available. However, all such therapies are quite expensive [3].

For the palliative therapy, large number of psoriatic patients in India often flocks around alternative stream of healing practices. Several herbal preparations are vastly used for the treatment of psoriasis and as a result the business opportunity available for several manufacturers in India to introduce several single to poly herbal oils for psoriasis have increased significantly and such market is growing exponentially and unabated.

Among various herbal oils from the alternative stream of healing practice for the treatment of psoriasis are available the oil prepared with the leaves of *Wrightia tinctoria* has occupied an exalted position in Indian market. Since the licensing system for the products from alternative stream of healing in India is not harmonized and is currently under the purview of respective state licensing authority. Further the quality norms for such products are not rigorous like the allopathic drugs. This has resulted in the entry of several herbal oils with *Wrightia tinctoria* as one of the ingredients for the treatment of psoriasis in Indian market [4].

In our earlier study we have established that the base oil such as coconut oil or gingelly oil used for preparing *Wrightia tinctoria* based preparation by different manufacturers showed high acid value due to rancidity. The rancid oils are bound to worsen the problem of psoriasis and would further trigger the inflammatory response. The *Wrightia tinctoria* oil with high acid value is bound to harm psoriasis than act as drug. However such side effects are seldom noticed or reported may be due to the acceptance of the fact that psoriasis is incurable and herbal drugs are always safe.

The frequency of inflammatory episode vis-à-vis use of oils with high acid value for the treatment of psoriasis is not known due to lack of any focused clinical trial.

In the present paper we have studied and compared the rancidity status of several anti-psoriatic Siddha/ Ayurveda oils. Further the implication of the acid value in worsening the psoriatic condition was

assessed by a battery of in vitro tests such as DPPH assay, nitric oxide inhibition anti-glycation, and angiogenesis by CAM assay and fibroblast toxicity. Findings clearly show that rancidity is directly proportional to toxicity and inversely proportional to modifying the herbal constituents. Details are presented in the paper.

MATERIALS AND METHOD

Details of the products studied

Dr. JRK's 777 oil

Dr. JRK's 777 oil is a registered, licensed proprietary Siddha product of Dr. JRK's Research and Pharmaceuticals. The oil is prepared with the leaf extract of *Wrightia tinctoria*. The product has been studied for the complete toxicity profile in order to establish its safety by following OECD guidelines. The tests performed for Dr. JRK's 777oil were

- Skin Sensitization in Guinea pig
- Effect on Mucus membrane
- In vitro Mammalian cell gene mutation test-chinese hamster ovary K1 cell line
- Bacterial reverse mutation test using S. typhimurium and E.coli WP2 uvr A
- Mammalian erythrocyte Micronucleus testing swiss albino mice
- Acute lethal dose
- Reproduction developmental toxicity screening

Psorolin oil

Psorolin oil is a registered, licensed proprietary Siddha product of Dr. JRK's Research and Pharmaceuticals. The oil is prepared with the leaf extract of *Wrightia tinctoria*, *Indigofera tinctoria*, *Indigofera aspalathoides*.

Details of sample used for tests

S. No	Sample details	Herbal ingredients
1	Dr. JRK's 777 oil	*Wrightia tinctoria*
2	Psorolin oil	*Wrightia tinctoria, Indigofera tinctoria, Indigofera aspalathoides*
3	Market sample-1	*Wrightia tinctoria and Hydnocarpus igdhiana*
4	Market sample-2	*Wrightia tinctoria*
5	Market sample-3	*Wrightia tinctoria, Rubia cordifolia, Psorolia corylifolia*
6	Market sample - 4	*Wrightia tinctoria*
7	Market sample-5	*Holarrhena antidysenterica, Andropogon annulatus, Hemidesmus indicus, Psoralea corylifolia, Azadirachta indica*
8	Market sample-6	*Azadirachta indica, Pongamia pinnata, Curcuma longa, Piper nigrum*
9	Coconut oil with acid value of 1.4	
10	Rancid Coonut oil with acid value of 25	

DPPH assay

Anti-oxidant (DPPH) or Free Radical Scavenging Assay

The DPPH (2,2 diphenyl-1- picryl hydrazyl) assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives maximum absorption at 517 nm (purple colour). When antioxidants react with DPPH, the molecule is reduced to DPPHH and as consequence, the absorbance decreases. The reduction of DPPH to DPPH-H results in more or less decolorization (yellow colour) with respect to the number of electrons captured. More the decolorization, more is the reducing ability of the test material. This test has been the most accepted model for evaluating the free radical scavenging activity of many chemical compounds [5].

