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Full Length Research Paper

Evaluation of anti-acne activity of hydroalcoholic extract of *Punica granatum* Linn.

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Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Acne vulgaris is an extremely common disorder affecting many adolescents and adults throughout their lifetimes. The pathogenesis of acne is multifactorial and is thought to involve excess sebum, follicular hyperkeratinization, bacterial colonization, and inflammation. Pomegranate, *Punica granatum* Linn. (Punicaceae) is an ancient, mystical, unique fruit used in several systems of medicine for a variety of ailments. The synergistic action of the pomegranate constituents appears to be superior to that of single constituents. The current research was focused on evaluation of anti-acne activity of *Punica* extract followed by its biological screening. The fingerprinting and spectroscopic analysis of the extract was determined. The attempt was made to investigate the extract of *P. granatum* for the said activity with the goal of elucidating the active potential compounds.

Key words: *Punica granatum*, broth dilution, cup plate method, high performance thin layer chromatography.

INTRODUCTION

Acne has plagued humankind since antiquity. Acne vulgaris is a highly variable disease attracting a crisp social rebuttal. Acne is the most common skin disease of adolescence and few teenagers escape the experience. The severity of acne varies considerably and in some individuals acne persists beyond the teens for reasons that are not yet clear. Acne usually begins at puberty when the output of sebum (grease) by tiny hair follicles on the face and upper trunk increases substantially. The sebum acts as a nutrient for a resident skin bacterium called *Propionibacterium acnes* (or more familiarly the acne bacillus), which grows abnormally in follicles whose pores are blocked (Kumar, 2005). The factors important in the development of acne are plugging of the hair follicle with abnormally cohesive desquamated cells, sebaceous gland hyperactivity, proliferation of bacteria (especially *P. acnes*) within sebum and inflammation. The changes in the hair follicle occur when the follicular canal becomes blocked with abnormally keratinized desquamating cells. This plug starts above the opening of the sebaceous gland into the follicular canal and causes gradual expansion of cells and sebum within the canal (Jain, 2007).

The pomegranate is an ancient, mystical, unique fruit used in several systems of medicine for a variety of ailments. The synergistic action of the pomegranate constituents appears to be superior to that of single constituents. The potential therapeutic properties of pomegranate are wide-ranging and include treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, and protection from ultraviolet (UV) radiation. This research indicates the most therapeutically beneficial pomegranate constituents are ellagic acid, ellagitannins (including punicalagins), punicic acid, flavonoids, anthocyanidins, anthocyanins, and estrogenic flavonols and flavones (Jurenka, 2008).

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It has a variety of uses such as blood purifier, in various skin diseases, immunomodulant, anti-inflammatory and anti-platelet-activating factor (PAF). The traditional claim on this plant for the fruit rind and bark reveals the anti-acne effect which can reduce the sebum production. That is why this research work is focused on carrying out anti-acne activity.

MATERIALS AND METHODS

Plant collection and extraction

The fresh fruit rinds of the plant *Punica granatum* Linn were collected from the local region of Pune. It was authenticated by Department of Botany, University of Pune (Voucher No. Bot /35/11). The powder of fruit rind prepared was extracted by maceration with hydroalcoholic mixture (60:40) for 72 h.

Histology

Histology was performed as to know the various microscopical aspects. The plant specimen of fruit rind were cut and fixed in formalin acetic acid (FAA) and then infiltration of specimen is carried out by paraffin wax (Sass, 1940). The specimens were sectioned with the help of microtome with thickness of 10 to 12 µm. The sections were stained with toluidine blue (O' Brien et al., 1964). Photographs of different magnifications were taken with Nikon Laboratory photo 2 microscopic units (Esau, 1964).

Preliminary phytochemical screening

The extract was then subjected to preliminary phytochemical screening to detect the presence of various phytoconstituents by various chemical tests (Mukherjee, 2002).

