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DEVELOPMENT AND EVALUATION OF HERBAL FORMULATION FOR THE TREATMENT OF ACNE

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ABSTRACT: *Acne vulgaris* is a most common skin disorder of pilosebaceous unit that affect areas containing the largest oil glands, including the face, back, and trunk. It is generally characterized by formation of seborrhea, comedone, inflammatory lesions. *Propionibacterium acnes* and *Staphylococcus epidermidis* have been recognized as pus-forming bacteria triggering an inflammation in acne. *Staphylococcus aureus* support to cause inflammation in acne. The present research work deals with formulation and evaluation of herbal gels against this etiologic agent of acne vulgaris. The ethanolic extract of Neem (leaves), Nutmeg (fruit), and Black pepper (fruit) were prepared and formulated into a topical gel. In vitro antibacterial activity was performed against *P. acnes*, *S. epidermidis* and *S. aureus*, using agar well diffusion method. The measured zones of inhibitions of the prepared formulations were compared with standard antibiotic (Clindamycin) and standard marketed topical herbal formulation. The prepared gels were evaluated for pH, viscosity, spreadability, stability, drug content, acute skin irritancy activity and *in vitro* diffusion. The results from the agar well diffusion showed that Neem, Nutmeg and Black pepper would inhibit the growth of *P. acnes*, *S. epidermidis* and *S. aureus* and the prepared polyherbal gels showed comparable antimicrobial activity against these bacterias with the marketed preparation. However, the standard Clindamycin was more active than that of prepared polyherbal gels, marketed herbal anti-acne preparation and extracts of Neem, Black pepper and Nutmeg. Taken together, our data indicated that Neem, Black pepper and Nutmeg had inhibitory and synergistic effect against *P. acnes*, *S. epidermidis* and *S. aureus*.

INTRODUCTION: Acne vulgaris is a most common chronic inflammatory skin disorder of pilosebaceous unit that affect areas containing the largest oil glands, including the face, back, and trunk [1].

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It is almost a universal disease occurring in all races and affecting 95% of boys and 83% of girls.

Acne vulgaris is generally characterized by formation of seborrhea, comedone, inflammatory lesions and presence of bacteria *Propionibacterium acnes, Staphylococcus epidermidis and Staphylococcus aureus* in the follicular canal and sebum production [2]. *P. acnes* have been described as an obligate anaerobic microorganism. It is implicated in the development of inflammatory

acne by its capability to activate complements and by its ability to metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils. On the contrary, *S. epidermidis*, an aerobic organism, usually involves in superficial infections within the sebaceous unit [3]. When the chemicals produced by P. acnes destroy the cellular structure of skin cells, Staphylococcus aureus, grows causing acne lesions. These factors provide a potential target for treatment. *P. acnes*, *S. epidermidis* and *S. aureus* are the target sites of antiacne drugs [4, 5].

With the excessive use of antibiotics for long periods has led to the increased resistance in acne causing bacteria i.e. *P. acnes*, *S. epidermidis* and *S. aureus* [6-9]. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics, and environmental factors. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases [10, 11].

In the present study, three medicinal plants, which have been traditionally used as antimicrobial and anti-inflammatory agents were examined for antimicrobial activities against microorganisms frequently involved in acne inflammation, such as *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus.*

MATERIALS AND METHODS:

Plants Material:

Collection and drying of Plant parts: The fresh leaves of Neem (*Azadirachta indica*) were collected from the local area of Nagpur district in the month of June 2011. While the fruits of Nutmeg (*Myristica fragrans*) and Black pepper (*Piper nigrum*) were purchased from the local market of Nagpur district. The plant specimens were dried and their herbarium sheets were prepared as shown in **Figure 1, 2 and 3**.

It was authenticated by Dongarwar Sir; Head of Department of Botany, R.T.M. Nagpur University, Nagpur.