When a solution of DPPH is mixed with the in-study substance, it can donate a hydrogen atom giving rise to the reduced form of di phenyl picryl hydrazine; a non-radical with the loss of violet colour (although there would be a residual pale yellow colour from the picryl group if present).

Different concentrations (1,2, 3 mg/ml) of the samples were added to toluene solution of DPPH radical. The final concentration was adjusted to 0.1mM. The mixture was shaken vigorously for 1 minute by vortexing and left to stand at room temperature in dark condition for 30 minutes. The absorbance of the samples was measured using UV 160 spectrophotometer at 517nm against ethanol blank. A negative control was taken after adding DPPH solution to 0.2ml of water. The percent of DPPH discoloration of the sample was calculated according to the equation

$$\% \text{ discolouration} = [1 - (\text{sample/control})] \times 100.$$

Inhibition of Nitric Oxide (NO) Production

Nitric oxide (NO) is a diatomic free radical produced from L-arginine by constitutive and inducible nitric oxide synthase (cNOS and iNOS) in numerous mammalian cells and tissues. Nitric oxide (NO), superoxide (O2-) and their reaction product peroxynitrite (ONOO-) may be generated in excess during the host response against viral and antibacterial infections and contribute to some pathogenesis by promoting oxidative stress, tissue injury and even cancer.

Oxidative damage, caused by action of free radicals, may initiate and promote the progression of diseases that indicates inflammation. The mechanism of inflammation injury is attributed, in part to the release of reactive oxygen species (ROS) from the activated neutrophils and macrophages. ROS propagate inflammation by stimulating the release of mediators such as NO and cytokines.

Macrophages were seeded at the density of 5 x 10^3 cells per well in a micro titer plate. The plate was incubated for 12 h at 37˚ C with 5% CO_2. Then media of each well was then aspirated, added and fresh FBS-free DMEM media was replaced. Different concentrations (5, 10 and 15 mg/ml) of the samples oils were used for testing [5].

After 1 h treatment, the cells were stimulated with 1 µg/ml of LPS for 24 h. The presence of nitrite was determined in cell culture media using commercial NO detection kit. After 10 minute incubation, the absorbance was measured at 540 nm using microplate reader. The amount of nitrite in the media was calculated from sodium nitrite (NaNO2) standard curve.

Anti-Glycation Assay

Glycation is the key molecular basis of several diabetic complications like diabetes retinopathy, nephropathy, neuropathy and some cardiovascular diseases. This is a non-enzymatic reaction between amino groups of proteins and carbonyl groups of reducing sugars forming a fluorescent, insoluble advanced glycation end product that accumulate as long living protein thus compromising the physiological function. Glycation is a non-enzymatic condensation reaction between reducing sugars and amino groups of proteins that undergo rearrangements to stable ketoamines, leading to the formation of advanced glycation end products (AGEs) [5].

Antiglycation activity was determined using the bovine serum albumin assay with slight modification. In the experiment, the final reaction volume was 1.0 ml and carried out in 1.5 ml Eppendorf tubes. Bovine Serum Albumin 500 µl (1 mg/ml concentration) was incubated with glucose 400 µl (500 mM final concentration) and different concentrations of the samples (50, 40, 30, 20, 10 mg/ml) in 100 µl of phosphate buffer saline was used and 100 µl Arbutin was used as standard. A negative control was carried out at the same time with BSA 500 µl (1 mg/ml concentration), 400µl phosphate buffer saline and the different concentrations of the sample (as described above) incubated under the same conditions.

The reaction was allowed to proceed at 60 °C for 24 hours and the reaction was terminated by adding 10 µl of 100% (W/V) trichloroacetic acid (TCA). The TCA added mixture was kept at 4 °C for 10 minutes and then centrifuged for 4 minutes at 13000 rpm. The precipitate was re-dissolved in alkaline phosphate buffer saline (pH 10) and was quantified for the relative amount of glycated BSA based on fluoresce intensity by Fluorescent Microplate Reader. The excitation and emission wavelength that used were 370 nm and 440 nm respectively. Each concentration of the sample was analyzed thrice. Percentage of inhibition was calculated and the sample concentration required for the 50% of inhibition was calculated using the formula

% of inhibition = OD blank – (OD sample – OD sample negative)/OD blank x 100

Angiogenesis- CAM assay

Chick chorio allontoic membrane is an extra embryonic membrane which is rich in blood vessels and is widely used in assessment of angiogenic and anti - angiogenic products. In the present study we have used 9 days old fertilized eggs. The eggs were surface sterilized and then incubated at 37^0C for one day. Then the eggs were observed under egg candling box to visualize the presence of blood vessels. A small hole was drilled in the air space to drain out the air. Further a small square shaped window was drilled out on the egg shell without damaging the membrane beneath. 10 eggs were used for each experiment [6].

