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Anti-acne activity of *Achillea* 'Moonshine' petroleum ether extract

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Achillea millefolium **(yarrow) is a traditionally used plant to treat wounds. The present study was conducted to evaluate the anti-acne activity of *Achillea* 'Moonshine', a hybrid variety of *Achillea*. The plant was extracted in four solvents - petroleum ether, ethyl acetate, ethanol and water. These extracts were screened for anti-microbial, free radical scavenging, anti-tyrosinase, anti-inflammatory activity and cytotoxicity assays necessary to characterize its anti-acne activity. The most promising activity was determined in the petroleum ether extract. The minimum inhibitory concentration (MIC) value for the petroleum ether extract was 0.83 mg/ml against *Propioni bacterium acnes* and 0.37 mg/ml against *Staphylococcus*. The minimum bactericidal concentration (MBC) value for petroleum ether was 0.83 and 0.75 mg/ml for *P. acnes* and *Staphylococcus epidermidis*, respectively. Though the ethyl acetate had a high flavonoid and phenolic content it was observed that the IC_{50} values for the petroleum ether extract for free radical scavenging activity was 64.81 µg/ml, which was higher than ethyl acetate. Petroleum ether also showed tyrosinase inhibition at 0.033 mg/ml. The extract was also able to decrease the inflammatory cytokines like TNF-α and IL-8, and showed no cytotoxicity against dermal fibroblasts. These results suggest presence of active anti-acne phytochemicals in the petroleum ether extract, making it a novel plant candidate for the treatment of acne.**

Key words: Anti-acne, *Achillea* 'Moonshine', petroleum ether extract.

INTRODUCTION

Achillea millefolium Linn. commonly known as yarrow is a flowering plant from the Asteraceae family, and is represented by about 85 different species. It is widely found in Asia, North America and Europe (Li et al., 2011; Moradi et al., 2013). Traditionally, yarrow is purported to be a diaphoretic, astringent, tonic, stimulant and mild aromatic (Benedek et al., 2007). The plant has a long history as a powerful 'healing herb', used topically for wounds, cuts and abrasions (Benedek et al., 2007). Previous studies have shown yarrow to have potential

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cosmetic uses (Chandler et al., 1982) including skin surface rejuvenation effects. However, the majority of the studies have been performed on the flowers of yarrow. Various species of *A. millefolium* used in garden traditions have been superseded with cultivars and hybrids with improved qualities. One such hybrid is *Achillea* 'Moonshine' which is a cross between *Achillea* 'Taygetea' and *Achillea clypeolata. Achillea* 'Taygetea' is an ornamental plant native to Europe while *Achillea clypeolata is* native to Balkan Peninsula. Both the plants have yellow colored flowers and are drought resistant. However, there has not been much research on individual species. Over and above, hybridization and intraspecific variability have complicated the taxonomy. As a result of this, most of the plants of genus '*Achillea*' are referred to as Yarrow and there is no clear identification of individual species. For the following study, the most popular *Achillea* that is used in the North American gardens *Achillea* 'Moonshine' was used.

Achillea 'Moonshine' is an herbaceous perennial plant, 1 to 2 ft high and blooms between June to September, and is resistant to drought and dry soil. It is known for its fern-like, aromatic, silvery to gray-green foliage and its tiny, long-lasting, bright lemon-yellow flowers which appear in dense, flattened, compound corymbs (to 2 to 3" across). The present research is aimed towards the study of anti-acne activity in the leaves and stem extracts of *Achillea* 'Moonshine'.

Acne vulgaris (cystic acne or simply acne) is a common human skin disease, occurring mostly during the onset of puberty. It is generally characterized by areas of skin with seborrhea, comedones, papules (pinheads), pustules (pimples), nodules and possibly scarring. The known causes for acne are infection by bacteria such as *Propionibacterium acnes* and *Staphylococcus epidermidis*, hormonal changes, genetic predisposition to acne, adverse effects of cosmetics, medications, or other factors. Hence, all scientific attempts in developing anti-acne drugs focus on addressing the above mentioned causes of acne (Toyoda and Morohashi, 2001).

Acne occurs due to blockage in the follicles resulting in the formation of comedones. The sebaceous glands become clogged with sebum and dead skin cells. Under these conditions, bacterium *Propionibacterium acne* can cause inflammatory lesions in the dermis around the comedones or microcomedo resulting in redness and scarring. The inhibition of *P. acnes* decreases the rupturing of comedones into the surroundings and helps to inhibit the progression of acne. Another organism which is implicated in the etiology of acne is a common skin micro flora, *Staphylococcus epidermidis* which promotes pustules and nodule formation. Thus, for screening anti-acne compounds the inhibition of both these organisms is of prime consideration in treating acne (Knor, 2005; Tanghetti, 2013).

