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STUDY OF PHYTOCHEMICALS AND ANTI ACNE ACTIVITY OF ETHANOLIC EXTRACT OF *WRIGHTIA TINCTORIA*

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Abstract

Acne vulgaris, a multifarious chronic inflammatory state and mostly happen within a pilosebaceous unit including hair, hair follicles, sebaceous gland of the skin. Acne is categorized by non-inflammatory lesions that are whiteheads, blackheads, and inflammatory lesions that are papules, nobles, pustule, podules, and cysts. The most common form of the disease in adolescents in is called acne vulgaris. Patients experience psychological burdens like depression, anxiety, and low self-esteem because of acne The therapies which are commonly used to the treatment of Acne vulgaris include are hormonal, systemic, topical, herbal and some drugs with combination therapy But, the use of these drugs produces a numeral of potential side effects and consumption of antibiotics will usually make the bacteria become resistant and permanent nature multiplies within its host. Herbal therapies, which have been in use from ancient times for the treatment of acne, include various herbal extracts, oil and their ayurvedic *Wrightia tinctoria* which is commonly known as 'Danthapala' is a known potential medicinal plant, the leaves of which is traditionally used in the treatment of psoriasis and non-specific dermatitis in Siddha and Ayurvedic systems of medicine formulation. So the aim of present study is to evaluate phytochemicals and anti acne effect of *Wrightia tinctoria* . The leaves of plant were collected and subjected to extraction by pet ether & ethanol. Further qualitative & quantitative estimation of phytochemical was performed. The antimicrobial activity was also evaluated.

The results showed that the percentage yield for pet ether extract & Ethanolic extract for leaves of *Wrightia tinctoria* was found to be 1.52% 7.54% respectively suggesting ethanol as better solvent. The phytochemical screening revealed presence of flavonoid, carbohydrate, proteins & diterpenes. The total flavonoid & total protein content of ethanolic extract of *Wrightia tinctoria* was found to be 0.762mg/100mg and 0.541 mg/100mg of dried extract. The antimicrobial activity was evaluated by well diffusion method. Four bacterial species *Streptococcus mutans, Klebsiella pneumoniae, Candida albicans, Propionibacterium acnes* were tested for effect of Wrightia tinctoria extract. At concentration of 100mg/ml the zone of inhibition for Streptococcus mutans, *Candida albicans, Propionibacterium acnes* was found to be 9±0.94mm, 15±0.47mm, 17±0.74mm respectively. The zone of inhibition were not seen in case of Klebsiella pneumoniae The result of antimicrobial acytivity suggest that the ethanolic extract of Wrightia tinctorial was highly effective against *Propionibacterium acnes.* From the results it can be conclude that *Wrightia tinctoria* exhibit significant anti acne potential.

Introduction

The term acne is derived from Greek word ―acme‖ which means ―prime of life. Acne is a skin disorder that leads to an outbreak of lesions called piples or ―Zits‖. Propionibacterium acnes and Staphylococcus epidermidis are common pus-forming microbes responsible for the development of various forms of acne. Acne vulgaris, a multifarious chronic inflammatory state and mostly happen within a pilosebaceous unit including hair, hair follicles, sebaceous gland of the skin. Acne is categorized by non-inflammatory lesions that are whiteheads, blackheads, and inflammatory lesions that are papules, nobles, pustule, podules, and cysts. The most common form of the disease in adolescents in is called acne vulgaris. Patients experience psychological burdens like depression, anxiety, and low self-esteem because of acne [1,2].

Acne vulgaris is inflammation of the sebaceous (oil) gland of the skin. At first, there is excessive sebum production due to the action of androgens. These glands become blocked causing blackheads and whiteheads due to increased keratinisation of the sebaceous duct. Secondary bacterial infections occur due to *Propionibacterium* acnes *(P. acnes*) in the sebum. Lipases are produced which in turn produce free fatty acids (FFA) from the triglycerides in the sebum. There is a normal role of *Propionibacterium* acnes *(P. acnes*) to play in the upper layers of the skin with the resultant FFA keeping the skin pH between 5 and 6 which acts as a barrier against other bacteria and viruses. Further down in the follicle close to the dermis the free fatty acids (FFA) become pro-inflammatory, thus provoking inflammation characterised by pimples (red papules, pustules), and deeper lumps (cysts or nodules) On the word of statistics, globally around 85% of young adults aged 12–25 years old, approximately 8% of adults aged 25–34 years old, and 3% of adults aged 35–44 years old experience certain degree of acne. On an average 42.5% of men and 50.9% of women continue to suffer from the disease in their twenties [3-5].

