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Anti-Acne and Anti-Inflammatory Potential of Fruit's Pulp, Peels and Juices

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Abstract

Present study was designed to explore the anti-acne and anti-inflammatory potential of ethanolic extracts and fresh juices of different dietary and nutritive fruits. The Disc Diffusion (DD) and Minimal Inhibitory Concentration (MIC) methods were used for microbial analysis of all tested 95% ethanolic extracts and fresh juices against four strains (S1, S2, S3, S4) of *Staphylococcus aureus*. Anti-inflammatory assessment was done through skin irritation and dermal toxicity test for active extracts and fresh juices by using albino mice. Pomegranate peel extract, pomegranate arils extract and fresh lemon juice substantiated as more effective and competent anti-acne agents with growth of inhibition zones up to 24 mm, 17 mm and 18 mm respectively. As well as pomegranate peel extract showed minimum inhibitory concentration at 10% whereas both pomegranate arils extract and fresh lemon juice showed minimum inhibitory concentration at 20 % with anti-inflammatory competency by showing no edema and erythema.

Keywords: Acne vulgaris, Stayphylococcus aureus, Anti-acne, Anti-inflammatory, Ethanolic fruit extracts

Introduction

In our society flawless skin is appreciated and praised. In other words, it increases the level of self-valuation, self-esteem, and self-confidence of a person (Whaley, 2007). Basically, skin is the major introductory organ of human body as well as main boundary between body and external environment, so it protects our body from many environmental impacts like ultra violet rays of sun and several kinds of microorganisms. (Baroni et al., 2012; Dermawan et al., 2015). Despite the defensive role of skin, most often it can become susceptible to the infectivity caused by micro-organisms. Acne (acne vulgaris) is one of the most common and widespread skin related problem (Baroni et al., 2012). According to the survey of Global Burden of Disease study 2010, there are

ten most universal diseases and among these diseases' acne vulgaris is placed at number eight. According to the survey of Global Burden of Disease study 2010, Acne vulgaris is placed at number eight among top ten universal diseases (Tuchayi et al.2015).

As the acne vulgaris is considered a most frequent and common skin disease. So, it can affect high populace of youth including both teenage girls and boys (Fabbrocini et al. 2018). It was reported by Chaudhari (2016) that about 95 % of 16 years old boys and 83 % of girls are suffering from it. Moreover, it is stated by Raza et al. (2012) that boys are at high risk to become suffer from acne vulgaris than girls during puberty whereas girls suffered from acne vulgaris more than boys in adulthood. Acne vulgaris is also accountable to increase the stress and anxiety level mostly in adolescence which might be eventually leaded towards suicidal affinities. (Webster, 2001) According to Olutunmbi et al. (2008), development of acne vulgaris can be sum up in four key points such as:

- Over activation of oil glands which results in increasing production of sebum.
- Blockage of skin pores due to hyperkeratosis through retention.
- Unusual functioning and colonisation of normal skin flora including Propionibacterium acnes and Stayphylococcus aureus.
- Irritation and inflammation of skin.

To deal with acne vulgaris many people use many types of very expensive antibiotics, prebiotics and probiotics which are considered very effective but these drugs are found to be responsible for bacterial resistance, cross resistance and other many side effects.

Therefore, now a days use of many natural things such as plants (fruits, herbs) have gotten attention due to their anti-bacterial and anti-inflammatory potential with no or less side effects rather than antibiotics (Banerjee et al., 2009). As Shahbazi et al. (2017) stated that fruit comprises of several imperative phytochemicals which are synthesized naturally by plants such as flavonoids, secondary metabolites, tarpenes and alkaloids which are responsible for antimicrobial, anti-inflammatory properties. Kim et al. (2008) stated that many researches documented those juices of various citrus fruits possess anti-acne potential. Furthermore, it was investigated by Chaudhari et al. (2016) that peel of fruits also possesses dietary fibres, polyphenols, phenolic components, organic acids and minerals which are responsible for their anti-viral, anti-bacterial, anti-inflammatory and anti-oxidant properties. Fruits are more diverse group of plant foods that is remarkable reward for the human beings because they are great source of minerals, vitamins, soluble dietary fibers, simple sugars, phytoestrogens, anti-oxidant, anti-inflammatory and anti-microbial agents. In particular, a number of studies have shown that certain dietary fruits including pomegranate, papaya, citrus fruits (lemon, orange, sweet lime, grape fruit), tomato, banana, bakain fruit and amla are excellent source of anti-bacterial agents. Until now many studies have been conducted to evaluate the anti-microbial and anti-inflammatory potential of different dietary fruits against Staphylococcus aureus in context of different diseases but very less research work was present regarding skin disease such as acne vulgaris. The aim of the present study is to explore the anti-acne and anti-inflammatory potential of fruit's pulp, peels and juices against Staphylococcus aureus.

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Materials and Methods

Sampling: Amla (Emblica oficinalis), tomato (Solanum iycopersicum), pomegranate (Punica granatum), orange (Citrus sinensis), mosambi (Citrus limetta), lemon (Citrus limon), sweet lime (citrus limetta), grape fruit (Citrus paradise) and papaya (Carcia papaya) were purchased from local market and bakain (Melia azedarach) fruit was collected from its tree. Peels of pomegranate (Punica granatum) and banana (Musa paradisiace) were collected from local shop of juice corner.