Different concentrations of the samples (1,2 mg/ml) were used for the study. The sample was loaded in sterilized methyl cellulose disc which was then carefully placed in the window made in the egg. The eggs were then incubated for further 3 more days at 37 C. After incubation the egg was opened and the extent of angiogenesis was observed. Saline was used as negative control and SLS was used as positive control.

Fibroblast Toxicity Assay

MTT assay - Principle

The cell proliferation occurs as a result of the mitochondrial succinate-terazolium reductase system that convert 3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) to a blue colored formazan. The test denotes the survival cells after exposure to the test samples.

Cell Culture Study

Fibroblast cell line was used for the present study. The cells were maintained and sub cultured in 25mm2 tissue culture flasks using 5 ml of minimum

essential medium (MEM) supplemented with 10% fetal bovine serum, 3% L-glutamine, penicillin (100 IU/ml), streptomycin (100 µg/ml), Amphotericin B (20 µg/ml), phenol red. The pH of the medium was adjusted to 7.2-7.4 with 7.5% sodium bicarbonate and all flasks were incubated at 37˚C in a humidified 5% CO2 /95% O2 incubator.

Cells were grown in an appropriate growth media and seeded at the concentration of 6 × 104 cells per ml of media in a 24 well plate. Cells were allowed to adhere to the bottom for 12 hours followed by treatment with different concentrations of the oils and incubated for 48 hours at 37 °C with 5 % CO_2. After treatment of 48 hours, cells were treated with 10% MTT in media for 4 hours in 37 ℃ and 5 % CO2. Media was then aspirated and the adherent cells were treated for formazon product that was dissolved in DMSO, centrifuged at an RPM of 5000 for 15 minutes to remove debris and the spectrophotometric absorbance of the sample was measured using a microplate reader. The wavelength used for measuring the absorbance of formazan product was 570 nm and the percentage of reduction in cell proliferation was determined.

RESULTS

Rancidity Assay

Among the eight anti-psoriatic oils tested for acid value, Dr. JRK's 777 oil and Psorolin oil showed least acid value, whereas other 6 products procured from the market showed high acid value which was above BIS () standard for coconut oil Table-1.

Table-1: Acid value of various anti –psoriatic oils

S. No	Product name	Category	Acid Value
1	Dr. JRK's 777 oil	Siddha product	1.2
2	Psorolin oil	Siddha product	1.1
3	Market sample 1	Siddha product	9.63
4	Market sample 2	Siddha Product	26.3
5	Market sample 3	Ayurveda product	9.46
6	Market sample 4	Ayurveda product	22.95
7	Market sample 5	Ayurveda product	11.73
8	Market sample 6	Ayurveda product	16.6
9	Coconut oil	-	0.85
10	Rancid coconut oil	-	25.2

DPPH Assay

Among the 8 anti-psoriatic oils tested for free radical scavenging (anti-oxidant) activity Dr. JRK's 777 oil and Psorolin oil showed high anti-oxidant effect in concentration dependent manner. The percentage of inhibition of free radical generation exhibited by the above two oils were 83.5 and 88. 5 respectively. Other six market samples showed poor anti-oxidant activity irrespective of the concentration range from 1 to 3 mg/ml. The coconut oil with acid value of 0.85 showed 88 % inhibition of free radicals whereas the coconut oil with acid value of 25 showed 35.5 % inhibition of free radical generation Table-2.

Table-2: Free radical scavenging assay of various anti-psoriatic oil

Sample	% inhibition/ Concentration in mg/ml		
	1	2	3
Dr. JRK's 777 oil	78.0	82.0	83.5
Psorolin oil	85.5	86.8	88.5
Market sample 1	42.5	46.0	48.2
Market sample 2	40.5	42.5	44.8
Market sample 3	32.3	36.5	37.2
Market sample 4	38.5	39.2	39.8
Market sample 5	44.2	45.6	46.1
Market sample 6	28.5	29.2	30.1
Coconut oil	85.0	88.3	88
Rancid coconut oil	40.5	38.5	35.5

Inhibition of Nitric Oxide (NO) Production

The effect of various oils on NO production inhibition is presented in the Table-6.

