KOROSANAI- AN ANIMAL BASED SILVER NANO CREAM FOR ACNE TREATMENT

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ABSTRACT

Acne vulgaris is a common chronic inflammatory skin disorder primarily caused by *Staphylococcus aureus* and *Micrococcus luteus*. Traditional animal-derived products have long been employed in various medical systems, including Siddha and Traditional Chinese Medicine. In this study, an anti-acne cream was developed using *Calculus bovis* (Korosanai), an ox-bile derivative, incorporated with biosynthesized silver nanoparticles. Pharmacognostical, physicochemical, and phytochemical evaluations confirmed the presence of amino acids, carbohydrates, tannins, flavonoids, alkaloids, and terpenoids. Quantitative analysis revealed significant phenolic (17 mg/g), tannin (21.89 mg/g), and flavonoid (79.3 mg/g) content. Silver nanoparticles were successfully synthesized and characterized by UV–Vis spectroscopy and SEM, showing spherical morphology with particle sizes ranging 200–300 nm. The formulated cream demonstrated desirable physicochemical properties (pH 7.4, smooth consistency, non-irritant, and washability) with no adverse effects. Antibacterial studies confirmed potent inhibition of acne-causing bacteria, with silver nanoparticle-loaded formulations exhibiting greater activity than crude extracts. These findings suggest that *Calculus bovis*-based silver nanoparticle cream could serve as a promising natural alternative for acne management.

KEYWORDS: Korosanai (*Calculus bovis*), silver nanoparticles, anti-acne cream, acne vulgaris, antimicrobial activity, traditional medicine, phytochemical analysis, nanotechnology.

INTRODUCTION

Animal products derived from different organs of their bodies have constituted part of the inventory of medicinal substance used in varies cultures since ancient times. Such uses still exit in traditional medicine[1]. Traditional Chinese Medicine (TCM) has been increasingly practiced in many countries of the world. Some recent textbooks of TCM still recommend formulas containing various animal tissues. Usually, animal tissues are combined with medical herbs. In most of the case, the medical use of the preparations is justified in terms of the rules of TCM[2]. Animal parts were also used for making different instruments used for diagnostic purpose as well surgical management for example horns of different animals are used for purpose of bloodletting[3,4]. These animal derived natural products hold much promise in extensive areas, example – controlled drug delivery. Animal products are used as drug in therapeutics. The major groups of animal products certain animal used in medicine are hormones, enzymes, animal extractive, organs and bile acids. Bile is a natural secretion of liver which passes into the intestinal tract and aids in the digestion of fats by emulsifying them and promoting their absorption. Bile contains sodium salts of bile acids-hydrochloric acid, taurocholic acid and deoxycholic acid. These acids are obtained chiefly from ox bile[5,6].

ACNE

Acne vulgaris is a chronic inflammatory dermatitis of the pilosebaceous unit, which affects approximately 85% of adolescents. It develops in the middle part of the sebaceous follicle channel, where they are dense on the face, scalp, chest neck, upper back and upper arms. Increase of the androgen levels in both sexes, especially during puberty causes the sebaceous glands to expand and the production of sebum to increase. The pathogenesis of acne vulgaris involves the interaction of multiple factors that ultimately lead to the formation of its primary lesion, which is known as “Comedo”[7]. The causative agent of acne is Staphylococcus aureus and Micrococcus luteus.

PATHOPHYSIOLOGY

The pathogenesis of acne vulgaris is multifactorial. It involves the interaction of several host factors, including the stimulation of sebaceous glands by circulating androgen, symbiosis of the pilosebaceous follicle micro biome and cellular immune responses. The microcomedo serves as the primary lesion and is the precursor for all clinical manifestations of acne vulgaris[8]. It is characterized by a small hyperkeratosis plug primarily composed of coenocytes and located in the lower region of the follicular infundibulum. Microcomedones gradually evolve and develop into other acne lesions, which include closed comedons (whiteheads), open comedons(blackheads), and inflammatory papules, pustules, and nodules. Acne develops as a result of an interplay of the following four factors:

1) Release of inflammatory mediators into the skin
2) Follicular hyper keratinization with subsequent plugging of the follicle
3) Cutibacterium acnes (formerly Propionibacterium acnes) follicular colonization
4) Excess sebum production[9,10,11].