Extraction Method: Collected fruits and peels were oven-dried at 70c° and then ground with the help of electric grinder to get very fine powder and passed through a mesh no. 14. After that stored each powder sample in air-tight bottles under cool and shady place until use. The method of extraction described by Biswas et al. (2013) was followed with little modification to prepare ethanolic extracts of each sample. 20g powder of each sample was introduced in 95 % ethanol by using conical flasks. Then each flask was wrapped with aluminium foil and left for 3 days at room temperature. After 3 days, all flasks were placed in incubator shaker at 70 rpm for 24 hours. Then each sample was centrifuged at 4000 rpm at 25 c° for ten minutes. After that collected the supernatant and further evaporated in oven at 70 c° to get more concentrated extract of each sample. Juices were freshly squeezed from fruits to determine their anti-bacterial activity against tested bacteria.

Method of Disc Diffusion: Discs were made and autoclaved at 121c° for 15 minutes. Then 20 ml of medium of nutrient agar was poured in sterilized petri plates and allowed to solidify. After it, 500 ul of test bacterial strain was spread on each petri plate. Then 10 ul of each ethanol extract was loaded on sterilized filter paper discs with the help of micropipette and allowed to dry. When discs got dried, one by one each disc was picked up and placed on Petri plates with sterilized forceps. Then placed all Petri plates in inverted position in incubator at 37 c° for 24 hours.

Measurement of Zone of Inhibition: After 24 hours of incubation, zones of inhibition were measured in term of millimetre (mm) to determine the anti-bacterial activity of each extract against four strains of Staphylococcus aureus.

Minimum Inhibitory Concentration: To determine Minimum Inhibitory Concentration (MIC) of each active extract, broth dilution method was followed. Six test tubes were used and rinsed with distilled water. After it poured 5ml nutrient agar in each test tube and sealed with aluminium foil. Then autoclaved at 121 c° for 15 minutes and left at room temperature for 24 hours. After its stock solution was prepared for each active extract by adding 1 g of concentrated extract in 1 ml of distilled water. 10 ul of nutrient broth was taken out from 1st test tube and 10 ul of extract was added in it with the help of micropipette. Then 20 ul of nutrient broth was taken from 2nd test tube and 20 ul of extract solution was added in same test tube. Then 30 ul of nutrient broth was taken from 3rd test tube and 3ul of extract solution was added in same test tube. Same procedure was followed from 4th test to 6th test tubes. Then each tube was sealed with aluminium foil and placed in incubator at 37 c° for 24 hours.

Skin Inflammatory Test: Anti- inflammatory effect of each active fruit extract was determined by following skin irritation and dermal toxicity test reported by Bhatt et al. (2011). The hair of each mouse was shaved from right and left lateral sides of body. About 2 cm area of body was reserved

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for inflammatory test. Fix doses of about 0.05ml of each active extract was applied on the right side of each mouse followed by increasing concentrations as 0.5 and 5ml. Eight mice were used for this test. Left side of each mouse was used as control and left untreated. The mice were observed after 4 hours then after 24 hours and at the end after 72 hours to check the presence or absence of edema and erythema.

Statistical analysis: Mean and standard deviation was calculated by using MS excel 2013.

Results

Recent research work was conducted to investigate the anti-acne and anti-inflammatory potential of fruit's pulp, peels and juices. Eleven fruits were employed for this purpose. These fruits were obtained from different areas of Kasur within a week. Checklist of these fruits with their scientific name, family, genus was given as in Table 1.

Table 1. Checklist of samples selected for this research work

Sr. No.	Scientific name	Common name	Family	Genus	Selected Parts	Juice Used
1	*Melia Azedarach*	Bakain	Meliaceae	Melia	Fruit	-
2	*Emblica Officinalis*	Amla	Phyllanthaceae	Phyllathus	Fruit	-
3	*Solanum Lycopersicum*	Tomato	Solanaceae	Solanum	Fruit	-
4	*Punica Granatum*	Pomegranate	Lythraceae	Punica	Peel, arils	Fresh juice
5	*Carica Papaya*	Papaya	Caricaceae	Carica	Peel, pulp	-
6	*Musa Paradisiace*	Banana	Musaceae	Musa	Peel	-
7	*Citrus Limon*	Lemon	Rutaceae	Citrus	Pomace	Fresh juice
8	*Citrus Paradise*	Grape fruit	Rutaceae	Citrus	Pomace	Fresh juice
9	*Citrus Limetta*	Sweet lime	Rutaceae	Citrus	Pomace	Fresh juice
10	*Citrus Limetta*	Mosambi	Rutaceae	Citrus	Pomace	Fresh juice
11	*Citrus Sinensis*	Orange	Rutaceae	Citrus	Pomace	Fresh juice

Anti-Acne Potential of the Fruit Extract

Organic extract was made and used to find inhibitory effect against four strains (S1, S2, S3, S4) of Staphylococcus aureus. Some of the fruit extracts such as pomegranate peel, pomegranate arils and fresh lemon juice exerted antagonistic effects on the Staphylococcus aureus, as was assessed by the disc diffusion method and expressed with growth of inhibition which measured in mm.

Table 2. Comparison of growth Inhibition zones of all tested ethanol fruit extracts in mean ± SD for four strains (S1, S2, S3, S4) of *stayphylcoccus aureus*.