In order to reconfirm the antioxidant effect of various anti-psoriatic oils tested by DPPH, nitric oxide inhibition assay was performed. LPS was used as a positive control to induce NO production. The values of NO production after treatment with different anti-psoriatic oils were subtracted from the NO value after treatment with LPS to arrive the percentage inhibition of NO. Dr. JRK's 777 oil and Psorolin oil showed high activity in inhibiting NO production with 84 and 89 % respectively. Other six anti-psoriatic oils showed poor activity with reference to NO inhibition Table-3. A marginal co-relation between activity and concentration was observed.

Table-3: Inhibition of Nitic oxide

Sample Details	Concentration of sample in mg/ ml and % of NO production [Values in the parenthesis denote % inhibition]		
	5	10	15
Only LPS (Lipo Poly Saccharide Positive control) [83% production of NO]			
Dr. JRK's 777 oil	43 [48]	21 [75]	13 [84]
Psorolin oil	33 [6]	18 [78]	9 [89]
Market sample 1	56 [33]	49 [41]	38 [54]
Market sample 2	63 [24]	69 [17]	76 [8]
Market sample 3	89 [-7]	91 [-10]	86 [–4]
Market sample 4	71 [14]	82[1]	88 [-6]
Market sample 5	73 [12]	74 [11]	84 [-1]
Market sample 6	54 [35]	68 [18]	81 [2]
Coconut oil	20 [76]	44 [47]	59 [29]
Rancid coconut oil	29 [65]	32 [61]	48 [42]

Anti-glycation Assay

Anti-glycation effect of various oils, i.e., IC50 concentration. IC50 concentration of Arbutin was at 0.8 mg/ml. Dr. JRK's 777 oil and Psorolin oil at 10 and 5 mg/ml respectively exhibited IC50 value in inhibiting the glycation event. Interestingly the coconut oil with acid value of 0.85 also showed IC50 activity at 10 mg/ml. other anti psoriatic oils procured from the market showed poor anti-glycation activity Table-4.

Table-4: Anti-glycation effect of various anti- psoriatic oils

Sample details	Inhibition of glycation event - IC 50 concentration in mg/ml
Dr. JRK's 777 oil	10
Psorolin oil	5
Market sample 1	30
Market sample 2	50
Market sample 3	20
Market sample 4	30
Market sample 5	50
Market sample 6	50
Coconut oil	10
Rancid coconut oil	20

CAM Assay

Dr. JRK's 777 oil and Psorolin oil did not induce angiogenesis. Neither secondary nor tertiary blood vessels had increased due to the treatment of Dr. JRK's 777 oil or Psorolin oil. The concentration of the above oils did not have any effect on angiogenesis. Rest of the anti-psoriatic oils showed significant effect in inducing angiogenesis in both at secondary and tertiary blood vessels Table-5.

The Wrightia tinctoria extract did not induce angiogenesis however when the extract was treated in rancid oil showed significant impact in inducing angiogenesis in chick embryo CAM assay. Interestingly although rancid oil induced angiogenesis the extend of its angiogenesis was lower than that of Wrightia extract treated in rancid oil.

Table-5: Effect of various anti-psoriatic oil in inducing angiogenesis (CAM assay)

Sample	Concentration (mg/ml)	Number of blood vessels/ hour after treatment					
		Secondary			Tertiary		
		48 h	72 h	96 h	48 h	72 h	96 h
Control (Normal saline)	-	16	17	16	172	172	169
Dr. JRK's 777 oil	1	18	18	19	160	165	162
	2	17	18	18	162	167	165
Psorolin oil	1	16	18	16	160	164	166
	2	16	17	17	162	161	162
Market sample 1	1	19	18	18	170	173	181
	2	19	18	17	177	172	185

Market sample 2	1	21	23	27	180	194	200
	2	22	25	28	185	182	202
Market sample 3	1	23	24	28	189	192	213
	2	24	26	29	190	194	211
Market sample 4	1	30	32	38	200	204	218
	2	32	35	39	201	208	220
Market sample 5	1	32	38	40	211	211	218
	2	34	39	42	215	212	222
Market sample 6	1	38	41	47	220	222	230
	2	39	42	48	221	222	232
Wrightia tinctoria extract	1	15	15	16	169	170	172
	2	16	17	16	172	170	170
Wrightia tinctoria extract in Rancid coconut oil	1	33	32	34	190	200	211
	2	32	34	35	199	211	213
Coconut oil	1	16	16	16	171	170	172
	2	15	15	17	172	171	170
Rancid Coconut oil	1	21	22	23	180	184	187
	2	22	24	25	190	186	189

Fibroblast Toxicity Assay

Dr. JRK's 777 oil and Psorolin oil did not show any cytotoxic effect on fibroblast whereas all the 6 market samples showed very high fibroblast toxicity at low concentration. The fibroblast toxicity was directly proportional to acid value Table-6.