FIG. 1: THE SPECIMEN VOUCHER NUMBER OF NEEM (*AZADIRACHTA INDICA*) IS 9306

FIG. 2: THE SPECIMEN VOUCHER NUMBER OF NUTMEG (*MYRISTICA FRAGRANCE*) IS 9307

FIG. 3: THE SPECIMEN VOUCHER NUMBER OF BLACK PEPPER (*PIPER NIGRUM*) IS 9308

The fresh leaves of neem and the fruits of nutmeg and black pepper were dried under shade and were powdered.

Preparation of extracts: The powdered plant materials were deffated with petroleum ether and then subjected to Soxhlet extraction till discoloration to obtain ethanolic extracts of neem, nutmeg and black pepper alternatively. The extracts thus obtained were filtered, concentrated on water bath to a thick paste and dried under vacuum.

METHODS:

Procedure: 1 g of Carbopol 934 was dispersed in 50 ml of distilled water with continuous stirring. The 5 ml of distilled water was taken and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath. The solution was cooled, then propylene glycol 400 and polyethylene glycol 200 were added as shown in **Table 1**.

Further required quantity of ethanolic extracts of Neem, Nutmeg and Black pepper as shown in **Table 2** were mixed to the above mixture and volume was made up to 100 ml by adding remaining distilled water. Finally all ingredients were mixed properly to the Carbopol 934 gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel of required consistency. The same method was followed for preparation of control sample without adding any extract [12-18].

TABLE 1: FORMULATION OF GEL

Sr. No.	Contents	Quantity (100 gm)
1	Carbopol 934	1.0 gm
2	Methyl paraben	0.15 gm
3	Propyl paraben	0.03 gm
4	Propylene glycol	5.0 ml
5	Polyethylene glycol	15.0 ml
6	Distill water	Upto100ml

TABLE 2: CONCENTRATION OF EXTRACTS USED IN ANTIACNE GEL FORMULATION

Sr. No.	Plant Extracts	Concentration of extracts (100gm gel)	
		G 1	G 2
1	Black pepper	1.75 gm	3.5 gm
2	Neem	1.5 gm	3.0 gm
3	Nutmeg	0.5 gm	1.0 gm

The test organisms used in this study were as followed: *Propionibacterium acnes* (MTCC 1951), Staphylococcus epidermidis (MTCC 931) and Staphylococcus aureus. These bacteria were obtained from the Microbial Type Culture Collection and Gene Bank, Chandigarh, India. Brain Heart Infusion medium, Tryptone Soya Broth and Nutrient Agar medium all these medias were purchased from Hi-media.

Antibacterial activity testing:

Sample Preparations: Solutions of extracts of Neem, Nutmeg, Black pepper, prepared polyherbal gels and marketed formulation were prepared using 100 mg of formulation in 10ml of dimethyl sulfoxide (DMSO). Clindamycin (10mg/ml) was used as a positive control and DMSO as a negative control.

Antibacterial Assay: The antibacterial activity of different extracts and formulations were determined by modified agar well diffusion method in which nutrient agar plates were seeded with 0.2 ml each of 24 h broth culture of *S. aureus*, plates of soyabean caseine digest media plate were seeded with 0.2 ml each of 24 h broth culture of *S. epidermidis* and plates of brain heart infusion media were seeded with 48 h broth culture of *P. acnes.*

The plates were dried for 1 h. With a sterile 8 mm borer four wells of equidistance in each of plates were cut into which 0.5 ml of solutions of extracts, prepared polyherbal gels, Clindamycin, marketed herbal formulation and allopathic Clarithromycin gel were introduced in each plate. The plates of *S. epidermidis* and *S. aureus* were incubated at 37^{o}C for 24h and of *P. acnes* were incubated for 48h. The antibacterial activity was evaluated by measuring the diameter of zones of inhibition (in mm). The experiments repeated four times and the mean was calculated. The results are depicted in **Table 5**.

