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IN-VIVO ANTIACNE ACTIVITY OF HYDROALCOHOLIC EXTRACT OF PTEROCARPUS SANTALINUS

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Abstract:
Background: In the present study we have selected Pterocarpus santalinus (Leaves) which have been utilized by the villager for acne or the other skin disorders. In this view the survey of literature has been carried out and in Ayurveda, it has been mentioned that this drug is useful in skin disease. Hence, the present work has been selected to evaluate the anti acne activity and scientific validity to traditional claims.
Methods: standard test and experimental procedure was used for the estimation of TPC, TFC and evaluation of Anti-Acne activities of Pterocarpus santalinus leaves extract was studied. The acne like inflammatory model was produced in the ears of male Sprague Dawly rats (180-220g) by subcutaneous injection of heat-killed bacteria Propionibacterium acnes (65^0C for 30 min) which was evaluated by measuring ear thickness (vernier calliper) and % inhibition of acne in different groups.
Results: The total phenols and flavonoids were found to be 18.01 and 25.14 mcg/ml respectively. The Hydroalcoholic extract of Pterocarpus santalinus leaves showeddecrease in ear thickness and % inhibition at dose dependent manner(200 and 300 mg/kg).
Conclusions: The presented data indicate that the administration of Hydroalcoholic extract of Pterocarpus santalinus (Leaves) at dose of 200 and 300mg/kg decreased inflammation and showed antibacterial activity. The Hydroalcoholic extract of Pterocarpus santalinus (Leaves) have potent anti-acne activity.

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INTRODUCTION:

Acne vulgaris, a chronic inflammatory disorder in adolescent consist of the pilosebaceous follicles, characterized by comedones, papules, cyst, and nodules and often scars, chiefly on face, neck etc. The microorganism involved includes *Propionibacterium acnes* (P. acne) and Staphylococcus epidermidis. The inflamed glands caused by stress, hereditary factors, hormones, drugs and bacteria. Cause of acne includes the action of sebum synthesized and secreted by the androgen-sensitive sebaceous glands, Increase in hormones called androgens in both girl and boy during puberty, Hormonal change related to pregnancy or starting or stopping birth control pills, stress, skin irritation and Heredity[1].

Propionibacterium species are inhabitants of the skin and usually are nonpathogenic. As a result, they are common contaminants of blood and body fluid cultures. *Propionibacterium* resembles *Corynebactrium* in morphology and arrangement. *Propionibacteriumacne* is the pleomorphic, Grampositive, non- spore forming anaerobic to aero tolerant diphtheroid bacillus that produces propionic acid, as its name suggests, it also has the ability to produce of fermentation. *P. acne* and *P. granulose* may also isolate from the gastrointestinal tract[2].

Thus, the germ theory of this century enabled the eradication of most microbial infection through the use of antibiotics and anti-viral drugs. Thus, the rapid and accurate analysis of *P. acne*, an invading organism, is needed. The medication have several adverse effects like birth defects, erythema, photosensitivity, allergic dermatitis, excessive skin irritation, urinary problem, joint and muscle pain, headache, depression etc. Many remedies have been employed to treat acne from long period. Most of the remedies were taken from plants and proved to be useful, scientifically established except for a few plants and some proprietary composite herbal drugs. The cosmetics available in the market are not reasonable for everyone thus an effort has been made to study their properties for anti acne activity and to incorporate these extracts in the formulations. The product may be cost effective. This has given rise to stimulation in the search for investigating natural resources showing anti-acne activity [3,4,5].

Ayurveda, the traditional system of Indian medicine described a number of plants which are utilized in skin care preparations. In the present study we have selected *Pterocarpus santalinus* (Leaves) which have been utilized by the villager for acne or the other skin disorders. In this view the survey of literature has been carried out and in Ayurveda, it has been mentioned that thisdrug is useful in skin disease. Hence, the present work has been selected to evaluate the anti-acne activity and scientific validity to traditional claims.