Sr. No.	Fruit extract	Zone of inhibition (mean ± SD)			
		Strain S_1	Strain S_2	Strain S_3	Strain S_4
1	Amla	12.5±0.577	13±0.513	13±0.577	13±1
2	Bakain	13.5±0.503	13±0.5	11.6±0.577	13.5±0.5033
3	Tomato	13.5±0.5	13.3±1	13.3±0.577	12.8±0.289
4	Pomegranate Arils	17± 0.288	17±1	17 ±0.577	17±1.04

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5	Papaya peel	-	-	-	-
6	Pomegranate Peel	24 ±0.252	24 ±0.577	24 ±0.288	24 ±0.288
7	Banana peel	-	-	-	-
8	Sweet lime pomace	-	-	-	-
9	Grape fruit pomace	-	-	-	-
10	Orange peel	-	-	-	-
11	Lemon pomace	12±0.577	12 ±0.577	14.3±0.577	13±0.503
12	Tomato juice	-	-	-	-
13	Sweet lime juice	-	-	-	-
14	Mosambi juice	-	-	-	-
15	Lemon juice	18±0.577	18±0.577	18±0.577	18±0.577
16	Grape fruit juice	-	-	-	-
17	Pomegranate juice	14.4 ±0.577	14.4±0.577	14.6±1.154	14.4±0.577
18	Papaya pulp	-	-	-	-

Note: Values represented as mean ± SD of triplicate experiments, (-) No inhibition

Measurement of Zone of Inhibition: Zones of inhibition were measured in term of millimetre (mm) to determine the anti-bacterial activity of each extract against four strains of Staphylococcus aureus as shown in figures no: 1-16.

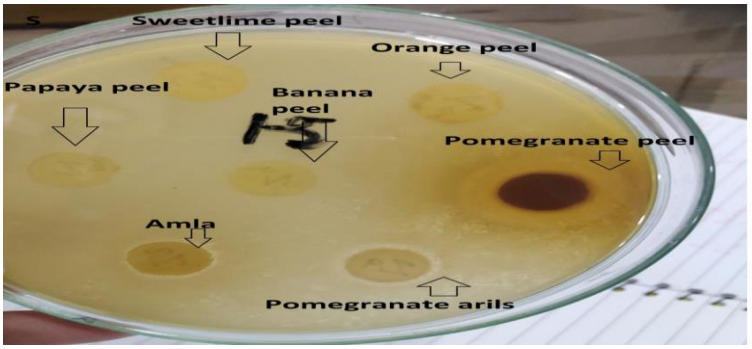

Figure 1. Zone of growth inhibition for fruit extract against S $_1$ Strain of *S.aureus*

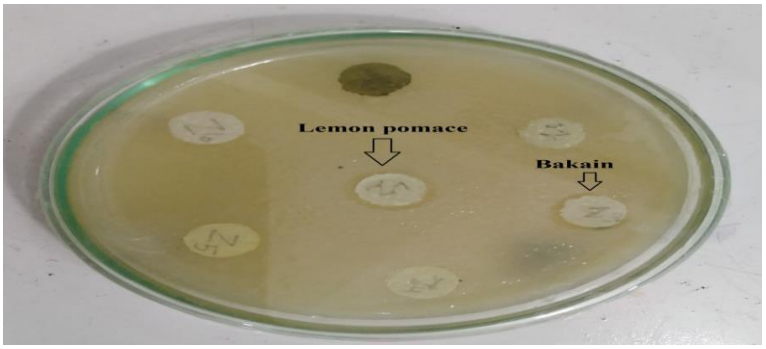

Figure 2. Zone of growth inhibition for fruit extracts against S $_1$ Strain of *S.aureus*

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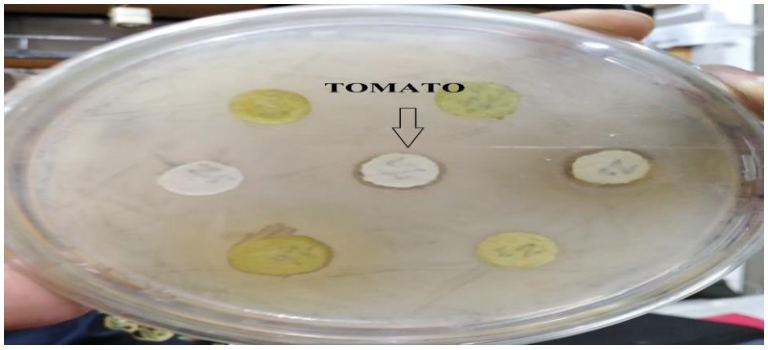

Figure 3. Zone of growth inhibition for fruit extract against S_1 Strain of *S.aureus*

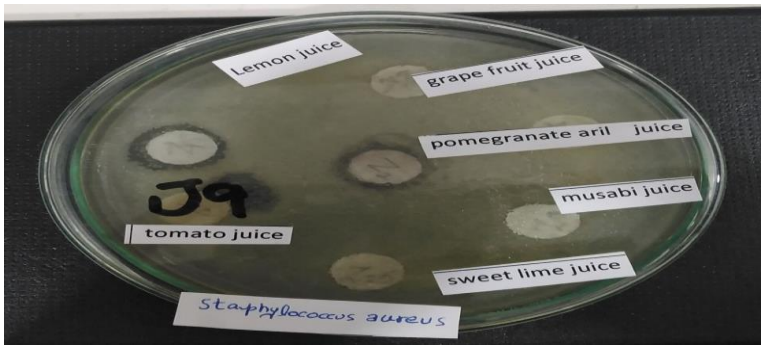

Figure 4. Zone of growth inhibition for fruit extract against S1Strain of *S.aure*

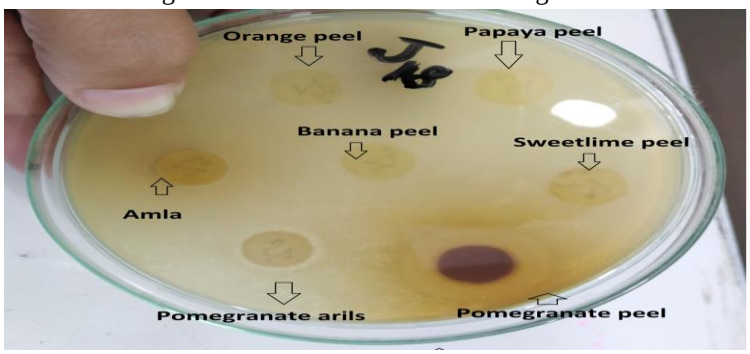

Figure 5. Zone of growth inhibition for fruit extract against S2 Strain of *S.aureus*