Anti-acne activity of the extract

The lyophilized cultures of bacteria *P. acne* (MTCC No. 1951) were procured from Indian Institute of Microbial Technology (IMTECH), Chandigarh. The dilutions of the extract were prepared and brain heart infusion broth was prepared. Tween 80 and 0.03 ml thioglycollic acid per 100 ml was added in the prepared broth as a reducing agent (Cunliffe, 1997). The 25 ml of the medium was poured in the ten test tubes followed by sterilization with autoclave at 15 lb pressure and 121°C for 30 min. Using sterile pipette exact amount of extract was added as indicated in the Table 1 and the final volumes were adjusted to 10 ml with medium followed by inoculation of cultures and incubation at 37°C for 48 h (Kumar, 2001). The growth in the tubes was monitored by turbidity method and minimum inhibitory concentration (MIC) of the extract was determined (Feldman, 2004; Fogdall, 1974). The extract was also subjected to anti-acne activity by cup plate diffusion method using clindamycin as internal standard and MIC was determined by zone of inhibition (Gemmell, 2007). Both analyses were performed thrice to confirm the efficacy of the result.

RESULTS

Pharmacognostic study

The quality control parameters were established and proximate analysis was found to be significant. Preliminary phytochemical screening revealed the presence of tannins and alkaloids.

Histology

The fruit or the pericarp is thick and fleshy and consist of less prominent epidermis or epicarp. Sclerides are distributed throughout in Mesocarp (Figures 1 and 2). Vascular strands have radial files of small xylem elements with phloem masses (Easu, 1964; Gambe, 1935).

Screening of extract for anti-acne activity

To screen the plant material for their anti-acne activity *in vitro* experiments were carried out by using the organism *P. acnes* (Rosso, 2004). The results as shown in Table 2 depict that the MIC values of hydroalcoholic extract of *P. granatum* was found to be 100 mg/ml (Leyden, 2001). The zone of inhibition was determined by cup plate diffusion method (Tables 1 and 2), where an increase in anti-acne activity was observed from zone of lysis emphasizing that the lysis may be due to the active components present in the extract of the plant (Kumar, 2001).

DISCUSSION

The results of the zone of inhibition for *P. granatum* are shown in Tables 1 and 2 (Kumar, 2001). The hydroalcoholic extract shows good anti-acne activity as compared to standard drug clindamycin. Thus the targets in the microbial cell could be surface exposed adhesion, cell wall peptides and membrane bound enzymes (Gnanamani, 2003).

Here tannins are the major phytoconstituent present in this plant which is responsible for anti-acne as in plant *Portulaca oleracea* containing the tannins possesses the said activity, also ethanolic extract of *Vernonia scorpioides* possess anti-acne action by improving regeneration due to the presence of tannins. Thus the experimental findings suggest the plant was found to be effective as to inhibit the effects caused by the *P. acnes.*

Conclusion

This study thus demonstrates the anti-acne activity of hydroalcoholic extract which may be effective in the treatment of acne vulgaris.

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Table 1. Protocol for evaluation of MIC by broth dilution method.

S/N	Amount of extract/ml	Amount of medium	Total volume of solution (ml)	Concentration of extract in final sol (ml)
1	0.1	9.9	10	0.1
2	0.2	9.8	10	0.2
3	0.3	9.7	10	0.3
4	0.4	9.6	10	0.4
5	0.5	9.5	10	0.5
6	0.6	9.4	10	0.6
7	0.7	9.3	10	0.7
8	0.8	9.2	10	0.8
9	0.9	9.1	10	0.9
10	1.0	9.0	10	1.0

Table 2. Zone of inhibition by cup plate method.

S/N	Amount of extract/ml	Zone of inhibition in mm for *Punica* extract (including borer size)
1	0.1	12
2	0.2	13
3	0.3	14

Diameter of standard borer 6 mm.