MATERIAL AND METHODS:

Collection of plant material:

The Flowers of selected plant namely *Pterocarpus santalinus*was purchased from Akshat nursery, Bhopal (M.P.). The leaves were further identified by Botanist Department of Botany, Safiya Science College, Bhopal, and specimens have been submitted and preserved in the Department of Botany.

Extraction and phytochemical analysis [6,7]:

The plant Material (Leaves) was defatted with petroleum ether (40°-60°C) for about 12 hrs separately & complete defatting was ensured by placing a drop from the thimble on a filter paper which did not exhibited any oily spot. The defatted material was removed from the Soxhlet apparatus and air dried to remove last traces of petroleum ether. The defatted plant drug was subjected to extraction by (ethanol: water; 70:30) as solvent.

Qualitative Phytochemical Analysis:

Preliminary chemical tests were carried out for methanolic extract to identify different phytoconstituents such as Alkaloids, Flavonoids, Cardiac glycosides, saponins, steroids, Tannins, Triterpenes, carbohydrates, Protein and amino acids.

Quantitative Phytochemical Analysis:

Determination of total phenol content [8]:

The amount of total phenol content was determined by Folin-Ciocalteu reagent method . 0.5 ml of extract and 0.1 ml of Folin-Ciocalteu reagent (0.5 N) was mixed and incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate was added. The mixture was allowed to stand for 30 min at room temperature and the absorbance of the reaction mixture was measured at 760 nm. The total phenolic content is expressed in terms of gallic acid equivalent (mg/g of extracted compound).

Determination of flavonoids content [9]:

The amount of total flavonoids content was determined by Aluminium chloride method. The reaction mixture consisted of 1.0 ml extract, 1 ml ethanol, 0.5 ml Aluminium chloride (1.2%) and 0.5 ml potassium acetate (120 mM). The mixture was allowed to stand for 30 min at room temperature and the absorbance of the reaction mixture was measured at 415 nm. The flavonoids content is expressed in terms of Quercetin equivalent (mg/g of extracted

compound).

In -*Vivo* Anti acne Activity on herbal extract:

Animal:

Wistar albino mice of either sex (25-30gm) were selected for acute toxicity studies and SpragueDawley male rats (180-220gm) were selected for Invivo anti-acne activity. The animals were acclimatized to standard laboratory conditions of temp (25±20C) and maintained on 12:12 h light: dark cycle. Procured from the animal house, College of Veterinary Sciences and Animal Husbandry, Mhow, Indore They was provided water ad libitum. The animal care and experimental protocols were in accordance with CPCSEA/IAEC guidelines.

Acute Oral Toxicity [10]:

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Cooperation and Development (OECD) revised draft guidelines 423 B ("Up and Down" method) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Assessments of Anti acne In-vivo Models[11]:

Based on the pilot screening the following protocol was carried out. In pilot screening 6 rats were taken under study which showed that the granulomatous inflammation remain constant from day 6th day to 10th day (Table No.1).

Table 1: Protocol study for in-vivo anti acne activity on male Sprague Dawley rats

Groups	Induction of Acne	Treatment
1) Normal control	No induction	Vehicle
2) control (acne induced)	Heat killed P. acnes in PBS	Vehicle
3) Treated with Standard	Heat killed P. acnes in PBS	Clindamycin200mg/kg b.w.,p.o.
4) Treated with HEPS	Heat killed P.acnes in PBS	HEPS 200mg/kg b.w.,p.o
5) Treated with HEPS	Heat killed P.acnes in PBS	HEPS 300mg/kg b.w.,p.o

Thus, depending on the protocol given, the animals were divided into 5 groups containing 6 in each and kept in metabolic cages. All animals had free access to regular rat pallets diet and drinking water ad libitum during the study.

Induction of acne by *Propionibacterium acnes:*

The acne like inflammatory model was produced in the ears of male Sprague Dawly rats (180-220g) by subcutaneous injection of heat-killed bacteria (650C for 30 min).

