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Phytochemical Investigation and Evaluation of anti-acne activity *of Gardenia thunbergia* Plant leaves

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ABSTRACT

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Secondary constituents contain alkaloids, flavonoids, phenol, sapon in, steroids and tannins. Medicinal plants have anticancer, antimicrobial, antidiabetic, antidiuretic and anti-inflammation activities. The increasing interest in powerful biological activity of secondary metabolites outlined the necessity of determining their contents in medicinal plants. In Indian Ayurvedic system, *Gardenia thunbergia* are well-known plants used for major and minor ailments. The aim of the present study is to examine leaf of *Gardenia thunbergia* for phytochemical profile, antioxidant potential and anti acne activity. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins Ciocalteau reagent method and aluminium chloride method respectively. The findings of the present study will be helpful to phytochemists, pharmacologists and pharmaceutical industries.

Keywords: *Gardenia thunbergia*, Phytochemical, Antioxidant, Antiacne.

1. INTRODUCTION TO ACNE

Acne is the most common skin disease worldwide.[1] It is estimated that 80-95% of all adolescents will have acne at some point in their lives and in some cases the acne will continue into adulthood. Genetics plays a role in the development of acne and thus there is a tendency for the skin disease to run in families.[2] Males and females are equally affected, but males tend to have more severe cases. A number of factors contribute to the development of acne lesions. These include internal hormones, bacteria, some medications, certain chemicals that come in contact with the skin, localpressure to the skin surface, and stress. While acne cannot be cured, it can be controlled[3]. The goal of treating acne is to reduce the symptoms and to prevent permanent scarring.

Components propelling change of skin break out are: extended sebum creation, ductal cornification, bacterial colonization of pilosebaceous channels and disturbance.[4] Disregarding reality that earnestness of skin break out vulgaris is joined with seborrhoea, disease is one of follicular infundibulum.[5] In smooth skin aggravation, keratinocytes of infundibulum hypercornify, hyperkeratinize and hypodesquamate to convey comedones.[6] Acne is a skin condition that occurs when your hair follicles become plugged with oil and dead skin cells. It causes whiteheads, blackheads or pimples.[7] Acne is most common among teenagers, though it affects people of all ages. Effective acne treatments are available, but acne can be persistent. The pimples and bumps heal slowly, and when one begins to go away, others seem to crop up. Depending on its severity, acne can cause emotional distress and scar the skin. The earlier you start treatment, the lower your risk of such problems.[8]