HISTOPATHOLOGY

The histopathologic evaluation of an acne lesion demonstrates a ruptured pilosebaceous apparatus with mixed inflammatory infiltrate of neutrophils, lymphocytes, and histiocytes[12]. Neutrophils may collect at the site of the rupture and form occasional intraepidermal pustules. Follicular plugging by keratin is also a common feature[13].

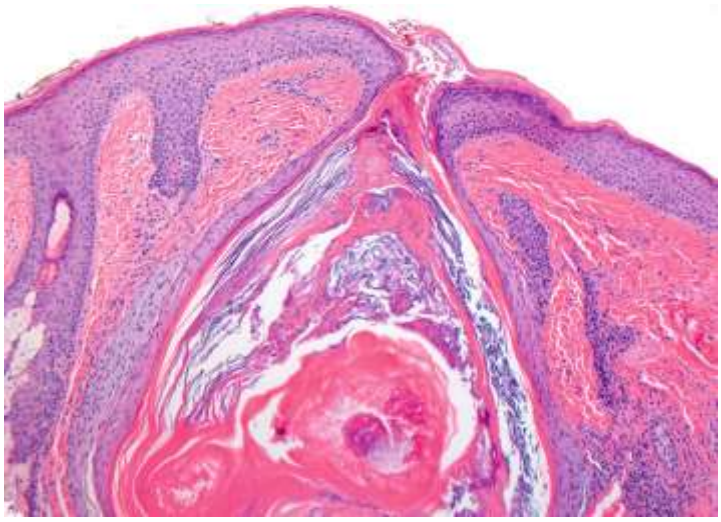


Figure1: Acne Vulgaris

Acne presents as a variety of polymorphic lesions from grade 1 to grade 4, starting with comedones, as listed below:

- ✓ Grade 1: Also known as "comedons" and is categorized into two types, open and closed. Open comedones from when the pilosebaceous orifice becomes plugged with sebum and appears as papules with a central, dilated follicular orifice containing grey, brown, or black keratotic material. On the other hand, closed comedones form when keratin and sebum block the pilosebaceous orifice beneath the skin surface. They appear as dome-shaped, smooth papules that can be skin-coloured, whitish, or greyish in appearance[14].
- ✓ Grade 2: Inflammatory lesions present as a small papule with erythema.
- ✓ Grade 3: Pustules.
- ✓ Grade 4: Many pustules coalesce to form nodules and cysts called nodulocystic acne[15].

DRUG PROFILE:

Ox gallstones also known as ox bezoars or "*Calculus bovis"* in the literature, were first recorded in Shen Nong's Herbal Classic, during the Qin (221-206 BCE) and the Western Han Dynasties (206 BCE-25 CE). These gallstones were considered a superior (i.e., top-grade) therapeutic drug, and have been employed for over two thousand years in TCM, mainly as a tranquilizer and sedative. Ox gallstones were believed to be successful in curing a number of childhood diseases. This was especially the case with febrile infantile convulsions, which in the ancient literature were characterized quite accurately by sudden onset, upward gazing of the eyes, lockjaw, rigidity of the neck, limb convulsions, frothy salivation, "rattling" of pharyngeal phlegm and eventually coma[16,17]. Ox gallstones were also prescribed for children

as a remedy for polydipsia, diarrhea, and vomiting “caused by fright”, and nocturnal crying “induced by fear” (most-likely febrile nightmares). Furthermore, in TCM, Ox gallstones were also used to treat apoplexy, with aphasia, and coma. In addition, ox gallstones were mixed and prescribed together with other herbal remedies[18].

The fresh ox gall secreted by the liver and collected in the gall-bladder. It is a dark or yellowish green viscid liquid of a peculiar unpleasant odour and bitterish taste. It is a neutral or faintly alkaline in reaction, soluble in water and alcohol[19,20].

Synonym - Bovine bile, Ox gall powder, dried ox gall, Ox bile extract
Scientific name – *Fel bovinum purifactum*

VERNACULAR NAMES:

English : Purified ox-gall or ox-bile, cow gallstones, ox gallstone, serpent stone
Arab : Hajr-ul-bahr
Latin : *Calculus bovis* seu bubali
Hindi : Gorochan
Sanskrit : Gorochanam
Tamil : Gorojanai
Telugu : Gorojanamu
Singh : Visagul
Pharmaceutical Name : *Calculus bovis*
Other names : Rosanam , Gomantram , Vachalamani , Jathi

MATERIALS AND METHODS:

- **PHARMACOGNOSTICAL STUDIES:**

1) Collection of drug

Crude animal based powdered material was purchased from truelife.com and check basic quality control parameter then powdered material was subjected to further process.

