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Anti- acne activity of Piper nigrum fruit extract

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ABSTRACT:

Many people in the world are affected by acne. Acne affects the patients physically as well as psychologically. There are various factors contributing to acne. There are more chances of acne development in patients with its family history. Piper nigrum, which is a commonly found fruit, possesses anti acne activity. In this study, the extract of Piper nigrum is tested for its anti-acne activity. The whole dried fruits of Piper nigrum were collected from the local market of the Valsad district (Gujarat) and authenticated. Preliminary phytochemical tests and pharmacognostic evaluation were performed on the dried fruits of Piper nigrum. The microscopic characters were also studied. The extracts of Piper nigrum were prepared. The extract with the highest yield was selected and agar cup plate method was used to analyse the antimicrobial activity of Piper nigrum. Propionibacterium acnes was used for performing this antimicrobial study, while using clindamycin as the standard drug. The alcoholic extract gave the maximum percentage yield. Phytochemical analysis showed that the alcoholic and aqueous extracts of Piper nigrum contains alkaloids, carbohydrates, proteins, amino acids, phenols, resins, tannins, volatile oil and resins. The zone of inhibition was found to be 11.3 mm, which was significantly closer to that shown by clindamycin. It was concluded from this study that Piper nigrum is an effective drug which can be used in the management of acne.

KEY WORDS: Anti- acne, Piper nigrum, Clindamycin, pilosebaceous glands, Propionibacterium acnes

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INTRODUCTION:

Acne is a very common problem of skin which affects population worldwide. It has psychological impact on patients. People have a lot of misunderstandings and wrong beliefs about this disease¹. The studies groups of a French study on acne in adolescents thought that eating dairy products, gender, physical activity and excess weight have no influence on acne. They also believed that acne can be improved by frequent washing. They concluded that smoking cigarettes, eating chocolate and snacks, sweating, touching or squeezing the spots, not washing, using make-up, eating fatty foods, menstruation and pollution worsen acne. Many of these beliefs are not true. About 80.8% of the patients involved in the study believed that acne is not a disease and it is just a normal phase of adolescence. Whereas, 69.3% believed that acne should be treated². An another study was conducted in Turkey which indicated that there is an urgent requirement to educate the people about the potential complications, etiopathogenesis and importance of appropriate acne treatment because there is high prevalence but deficient knowledge and false beliefs regarding acne³. Recently, an association has been studied between certain dietary factors and acne. A common perception has been seen among acne patients that foods such as chocolates, sweets, and nuts, oily and fried foods are responsible for

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exacerbation of acne. Many studies have been performed for the determination of association between diet and acne. But unfortunately, inconclusive results have been obtained. Acne patients should avoid milk consumption and diet with high glycemic load, including refined sugar, processed food etc. Calcium and vitamin D supplementation is advised along with the maintenance of healthy weight⁴. Stress is also considered as one of the major causes of acne. According to a study conducted in Saudi Arabia, acne got worse in 58.4% patients due to stress. There was one uncommon cause which came up in this study. 3% of the patients believed that the use of computer screen can make acne worse⁵. Diet induced hyperinsulinemia triggers the hormonal cascade which in turn elicits an endocrine response. Simultaneously, this hormonal response promotes the growth of unregulated tissue and also enhances the synthesis of androgen. Thus it can be concluded that hyperinsulinemic diet is involved in acne development as it has an influence on the growth of follicular epithelium and keratinization and also on the sebum secretion mediated by androgen. Acne may be classified in four grades depending upon their severity and symptoms. In grade 1 acne, open or closed comedones are present with few papules. Comedones and papules are present along with few pustules in grade 2 acne. In grade 3 acne, along with comedones, pustules and papules, few nodules are also present. And in grade 4 acne, pustules, comedones, nodules, papules and cysts are present⁶.

Approximately 20% of young population is affected by moderate to severe acne condition. Its severity is dependent on pubertal maturity. The association of ethnicity with acne is unclear. The effect of post inflammatory hyperpigmentation is more on back individuals. The chances of acne inheritance in first degree relatives is about 80%. Acne develops at an early age and more severely in people having positive family history. People suffering from severe acne have higher suicidal tendencies as compared to those with mild acne condition⁷. There is a heavy impact of acne on the quality of life of the patients. Patients suffering from acne vulgaris have possibility of having depression, anxiety, stigmatization, embarrassment, lower self esteem, social withdrawal, eating disorders, unemployment and body dysmorphic disorders⁸.

Acne is a disease of the hair follicles of the skin which are associated with oil glands. The clinical symptoms of acne are inflammatory lesions (papules and pustules), non-

inflammatory lesions (open and closed comedones), various degrees of scarring and seborrhoea (excess grease). Acne mostly affects the body parts having maximum density of the pilosebaceous units, such as, face, neck, shoulders, upper chest and back⁹. Acne have a complex pathogenesis. It is dependent on four important factors. These factors are follicular hyperkeratinisation, leading to comedones stimulation of sebaceous gland activity mediated by androgen, inflammatory mediators released into the skin and follicular colonization of bacterium *Propionibacterium acnes*. *Propionibacterium acnes* is an anaerobic bacterium and is a normal constituent of skin microbial flora. It gets proliferated in the pilosebaceous ducts due to the high level of sebum which is elicited by androgen. This proliferation activates the inflammatory response of the host. Thus interleukin-1b (IL-1b), IL-8, proinflammatory cytokines, tumor necrosis factor- α (TNF- α), granulocyte macrophage colony stimulating factor (GM-CSF) and complement factors are discharged. *Propionibacterium acnes* is the main causative microorganism, but *Pityrosporum ovale* and *Staphylococcus epidermidis* are also present in acne lesions¹⁰.