Measurement of ear thickness:

Ear thickness was measured as an index of inflammatory strength and acne. Thickness was measured by using a vernier calliper. Thickness was measured once every day for the first week of induction, then every other day until 35th day.

Histopathology:

On the 10th day after the induction of acne, three animals from each group were sacrificed and ears were excised and fixed in 10% formalin (pH 7.2) and then embedded in paraffin and thick sections were taken to stain using hematoxylin-eosin dye and mounted in diphenyl xylene and observed for the changes. And other 3 animals were observed till 35th day.

Results:

The plant *Pterocarpus santalinus* leaves were powdered and subjected to Soxhletion with ethanol: water (70:30) as a solvent for 12 hours. The results of the phytochemical screening of leaves extract of *Pterocarpus santalinus* leaves were presented in Table-1. Different types of secondary metabolites such as flavonoids, glycoside, phenol, saponins, sterols and tannins were present while alkaloids, carbohydrate and resinswere absent in *Pterocarpus santalinus* leaves

Table 2: Preliminary phytochemical screening of *Pterocarpus santalinus* leaves

S.N.	Phytoconstituents	Test Name	Hydroalcoholic Extract
1	Alkaloids	Mayer's Test	**Absent**
2	Glycosides	Raymond's Test	Present
3	Carbohydrates	Molisch's Test	**Absent**
4	Tannins	Vanillin- HCl Test	Present
5	Flavonoids	Lead acetate	Present
6	Resins	Color detection with ferric chloride	**Absent**
7	Steroids	Libermann- Bur chard Test	Present
8	Proteins & Amino acids	Biuret Test	Present
9.	Phenols	Ellagic Acid Test	Present

Estimation of total phenolic content:

Total phenolic content was estimated by gallic acid and expressed as milligrams of gallic acid equivalent (GAE). All the extracts contained a considerable amount of phenolic contents of GAE/g of extract .

Table 3: Total Phenolic Content of Hydroalcoholic extract of *Pterocarpus santalinus*

Sample	Total phenolic content GAE mcg/ml
Hydroalcoholic extract 100µg/ml	23.11± 0.001

n=3, values are given in SEM

Estimation of total flavonoids content:

Flavonoid content was calculated from the regression equation of the standard plot (y=0.031x+0.123, R^2 =0.995) and is expressed as quercetin equivalents (QE) (fig.). Total Flavonoid content was 22.81 ± 0.001mg/g quercetin equivalent in MELP. Flavonoids are the most common and widely distributed group of plant's phenolic compounds,.

Table 4: Total Flavonoid content of Hydroalcoholic extracts of *Pterocarpus santalinus*

Sl. No.	Extracts 100µg/ml	Flavonoid content Quercetin equivalent mcg/ml
1	Hydroalcoholic extract (100µg/ml)	22.81 ± 0.001

n=3, values are given in SEM

In -*Vivo* Anti acne Activity on herbal extract:

The acne-like inflammatory model was produced in the ears of rats by subcutaneous injection of 140 µg of heat killed *Propionibacterium acnes*. Ear thickness was measured as an index of inflammatory strength, using a micro indicator once every others day for the first week, then every other day until the 35th day.

The result of the extract was comparable with standard. The data resulted from anti-acne effect of Hydroalcoholic extract of *Pterocarpus santalinus* decreased the inflammation in rats ear. On the10th day there was a significant decrease ($p<0.01$) in inflammation (0.192±0.0024 and 0.294±0.0260) respectively by Hydroalcoholic extract of *Pterocarpus santalinus* at the dose of 300 and 200mg/kg.