2) Morphological studies

The drug is studied for its morphological characters like colour, odour, taste, shape.

3) Physio Chemical Parameters

The drug is subjected to various physio-chemical parameters such as foreign organic matter, loss on drying and ash value.

4) Determination of Foreign organic matter

An accurately weighed 25g of drug and spread out in a thin layer. The drug was inspected with the unaided eye or with the use of 6x lens and the foreign organic matter was separated manually as completely as possible and weighed. The percentage of foreign organic matter was calculated with reference to the weight of the drug taken[21]

5) Determination of loss on drying

For the determination of loss on drying, the method describe by Wallis was followed. 1g of drug was accurately weighed in a tarres petri dish, previously dried under the conditions specified in IP'96. The powder was distributed as evenly as practicable, by gentle sidewise shaking. The dish was dried in an oven at 100-105ºC for 1 hour. It was cooled in desiccator and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken[21].

6) Determination of total ash value

2g of the drug was accurately weighed in a silica crucible separately. The powder was scattered into a fine even layer on the bottom of the crucible and incinerated by gradually increasing the temperature not exceeding 450ºC, until free from carbon. Then it was cooled and weighed for constant weight. The percentage of ash with reference to the drug was calculated[21].

- **CHEMICAL STUDIES**

Qualitative analysis (Procedure)

Aqueous solution of *calculus bowis* was subjected to chemical tests for the identification of their active constituents[22,23,24].

1)Test for amino acids and proteins

a) **Biuret test:** To 1ml of sample solution, add 1ml of 5% sodium hydroxide and 2 drops of 1% copper sulphate solution. A violet color develops the presence of Amino Acids.

b) **Ninhydrin test:** To 1ml of sample solution, add 10 drops of Ninhydrin reagent. Heat to boiling. A blue or purple color develops the presence of Amino Acids.

c) **Millon's test:** To the 1ml of sample solution add 1ml of mercuric sulphate solution and boil for 30 sec, add 2 drops of 1% sodium nitrate. Red color was produced, presence of Amino acids.

d) **Pauly's test:** To 0.5ml of sulphanilic acid add equal amount of 0.5% sodium nitrate mix well, after few mins add 1ml of sample solution mix vigorously and add 2ml of 10% sodium carbonate. Red color was produced, presence of amino acid.

e) **Sulphur test:** To 1ml of sample solution add an equal volume of 40% sodium hydroxide. Boil at least for 3 minutes and allow it for cool. Add 2-3 drops of lead acetate solution with mixing. A black or brown colored precipitate develops the presence of Amino Acids.

f) **Molisch test:** To 1ml of sample solution, add 2-3 drops of 1% Alpha-naphthol solution in ethanol. Add carefully through the side of the test-tube, 2.0ml of concentrated H_2SO_4. A reddish violet ring forms at the junction of the two phases the presence of Amino Acids.

2)Test for Carbohydrate

a) **Molisch test:** Aqueous solution of sample was mixed with few drops of molisch reagent (Alpha-naphthol) and concentrated H_2SO_4 was added from sidewall of test-

tube. Formation of purple coloured ring at junction of the two phases the presence of Carbohydrates.

b) **Iodine test:** Few drops of Iodine solution was added to aqueous solution of sample. Formation of blue colour, which disappears on heating and reappears on cooling, the presence of starch.

c) **Seliwanoff's test:** To 1ml of aqueous solution of sample, 5ml of Seliwanoff's reagent was added and boiled. Formation of cherry red colour the presence of carbohydrates.

3) Test for reducing sugars

a) **Fehling solution test:** Equal volumes of Fehling A and Fehling B solutions were mixed 1ml each and 2ml of aqueous solution of sample was added followed by boiling for 5-10 minutes on water bath. Formations of reddish-brown coloured precipitate the presence of reducing sugars.

b) **Benedict's test:** To the 4ml of aqueous solution of sample, 1ml of Benedicts solution was added and heat to boil. Formation of green color develops the presence of reducing sugars.