The therapies which are commonly used to the treatment of Acne vulgaris include are hormonal, systemic, topical, herbal and some drugs with combination therapy. The topical drugs used for the treatment and management of Acne vulgaris are benzoyl peroxide,

antibiotics, and retinoids. The systemic agents are also being used and they are commonly known as antibiotics and isotretinoin. For the past many year's the researchers prove that the antibiotics and retinoids have been used mostly and they remain a very good choice for the topical and systemic treatment of *Acne vulgaris*. But, the use of these drugs produces a numeral of potential side effects and consumption of antibiotics will usually make the bacteria become resistant and permanent nature multiplies within its host [6,7].

Herbal therapies, which have been in use from ancient times for the treatment of acne, include various herbal extracts, oil and their ayurvedic formulation. The introduction of novel herbal formulations for the treatment of acne may produce many advantages over previously formulated therapies. These herbal drugs are effective against a variety of Gram-positive and Gram negative Bacteria. Sunder Vati, which is an ayurvedic formulation, was found to be orally effective and well tolerated for the treatment of acne vulgaris. Purintablets and klarina cream formulations, which contain many herbal extracts and have negligible adverse effects compared with modern medicine, are commonly indicated for moderate and severe forms of acne. There are certain herbal extracts, such as *A. dahurica, R. coptidis and Psidium quajava*, that are more effective than antibiotics and retinoids. The efficacy of these herbal agents in acne treatment is not only based on antimicrobial activity but on their possessed antioxidant and anti-inflammator properties by which they inhibit neutrophil migration and generation of ROS. Herbal extracts or oil may be used as monotherapy or in combination therapy. The concerned side effects of herbal drugs are much less compared with modern drugs. Thus, natural substances, which are obtained from the medicinal plant, having antibacterial and anti-inflammatory activity, are commonly employed for the treatment of acne [8-10].

Wrightia tinctoria which is commonly known as 'Danthapala' is a known potential medicinal plant, the leaves of which is traditionally used in the treatment of psoriasis and non-specific dermatitis in Siddha and Ayurvedic systems of medicine and distributed in tropical region belongs to the family *Apocynaceae* The plant of *W. tinctoria* is widely distributed in Asia, Africa and Australia and are known to be the native of Australia,

India, Myanmar, Nepal and Vietna Numerous traditional uses have been recorded for *W. tinctorial*. In Karnataka and Tamilnadu (states of India), the tree named as "jaundice curative tree", since the juice of its tender leaves is used as an effective drug for treatment of jaundice [11,12]

Fresh leaves are pungent and when crushed fresh leaf filled in the cavity of decayed tooth or chewed under tooth relieves toothache. Juice of leaves is also employed against serpent bite and considered febrifuge, stomachic and tonic ark is used as tonic, anthelmintic, antidiarrheal, febrifuge and aphrodisiac; it is used in treatment of seminal weakness, flatulence, colic diarrhea, leprosy, psoriasis, haemorrhoids dipsia, helminthiasis fever, digestive, stomachic, constipating, depurative, febrifuge, burning sensation and dropsy. Seeds are useful as a tonic, carminative, anthelmintic, astringent, aphrodisiac and febrifuge and for treatment of stomach disorder. In Unani medicine, the seeds of Wrightia are differently known as "Lisanul-e-Asafir", Inderjao Shireen, and Meetha Inderjao and have been reported to have used for cure disorders of central nervous system and claimed to have analgesic, aphrodisiac, tonic and emmenagogue actions. *W. tinctoria* have been recommended for the treatment of infections of the chest (in asthma), colic and as diuretic. Extracts of the root and leaves possess hypotensive activity [13,14]. Keeping the uses of this plant in mind this study deals with the evaluation of anti -acne activity of *W. tinctoria.*

Experimental

Materials

Collection of plant material

Leaves of *Wrightia tinctoria* were collected from local area of Bhopal (M.P.) in the month of February, 2022. The leaves of plants sample were separated and washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. Drying of fresh plant part was carried out in sun but under the shade. Dried leaves of *Wrightia tinctoria* were preserved in plastic bags and closed tightly and powdered as per the requirements.

Methods

Extraction of plant material

63.57 gram shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration [81]. The extraction was continued till the defatting of the material had taken place. Defatted leaves powder of *Wrightia tinctoria* has been extracted with ethanol using maceration for 48 hrs, filtered and dried using vaccum evaporator at 40^0C.

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage.

Phytochemical screening

Phytochemical examinations were carried out for all the extract as per the standard methods [15].

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method [83]. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm [16].