Another factor that causes increased chances of acne is oxidative stress. The skin is exposed to oxidative stress induced by reactive oxygen species (ROS) both endogenously and externally. ROS play an important role in production of inflammatory mediators such as interleukin-8 (IL-8) and tumor necrosis factor- α (TNF-α) by monocytes/macrophages during the progression of acne. Also, excessive and repeated stimulation by invading organisms such as *P. acnes* and *S. epidermidis* causes over production of ROS (Akamatsu and Horio, 1998). These ROS causes considerable damage to the tissues leading to increase in inflammatory responses. Thus, reduction of ROS by antioxidants and free radical scavengers as well as inhibition of these inflammatory mediators is necessary to prevent damage at the cellular and the tissue levels. Post inflammatory hyper pigmentation (PIH) characterized by pigmentation in dermis or epidermis is commonly seen in acne sufferers. It causes discoloration of skin following the inflammation caused during acne. Increase in pigmentation is due to alternations in the melanocytes, and thus, post-acne treatment requires clearing of affected skin area (Davis and Callender, 2010). A potential anti-acne candidate should thus also have an ability to clear the melanin depositions.

Many oral and topical antibiotics are used for the management and treatment of acne vulgaris. However, the prolonged use of these antibiotics has created a problem of antibiotic resistance among the acne inducing bacteria (Humphrey, 2012). Also, the long term use of topical agents causes skin irritation, leading to decrease in patient compliance and treatment failure. To overcome these drawbacks, natural products have been studied extensively as alternatives to complement the existing therapies for the treatment of acne because they provide a largely unexplored source of drug discovery and development.

In this study, the antibacterial activity of *Achillea* 'Moonshine' extracts against the two acne causing organisms (*P. acnes* and *Staphylococcus epidermidis*), free radical scavenging activity, anti-tyrosinase, anti-inflammatory potential as well as cytotoxicity against human skin cells to identify the most potent extract possessing anti-acne activity were determined.

METHODOLOGY

Collection of plant material

Eight full bloomed *Achillea* 'Moonshine' plants were collected from Holly Days Nursery Inc. near Philadelphia (United States) in June, 2013.

Preparation of extracts

The leaves and stems were washed three times with distilled water and grounded into fine powder using a simple grinder after they were air dried for 3 days. The plant material was then extracted with four solvents of increasing polarity (petroleum ether, ethyl acetate, ethanol and water) by maceration. The solvent was evaporated and the extracts were stored at 4°C till further use.

Determination of anti-microbial activity against *P. acnes* and *S. epidermidis*

Bacterial strains and media

The bacterial strains incorporated in this study were *P. acnes* (ATCC) and *S. epidermidis* (ATCC). *P. acnes* was incubated in brain-heart infusion media for 48 h at 37°C under anaerobic conditions in an anaerobic jar, while *S. epidermidis* was incubated in Muller Hinton broth for 24 h at 37°C, and their densities were adjusted to approximately 0.5 McFarland Standard.

Anti-microbial activity by disc diffusion method

Sterile 5 mm-Whatman No. 1 filter paper discs were used in the disc diffusion assay. The discs were soaked separately with 30 µl of each of the extract at a concentration of 6 mg/ml in dimethyl sulfoxide (DMSO) for organic solvents and sterile water for the water extract. Negative control discs were soaked in DMSO and distilled water for organic solvent and water extracts respectively. Erythromycin (60µg/disc) was used as positive control. These discs were placed on Mueller-Hinton agar plates and brain heart infusion agar plates, previously swabbed with *S.epidermidis* and *P. acnes* respectively at a concentration of 10^6Colony Forming Units (CFU)/ml. The plates were incubated at 37°C for 24 h in case of *S. epidermidis* and 48 h anaerobically for *P. acnes.* Anti-bacterial activity was defined as the diameter (mm) of the clear inhibitory zone formed around the discs.

Minimum inhibitory concentration by tube dilution method

Tube dilution method was used to determine the minimum inhibitory concentration (MIC) against *P. acnes* and *S. epidermidis*. The extracts were dissolved in DMSO and were serially diluted from concentration 5 to 0.1 mg/ml in sterile culture tubes containing 0.2 ml sterile nutrient broth. The tubes were inoculated with 100 µl of bacterial suspension (approximately 10^6 CFU/ml) in their respective broths and incubated at 37°C for 24 h in the case of *S. epidermidis* and 48 h anaerobically for *P. acnes*. After incubation, the tubes were examined for growth by visually observing the turbidity. MIC was defined as the minimum concentration that resulted in no growth of the bacteria. The test was carried out in triplicates (Chomnawang et al., 2005; Kim et al., 2008).

Minimum bactericidal concentration (MBC)

The MBC was determined by pipetting out 0.1 ml bacterial culture from MIC tubes which had no bacterial growth and plating it on to Muller Hinton agar for *S. epidermidis* and on Brain heart infusion agar plate in case of *P. acnes* for 24 h and 48 h respectively. After incubation, the concentration at which there was no single colony of bacteria was taken as MBC. The tests were carried out in triplicates.

Determination of total phenolic contents in the plant extracts

The concentration of phenolics in plant extracts was determined using Folin-Ciocalteu method (Pourmoradet al., 2006). The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract in concentration of 1mg/ml, 5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 4 ml 1M $NaHCO_3$. The absorbance was measured at 765 nm. The total phenolic content was calculated from a linear calibration curve with commercial gallic acid. The content of phenolics in extracts was expressed in terms of gallic acid equivalent (µg of gallic acid/ml of extract). The experiments were carried out in triplicates.