4)Test for Glycosides

a) **Borntrager's test:** To the aqueous sample solution was treated with chloroform and the chloroform layer was separated. To this equal volume of dilute ammonia solution was added. Ammonia layer acquires pink color shows the presence of Anthraquinone Glycosides.

b) **Keller kiliani test:** To 0.5ml containing glacial acetic acid add 2-3 drops of ferric chloride mix well add 2ml of sample solution and add 1ml of concentrated sulphuric acid. No deep blue color was produced, absence of cardiac glycosides.

5)Test for Alkaloids

a) **Dragendorff's test:** To the sample solution add Dragendorff's reagent (potassium bismuth iodide), formation of orange red color. Presence of Alkaloids.

b) **Mayers's test:** To the sample solution add few drops of Mayer's reagent, no formation of white precipitate. Absence of Alkaloids.

6)Test for tannins

a) **Test with iron salts:** To the aqueous solution of sample add few drops of Ferric chloride solution forms greenish brown colored precipitate. Presence of tannins.

7)Test for flavonoids

a) **Shinoda's test:** To the sample add few pieces of magnesium turnings and add drop by drop of concentrated hydrochloric acid and heat. Appearance of magenta red color presence of flavonoids.

8)Test for terpenoids

a) **Salkowski test:** To the 1ml of sample dissolved in chloroform add few drops of concentrated sulphuric acid. Appearance of brown color presence of terpenoids.

9)Test for Lipids

a) Grease spot test: To the 3ml of ether in a test tube add 5 drops of aqueous sample solution. To the filter paper few drops of above solution was placed. Appearance of greasy spot presence of lipids.

b) Test for coumarins: 3ml of 10% NaOH was added to 2ml of aqueous extract, formation of no yellow color indicates. The absence of coumarins.

Quantitative analysis

Aqueous solution of *Calculus bovis* was subjected to estimation for the identification of totalphenols, tannins, flavonoids content present[25].

1) Determination of total phenolic content:

The total phenolic content of *Calculus bovis* aqueous extract was estimated by the Folin–Ciocalteu method using UV–Visible spectrophotometry. The reaction mixture contained 1 mL of sample, 4 ml of distilled water, 1 mL of Folin–Ciocalteu reagent, and 1 mL of 7% sodium carbonate solution. After 30 min incubation, absorbance was measured at 550 nm. Gallic acid (20–100 µg/mL) was used for standard calibration.

Instrument used:

UV–Visiblespectrophotometer

Chemicals used:

- Gallic acid,
- Folin–Ciocalteu reagent,
- 7% sodium carbonate.



Figure 2: **Determination of total phenolic content**

2) Determination of total tannin content:

Total tannin content was estimated using the Folin-Ciocalteu method. Briefly, 0.1 ml of sample was mixed with 7.5 ml distilled water, 0.5 ml Folin-Ciocalteu reagent, and 1 ml of 35% sodium carbonate, then diluted to 10 ml. Standard gallic acid solutions (20–100 µg/ml) were prepared similarly. Absorbance was measured at 725 nm using a UV–Vis spectrophotometer.

Instrument used:

UV–Vis spectrophotometer

Chemicals used:

- Gallic acid,
- Folin-Ciocalteu reagent,
- 35% sodium carbonate.



Figure 3: **Determination of total tannin content**

3) Determination of total flavonoid content:

Total flavonoid content was estimated by aluminium chloride colorimetric assay. Briefly, 1 ml of sample and 4 ml of distilled water were mixed, followed by 0.3 ml of 5% sodium nitrite. After 5 min, 0.3 ml of 10% aluminium chloride was added. After another 5 min, 2 ml of 1 M sodium hydroxide was added and the mixture diluted to 10 ml with distilled water. Standard quercetin solutions (20–100 µg/ml) were prepared similarly. Absorbance was measured at 510 nm against reagent blank using a UV–Visible spectrophotometer.

Instrument used:

UV–Vis spectrophotometer.

Chemicals used:

- Quercetin,
- sodium nitrite,
- aluminum chloride,
- sodium hydroxide.



Figure 4: **Determination of total flavonoid content**

- **FORMULATION AND EVALUATION STUDIES**

Synthesis of silver nanoparticles

About 0.1g of the powdered sample was dissolved in 100ml of distilled water art 60ºC for 1 hr; it was allowed to cool and then filtered with Whatman No.1 paper. The filtrate was centrifuged at 4000 rpm for 15 min. Thereafter, 1ml of the centrifuged filtrate was transferred into 40 ml of 1mM,0.01 aqueous silver solution an was observed for colour change[26].