Estimation of total protein content

The amount of protein was estimated by Lowry's method. 1 ml of each BSA (Bovine serum albumin) working standard 50-250 µg/ml or test in test tubes. The test tube with 1 ml distilled water was serving as blank. Added 4.5 ml of reagent I and incubated for 10

minutes. After incubation added 0.5 ml of reagent II and incubated for 30 minutes. Measure the absorbance at 660 nm and plot the standard graph [17].

Antimicrobial testing

The well diffusion method was used to determine the antimicrobial activity of the extract prepared from the *Wrightia tinctoria* using standard procedure [18]. Four bacterial species *Streptococcus mutans, Klebsiella pneumoniae, Candida albicans, Propionibacterium acnes* were tested for effect of *Wrightia tinctoria* extract. Four standard drug Ofloxacin, Ciprofloxacin, Fluconazole, Clindamycin was also used. There were 3 concentration used which are 25, 50 and 100 mg/ml for extracted phytochemicals in antibiogram studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37^{o}C for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

Results & Discussion

The percentage yield for pet ether extract & Ethanolic extract for leaves of *Wrightia tinctoria* was found to be 1.52% 7.54% respectively suggesting ethanol as better solvent. The phytochemical screening revealed presence of flavonoid, carbohydrate, proteins & diterpenes. The total flavonoid & total protein content of ethanolic extract of *Wrightia tinctoria was found to be 0.762mg/100mg amd 0.541 mg/100mg of dried extract. The antimicrobial activity was evaluated by well diffusion method. Four bacterial species Streptococcus mutans, Klebsiella pneumoniae, Candida albicans, Propionibacterium acnes* were tested for effect of *Wrightia tinctoria extract.* At concentration of 100mg/ml the zone of inhibition for *Streptococcus mutans, Candida albicans, Propionibacterium acnes* was found to be 9±0.94mm, 15±0.47mm, 17±0.74mm respectively. The zone of inhibition were not seen in case of *Klebsiella pneumoniae* The result of antimicrobial acytivity suggest that the ethanolic extract of *Wrightia tinctorial* was highly effective against *Propionibacterium acnes.*

Table 1: % Yield of *Wrightia tinctoria*

S. No.	Extracts	% Yield (W/W)
1.	Pet. ether	1.52%
2.	Ethanolic	7.54%

Table 2: Phytochemical screening of *Wrightia tinctoria* extract

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Dragendroff's test	-ve
	Wagner's test	-ve
	Mayer's test	-ve
	Hager's test	-ve
2.	**Glycosides**	
	Legal's test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenol**	
	$Fecl_3$	-ve
5.	**Carbohydrates**	
	Fehling's test	+ve
6.	**Tannins**	
	Gelatin test	-ve
7.	**Proteins**	
	Xanthoproteic test	+ve
8.	**Saponins**	
	Foam test	-ve
9.	**Diterpenes**	
	Copper acetate test:	+ve

Table 3: Total bioactive constituents content of *Wrightia tinctoria*

S. No.	Extract	Total flavonoid (mg/100mg)	Total protein (mg/100mg)
1.	Ethanolic extract	0.762	0.541

Table 4: Antimicrobial activity of standard drug against selected microbes

S. No.	Name of drug	Microbes	Zone of inhibition		
			10 µg/ml	20 µg/ml	30 µg/ml
1	Ofloxacin	*Streptococcus mutans*	12±0.15	15±0.13	17±0.19
2	Ciprofloxacin	*Klebsiella pneumoniae*	10±0.57	18±0.47	20±0.57
3	Fluconazole	*Candida albicans*	26±0.5	30±0.74	32±0.94
4	Clindamycin	*Propionibacterium acnes*	13±0.28	16±0.57	19±0.86

Table 5: Antimicrobial activity of ethanolic extract of *Wrightia tinctoria* against selected microbes

S. No.	Name of microbes	Zone of inhibition		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Streptococcus mutans*	6±0	8±0.86	9±0.94
2.	*Klebsiella pneumoniae*	-	-	-
3.	*Candida albicans*	10±0.5	12±0.57	15±0.47
4.	*Propionibacterium acnes*	11±0.86	14±0.94	17±0.74

Conclusion

The present research work concludes that *Wrightia tinctoria* is important medicinal plant with varied antimicrobial spectrum. Current study on the evaluation of anti-acne property exhibited by the medicinal plant *W. tinctoria* would in near future greatly assist the mankind in the skin disorders & ailing diseases. Qualitative & quantitative stimation of phytochemical revealed that plant is enriched with flavonoid & protein. Further studies are needed to explore the potential compounds responsible for the biological activity from *W. tinctoria* for curing skin related disorders.

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