Determination of total flavonoid content in the plant extracts

The content of flavonoids in the examined plant extracts was determined using spectrophotometric method (Pourmorad et al., 2006). Briefly, 0.5 ml of methanolic solution of the extract in the concentration of 1 mg/ml was mixed with 1.5 ml methanol, 0.1 ml of 10% $AlCl_3$ solution, 0.1 ml of 1M potassium acetate and 2.8 ml distilled water. The samples were incubated for 30 min at room temperature, and the absorbance was measured at 415 nm.The amount of flavonoid was calculated from a liner calibration curve with commercial quercetin. The flavonoid content was expressed in terms of quercetin equivalent (µg of quercetin/ml of extract). The experiments were carried out in triplicates.

Determination of free radical scavenging activity

The free radical scavenging activity of the extracts was determined on the ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals(Wu et al., 2009). The stock solution of extracts was prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.62 µg/ml respectively. 0.1 ml from each dilution was mixed with 0.9 ml of methanolic solution of DPPH (0.4% w/v). After 30 min incubation in dark at room temperature, the absorbance was recorded at 517 nm. Control sample contained all the reagents except the extract. Percentage scavenging was calculated using equation:

% Scavenging = [Ac - As/Ac] × 100

Where, Ac is absorbance of control (DPPH in methanol); As is the absorbance of the sample/extract. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of percentage scavenging against extract concentration. Tests were carried out in triplicate.

Determination of anti-tyrosinase activity

Tyrosinase inhibition activity in order to evaluate the ability of the extracts to reduce the post inflammatory hyper pigmentation was determined by spectrophotometric assay in a 96 well plate (Baurin et al., 2002). 70 µl of extract in DMSO was mixed with 30 µl mushroom tyrosinase (313 Units/ml). The mixture was incubated for 10 min at 37°C. L-tyrosinase (2 mM) in potassium phosphate buffer (50 mM, pH 6.5) was added and the resultant solution was again incubated for 20 min at 37°C. The final absorbance was read at 490 nm. Percentage inhibition was calculated by

% Inhibition = [Ac - As/Ac] X 100

Where, Ac is absorbance of control; As is the absorbance of the sample. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of percentage inhibition against extract concentration. Tests were carried out in triplicate.

Cell line and culture media

Human monocytic THP-1 cells (ATCC TIB-202) were cultured in RPMI supplemented with heat inactivated fetal bovine serum while normal human dermal fibroblasts (Lonza CC2155) were cultured in FGM™-2 BulletKit™ (Lonza) and were incubated in a humidified

Table 1. Antimicrobial activity of various *Achillea* 'Moonshine' extracts by disc diffusion assay.

Extract	*P. acnes*	*S. epidermidis*
PE	13.6 mm±0.5	13.6 mm±0.5
EA	18 mm±1.73	15.3 mm±1.52
ET	17.1 mm±0.25	NA
Water	NA	NA
Erythromycin	23.8 mm±0.2	21.1 mm±1.25

Values indicate zone of inhibition in mm. Values expressed as mean ± SD (n=3). PE: Petroleum ether extract; EA: Ethyl acetate extract; ET: Ethanol extract; NA: No activity.

Table 2. Antimicrobial activity against *P. acnes* and *S. epidermidis.*

Extract	*P. acnes*		*S. epidermidis*	
	MIC	MBC	MIC	MBC
PE	0.83	0.83	0.37	0.75
EA	1.25	1.25	0.53	1.06
ET	1.00	2.00	2.00	4.00

MIC- Minimum inhibitory concentration (mg/ml); MBC-Minimum bactericidal concentration (mg/ml), PE: Petroleum ether extract; EA: Ethyl acetate extract; ET: Ethanol extract.

atmosphere with 5% CO_2 at 37°C.

Measurement of cytokine production

To determine the effect of the petroleum ether extract on production of pro inflammatory cytokines (TNF-α and IL-8), human monocytic THP-1 cells (10^6 cells/ml) in a serum free media was stimulated with 100 µg/ml *P. acnes* (wet weight), alone or in combination of different concentrations (50 and 100 µg/ml) of the extract and were incubated for 18 h at 37°C. The culture supernatants were harvested. The concentrations of IL-8 and TNF-α in the supernatant were measured by ELISA (Kim et al., 2008).

Cytotoxicity assay

Normal human dermal fibroblasts (6000cells/well) were seeded in a 96 well plate and the extract treatment began 10 h after seeding. The general viability of the cultured cells was determined by XTT assay in which XTT is reduced to its formazan derivative. The cells were incubated with 50 and 100 µg/ml petroleum ether extract in DMSO for 18 h in a humidified atmosphere with 5% CO_2 at 37°C. 20µl of activating reagent was added to 1ml XTT and each well was treated with 50 µl of this activated XTT and was incubated for 5 h. The absorbance of the wells was measured at 475 nm and 660 nm. The tests were carried out in triplicates (Kim et al., 2008).