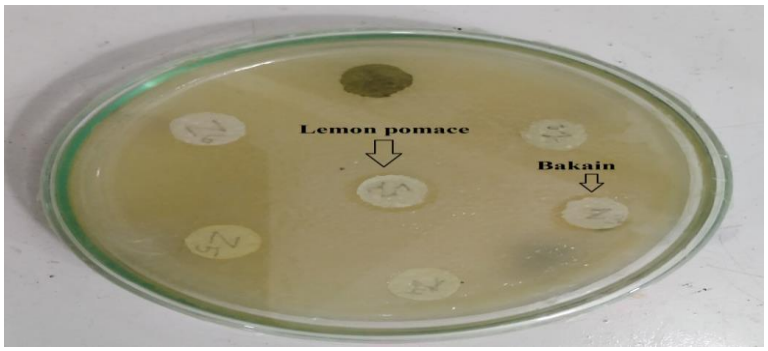

Figure 6. Zone of growth inhibition for fruit extract against S2 Strain of *S.aureu*

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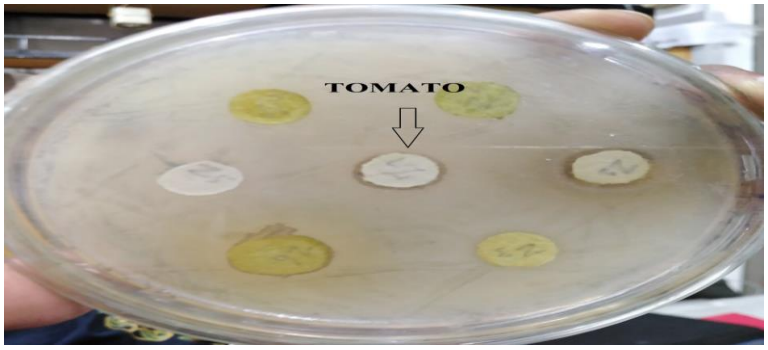

Figure 7. Zone of growth inhibition for fruit extract against S2 Strain of *S.aureus*

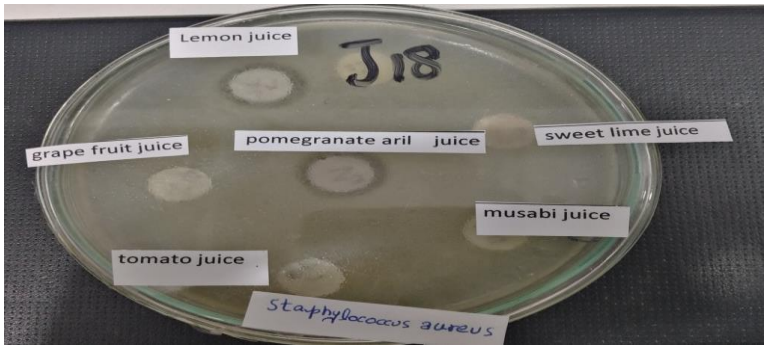

Figure 8. Growth of inhibition zone for fruit extract against S2 Strain of *S.aureus*

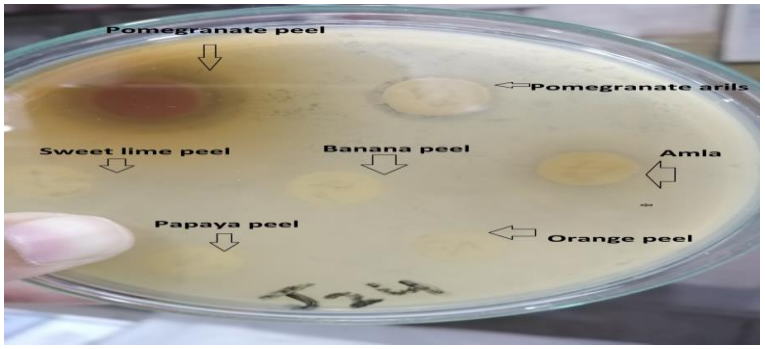

Figure 9. Growth of inhibition zone for fruit extract against S3 Strain of *S.aureus*

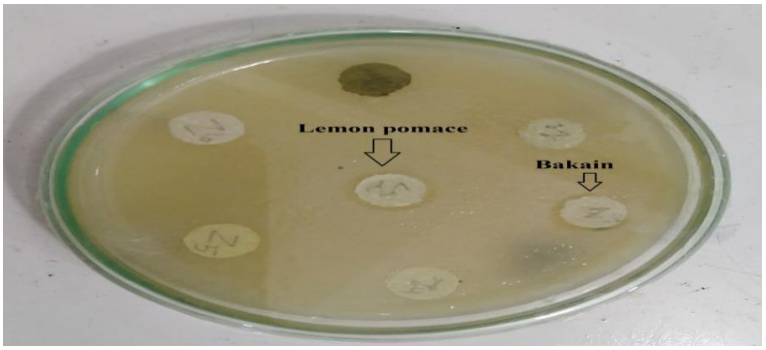

Figure 10. Growth of inhibition zone for fruit extract against S3 Strain of *S.aureus*