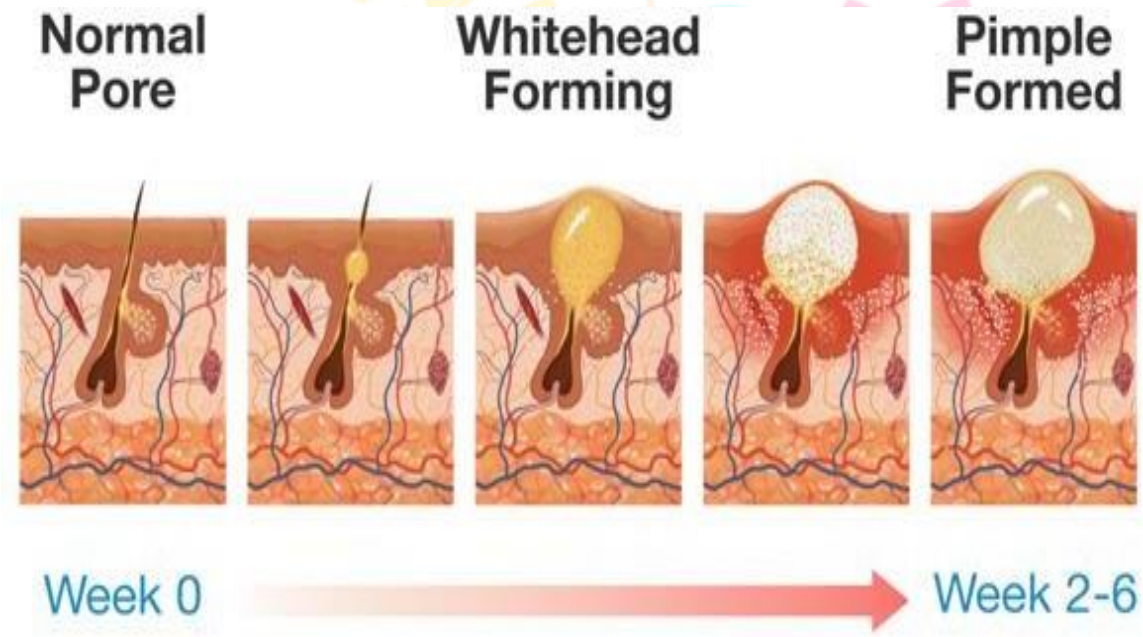

Figure: 1.1 Formation of Acne

1.2 Pathophysiology

Acne arises from the interaction of the following four factors:

a.Increased sebum production caused by androgenic stimulation of sebaceous glands in puberty or adulthood.[9,10]

b. Outlet obstruction of the sebaceous follicle arising from an abnormal keratinization process characterized by increased cohesiveness and turnover of follicular epithelial cells.[11,12]

c. Proliferation of *P. acne*, an anaerobic diphtheroid residing in the pilo-sebaceous follicle *P. acne* produces free fatty acids that irritate the distended follicularwall.[13,14,15]

d. Inflammation mediated by the irritant action of sebum leaking into the dermis aswell as the presence of chemo tactic factors and pro-inflammatory mediators generated by *P. acne*.[16,17]