Data analysis

Data are expressed as mean ± standard deviation or as percentages for anti-microbial, free radical scavenging, total phenolic and flavonoid content determination and anti-tyrosinase activity. Statistical significance was determined by one way ANOVA using GraphPad Prism 6 software for free radical scavenging, anti-tyrosinase, anti-inflammatory activity and cell viability assay (Graphpad Software, Inc, La Jolla, California). Differences were considered significant at P value of less than 0.05.

RESULTS

Determination of anti-microbial activity

Disc diffusion assay

Disc diffusion assay was performed to determine the antibacterial activity of the extracts against *P. acnes* and *S. epidermidis* (Table 1). The assay was performed as a pilot study to identify the active extracts. The water extract did not show any anti-microbial activity against both the organisms, and so was eliminated from further studies. The ethanolic extract did not show any activity against *S. epidermidis* but was active against *P. acnes.*

MIC determination by tube dilution

The anti-microbial activity of the extracts against the two acne causing organisms, *P. acnes* and *S. epidermidis* is shown in Table 2. All the extracts showed anti-microbial activity against the two acne causing organisms. The lowest MIC against *P. acnes* was observed in the petroleum ether extract (0.83 mg/ml) whereas that for *S. epidermidis* was also observed in the petroleum ether extract (0.37 mg/ml). MIC is the lowest concentration of the extract that will inhibit the visible growth of the bacteria while MBC is the lowest concentration of the plant extract that is required to kill the bacteria. As shown in the results, all the extracts show MBC value no more than four times the MIC values, and so all the extracts were considered to have antimicrobial properties. The results reported are performed in triplicates (French, 2006).

Total phenolic and flavonoid content

Total phenolic content was estimated by gallic acid (Table 3), and expressed as micrograms of gallic acid equivalent (GAE)/ml of extract. The highest phenolic content was determined in the ethyl acetate extract (360.3±0.082µg GAE/ml extract). The petroleum ether extract had the lowest amount of phenolic content. Total flavonoid content was estimated by quercetin (Table 3) and expressed as micrograms of quercetin equivalent (QE)/ml of extract. The highest flavonoid content was determined in the ethyl acetate extract (308±0.015 µg QE/ml extract). The petroleum ether extract had the lowest amount of flavonoid content.

Table 3. Total phenolic and flavonoid content in various extracts.

Extract	µg gallic acid/ ml extract	µg quercetin/ ml extract
PE	107.6±0.020	52±0.279
EA	360.3±0.082	308±0.015
ET	226.67±0.037	66.6±0.007

All experiments are performed in triplicates. Values expressed as mean ± SD (n=3). PE: Petroleum ether extract; EA: Ethyl acetate extract; ET: Ethanol extract.

Table 4. % Free radical scavenging activity of various extracts.

Concentration (µg/ml)	BHT	PE	EA	ET
500	96.08±0.035	51.25±0.908	60.24±1.45	93.29±0.460
250	94.94±0.767	54.32±2.1	43.57±0.121	80.78±2.62
125	90.34±1.940	54.68±0.126	44.06±0.539	62.74±2.42
62.5	76.74±0.862	49.85±1.466	40.02±1.204	49.15±0.863
31.25	49.96±1.026	47.21±0.364	34.76±3.64	48.18±1.99
IC_{50}	31.05	64.81	346.36	66.86

All experiments are performed in triplicates. Values expressed as mean ± SD (n=3), PE: Petroleum ether extract; EA: Ethyl acetate extract;.ET: Ethanol extract.

Table 5. % Tyrosinase inhibition of various extracts.

Concentration (mg/ml)	Benzoic acid	PE	EA	ET
0.67	98.82±0.22	89.54±1.68	85.41±0.64	98.69±0.19
0.34	98.86± 0.29	90.28±0.47	75.86±0.67	98.18±0.43
0.17	97.99±0.99	76.35±0.89	59.03±0.68	77.27±3.72
0.09	93.70±0.44	65.81±0.91	57.75±0.24	65.76±0.24
0.04	78.16±4.36	58.29±0.387	52.80±1.79	52.45±0.17
0.02	48.1±0.017	34.18±0.060	33.51±0.008	10.54±0.019
IC_{50}	0.021	0.033	0.037	0.038

All experiments are performed in triplicates. Values expressed as mean ± SD (n=3), PE: Petroleum ether extract; EA: Ethyl acetate extract; ET: Ethanol extract.

Determination of free radical scavenging activity

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay was performed to evaluate the free radical scavenging activity. The % radical scavenging for all the three varieties is shown in Figure 1 and Table 4. DPPH is a stable free radical. On accepting hydrogen from a corresponding donor, its solutions lose the characteristic deep purple (λ_{max} 515–517 nm) color. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. The petroleum ether extract had the highest free radical scavenging activity (IC_{50}: 64.81 µg/ml). BHT (butylated hydroxyl toluene; IC_{50}:31.05 µg/ml) was used as a reference compound. The extract concentrations have a significant level ($P < 0.05$) of free radical scavenging activity compared with the control.