Determination of Minimum Inhibitory Concentrations (MIC): The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. In order to determine the relative minimum inhibitory concentration values extracts were dissolved in DMSO to make a concentration of 100 mg/ml.

The extracts were then diluted with DMSO to make different concentrations. 100µl of these extracts were then added separately to each cup. All the tests were repeated in duplicates and its mean were calculated and are depicted in **Table 6** [19].

EVALUATION OF EXTRACTS:

Characteristics of extracts: The ethanolic extracts of Neem, Nutmeg and Black pepper were evaluated for its physical state, colour, odour, taste and percent yield and results are shown in **Table 3**.

Phytochemical screening of extracts: The ethanolic extracts of Neem, Nutmeg and Black pepper were subjected to preliminary phytochemical testing for the detection of major phytoconstituents such as phenols, tannins, steroids, alkaloids, glycosides and flavonoids and the results are depicted in **Table 4**.

EVALUATION OF GEL: These topical formulations were tested for colour, appearance, pH, viscosity, spreadability, stability, drug content and *in vitro* diffusion.

Physical Evaluation: Physical parameters such as colour and appearance were checked visually and results are depicted in **Table 7**.

Measurement of pH [20]: The pH of various formulations was determined by using Digital pH meter. One gram of gel was dissolved in 100 ml of distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values are depicted in **Table 7**.

Viscosity: The measurement of viscosity of prepared gels were carried out with Brookfield Viscometer The measurements was over speed setting of 100 rpm at 25 ºC using Brookfield viscometer. The values of each formulation are depicted in **Table 7**.

Spreadability [21]: Spreadability denotes the extent of area to which the gel readily spreads on application to skin or the affected part. The bioavailability efficiency of a gel also depends on its spreading value. The Spreadability was expressed in terms of time in seconds taken by two slides to slip off from the gel, placed in between the slides, under certain load. Lesser the time taken for separation of the two slides, better the Spreadability. Two sets of glass slides of standard dimensions were taken. The gel formulation was placed over one of the slides.

The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 6.0 cm along the slide. 100 gm weight was placed upon the upper slides so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only the upper slide to slip off freely by the force of weight tied to it. A 20 gm weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 6.0 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated by three times and the mean time taken for calculation.

Spreadability was calculated by using the following formula:

$$S = M \times L / T$$

Where, S= Spreadability, M = Weight in the pan (tied to the upper slide), L = Length glass slide and T = Time (in sec.) taken to separate the slides.

Stability Study [22]: The stability study was performed as per ICH guidelines. The formulated gels were filled in the collapsible tubes and stored at fixed temperature and humidity condition, viz. $40^{o}C \pm 2^{o}C$/ 75% ± 5% RH for a period of three months and studied for appearance, pH, viscosity and spreadability. Results are depicted in **Table 9**.

Drug content: Each formulation (1g) was accurately weighed and transferred to 100 ml volumetric flask to which about 70 ml of methanol was added. After shaking, the volume was made up to 100 ml with methanol. The content was filtered through a suitable filter paper. 1ml filtrate was taken and suitable diluted and the drug content (extract) was estimated by using UV/Visible spectrophotometer at 250nm for neem, 276nm for nutmeg and at 330 nm for black pepper. Results are shown in **Table 7**

Drug release: The *in vitro* diffusion studies of the gels were performed using a vertical Franz diffusion cell whose diffusion area was 1.59 cm^2, and by using dialysis membrane (Sigma Inc. MO,

USA; dry, unwashed, pre-cut and open ended; fiat width: 35 mm; inflated diameter, 21mm; Length: 30mm). The membrane soaked in phosphate buffer pH 7.4 for 6-8 h was clamped carefully to one end of the hollow glass tube of dialysis cell (2.3 cm diameter, 4.16 cm^2 area). 100 ml of phosphate buffer was taken in a beaker, which was used as receptor compartment for the study. 1gm of each formulation was spreaded uniformly on the membrane.