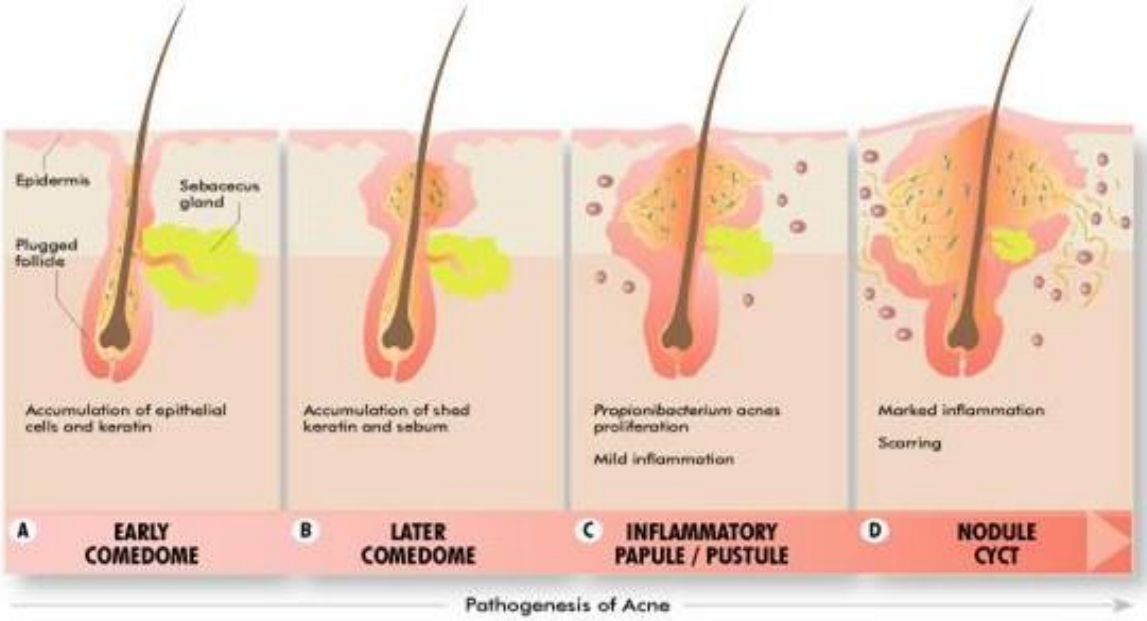

Figure 1.2 : Pathogenesis of Acne

1.3 Acne causes

Several factors contribute to the development of acne. The primary problem is that theabnormal flaking of cells inside the hair follicle leads to the formation of a plug. The plug can enlarge and even rupture the hair follicle.[18] A ruptured hair follicle spills its contents of oil and debris into the skin where it leads to swelling and causes redness and inflammation. Bacteria that normally live on the skin also play a role in acne development. The bacteria known as Propionibacterium acnes are responsible for causing acne. These bacteria produce substances that cause redness and irritation. They also make enzymes, which dissolve the sebum (oil from oil glands in the skin) into irritating substances. These substances also make the inflammation worse. Certain hormones called androgens are an additional factor in causing acne. Androgens are male hormones that are present in both men and women, but are higherin men. Androgens do two things: First, they enlarge the sebaceous glands in the skin. Second, they cause these glands to increase sebum (oil) production. The increased sebum leads to plug formation and serves as more "food" for the bacteria. Androgens surge at puberty, which is why teens develop armpit and pubic hair, and why boys develop facial hair and deeper voices. This hormonal surge also contributes to the development of acne in teens.[19]

Estrogens, which are the female hormones, actually can help to improve acne in gins. A woman's monthly menstrual cycle is due to changes in the estrogen levels in her body. This is why acne in a female may get better and then get worse as she goes through her monthly cycle.[20] A doctor may recommend acne treatment with birth control pills which contain the helpful estrogens. We also now believe that acne can run in some families. This may be due to some genetic factor that has not yet been discovered.[21]

Anatomy of the hair follicle: Hair follicles exist on virtually all skin except for the palms of the hands and soles of the feet. Inside the follicle, the hair extends up from the deep layers of the skin and comes out of a

pore. Near the surface, the oil gland (sebaceous gland) enters the hair follicle where it empties oil (sebum) at a relatively constant rate. The sebum lubricates the skin and provides a protective barrier to prevent drying. Skin on the face, chest, and back has an especially large number of sebaceous glands.[22]

2. MATERIAL AND METHOD

2.1 Selection of plant material

The plants have been selected on its availability and folk use of the plant.

2.2 Collection of plant material

Every part of the plant may contain active secondary metabolites, such as bark, leaves, flowers, roots, fruits and seeds. Fresh & healthy, disease free plant leaves of *Gardenia thunbergia* were collected from ruler area of Raisen (M.P.) in the month of September, 2021.

2.3 Percentage loss

The weight of the fresh sample and dried powder was determined, and the percentage loss was calculated due to drying and water loss.

The percentage loss was calculated by using following formula:

- $$\% \text{ Loss of drying } = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} x\ 100$$

2.4 Extraction procedure

2.4.1 Extraction by maceration process

100 gram of *Gardenia thunbergia* was exhaustively extracted with different solvent (methanol, aqueous) by maceration method. Over their boiling points the extract was evaporated. Finally, the percentage yields for the dried extracts are determined.

2.4.2 Determination of percentage yield

Following formula was adopted for determination of percentage yield of selected plant materials. The percentage yield of each extract was calculated by using following formula:

$$\textbf{Percentage Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Powder drug taken}} \text{x } 100$$

2.5 Phytochemical screening

Medicinal plants are traditional medicinal resources and many of the modern medicinal products are produced indirectly from plants. Phytochemical components consist of two primary bioactive components (chlorophyll, proteins, amino acids, sugar, etc.) and secondary bioactive components (alkaloids, terpenoids , flavonoids, etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods.