Determination of anti-tyrosinase activity

The % inhibition of tyrosinase by the extracts is shown in Figure 2 and Table 5. The IC_{50} value for benzoic acid, the reference compound, was 0.021 mg/ml. The petroleum

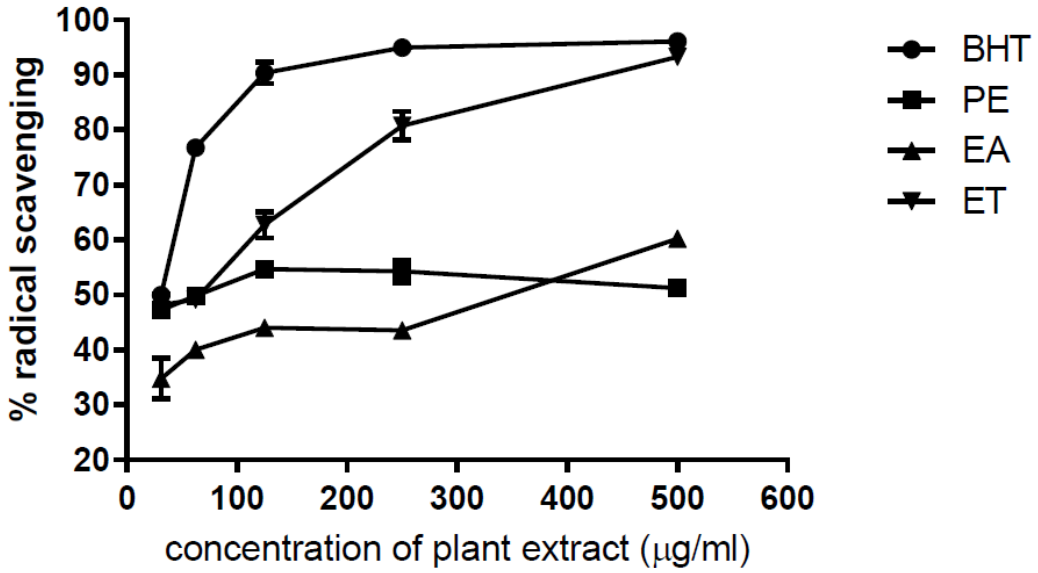

Figure 1. % radical scavenging activity of *Achillea* 'Moonshine' results expressed as the mean ± SD (n=3), PE: Petroleum ether extract; EA: Ethyl acetate extract; ET: Ethanol extract.

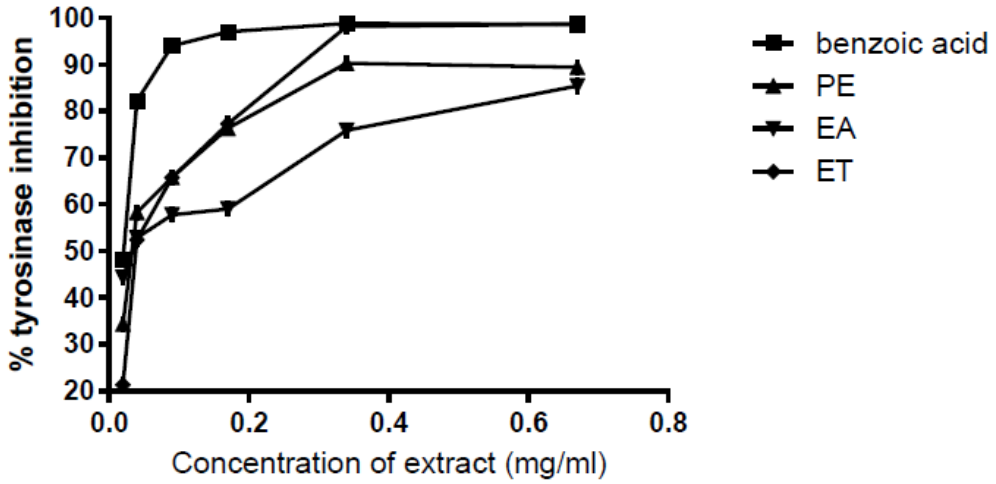

Figure 2. % tyrosinase inhibtion activity of *Achillea* 'Moonshine'. Results expressed as the mean ± SD (n=3)
PE: Petroleum ether extract; EA: Ethyl acetate extract; ET: Ethanol extract.

ether extract showed the lowest IC_{50} value for tyrosinase inhibition (IC_{50}: 0.033 mg/ml) compared to other extracts. The petroleum ether extract concentrations have a significant level ($P < 0.05$) of anti-tyrosinase activity compared with the control.

Measurement of cytokine production

The petroleum ether extract of *Achillea* 'Moonshine' was examined for its activity against inflammation induced by *P. acnes* in terms of inhibitory effects of cytokine production. As shown in the Figure 3, cells treated with bacteria showed an increase in TNF-α and IL-8 production. However, co-cultures of cells with bacteria and extracts (50 µg/ml) suppress the production of these cytokines. There was an elevated level of TNF-α cytokine production in 100 µg/ml of extracts which is was observed in some other studies as well (Kim et al., 2008).