The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37±0.50C. The solutions on the receptor side were stirred by externally driven Teflon-coated magnetic bars. At pre-determined time intervals, 2 ml of solution from the receptor compartment was pipetted out and immediately replaced with 2 ml fresh phosphate buffer solution. The drug concentration of the receptor fluid was determined spectrophotometrically at 250nm, 276nm and 330nm against appropriate blank. The amounts of drug permeation of all the formulations were calculated. This experiment was carried out in triplicate and the results are extrapolated in **Figure 7 and 8**.

Acute skin irritation study [21]: The primary skin irritation test was performed on albino rats and weighing about 150-200 gm. The animals were maintained on standard animal feed and had free access to water *ad libitum*. The animals were kept under standard laboratory condition. The total mass was divided into four batches, each batch containing seven animals. Two batches of each were used for control and test. Dorsal hairs at the back of the rats were clipped off one day prior to the commencement of the study. Animals showing normal skin texture were housed individually in cages with copography meshes to avoid contact with the bedding. 50 mg of the each formulation of different concentrations were applied over one square centimeter area of intact and abraded skin to different animals. A 0.8 %v/v aqueous solution of formalin was applied as standard skin irritant. The formulation was removed after 24 h and score of erythema was recorded and compared with standard. Score of erythema is read and recorded as: Score 0 for no erythema; Score 1 for Mild erythema (barely perceptible- light pink); Score 2 for Moderate erythema (dark pink); Score3 for Severe erythema (Extreme redness).Results are depicted in **Table 10.**

RESULTS:

EVALUATION OF EXTRACTS:

Characteristics of ethanolic extracts of neem, nutmeg and black pepper: The physical state, color, odor, taste and percent yield of all three extracts are as follows

TABLE 3: CHARACTERISTICS OF EXTRACTS

Sr. no.	Characteristics	Observations		
		Neem	Nutmeg	Black pepper
1	Physical state	Semisolid	Semisolid	Semisolid
2	Color	Greenish	Brownish	Blackish brown
3	Odor	Aromatic	Aromatic	Aromatic
4	Taste	Bitter	Characteristic	Characteristic
5	Percent yield	20.41%w/w	18.56%w/w	15.25%w/w

Phytochemical screening of ethanolic extracts of neem, nutmeg and black pepper: The following phytoconstituents were found to present in the ethanolic extracts of neem, nutmeg and black pepper respectively.

TABLE 4: PHYTOCHEMICAL SCREENING OF EXTRACTS

Test for Phytoconstituents	Plant extract		
	Neem	Nutmeg	Black pepper
Phenols	+	+	+
Tannins	+	+	+
Steroids	+	+	-
Alkaloids	+	+	+
Glycosides	+	+	-
Flavonoids	+	+	+

Antimicrobial activity of Extracts: The ethanolic extracts of neem, nutmeg and black pepper and there combination were examined for antimicrobial activities against microorganisms frequently involved in acne inflammation, *Propionibacterium acnes, Staphylococcus epidermidis* and *Staphylococcus aureus.*

From the results shown in Table 6, it was observed that the combination of all three extracts showed maximum zone of inhibition as compared to individual extracts of neem, nutmeg and black pepper but lesser zone of inhibition as compared to standard antibiotic clindamycin.

TABLE 5: ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANT EXTRACT

Bacteria	Zone of inhibition(mm)				
	Plant Extracts (20mg/ml)				Standard
	Black pepper (A)	Neem (B)	Nutmeg (C)	Combination of A, B and C	Clindamycin
P. acnes	16	20	18	23	36
S. epidermids	20	19	17	22	33
S. aureus	12	13	14	17	31

Concentration of the each extract used: 20 mg/ml, Clindamycin: 1.0 mg/ml

Antimicrobial activity against *Propionibacterium acnes*: Zone of inhibition of ethanolic extracts neem, nutmeg and black pepper against *Propionibacterium acnes* is shown in **fig. 4a, fig. 4b** and in **fig. 4c** respectively. The comparative study of extracts of neem, nutmeg and black pepper each of concentration 10 mg/ml and the combination of all three extracts is shown in **fig. 4d**.