Materials and Methods

Piper nigrum, in the form of whole dried fruits, were collected from the local market of the Valsad district from Gujarat. These whole dried fruits of *Piper nigrum* were authenticated by Dr. Sachin B. Narkhede, the head of pharmacognosy department in Smt. BNB Swaminarayan Pharmacy College, Salvav (Gujarat). Some preliminary phytochemical tests and pharmacognostic evaluation of dried fruits of *Piper nigrum* was done. The fruits were collected and sectioned, and then followed by safranin staining for their anatomical study¹¹. The microscopic characters were studied by using the powder of dried fruits of *Piper nigrum*¹².

The shade dried fruits of *Piper nigrum* were reduced to fine powder and around 400 gm of powder was subjected to successive continuous hot extraction (soxhlet) with petroleum ether, chloroform, alcohol and water. Each time before extracting with the next solvent the powder material was dried in a hot air oven at 50°C for one hour. After the effective extraction, the solvents were distilled off, the extracts were then concentrated on water bath and extracts obtained with each solvent were weighed. Its percentage were calculated. The extract with the highest yield was selected and agar cup

plate method was used to analyse the antimicrobial activity of *Piper nigrum*^{13, 14}. *Propionibacterium acnes* was the organism which was used for performing this antimicrobial study. Clindamycin was used as the standard drug.

RESULTS AND DISCUSSION

Macroscopy

The entire fruit of *Piper nigrum* is almost globular. It is about 4-6 mm in diameter and is of brownish to black in colour. The surface of the fruit is uneven. Its seeds are brown to black in colour. It has aromatic smell and pungent taste.

Microscopy

The epicarp has an outer layer composed of polygonal cells with distinct cuticle. It contains contents which are dark brown to black in colour. Below this, there are 2-3 layers of thin walled parenchymal cells. These parenchymal cells are intermingled with thick isodiametric to radically elongated stone cells. Mesocarp is comparatively broad zone which constitutes the greater area of pericarp. The outer 7-8 layers of cells are parenchymatous cells along with certain small starch grains. Large secretion sacs and oil/resin contents are scattered among these cells. Below this are several layers of compressed cells and fibrovascular bundles. A layer of larger oil cells which are having suberized walls are present below the compressed cells. Below this, a zone of two layers of small parenchymal cells are present. Endocarp consists of single layer of stone cells. The radius and inner walls of these stone cells are more strongly signified than the outer layer. Testa consists of 2-3 layers of the compressed elongated cells. A pigment layer containing dark brown tannin substance is present below testa. radically elongated perisperm cells in the inner zone are embedded largely with oleoresins along with starch and protein substances.

Powder microscopy

The powder of *Piper nigrum* fruit is brown to blackish in colour. It has pungent odour with bitter acrid taste. When the powder is stained with safranin, it shows isodiametric or slightly elongated stone cells interspersed with beaker shaped stone cells, thin walled polygonal hypodermal cells and numerous elongated, polyhedral cells from perisperm. These are tightly packed with many

minute compounds, trachieds and round to oval starch grains oil globules.

Physicochemical parameters

Following is the table with the results of physicochemical parameters of *Piper nigrum*, which includes parameters such as loss on drying, ash values, pH and moisture content.

Sr. No.	Parameters studied	<i>Piper nigrum</i>
01	Loss on drying	3.43%
02	Ash value	
	Total ash	4.41% w/w
	Acid insoluble ash	0.42% w/w
	Water soluble ash	4.22%w/w
03	pH	7.41
04	Moisture content	10.39%w/w

Percentage yield of extracts of *Piper nigrum*

The percentage yield of the different extracts of *Piper nigrum* are as follows:

Sr. No.	Solvents	Nature of extract	Color	% Yield
01	Pet-ether	Semisolid	Black	0.7
02	Chloroform	Semisolid	Black	1.6
03	Alcohol	Semisolid	Black	2.5
04	Aqueous	Semisolid	Black	2.1

The maximum yield was extracted from alcoholic extract of *Piper nigrum*.

Phytochemical parameters

Phytochemical analysis revealed the presence of alkaloids, carbohydrates, proteins, amino acids, phenols, resins, tannins, volatile oil and resins in ethanolic and aqueous extracts of *Piper nigrum*.

Sr. No.	Phytoconstituents	Ethanol	Aqueous
01	Alkaloids	+	+
02	Carbohydrates	+	+
03	Proteins	+	+
04	Amino acids	+	+
05	Flavonoids	-	-
06	Glycosides	-	-
07	Phenol	+	+
08	Saponins	-	-
09	Resin	+	+
10	Tannin	+	+

11	Volatile Oil	+	+
12	Resins	+	+

Antimicrobial activity

The antibacterial activity of the ethanolic extract of *Piper nigrum* was tested against *Propionibacterium acnes* by studying the parameter of zone of inhibition. Agar cup plate method was used for this study. The zone of inhibition was found to be 11.3 mm.

Drug	<i>Piper nigrum</i> extract	Clindamycin
Zone of inhibition (in mm)	11.3	18.9

CONCLUSION

This study may be used as a reference for identification of *Piper nigrum* on the basis of macroscopy and microscopy. This can also be used as a reference for the physicochemical and phytochemical records of *Piper nigrum*. The preliminary phytochemical investigation may assist for the isolation of some important compound in future studies. The antimicrobial analysis of *Piper nigrum* shows that it is an effective drug which can be used in the management of acne.

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