Cytotoxicity assay

A potential candidate to be used in an anti-acne

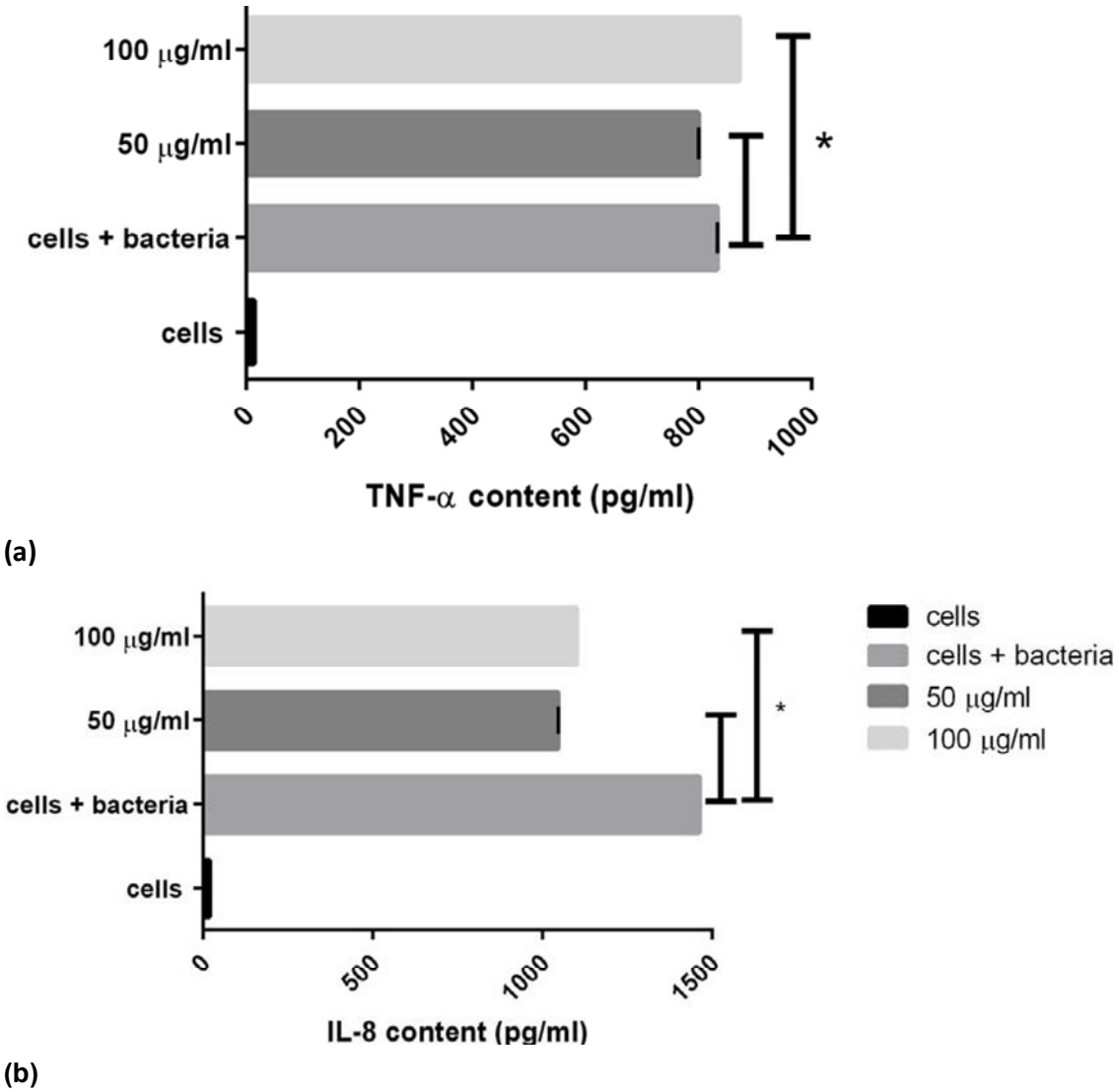

Figure 3. Inhibition of *P. acnes* induced pro-inflammatory mediators, TNF-α (top) and IL-8 (bottom) by petroleum ether extract. Data expressed as mean ± SD. *$p < 0.05$ (n=3).

formulation should not be cytotoxic to human skin when applied topically. Figure 4 shows that the cell viability for human dermal fibroblasts is not significantly different for the cells grown with and without the plant extracts, suggesting no cytotoxicity.

DISCUSSION

The anti-acne screening presented in this study provides a plant candidate that is commonly grown in major parts of the world. To be qualified as plant with anti-acne properties, several different properties such as MIC and MBC against *P. acnes* as well *S. epidermidis* was performed. Other tests such as free radical scavenging, cytotoxicity test and anti-inflammatory tests were used to determine the extract which had the most potential of reducing and curing acne. Some of the published anti-acne studies that are cited here have used plant material that is not easily available; hence our research provides data that gives insights to a unique property of yarrow that is known historically to treat wounds (Chandler et al., 1982). Anti-microbial activity assay, both by disc diffusion and tube dilution method for different extracts from *Achillea* 'Moonshine' showed that the petroleum ether extract had the highest potency against the main acne causing bacteria. Hence, the study is interested to know about the properties of petroleum ether extract in alleviating other causes and effects of acne.