2.6 Quantitative estimation of bioactive compounds

2.6.1 Total Phenolic content estimation

Procedure: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 2 ml (1mg/ml) of this extract was for the estimation of Phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

2.6.2 Total flavonoids content estimation

Procedure: Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 3 ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

2.7 Antioxidant activity of extracts by DPPH scavenging activity

DPPH scavenging activity was assessed with spectrophotometer. Storage solution (6 mg in 100 ml methanol) was prepared to give 1.5 ml of it in 1.5 ml of methanol as an initial absorption. Decline in absorbance was observed at different concentrations (10-100 µg / ml) after 15 minutes when sample extract was present. 1.5 ml of DPPH solution was taken and methanol volume was produced up to 3 ml, absorbance was taken immediately at 517 nm for control reading.. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

$$\% \textbf{ Reduction} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \text{x}100$$

2.8 *In-Vivo* Anti-acne activity

Wistar rats (150-200 g) were housed in a regular 12 h light / dark cycle group (n= 6) and regulated temperature and humidity conditions (25±2 ° C, 55–65 per cent). Rats received standard rodent chow *ad libitum* and water. Rats were acclimatized for 7 days before carrying out the experiments to the laboratory conditions. All the tests were carried out from 08.00 to 15.00 h in a noise-free environment. For each set of experiments separate group (n=6) of rats was used.

Induction of acne by *Propionibacterium acnes*

Groups of 6 Wistar rats weighing 150–200g are shaved in the interscapular area. Intradermal injection of killed *Propionibacterium acne* into the rat ear will be used to induce chronic acne like inflammation. Ear thickness was measured using vernier calipers.

Experimental designs

- Group –I: Control (acne induced)
- Group –II: Clindamycin
- Group -III: Treated with Hydro-methanolic *Gardenia thunbergia* 200 mg/kg b.w.,p.o
- Group -IV: Treated with Hydro-methanolic *Gardenia thunbergia* 400 mg/kg b.w.,p.o

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 every two day until the 10th day.

3. RESULTS AND DISCUSSION

3.1 Determination of Percentage Yield

Table 3.1: % Yield of hydro-methanolic extract of *Gardenia thunbergia*

S. No.	Part	% Yield(W/W)
1.	Leaves	12.45

3.2 Phytochemical screening of extract

Table 3.2: Result of Phytochemical Screening of extracts of *Gardenia thunbergia*

S. No.	Constituents	Hydro-methanol extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	 -Ve -Ve
2.	Glycosides A) Legal's Test:	 +Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	 +Ve +Ve
4.	Saponins A) Froth Test:	 +Ve
5.	Phenolics A) Ferric Chloride Test:	 +Ve
6.	Proteins and Amino Acids A) Xanthoproteic Test:	 +Ve
7.	Carbohydrate	

	A) Fehling's Test:	+Ve
8.	Diterpenes A) Copper acetate Test:	+Ve
9.	Tannin A) Gelatin test:	-Ve

3.3 Results of Estimation of Total Phenolic Contents

Table 3.3: Total Phenolic and Total flavonoid content of *Gardenia thunbergia*

S. No.	Extract	Total Phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	**Hydro-methanolic extract**	2.950	2.512

3.4 Results of Antioxidant activity using DPPH method

Variable DPPH activity was recorded for Hydro-methanol extract of *Gardenia thunbergia.* The DPPH scavenging activity of Hydro-methanolic extract of *Gardenia thunbergia* was showed IC50 value 55.92µg/ml as compared to positive control ascorbic acid 21.41µg/ml.

3.5 Results of *In-Vivo* anti-acne activity

The acne-like inflammatory model was produced in the ears of rats by subcutaneous injection of 140µg of heat killed *Propionibacterium acnes*. Ear thickness was measured as an index of inflammatory strength, using a micro indicator once every two day until the 10^{th} day. The results of the extracts were comparable with standard. The data resulted from anti-acne effect of Hydro-methanolic extract of *Gardenia thunbergia* was significantly decreased the inflammation in rats ear.

3.5.1 Induction of acne by *Propionibacterium acnes*

The acne like inflammatory model was produced in the ears of male Wister Albino rats (150-200g) by subcutaneous injection of heat-killed bacteria (65 ^{0}C for 30 min).

3.5.2 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 10^{th} day.