Zone of inhibition of neem, nutmeg and black pepper extract against *P. acnes*:

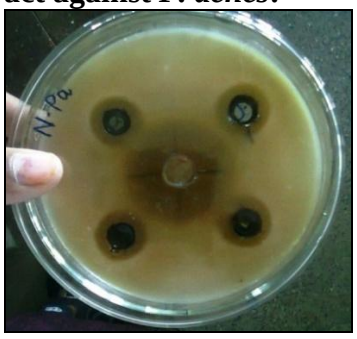

FIG. 4A: ZONE OF INHIBITION OF NEEM AGAINST *P. ACNES*

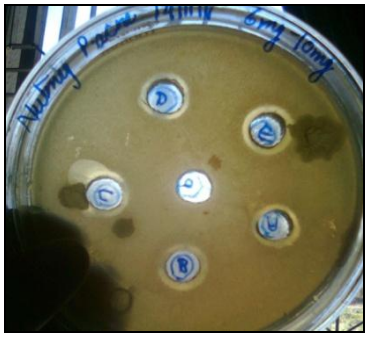

FIG. 4B: ZONE OF INHIBITION NEEM AGAINST *P.ACNES* AGAINST *P. ACNES*

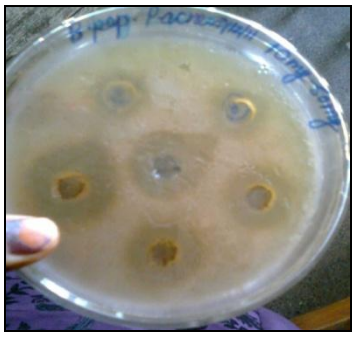

FIG. 4C: ZONE OF INHIBITION OF BLACK

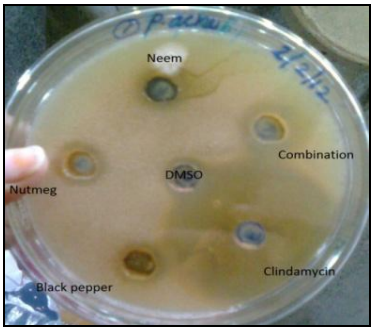

FIG. 4D: ZONE OF INHIBITION OF COMBINATION OF NEEM, NUTMEG AND BLACK PEPPER AGAINST *P. ACNES*

Antimicrobial activity against *Staphylococcus epidermidis*: Zone of inhibition of ethanolic extracts of neem, nutmeg and black pepper against *Staphylococcus epidermidis* are shown in **fig. 5a, fig. 5b** and in **fig. 5c** respectively. The comparative study of extracts of neem, nutmeg and black pepper each of concentration 10 mg/ml and the combination of all three extracts is shown in **fig. 5d**.

Zone of inhibition of neem, nutmeg and black pepper extract against *S. epidermidis*:

FIG. 5A: ZONE OF INHIBITION OF NEEM AGAINST *S. EPIDERMIDIS*

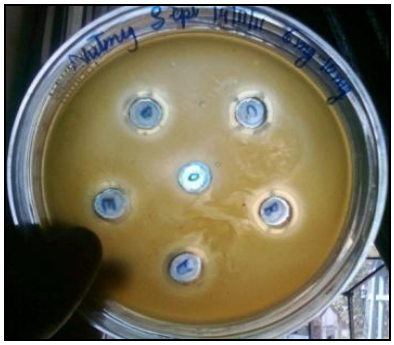

FIG. 5B: ZONE OF INHIBITION OF NUTMEG AGAINST *S. EPIDERMIDIS*

FIG. 5C: ZONE OF INHIBITION OF BLACK PEPPER AGAINST *S. EPIDERMIDIS*

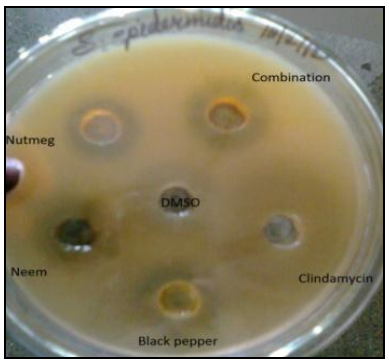

FIG. 5D: ZONE OF INHIBITION OF COMBINATION NEEM, NUTMEG AND BLACK PEPPER AGAINST *S. EPIDERMIDIS*

Antimicrobial activity against *Staphylococcus aureus*: Zone of inhibition of ethanolic extracts neem, nutmeg and black pepper against *Staphylococcus aureus* is shown in **fig. 6a, fig. 6b** and in **fig. 6c** respectively. The comparative study of extracts of neem, nutmeg and black pepper each of concentration 10 mg/ml and the combination of all three extracts is shown in **fig. 6d**.

Zone of inhibition of neem, nutmeg and black pepper extract against *S. aureus*:

FIG. 6A: ZONE OF INHIBITION OF NEEM AGAINST *S. AUREUS*

FIG. 6B: ZONE OF INHIBITION OF NUTMEG AGAINST *S. AUREUS*

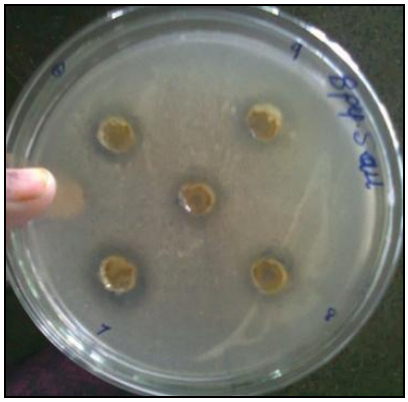

FIG. 6C: ZONE OF INHIBITION OF BLACK PEPPER AGAINST *S. AUREUS*

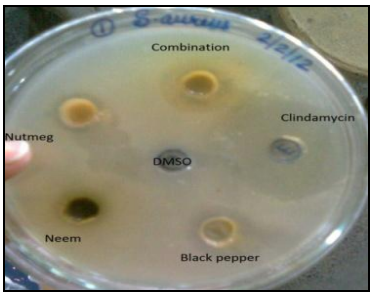

FIG. 6D: ZONE OF INHIBITION OF COMBINATION OF NEEM, NUTMEG AND BLACK PEPPER AGAINST *S. AUREUS*

Minimum inhibitory concentration of extracts: The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. The MIC of all the extracts is as shown in **Table 6**.

Evaluation of Formulations:

Physicochemical evaluations of different formulation of gels: The prepared polyherbal gels using different concentration of extracts of neem, nutmeg and black pepper were tested for appearance, pH, viscosity, spreadability, drug content and from the results it was found that prepared gel 2 showed good pH, viscosity, Spreadability and drug content as compared to gel 1.

TABLE 6: MINIMUM INHIBITORY CONCENTRATION OF THREE MEDICINAL PLANT EXTRACTS

Sr.no.	Bacteria	Minimum inhibitory concentration(mg/ml)		
		Neem	Nutmeg	Black pepper
1	*P. acnes*	6.0	2.0	10.0
2	*S. epidermidis*	6.0	0.3	3.0
3	*S. aureus*	4.0	2.0	0.7

TABLE 7: EVALUATION OF GELS

Sr. No.	Formulations	Appearance	pH	Viscosity (cps)	Spreadability (gm-cm/sec)	Drug content
1.	Gel 1	Brownish	6.67	3800	11.76	95.65%
2.	Gel 2	Brownish	6.79	3900	11.53	96.66%

***In vitro* diffusion study of different formulations of gel:** The *in vitro* diffusion studies of the gel 1 and 2 were performed by using dialysis membrane and the results were observed as shown in **fig. 7** and **fig. 8**. The study showed that the percent release of all the active ingredients of gel 2 was better as compared to gel 1.