Table-6: Fibroblast toxicity assay

Sample	IC90 concentration in µg/ml	Toxicity ranking
Dr. JRK's 777 oil	2000	Nil
Psorolin oil	2000	Nil
Market sample 1	300	High
Market sample 2	10	Very high
Market sample 3	10	Very high
Market sample 4	5	Very high
Market sample 5	10	Very high
Market sample 6	10	Very high
Coconut oil	2000	Nil
Rancid coconut oil	100	High

Free radical scavenging assay for various herbal extracts

The herbal extracts that were used in the formulation of all the eight anti-psoriatic oils were tested individually for their anti-oxidant effect.

All the 13 herbal extracts used in formulating different anti-psoriatic oil showed good anti-oxidant activity. However the activity of *Wrightia tinctoria* was the highest.

We have grouped all the herbs into three categories based on their individual activity under the scale – high, medium and low. 3/13 herbal extracts showed high activity and 4/13 showed medium activity and the rest fell under the category of low activity Table-7.

Table-7: Free radical scavenging assay of various herbal extracts

S.no	Sample	% inhibition/ Concentration in mg/ml		
		1	2	3
1	*Wrightia tinctoria* extract	85	88	92
2	*Indigofera tinctoria*	75	77	78.5
3	*Indigofera asphalathoides*	64.5	66.2	67.8
4	*Hydnocarpus igdhiana*	78.5	80.1	82.5
5	*Azadirachta indica*	80.5	82.5	86.0
6	*Pongamia pinnata*	68.2	70.5	71.2
7	*Curcuma longa*	72.5	76.2	78.0
8	*Piper nigrum*	65.3	67.5	69.5
9	*Rubia cordifolia*	58.5	62.5	68.5
10	*Psoralea corylifolia*	65.5	68.2	71.5
11	*Holarrhena antidysenterica*	68.5	72.5	73.5
12	*Andropogon annulatus*	55.5	58.5	60.2
13	*Hemidesmus indicus*	52.5	56.5	68.5

Free radical scavenging assay of various herbal extracts treated in rancid coconut oil

To understand the impact of rancidity upon the anti-oxidant effect of different herbal extracts the present study was under taken.

All the 13 herbal extracts showed great dip in the anti-oxidant activity when tested them after treating in rancid coconut oil with an acid value of 25. The coconut oil with least rancidity showed high DPPH activity whereas the coconut oil with high acid value showed poor DPPH activity Table-8.

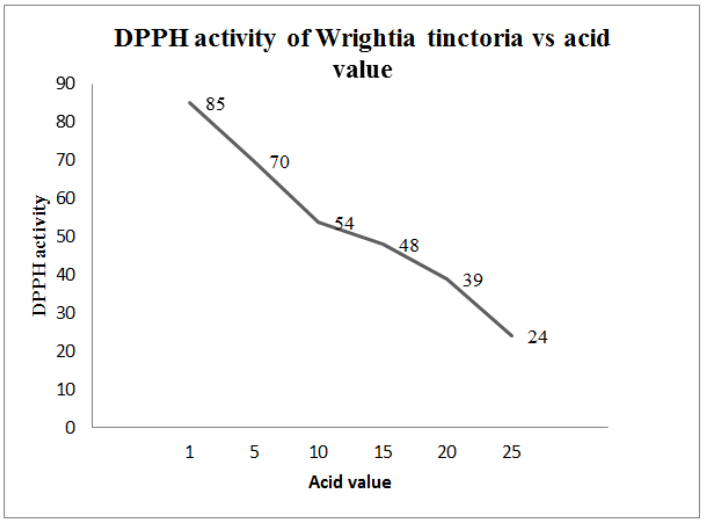
Higher the concentration of extract, lower was its anti-oxidant activity when treated in rancid oil. This finding totally contradicts our earlier observation on the increased concentration of extract showing increased activity when not treated in rancid oil.