Table 3.4: Effect of Clindamycin (standard) and Hydro-methanolic *Gardenia thunbergia* extract on acne induced by *Propionibacterium acnes* in rats

Groups	Treatment	Mean thickness ±SEM after 10th Days
Group-1 Control (acne induced)	Treated with *Propionibacterium acne*	0.615 ± 0.126
Group-2 Clindamycin (Standard)	Treated with *Propionibacterium acne* and standard clindamycin 100 mg/kg	0.110 ± 0.029
Group-3 Treated with Hydro-methanolic *Gardenia thunbergia*	Treated with *Propionibacterium acne* and HMGT 200mg/kg	0.485 ± 0.05
Group-4 Treated with Hydro-methanolic *Gardenia thunbergia*	Treated with *Propionibacterium acne* and HMGT 400mg/kg	0.292 ± 0.05

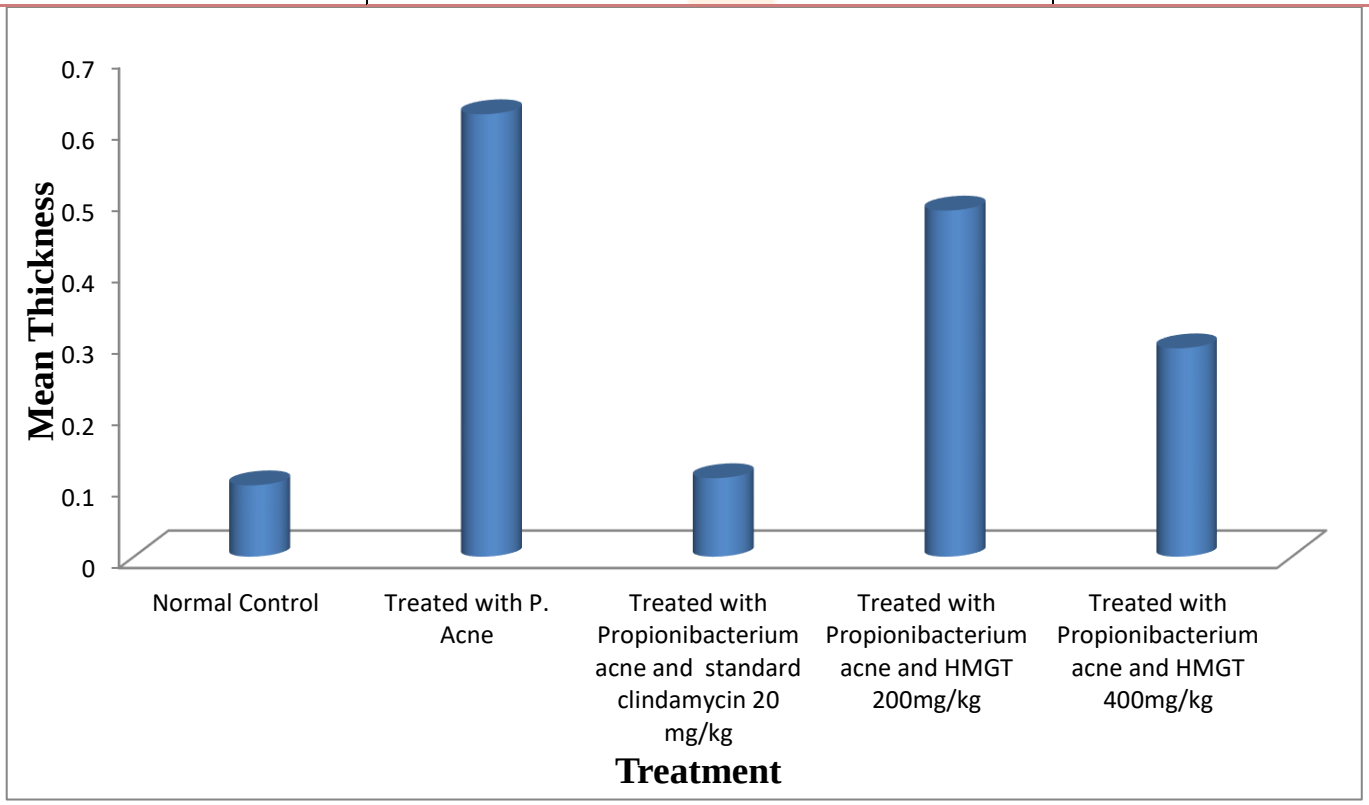

Figure 3.1: Effect of Clindamycin (standard) and Hydro-methanolic extract of *Gardenia thunbergia* induced on acne by *Propionibacterium acnes* in rats

3.6 Histopathology

In the present study the Hydro-methanolic extract of *Gardenia thunbergia* 200 mg/ kg and 400 mg/kg extracts were selected, after the acute toxicity studies. 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.