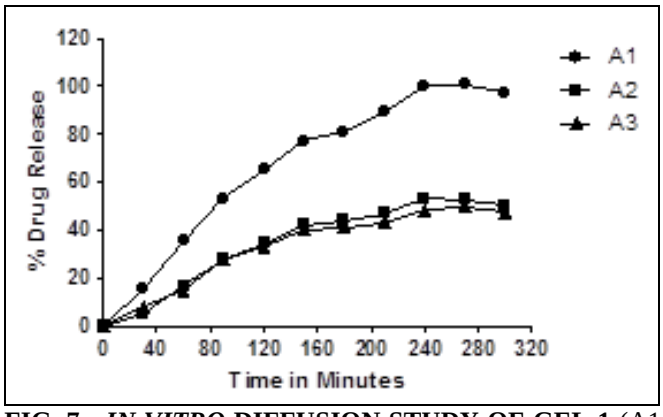

FIG. 7: *IN VITRO* DIFFUSION STUDY OF GEL 1 (A1- Neem, A2- Nutmeg, A3- Black pepper)

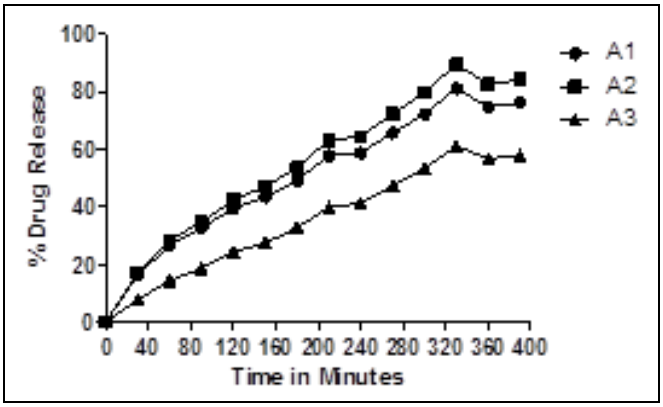

FIG. 8: *IN VITRO* DIFFUSION STUDY OF GEL 2 (A1- Neem, A2- Nutmeg, A3- Black pepper)

Comparative study of prepared gels with the marketed formulations and standard antibiotic: The comparative antimicrobial activity was performed on the basis of zone of inhibition of extracts of neem, nutmeg and black pepper, combination of all three extracts, prepared gel 1 and gel 2, marketed herbal formulation (Clarina cream), clarithromycin gel and standard antibiotic clindamycin. From the results obtained as below it was observed that prepared gel 2 showed more zones of inhibition than prepared gel 1 and marketed formulation.

TABLE 8: ANTIBACTERIAL ACTIVITY OF VARIOUS FORMULATIONS AGAINST *PROPIONIBACTERIM ACNES, STAPHYLOCOCCUS EPIDERMIDIS* AND *STAPHYLOCOCCUS AUREUS*

Sr. no.	Different formulations	Susceptibility of bacteria to different formulations, Zone of inhibition (mm)[a*]		
		P. acnes	***S. epidermidis***	***S. aureus***
1	*Azadirachta indica*	20	19	13
2	*Myristica fragrance*	18	17	14
3	*Piper nigrum*	16	20	12
4	*Combination (1,2 and 3)*	23	22	17
5	Prepared gel 1	11	14	12
6	Prepared gel 2	13	16	14
7	Marketed herbal formulation	12	18	15
8	Clarithromycin gel	23	26	25
9	Standard antibiotic (clindamycin)	36	33	31

Concentration of the each extract and formulation used : 20 mg/ml, Clindamycin: 1.0 mg/ml