The alcoholic fractions, usually rich in flavonoids have been used previously for anti-microbial treatments. For example, methanolic fraction from *Terminalia chebula*

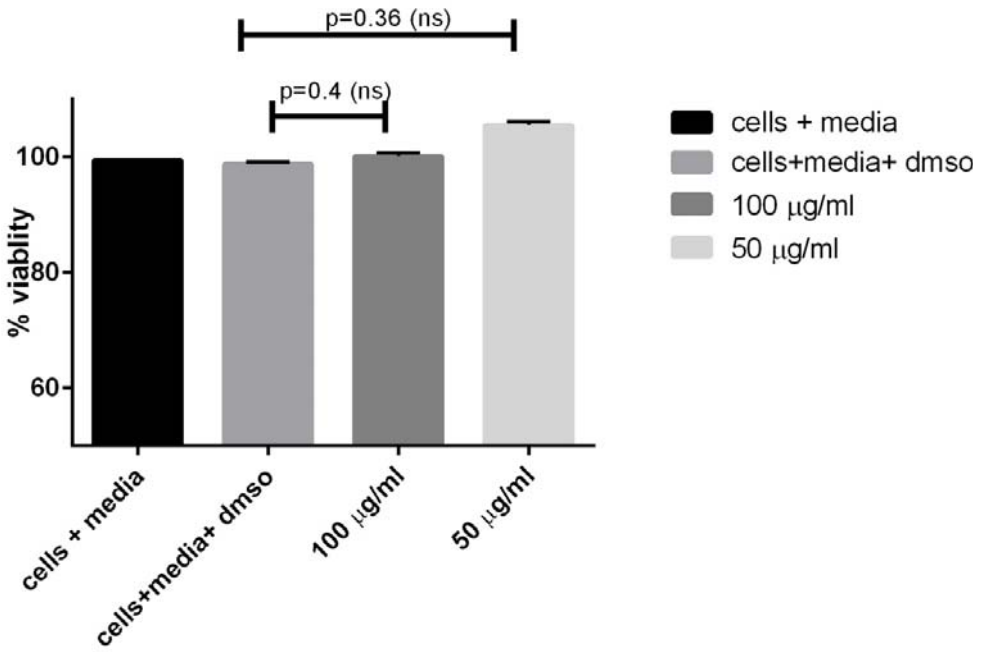

Figure 4. Cytotoxicity of petroleum ether extract against human dermal fibroblasts. Data expressed as mean ± SD. * $p < 0.05$ (n=3); ns- non significant.

and *Terminalia bellarica* used in comibination showed more anti-microbial activity against several microbes including *P. acnes* and *S. epidermidies* (Greeshma et al., 2006). Batubara et al. (2009) studies on anti-acne potency in Indonesian medicinal plants also showed higher anti-microbial activity in the alcohol fractions. However, this study report that the petroleum ether extracts from *Achillea* showed a high potency against acne causing organisms. Petroleum ether extracts from medicinal plants have been shown to contain majorly alkaloids and tannins (Ghumare et al., 2014). Tannins have natural astringent properties, and have been used topically to treat acne (Bedi et al., 2002). Alkaloids such as achilleine isolated from *A. millefolium L.* have been used as homeostatic and could be possibly one of the compounds in the petroleum ether extract contributing to the anti-acne effects (Miller et al., 1954).

This study also reported free radical scavenging activity more in the petroleum ether extract (Table 4). Generally, the free radical scavenging activity is attributed to flavonoids and phenolic compounds which are high in ethyl acetate extracts (Table 3). But, in this study the petroleum ether fraction had the highest radical scavenging activity, which indicates presence of certain potent non polar compounds such as fatty acids, certain flavones and steroids with high radical scavenging potential. Free radical scavenging activity was high in ethyl acetate extracts but this extract did not show high potency in killing *P. acnes* and *S. epidermidis* compared to petroleum ether extracts which is one of the most important factors in anti-acne treatment.

The tyrosinase inhibition activity was significant in the petroleum ether extract compared to the control. Compounds such as fatty acids and steroids easily solubilize in petroleum ether. These non-polar phytochemicals act as tyrosinase inhibitors by either competitively inhibiting the enzyme or by chelating the copper ion at the catalytic site of the enzyme. For some lipids, the inhibitory action is proposed due to binding of the compound to some site of the tyrosinase, except the catalytic site. Polyphenols are also considered to be potent tyrosinase inhibitors, but in this case, these compounds are not as effective as the non-polar phytochemicals because the petroleum ether extract which generally contains non polar compounds was more effective than the other extracts in which the polyphenols are soluble.