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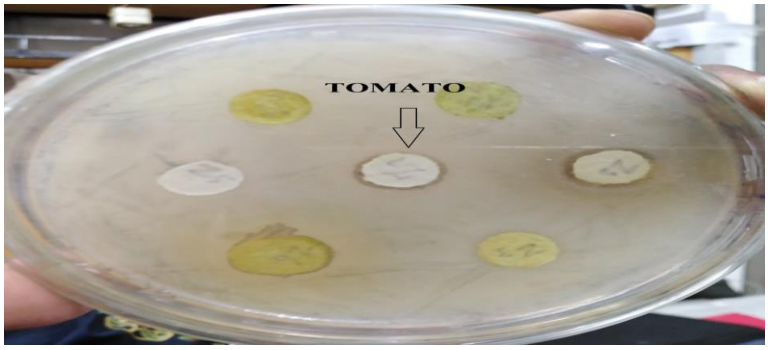

Figure 11. Growth of inhibition zone for fruit extract against S3 Strain of *S.aureus*

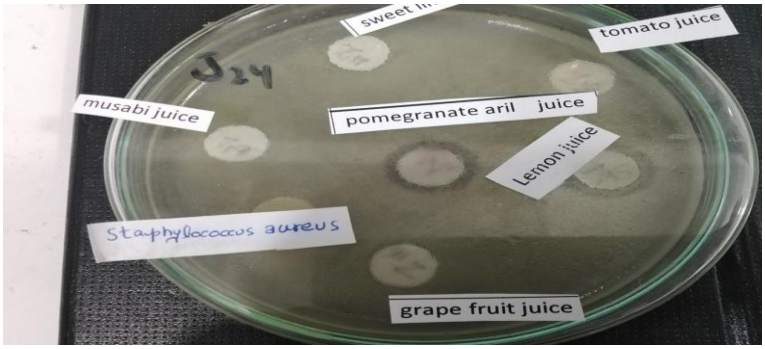

Figure 12. Growth of inhibition zone for fruit extract against S3 Strain of *S.aureus*

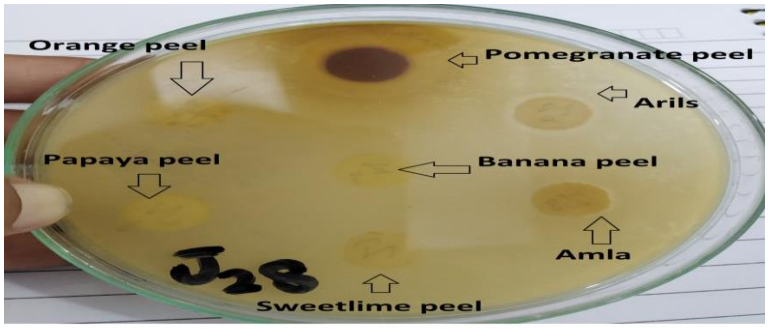

Figure 13. Growth of inhibition zone of fruit extract against S4 Strain of *S.aureus*

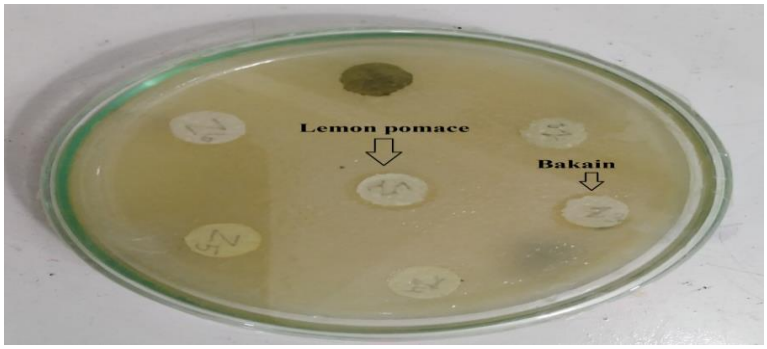

Figure 14. Growth of inhibition zone for fruit extract against S4 Strain of *S.aureus*

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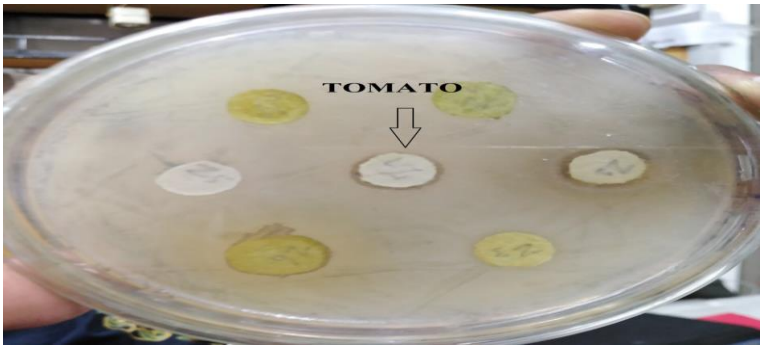

Figure 15. Growth of inhibition zone for fruit extract against S4 Strain of *S.aureus*

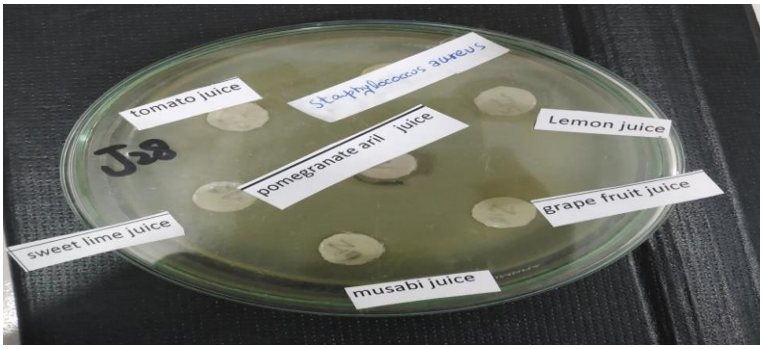

Figure 16. Growth of inhibition zone for fruit extract against S4 Strain of *S.aureus*

Minimum Inhibition Concentration (MIC)

Pomegranate peel extract showed minimum inhibition concentration at 10 % than pomegranate arils extract and fresh lemon juice against four tested strains (S1, S2, S3, S4) of stayphylococcus aureus. Whereas both pomegranate arils extract and fresh lemon juice exhibited minimum inhibition concentrations at 20 % as shown in Table no: 3 - 6.