UV-Visible analysis for the synthesized silver nano particles

2 ml of solution of nano particles was taken in Quartz cuvette. UV range was set between 200-800 nm and blank was neutralized with de-ionized water[27].

SEM analysis for synthesized silver nano particles

Thin films of magnetite nano particles were prepared on a coated copper grid by dropping small amount sample and then the film on the SEM grid were allowed to dry by putting under mercury lamp for 5 minutes[28].

Formulation of cream:

Oil phase was prepared with beeswax, propylene glycol in 1 beaker, then it was heated water bath until it was completely melted. Then aqueous phase was prepared with silver nano particle, white soft paraffin, glycerine, zinc oxide, sodium benzoate and distilled water in 2 beaker while heating in water bath. Oil phase was added into aqueous phase by continuous stirring was done until semisolid mass was formed[29].

S.NO	INGREDIANTS	QUANTITY
1.	Silver nano particle	0.25gm
2.	Beeswax	3.2gm
3.	Propylene glycol	1ml

4.	White soft paraffin	9ml
5.	Glycerine	1ml
6.	Zinc oxide	0.7gm
7.	Sodium benzoate	0.1gm
8.	Rosemary oil	Q.S
9.	Distilled water	Q.S

Table 1: **Formula of cream formulation**

EVALUATION OF CREAM

1) Physical appearance

Colour: The colour of cream was checked by visual inspection.

Odour: The odour of cream was checked by observing the smell of the cream.

Consistency: The consistency of cream was checked by applying on skin.

2) Measurement of pH:

The cream ph was measured by taking 1 gm of cream dissolved in 10 ml of distilled water and keep it apart for two 2hrs.The ph was measure by dipping the glass electrode completely into the cream three time.

3) Homogeneity:

Homogeneity of cream was test by visual inspection after the cream had been set into the container. They were tested for any aggregate appearance was presented.

4) Irritancy:

The area (1cm^2) on the left-hand dorsal surface. Then the cream was applied to the area and the time was noted. Then it is checked for irritancy, erythema if any for an interval up to 24 hour.

5) Washability:

A small amount of cream is applied on the hand and it is then washed with tap water.

6) Phase separation:

Prepared cream was kept in an closed container at temperature 25-100ºC away from light. Then phase separation was checked for 24 hours for 30 days.

7) Determination of emolliency:

The emolliency test was performed to check the amount of residue left after the application of specific quantity of cream.

8) Viscosity:

Viscosity of cream was done by using Brooke field viscometer at 100 rpm with the spindle no.7[30].

- **PHARMACOLOGICAL STUDY**

In-vitro antibacterial study for ***Calculus bovis***

Antibacterial assay:

1) Preparation of inoculum:

Stock cultures were maintained at 4ºC on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loopful of cells from the cultures to test

tube of Muller-Hinton broth(MHB) for bacteria that were incubated without agitation for 24 hrs at 37ºC. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0X 10 colony forming units (CFU/ml) For bacteria.

2) Preparation of sterile swabs:

Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or by dry heat (only for the wooden swabs).It was sterilized by packing the swabs in culture tubes, paper or tins etc.

3) Sterilization of forceps:

Forceps can be sterilized by dipping in alcohol and burning of the alcohol.

4) Preparation of Muller-Hinton agar:

38 milligram of Muller-Hinton agar powder was added into one litre of distilled water in a flat-bottomed conical flask. The mixture was heated with frequent agitation and boiled for one minute to completely dissolve the media. The flask was then tightly closed using cotton wool and further covered with aluminium foil. The mixture was autoclaved for 15 minutes at 121 degree Celsius after which it was left to cool down to room temperature. The media was poured in the petri dishes in laminar flow to give uniform depth of 3-4 millimetres. The petri dishes containing the medium were then placed in a sterile plastic bag and stored at a temperature of 2-8 degree Celsius before use.

5) Antibacterial assay using agar well diffusion Method:

The well diffusion method was used to screen the antimicrobial activity. *In vitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from HI media (Mumbai).The MHA plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates could solidify for 5 minutes and 0.1 % inoculums suspension was swabbed uniformly and the inoculums could dry for 5 minutes. Wells were cut and 20µ of the different concentration of test drug were added. The plates were then incubated at 37º C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Chloramphenicol disc was used as a positive control.

6) *In-vitro* anti-acne study for formulated silver nano particles.