Table-8: Free radical scavenging assay of various herbal extracts treated in rancid coconut oil

S.no	Sample	% inhibition/ Concentration in mg/ml		
		1	2	3
1	*Wrightia tinctoria*	55	52	50
2	*Indigofera tinctoria*	42	40	38
3	*Indigofera asphalathoides*	38	35	32
4	*Hydnocarpus igdhiana*	19.5	20	25
5	*Azadirachta indica*	44.8	42	40.5
6	*Pongamia pinnata*	28.5	25.0	22.0
7	*Curcuma longa*	35.0	32.0	28.0
8	*Piper nigrum*	18.5	15.5	12.5
9	*Rubia cordifolia*	22.5	18.5	17.1
10	*Psoralea corylifolia*	36.5	32.5	30.5
11	*Holarrhena antidysenterica*	25.0	22.0	20.2
12	*Andropogon annulatus*	20.2	19.5	17.9
13	*Hemidesmus indicus*	30.2	28.9	27.5
14	Coconut oil	85.0	88.3	88
15	Rancid coconut oil	40.5	38.5	35.5

Graph showing effect of acid value on DPPH activity of *Wrightia tinctoria*

In order to understand the effect of the acid value on DPPH activity of Wrightia tinctoria we have taken up the present study. 3mg/ml concentration of Wrightia tinctoria was incorporated in different sets of coconut oil with varying acid values and then DPPH activity was measured. The findings show that acid value is directly proportional to decreased DPPH activity of Wrightia tinctoria Graph-1.



Graph-1: DPPH activity of Wrightia tinctoria vs acid value

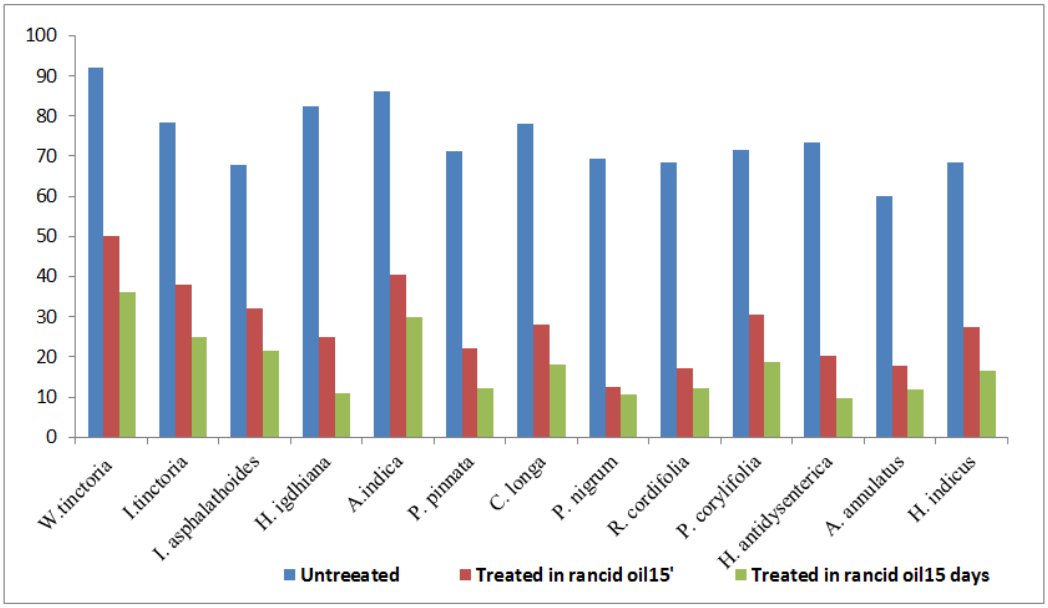
Time kinesis – effect of rancidity on DPPH activity of extracts

After establishing the definite role of rancidity in reducing the anti-oxidant effect of different herbal extracts, a study was planned to understand the impact of time of rancid oil in attenuating the anti-oxidant effect of various herbal extracts. Like the definite role of rancidity in retarding the anti-oxidant activity of herbal extracts, the duration of treatment of rancid oil also found to have strong negative impact on the anti-oxidant effect Table-9.