Table 3. Minimum inhibition concentration of most active fruit extracts against S_1 strain of *Staphylococcus aureus*

Sr. No.	Conc. of fruit's extracts used	10% (0.1)	20% (0.2)	30% (0.3)	40% (0.4)	50% (0.5)	60% (0.6)
1	Pomegranate peel	+	–	–	–	–	–
2	Pomegranate arils	+	+	–	–	–	–
3	Lemon juice	+	+	–	–	–	–

(+) Presence of turbidity, (−) Absence of turbidity

Table 4. Minimum inhibition concentration of fruit extract against S_2 strain of *Staphylococcus aureus*

Sr. No.	Conc. of fruit's extracts used	10% (0.1)	20% (0.2)	30% (0.3)	40% (0.4)	50% (0.5)	60% (0.6)
1	Pomegranate peel	+	–	–	–	–	–
2	Pomegranate arils	+	+	–	–	–	–
3	Lemon juice	+	+	–	–	–	–

(+) Presence of turbidity, (−) Absence of turbidity

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Table 5. Minimum inhibition concentration of fruit extract against S_3 strain of *Staphylococcus aureus*

Sr. No.	Conc. of fruit's extracts used	10% (0.1)	20% (0.2)	30% (0.3)	40% (0.4)	50% (0.5)	60% (0.6)
1	Pomegranate peel	+	–	–	–	–	–
2	Pomegranate aril	+	+	–	–	–	–
3	Lemon juice	+	+	–	–	–	–

(+) Presence of turbidity, (–) Absence of turbidity

Table 6. Minimum inhibition concentration of fruit extract against S_4 strain of *Staphylococcus aureus*

Sr. No.	Conc. of fruit's extracts used	10% (0.1)	20% (0.2)	30% (0.3)	40% (0.4)	50% (0.5)	60% (0.6)
1	Pomegranate peel	+	–	–	–	–	–
2	Pomegranate arils	+	+	–	–	–	–
3	Lemon juice	+	+	–	–	–	–

(+) Presence of turbidity, (–) Absence of turbidity

Skin Irritation and Dermal Toxicity Test

To assess the anti-inflammatory capability of active fruit extracts albino mice were selected and kept under required conditions. Doses were prepared with different concentrations such as 0.05 ml, 0.5ml and 5ml and applied on right side of all mice. First observation was made after 4 hours and second observation after 24 hours. Similarly, third observation was made after 48 hours and final results were recorded after 72 hours as shown in Table 7.

Table 7. Results of Skin irritation and dermal toxicity test for active ethanolic extract of pomegranate peel, ethanolic extract of pomegranate arils and fresh lemon juice

Sr. No.	Fruit extract	Doses (ml)	Shaving area(cm)	Observation after 4hrs	Observation after 24 hrs	Observation after 48 hrs	Observation after 72 hrs
1	Pomegranate peel	a. 0.05 b. 0.5 c. 5	1.5	_	_	_	_
2	Pomegranate arils	a. 0.05 b. 0.5 c. 5	1.5	_	_	_	_
3	Lemon juice	a. 0.05 b. 0.5 c. 5	1.5	_	_	_	_

(+) Presence of turbidity, (–) Absence of turbidity

Observations of Anti-Inflammatory Effect for Pomegranate Peel Extract on Mice

After the application of pomegranate peel extract on selected skin area of mice, first observation was made after 4 hours and second observation after 24 hours for pomegranate peel extract on selected area of mice skin Similarly, third observation was made after 48 hours and final results were recorded after 72 hours. At the end no edema and no erythema were noticed as shown in figure no:17(a, b, c, d)

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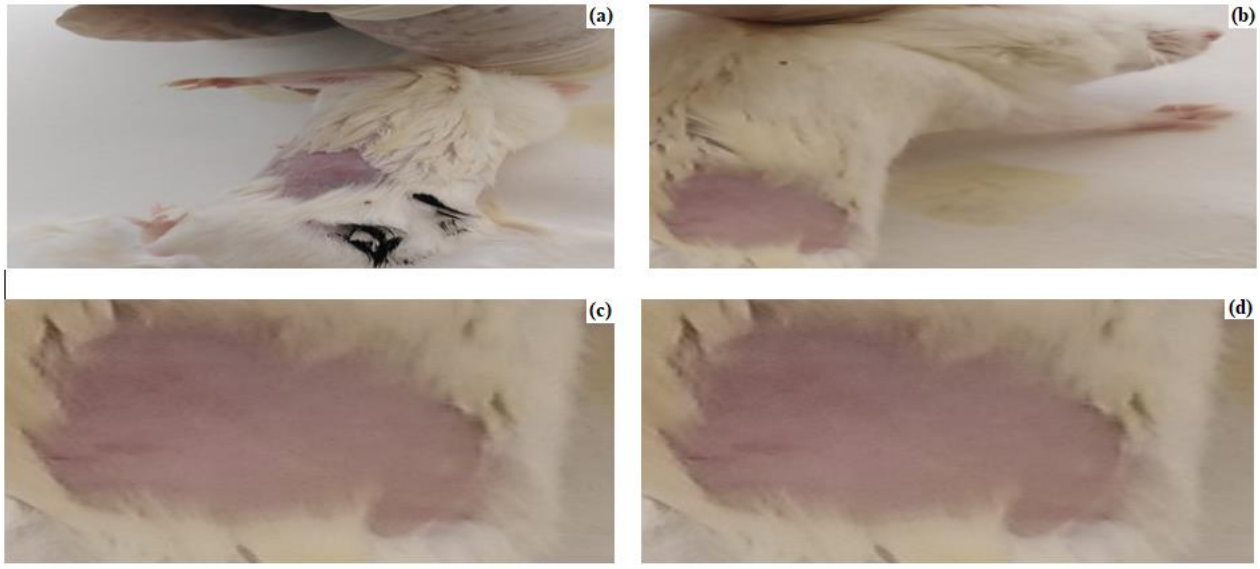