Table 5: Effect of Clindamycin (standard) and Hydroalcoholic extract of *Pterocarpus santalinus* at different dosages

S. No.	GROUP	Mean thickness ±SEM					
		Day1	Day3	Day5	Day6	Day7	Day10
1	Control	1.436±0.016	1.369± 0.012	1.271± 0.021	1.265± 0.026	1.265± 0.023	1.265± 0.020
2	Clindamycin	1.351±0.0054***	1.270± 0.0031***	0.1932±0.0018***	0.1052±0.0048***	0.1052±0.0047***	0.1052±0.0046***
3	HEPS 200mg/kg	1.411±0.0019*	1.336± 0.0042*	0.306±0.0040*	0.294±0.0263*	0.2940.02618*	0.294±0.0260*
4	HEPS 300mg/kg	1.389±0.0038**	1.303±0.0040**	0.263±0.0014**	0.192±0.0027**	0.192±0.0025**	0.192±0.0024**

***P<0.001, **P<0.01, *P<0.05

Thus the Table reveals that the maximum inflammation on ear was on 3rd day in all groups. In test group on 5th day there was a sudden decrease in inflammation which was constant till 10th day and at around 20th day the inflammation reduced and came to normal. The observations were made till 35th day where the thickness was found to be normal.

Histological changes As per the preliminary studies carried out in animals, the thickness of the ear (inflammation) was observed on 10th day. The thickness was found to be constant between 6th to 10th days. Hence, histopathology of the ear was assessed on 10th day. In histopathology study it was found that accumulation of neutrophils on inflammatory lesions site with subsequent rupture of the follicle and formation of a pustule in the dermis and the transmigration of lymphocytes into the wall of the follicle associated with increasing spongiosis of the follicular epithelium. During 24-72 hours, the accumulation of neutrophils within the follicle led to its distension and subsequent rupture. There was a localized loss of the granular layer in the region of the eventual rupture. This shows the difference of normal and acne- induced ear section. The treated ear section showed no infiltration in the case of standard drug which was similar to the normal . The histopathology study supports the results shown in Table.

Table 6: Percentage inhibition of P. acne induced granulomatous inflammation treated with std. and Hydroalcoholic extract of *Pterocarpus santalinus* at different dosages

S. No.	Test Material	Percentage Inhibition (%)					
		Day1	Day3	Day5	Day6	Day7	Day10
1	Clindamycin	5.92	7.23	84.79	91.68	91.68	91.68
2	HEPS 200mg/kg	1.74	2.41	75.13	76.01	76.01	76.01
3	HEPS 300mg/kg	3.27	4.82	79.20	84.13	84.13	84.13

DISCUSSION:

Acne vulgaris is a chronic inflammatory disease results in the formation of inflamed and/or no inflamed eruptions Propionibacterium acnes are the anaerobes, in the skin which grow in the sebaceous region. Various antibiotics like tetracycline, Clindamycin, and erythromycin etc and other drugs like benzoyl peroxide are used for acne treatment.
The various drawbacks of synthetic drugs are different side effects and resistant developed towards these drugs. Herbal therapy is required to overcome the above drawbacks and treat the acne. So in the present study Hydroalcoholic extract of *Pterocarpus santalinus* (Leaves) were selected for the anti acne activity.

The preliminary phytochemical study was carried out according to standard literature. This revealed that they contain various phytoconstituents which can be responsible for the anti acne activity. The extracts were subjected to antimicrobial activity against *Propionibacterium acnes* (1 µg/ml).

As per literature review various phytoconstituents like curcuminoids, alkaloids, triterpenoids, Phenols and flavonoids are proved to possess antibacterial activity. The acne like inflammatory activity was carried out by measuring the ear thickness and histopathological studies of the ear. Hydroalcoholic extract of *Pterocarpus santalinus* (Leaves) at dose of 200 and 300mg/kg showed significant reduction in the ear thickness.
The Hydroalcoholic extract of *Pterocarpus santalinus* (Leaves) contain alkaloids, Phenols, flavonoids etc. The above phytoconstituents were proved potent anti-oxidants. The presence of various phytoconstituents showed significant anti acne properties which are supported by the antimicrobial and histopathological studies.

CONCLUSION:

In conclusion, the presented data indicate that the administration of Hydroalcoholic extract of *Pterocarpus santalinus* (Leaves) at dose of 200 and 300mg/kg decreased inflammation and showed antibacterial activity. The Hydroalcoholic extract of *Pterocarpus santalinus* (Leaves) have potent anti-acne activity.

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