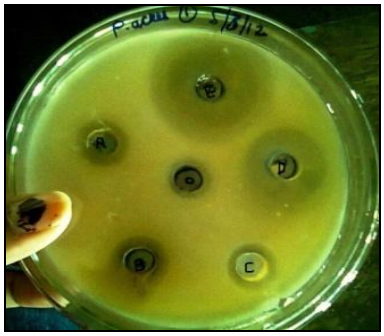

FIG. 9A: ZONE OF INHIBITION AGAINST *P. ACNES*

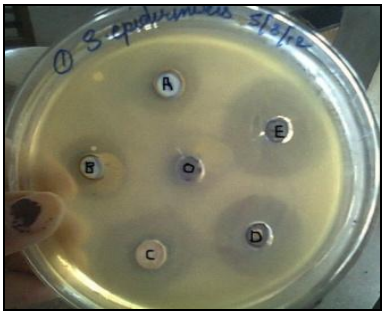

FIG. 9B: ZONE OF INHIBITION AGAINST *S. EPIDERMIDIS*

FIG. 9C: ZONE OF INHIBITION AGAINST *S. AUREUS*

FIG. 9: COMPARATIVE STUDY OF ANTI-MICROBIAL ACTIVITY OF PREPARED FORMULATIONS WITH THE MARKETED HERBAL FORMULATION AND STANDARD ANTIBIOTIC. A- Gel 1, B- Gel 2, C- Clarina Cream, D- Clarithromycin Gel, E- Clindamycin

Stability study of different formulations of gel: Stability study of the two formulations were carried out at 40^{o}C± 2^{o}C/ 75% ± 5% RH temperature and relative viscosity for the period of 3 months. The different parameters which were recorded for the change during this period were as follows:

a. Changes in appearance

b. Changes in pH

c. Changes in viscosity

d. Changes in Spreadability

Skin irritation study of gel: All the formulations did not produce any skin irritation, i.e., no erythema and oedema was seen for about a week when applied over the skin.

DISCUSSION: *Acne vulgaris* is an extremely common skin disorder that affects virtually all individuals at least once during life. The incidence of acne peaks at teenage, but substantial numbers of men and women between 20-40 years of age are also affected by the disorder. Herbal medication are considered safer than allopathic medicines as allopathic medicines are associated with side effects such as like contact allergy, local irritation, scaling, photosensitivity, itching, pruritus, redness, skin peeling, xerosis of the skin etc.

TABLE 9: STABILITY STUDY OF GELS AT 40^{O}C ± 2^{o}C/ 75% ± 5% RH AT 3RD MONTH

Sr.no.	Formulations	Appearance	pH	Viscosity (cps)	Spreadability (gm-cm/sec)
1.	F1	Brownish	6.61	3600	11.54
2.	F2	Brownish	6.74	3840	11.34

TABLE 10: SKIN IRRITATION STUDY

Formulations	Treatment						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	-	-	-	-	-	-	-
Gel 1	-	-	-	-	-	-	-
Gel 2	-	-	-	-	-	-	-

The present research work deals with formulation and evaluation of herbal antiacne gels. The plant material used for the formulations was ethanolic extract of leaves of *Azadirachta indica* (neem), ethanolic extract of fruits of *Myristica fragrance* (nutmeg) and *Piper nigrum* (black pepper). Although various topical herbal formulations for acne containing *neem* are available in the market, we propose to make use of *black pepper and nutmeg* extract for the first time in the developed formulations.

Neem, nutmeg and black pepper has been reported in the literature as a good antibacterial agent and anti-inflammatory agent. The developed formulations were evaluated for their *in vitro* anti-bacterial activity against *P. acnes, S. epidermidis and S. aureus*. The Zones of inhibitions for the antibacterial activity were compared with the standard Clindamycin, marketed herbal antiacne preparation, active ingredients used in the formulation (ethanolic extracts of neem, nutmeg and black pepper). The prepared ethanolic extracts of neem, nutmeg and black pepper showed antimicrobial activity against *P. acnes, S. epidermidis* and *S. aureus* but the combination of all three extracts showed more zone of inhibition as compared to individual extracts.

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