The acne-like inflammatory model was produced in the ears of rats by subcutaneous injection of 100 µg of heat killed Propionibacterium acnes. Ear thickness was measured as an index of inflammatory strength, using a micro indicator once until the 10th day. The result of the *Gardenia thunbergia* extracts were comparable with standard. The data resulted from anti-acne effect of Hydro-methanolic extract of *Gardenia thunbergia* decreased the inflammation in rats ear. On the10th day there was a significant decrease ($p<0.01$) in inflammation (0.485±0.05) by hydro-methanolic *Gardenia thunbergia* 200mg/kg Dose and 0.292 ± 0.05 by hydro-methanolic.

CONCLUSION

In this study, we evaluated the anti-acne activity Hydro-methanolic extract of *Gardenia thunbergia* commonly used traditional medicinal plants from India. Extract displayed a potent antibacterial activity in the dose-dependent manner. the presented data indicate that the administration of Hydro-methanolic extract of *Gardenia thunbergia* decreased inflammation and showed antibacterial activity. The Hydro-methanolic extract of *Gardenia thunbergia* have potent anti-acne activity. Indian history of medicinal plants has proven that herbs have been used to treat topical infections. From the long list of herbs in my research *Gardenia thunbergia* are some of the common herbs that are found in abundant. The chosen plants develop an effective Anti-acne active.

REFERENCES

1. Williams HC, Dellavalle RP, Garner S. Acne vulgaris. Lancet. 2012;379(9813):361-72.

2. Cong TX, Hao D, Wen X, Li XH, He G, Jiang X. Pathogenesis of acne vulgaris to anti-acne agents. Arch Dermatol Res. 2019;311(5):337-349.

3. Kolli SS, Pecone D, Pona A, Cline A, Feldman SR. Topical Retinoids in Acne Vulgaris: A Systematic Review. Am J Clin Dermatol. 2019;20(3):345-365.

4. Sanjay K Rathi. Acne vulgaris treatment: The current scenario. Indian J Dermatol. 2011; 56(1): 7-13.

5. Darren D Lynn,Tamara Umari, Cory A Dunnick, Robert P Dellavalle. The epidemiology of acne vulgaris in late adolescence. Adolescent Health, Medicine and Therapeutics 2016:7 13–25.

6. Mohamed L Elsaie. Hormonal treatment of acne vulgaris: an update. Clinical, Cosmetic and Investigational Dermatology 2016:9 241–248.

7. Harald P. Gollnick, Christos C. Zouboulis. Not All Acne Is Acne Vulgaris. Deutsches Ärzteblatt International | Dtsch Arztebl Int 2014; 111: 301-312.

8. Kristen M Whitney, Chérie M Ditre. Management strategies for acne vulgaris. Clinical, Cosmetic and Investigational Dermatology 2011;4:41-53.

9. Hammer KA. Treatment of acne with tea tree oil (melaleuca) products: a review of efficacy, tolerability and potential modes of action. Int J Antimicrob Agents 2015; 45(2):106-10.

10. Holzmann R, Shakery K. Postadolescent acne in females. Skin Pharmacol Physiol 2014;27(1):3-8.

11. Ismail NH, Manaf ZA, Azizan NZ. High glycemic load diet, milk and ice cream consumption are related to acne vulgaris in Malaysian young adults: a case control study. BMC Dermatol 2012;12:13.

12. Khayef G, Young J, Burns-Whitmore B, Spalding T. Effects of fish oil supplementation on inflammatory acne. Lipids Health Dis 2012;11:165.