Figure 17. Observations of Anti-Inflammatory Effect for Pomegranate Peel Extract on Mice (a) after 4 hours, (b) after 24 hours, (c) after 48 hours and (d) after 72 hours

Observations of Anti-Inflammatory Effect for Pomegranate Arils Extract on Mice

Pomegranate arils extract was applied on selected skin area of mice then first observation was made after 4 hours and second observation after 24 hours Similarly, third observation was made after 48 hours and final results were recorded after 72 hours. At the end no edema and no erythema were noticed as shown in figure no:18 (a, b, c, d)

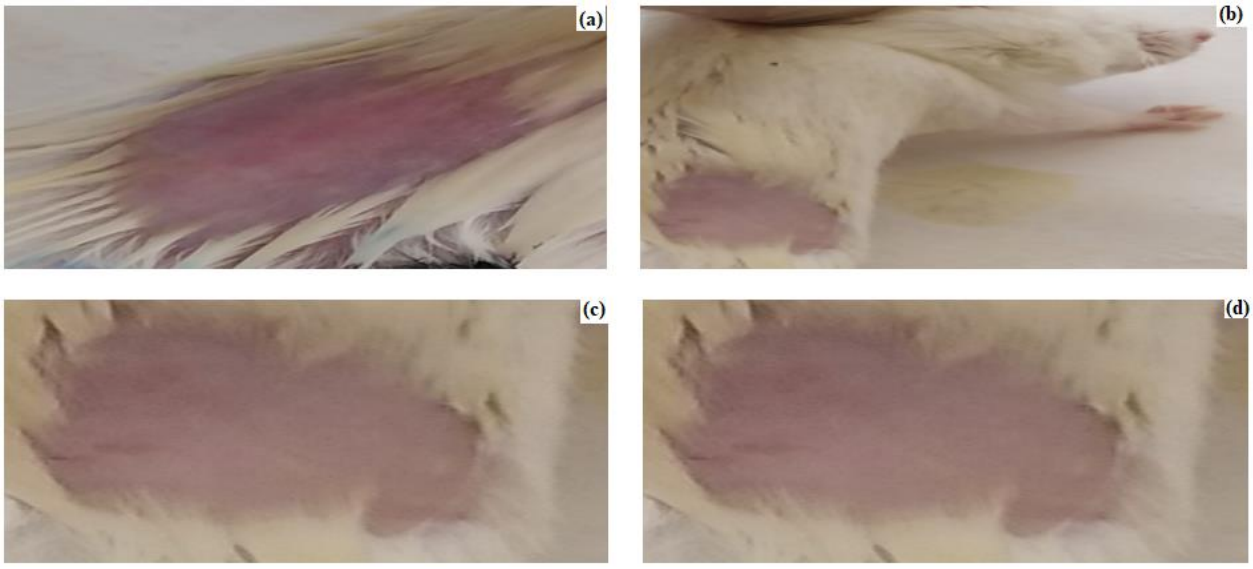

Figure 18. Observations of Anti-Inflammatory Effect for Pomegranate Arils Extract on Mice (a) after 4 hours, (b) after 24 hours, (c) after 48 hours and (d) after 72 hours

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Observations of Anti-Inflammatory Effect for Lemon Juice Extract on Mice

To assess the anti-inflammatory effect of fresh lemon juice extract on mice, first observation was made after 4 hours and second observation after 24 hours Similarly, third observation was made after 48 hours and final results were recorded after 72 hours. At the end no edema and no erythema were noticed as shown in figure no:19 (a, b, c, d)