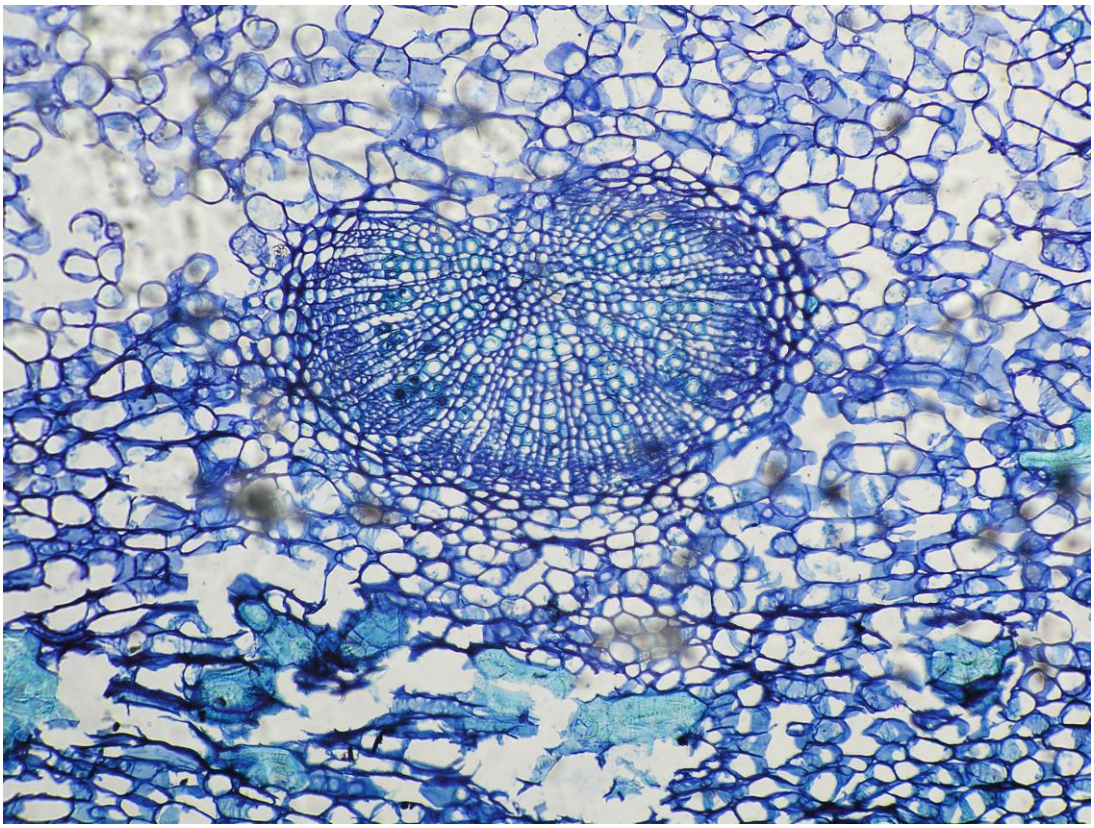

Figure 1. Histology showing sclerides throughout mesocarp region.

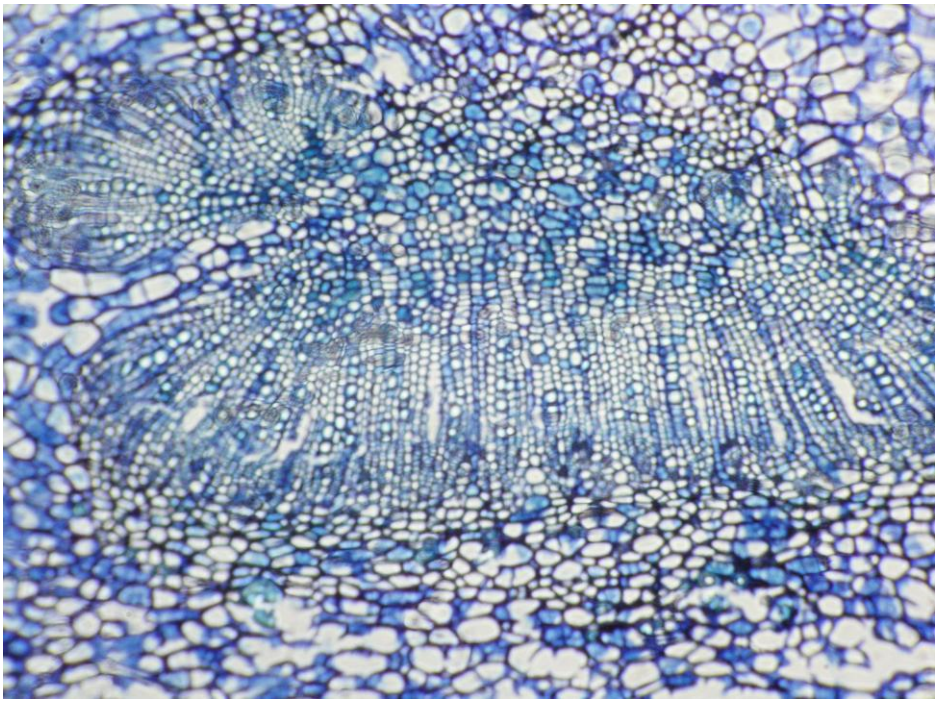

Figure 2. Histology showing vascular strands with xylem and phloem masses.

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