13. Khodaeiani E, Fouladi RF, Amirnia M, Saeidi M, Karimi ER. Topical 4% nicotinamide vs. 1% clindamycin in moderate inflammatory acne vulgaris. Int J Dermatol 2013; 52(8): 999-1004.

14. Kim KS, Kim YB. Anti-inflammatory effect of Keigai-rengyo-to extract and acupuncture in male patients with acne vulgaris: a randomized controlled pilot trial. J Altern Complement Med 2012;18(5):501–8.

15. Kong YL, Tey HL. Treatment of acne vulgaris during pregnancy and lactation. Drugs 2013;73(8):779–87.

16. Ma L, Xiang LH, Yu B, Yin R, Chen L, Wu Y, et al. Low-dose topical 5- aminolevulinic acid photodynamic therapy in the treatment of different severity of acne vulgaris. Photodiagnosis Photodyn Ther 2013;10(4):583–90

17. Maleszka R, Turek-Urasinska K, Oremus M, Vukovic J, Barsic B. Pulsed azithromycin treatment is as effective and safe as 2-week-longer daily doxycycline treatment of acne vulgaris: a randomized, double-blind, noninferiority study. Skinmed 2011;9(2):86–94.

18. Poulin Y, Sanchez NP, Bucko A, Fowler J, Jarratt M, Kempers S, et al. A 6- month maintenance therapy with adapalene-benzoyl peroxide gel prevents relapse and continuously improves efficacy among patients with severe acne vulgaris: results of a randomized controlled trial. Br J Dermatol 2011;164(6):1376-82.

19. Qin M, Landriscina A, Rosen JM, Wei G, Kao S, Olcott W, et al. Nitric oxide- releasing nanoparticles prevent propionibacterium acnes-induced inflammation by both clearing the organism and inhibiting microbialstimulation of the innate immune response. J Invest Dermatol 2015;135(11):2723–31.

20. Sakamoto FH, Torezan L, Anderson RR. Photodynamic therapy for acnevulgaris: a critical review from basics to clinical practice: part II. Understanding parameters for acne treatment with photodynamic therapy. J Am Acad Dermatol 2010;63(2):195–211.S

21. limonart T. Newer approaches to the treatment of acne vulgaris. Am J Clin Dermatol 2012;13(6):357–64.

22. Tan J, Stein Gold L, Schlessinger J, Brodell R, Jones T, Cruz A, et al. Short- term combination therapy and long-term relapse prevention in the treatment ofsevere acne vulgaris. J Drugs Dermatol 2012;11(2):174–80.

23. Trifu V, Tiplica GS, Naumescu E, Zalupca L, Moro L, Celasco G. Cortexolone 17alphapropionate 1% cream, a new potent antiandrogen for topical treatment of acne vulgaris. A pilot randomized, double-blind comparative study vs. placebo and tretinoin 0.05% cream. Br J Dermatol 2011;165(1):177–83.

24. Trivedi MK, Shinkai K, Murase JE. A review of hormone-based therapies to treat adult acne vulgaris in women. Int J Womens Dermatol 2017;3:44–52.

25. Weinstein M, Laxer R, Debosz J, Somers G. Doxycycline-induced cutaneous inflammation with systemic symptoms in a patient with acne vulgaris. J Cutan Med Surg 2013;17(4):283–6.

26. Williams HC, Dellavalle RP, Garner S. Acne vulgaris. Lancet 2012;379(13): 361–72.

27. Zaenglein AL, Shamban A, Webster G, Del Rosso J, Dover JS, Swinyer L, et al. A phase IV, open-label study evaluating the use of triple-combination therapy with minocycline HCl extended-release tablets, a topical antibiotic / retinoid preparation and benzoyl peroxide in patients with moderate to severe acne vulgaris. J Drugs Dermatol 2013;12(6):619–25.

28.Zaenglein AL, Pathy AL, Schlosser BJ, Alikhan A, Baldwin HE, Berson DS, et al. Guidelines of care for the management of acne vulgaris. J Am Acad Dermatol 2016; 74(5):945–973.