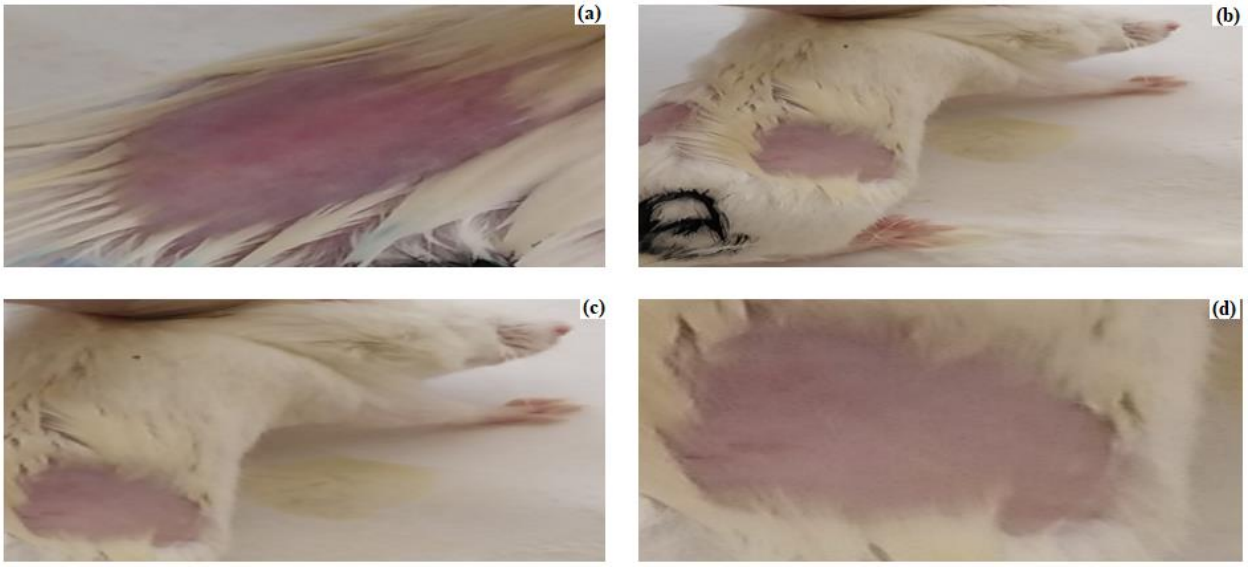

Figure 19. Observations of Anti-Inflammatory Effect for Lemon Juice Extract on Mice (a) after 4 hours, (b) after 24 hours, (c) after 48 hours and (d) after 72 hours

Discussions

From the past few years due to the continual pervasiveness of antibiotic resistance, there is a need to explore more promising and alternative sources to deal with acne vulgaris. So natural products obtained from fruits could be regarded as more effective and economical against acne causing microbes such as Staphylococcus aureus. As current study was meant to explore the anti-acne and anti-inflammatory potentiality of total eleven types of fruits different selected parts (peel, pulp, pomace, arils, juices) against four strains of Staphylococcus aureus as shown in Table 1.

Anti-bacterial activity: Among all tested ethanolic extracts and fresh juices only amla, bakain, tomato, pomegranate peel, pomegranate arils, lemon pomace, lemon juice, pomegranate juice showed anti-bacterial affinity against for strains (S1, S2, S3, S4) of Staphylococcus aureus. From comparison of inhibition growth zones of active ethanolic extracts and fresh juices it was explored that pomegranate peel, pomegranate arils and lemon juice appeared more effective anti-acne agents Staphylococcus aureus as shown in Table 2.

Findings of Nuamsetti et al (2012) showed that ethanolic extracts of both pomegranate peel and arils appeared effective against Staphylococcus aureus which supports the results of current study as 95 % ethanolic extracts of pomegranate peel and arils showed significant growth of inhibition zones 25mm and 17mm respectively.

This difference in antimicrobial potential of ethanolic extracts of pomegranate peel and arils may be justified by the findings of Choubey (2018) which described the presence of many important anti-bacterial, anti-inflammatory and anti-oxidant components in pomegranate peels.

Findings of Khan and Hanee (2011) supports the results of present study for ethanolic extract of pomegranates peel against Stayph.aureus with inhibition zone 26 mm which is comparable to the results pomegranate peel with growth of inhibition zones up to 24.73±0.252, 24.33±0.577, 24.83±0.288 and 24.66±0.288 for strains S1, S2, S3, S4 of Staphylococcus aureus respectively. Similarly, Dahham et al. (2010) also supports the findings of present study by showing zones of inhibition 25mm for pomegranate peel extract. Furthermore results of inhibitory potential of lemon juice against S.aureus of present study was supported by Abdullah (2009), as he reported that the lemon juice showed growth of inhibition zone 17mm. It was also reported by Chaudhari et al (2016) that lemon juice was tested against pathogens such as Staphylococcus aureus and Staphylococcus epidedermis exhibited inhibitory potential which supports the results of present study as it was showed inhibition zone 17 mm.

Kawaii et al (2000) claimed that presence of alkaloids increased the antimicrobial affinity of lemon juice. According to Okwu (2004) and Oyekunle et al. (2006) variations among findings of studies conducted by different researchers might be due to the opted method used for extraction of selected sample. Also, the selection of solvent for extraction purpose could be another reason for divergence of results. Furthermore, it was also documented by Bansod and Rai (2008) that growth of microbes also depends on the disclosure of microbes to the selected extract and as well as the solubility of dynamic components in selected solvent.

Minimum inhibition concentration was determined by broth dilution method for all tested strains of Staphylococcus aureus. Ethanolic extract of pomegranate peel showed lowest MIC value because no turbidity was observed at 20% concentration followed by the relatively low MIC values of pomegranate ethanolic extract of arils which showed absence of turbidity at 30% concentration. As Tianchai Nuamsetti (2012) stated that high ratio of phenolic components is responsible for low MIC values of pomegranate peel as compared to arils. As well as lemon juice also showed lower MIC value because no turbidity was observed at 30% concentration reported in Table 3-6.

Anti-inflammatory activity: Skin irritation and dermal toxicity test was applied to assess the anti-inflammatory ability of active fruit extracts by using albino mice. Doses were prepared with different concentrations such as 0.05 ml, 0.5ml and 5ml and applied on right side of all mice. First observation was made after 4 hours and second observation after 24 hours. Similarly, third observation was made after 48 hours and final results were recorded after 72 hours. Table 7

At the end no edema and erythema was noticed for all active fruit extracts and the findings of Bhatt et al. (2011) supports the results of present research for anti-inflammatory effect.

Conclusion

Now a days use of natural plants such as fruits have gained attention due to their anti-microbial and anti-inflammatory tendency and rationally less or no substantial side-effects as compared to chemically synthesized drugs. As the present study was conducted to verify anti-acne and anti-

inflammatory potential of nutritional parts of different fruits (pulp, peels, juices). So, this obtained information would be helpful to cure skin related problems especially acne vulgaris which is caused by Staphylococcus aureus.

Limitations and Future Studies

Photochemical analysis would also be implemented for all tested active extracts. This obtained information would be valuable to formulate anti-acne and anti-inflammatory products which would be economical with no side effects as compared to antibiotics.

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