Petroleum ether extracts also showed anti-inflammatory effects that contribute in the properties of *A.* 'Moonshine' to qualify as a treatment for acne. The cytotoxicity tests on human dermal fibroblasts also showed *Achillea* to be safe for topical use. Based on the different anti-acne screens the petroleum ether extracts demonstrated to have the best potential as an anti-acne agent.

CONCLUSION AND RECOMMENDATION

Based on the current findings, the petroleum ether extract of *Achillea* 'Moonshine' showed the most promising anti-acne activity. It had potent anti-microbial, free radical

scavenging, anti-tyrosinase and anti-inflammatory activity. The extracts were also nontoxic to the dermal fibroblasts making it a good candidate for topical application. The plant is easily available in North America, Asia and Europe. These results and the increased availability of *Achillea* 'Moonshine' makes it an ideal novel plant candidate for the treatment of acne. Further studies need to be focused on isolation and characterization of the phytoconstituents responsible for anti-acne activity.

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Conflicts of interest

The authors have none to declare.

REFERENCES

Akamatsu H, Horio T (1998). The possible role of reactive oxygen species generated by neutrophils in mediating acne inflammation. Dermatology 196(1):82-85.

Batubara I, Mitsumaga T, Ohashi H (2009). Screening antiacne potency of Indonesian medicinal plants: antibacterial, lipase inhibition, and antioxidant activities. J. Wood Sci. 55:230-235.

Bedi MK, Philip DS (2002). Herbal therapy in Dermatology. Arch. Dermatol. 138:232-242.

Baurin N, Arnoult E, Scior T, Do QT, Bernard P (2002). Preliminary screening of some tropical plants for anti-tyrosinase activity. J. Ethnopharmacol. 82(2-3):155-158.

Benedek B, Kopp B (2007). Achillea millefolium L. s.l. revisited: Recent findings confirm the traditional use. Wien. Med. Wochenschr. 157(13-14):312-314.

Chandler RF, Hooper SN, Harvey MJ (1982). Ethnobotany and phytochemistry of yarrow, Achillea millefolium, compositae. Econ. Bot. 36(2):203-223.

Chomnawang MT, Surassmo S, Nukoolkarn VS, Gritsanapan W (2005). Anti-microbial effects of Thai medicinal plants against acne-inducing bacteria. J. Ethnopharmacol. 101(1-3):330-333.

Davis EC, Callender VD (2010). Post inflammatory Hyperpigmentation: A Review of the Epidemiology, Clinical Features, and Treatment Options in Skin of Color. J. Clin. Aesthet. Dermatol. 3(7):20-31.

French GL (2006). Bactericidal agents in the treatment of MRSA infections-the potential role of daptomycin. J. Antimicrob. Chemother. 58(6):1107-17.

Greeshma A, Deepa M, Poornima EH (2012). A review on anti-acne potential of medicinal plant extracts against propionibacterium acnes. Int. J. Pharm. Biol. Sci. 3(3):987-997.

Ghumare P, Jirekar DB, Farooqui M, Naikwade SD (2014). Phytochemical analysis of petroleum ether extract of some selected medicinal plant leaves. Indian J. Adv. Plant. Res. 1(5):20-23.

Humphrey S (2012). Antibiotic resistance in acne treatment. Skin Ther. Lett. 17(9):1-3.

Kim SS, Kim JY, Lee J, Park D (2008). Antibacterial and anti-inflammatory effects of Jeju medicinal plants against acne-inducing bacteria. J. Gen. Appl. Microbiol. 54(2):101-6.

Knor T (2005). The pathogenesis of acne. Acta Dermatovenerol. Croat. 13(1):44-49.

Li Y, Zhang ML, Cong B, Wang SM, Dong M, Sauriol F, Huo CH, Shi QW, Gu YC, Kiyota H (2011). Achillinin A, a cytotoxic guaianolide from the flower of Yarrow, *Achillea millefolium*. Biosci. Biotechnol. Biochem. 75(8):1554-1556.

Miller FM, Chow LM (1954). Alkaloids of Achillea millefolium L. I. Isolation and characterization of Achillene. J. Am. Chem. Soc. 76:1353-1354.

Moradi MT, Rafieian-Koupaei M, Reza I, Nasiri J, Shahrani M, Rabeiei Z, Alibabaei Z (2013). Antispasmodic Effects of Yarrow (*Achillea millefolium* L.) Extract in the Isolated Ileum of Rat. Afr. J. Tradit. Complement. Altern. Med. 10:499-503.

Pourmorad F, Hosseinimehr SJ, Shahabimajd N (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. Biotechnol. 5(11):1142-1145.

Tanghetti EA (2013). The role of inflammation in the pathology of acne. J. Clin. Aesthet. Dermatol. 6(9):27-35.

Toyoda M, Morohashi M (2001). Pathogenesis of acne. Med. Electron Microsc. 34(1):29-40.

Wu N, Fu K, Fu Y , Zu Y, Chang F, Chen Y, Liu X, Kong Y, Liu W, Gu C (2009). Antioxidant Activities of Extracts and Main Components of Pigeonpea [*Cajanus cajan* (L.) Millsp.] Leaves. Molecules 14(3):1032-1043.