Table-9: Time kinesis of rancidity on DPPH activity of extracts

S. No	Sample	% inhibition/ Concentration in 3 mg/ml/ duration of treatment in days		
		5	10	15
1	*Wrightia tinctoria*	49	40	36
2	*Indigofera tinctoria*	38	29	25
3	*Indigofera asphalathoides*	30	28.2	21.5
4	*Hydnocarpus igdhiana*	15	12	11.0
5	*Azadirachta indica*	38	32	30
6	*Pongamia pinnata*	18	15.5	12.2
7	*Curcuma longa*	25.0	22	18
8	*Piper nigrum*	12.5	11.2	10.5
9	*Rubia cordifolia*	17.5	14.2	12.3
10	*Psoralea corylifolia*	21.6	20.2	18.7
11	*Holarrhena antidysenterica*	19.2	12.6	9.7
12	*Andropogon annulatus*	17.1	14.6	11.9
13	*Hemidesmus indicus*	25.1	21.9	16.5

The graph shows the progressive reduction in the anti-oxidant effect of herbal extract vis-à-vis the treatment time of rancid oil. The treatment time of rancid oil was found to have a definite effect in the activity of various herbal extracts Graph-2.



Graph-2: Reduction of anti-oxidant activity versus time of treatment in rancid oil

DISCUSSION

The present study has unraveled several mysterious facets of the acid value – herbal interaction. Due to the globally accepted limitation in treating psoriasis, the patients often go panic and would run from pillar to post in search of newer and newer therapies/treatment products. As a result of the above scenario, several manufacturers of various Siddha and Ayurveda products have come up with many single or poly herbal oil preparations for the management of psoriasis.

Among the various herbs used in the preparation of anti-psoriatic oils, the leaves of plant *Wrightia tinctoria* enjoys great patronage and credibility. Several 'touch and go' experiments have shown that *Wrightia tinctoria* is likely to possess some anti-psoriatic effect. However various nuances and nitty-gritty of the oil base and its quality that might either boost or impair the therapeutic excitement for the treatment of psoriasis is not clearly understood.

Our earlier study has shown that *Wrightia tinctoria* possess activity against keratinocyte proliferation and the activity was found to be non – cytotoxic in nature. After the recognition of *Wrightia tinctoria* for the treatment of psoriasis, several manufactures in India started to exploit the plant and have brought out several preparations. Oils with *Wrightia tinctoria* alone and or in combination with several other herbal extracts are available in the market today. Despite the availability of large number of *Wrightia tinctoria* based oils in India still the incidence and prevalence of psoriasis is on increase. Therefore naturally the question would emerge on the real therapeutic benefit of *Wrightia tinctoria.*

We have established earlier the acid value of the oil that was used for preparing products for psoriasis has a definite role in worsening the problem than offering any benefit. However such broad hypothesis was arrived purely from the per see of acid value without including and considering the multi-various therapeutic benefits of various herbs [7, 8].

In our previous study we have established that several herbs can act as metal chelators to nullify the metal toxicity. Similarly whether some herbs can nullify the effect of acid value of the base oil and offer therapeutic benefit remains unclear. To answer the above question we have undertaken the present study.

Along with Dr. JRK's 777 oil and Psorolin oil, six anti-psoriatic oils of Siddha or Ayurveda origin were directly procured from the market and were subjected to intense research.

The acid value of Dr. JRK's 777 oil and Psorolin oil were the lowest when compared to the acid value of six market products. In order to answer whether the acid value has any bearing on the free radical scavenging effect, we have studied all the oils for DPPH assay.

Dr. JRK's 777 oil and Psorolin oil showed very high anti-oxidant activity when compared to other 6 market products. However, the anti-oxidant activity of the coconut oil with acid value of 25 was appreciable than the activity of 6 market products. This led us to presume that the acid value alone may not be contributing negatively towards the DPPH activity. It means, the high acid value may perhaps modify the herbal constituents and as a result show poor anti-oxidant activity.

To validate the above hypothesis we have studied the anti-oxidant activity of various herbs that were used in the formulation of 6 market products including Dr. JRK's 777 oil and Psorolin oil. Most of the herbal extracts individually showed good anti-oxidant effect however *Wrightia tinctoria* ranked top. When the above herbal extracts treated in rancid oil, we found significant dip in the anti-oxidant value suggesting the definite role of acid value in diminishing the therapeutic potential of various herbal extracts.

To affirm the above postulate through an experimental design we have further treated the herbal extracts in rancid oil and tested for anti-oxidant activity at different time points. With increase in time, decreased anti-oxidant activity of most of the herbs was observed. Similarly the role of different acid value numbers on the antioxidant effect of the herbal extracts was also studied. Both the duration of treatment as well as the acid value number was contributing equally to diminish the anti-oxidant activity of all the herbs tested. On the contrary the concentration of the extract was seems to have less resisting power against acid value. This suggests that however high the concentration of herbal extract (s) in the formulation has less effect if the base oil is rancid.

The question we could not resolve was about that end product that formed due to the interaction between acid value and herbal constituents. The coconut oil with very high acid value of 25 showed better activity than many of the anti-psoriatic oils procured from the market with acid value less than 25. This may be due to the end product that form might be far more toxic than mere acid number. The coconut oil with acid value of 0.85 possessed strong anti-oxidant effects and this finding goes in line with the findings of earlier study.

To further establish the co-relation between the rancidity of the base oil and herbal interaction versus therapeutic benefits we studied Dr. JRK's 777 oil and Psorolin oil along with 6 market products for Nitric Oxide (NO) inhibition. The findings clearly show that Dr. JRK's 777 oil and Psorolin oil showed greater effect in inhibiting NO production when compared to the 6 market products. Similarly the concentration of Dr. JRK's 777 oil and Psorolin oil in inhibiting 50% (IC50) the glycation end product was much lower than that of 6 market products.

The above tests clearly show a definite co-relation between rancidity- herbal interaction as most of the anti-psoriatic oils procured from the market with high acid value showed poor efficacy in inhibiting both NO or glycation end product.

We further intrigue the above co-relation to unearth the possible science and hence performed CAM assay. Dr. JRK's 777 oil and Psorolin oil did not induced angiogenesis in secondary or tertiary blood vessels in chick embryo up to a period of 96 hours. On the contrary, all the market products that had high acid value had induced angiogenesis significantly proving the definite toxic side effect of the rancidity of the oil preparations. *Wrightia tinctoria* extract did not induce angiogenesis. Interestingly *Wrightia tinctoria* treated in rancid oil induced angiogenesis significantly when compared to rancid coconut oil.

CAM assay being a semi-in vivo method, the result obtained by us assumes great significance. The question of why the herbal oil with the acid value of 11 had induced angiogenesis significantly than the coconut oil with acid value 25. The possible explanation could be the end product of herbal- rancid oil interaction which we presume may be extremely toxic than acid value.

The fibroblast toxicity assay give further support to our postulate that rancidity is directly linked

to toxicity and may be indirectly linked to modifying the bio-active molecules of various herbs. However this postulates requires further study.

We are not surprised with the superior quality and the safety bio-data of Dr. JRK's 777 oil.

Dr. JRK's 777 oil may be the first and the only Siddha product having studied thoroughly and elaborately for various safety parameters such as:

- Skin Sensitization in Guinea pig
- Effect on Mucus membrane
- In vitro Mammalian cell gene mutation test-chinese hamster ovary K1 cell line
- Bacterial reverse mutation test using S. typhimurium and E.coli WP2 uvr A
- Mammalian erythrocyte Micronucleus testing swiss albino mice
- Acute lethal dose
- Reproduction developmental toxicity screening as per OECD guidelines.

Psoriasis being an auto-immune disorder the etio-pathalogy of the disease also includes free radical generation and glycation end products [9, 10]. Therefore use of anti-oxidants also as one of the medicaments for the management of psoriasis is well known. In the present context the strong anti-oxidant effect of Dr. JRK's 777 oil and Psorolin oil shows its superior therapeutic value for psoriasis. The anti-angiogenesis effect further reaffirms the possible value of both Dr. JRK's 777 oil and Psorolin oil in the management of inflammatory stage of psoriasis. Neo-genesis of blood vessel and further ramification are considered as one of the precursors that prelude inflammatory responses. Angiogenesis is the process where the formations of secondary and tertiary blood vessels are subdued. Dr. JRK's 777 oil and Psorolin oil does not possess fibroblast toxicity however they show strong keratinocyte proliferation inhibition. The above findings suggest that the therapeutic effect of Dr. JRK's 777 oil and Psorolin oil are not based on producing cytotoxicity upon the keratinocytes but may be targeting early or mid-events of cell multiplication at nuclear level.

Our study clearly establishes that therapeutically effective herbal preparations can countermand the treatment success if the base oil quality is not ascertained or followed. During processing whether the base oil turns rancid also needs to be understood. However sacred and sacrosanct the ancient wisdom may be judicious integration of modern science is inevitable to offer superior therapeutic benefit and prevent side effects.

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