The procedure followed for *In- vitro* anti-acne studies of korosanai has been adopted for *In-vitro* anti-acne study for formulation silver nano particles.

RESULT AND DISCUSSION:

- **PHARMACOGNOSTICAL STUDIES**

Physio-chemical parameters:

Physiochemical parameters such as foreign matters, Loss on drying and ash value were determined for the powdered drug and the results were presented in the table;

S.NO	Physiochemical constants	Result (%W/W)
1	Foreign matter	Nil
2	Loss on drying	3.47±0.11
3	Ash value (Total ash)	11.45±0.227

Table 2*: Physiochemical characteristics of Calculus *bovis

- **CHEMICAL STUDIES**

QUALITATIVE ANALYSIS:

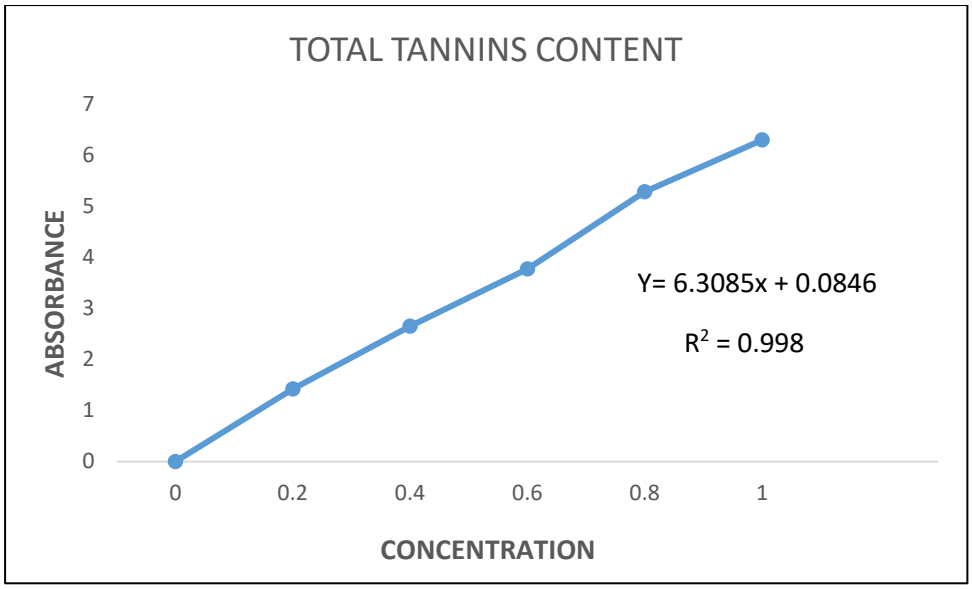
Qualitative analysis of aqueous solution of *Calculus bovis* for the presence of various chemical constituents (Preliminary chemical screening) and the results are shown in the table.

S.NO	Name of the test	Inference
1	**Test for Amino acids and proteins**	
	A) Biuret test	+
	B) Ninhydrin test	+
	C) Millon's test	+
	D) Pauly's test	+
	E) Sulphur test	+
	F) Molisch test	+

2	**Test for carbohydrates**	
	A) Molisch test	+
	B) Iodine test	+
	C) Seliwanoff's test	+
3	**Test for reducing sugars**	
	A) Fehling solution test	+
	B) Benedict's test	+
4	**Test for Glycosides**	
	Borntrager's test	-
	Keller killani test	-
5	**Test for Alkaloids**	
	Dragendroff's test	+
	Mayer's test	-
6	**Test for tannins**	
	Test with iron salts	+
7	**Test for flavonoids**	
	Shinoda's test	+
8	**Test for terpenoids**	
	Salkaowski test	+
9	**Test for lipids**	
	Grease spot test	+
	Test for coumarins	-

Table3*: Phytochemical test for *Calculus bovis

➢ DETERMINATION OF TOTAL TANNIN CONTENT:

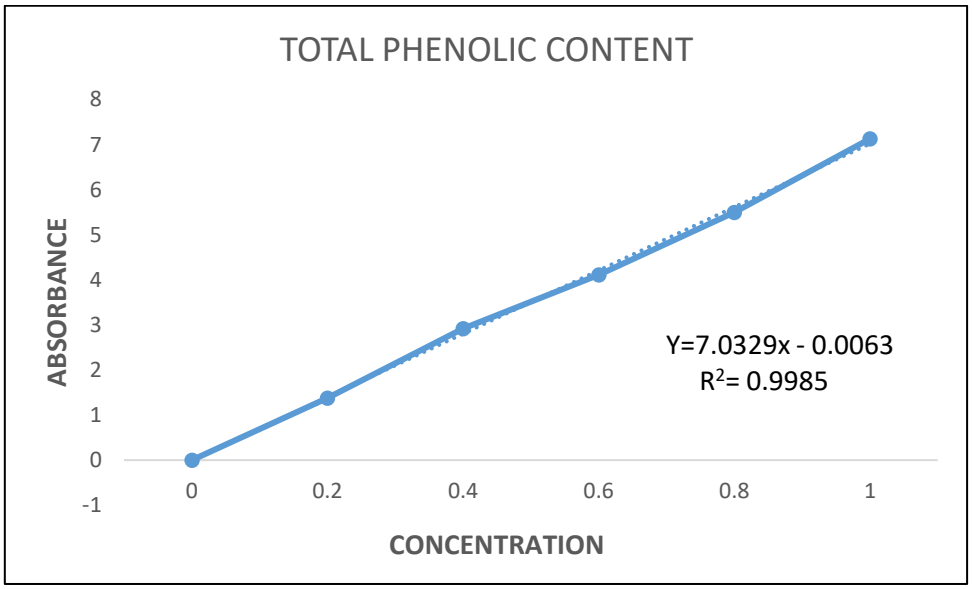


***Graph 1*: Calibration curve of Gallic acid in Total Tannin content**

S.NO	CONCENTRATION	ABSORBANCE	
	Gallic acid & Korosanai (MCG/ML)	**Gallic acid Mean ± SEM**	**Korosanai Mean± SEM**
1	0.2	1.4243 ± 0.00245	0.1567 ± 0.00198
2	0.4	2.6583 ± 0.00212	0.2417 ± 0.00201
3	0.6	3.7751 ± 0.00298	0.3297 ± 0.00210
4	0.8	5.2813 ± 0.00276	0.3871 ± 0.00192
5	1	6.2943 ± 0.00207	0.4211 ± 0.00194
		GAE	21.89 mg/g

***Table 4:* Total Tannin content in korosanai**

➢ DETERMINATION OF TOTAL PHENOLIC CONTENT:

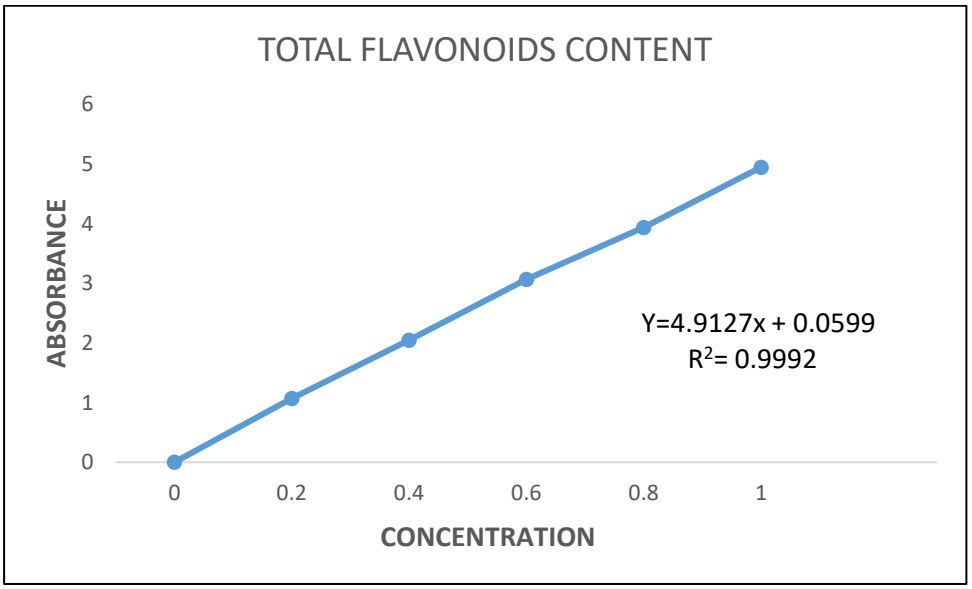


***Graph 2*: Calibration curve of Gallic acid in Total Phenolic content**

S.NO	CONCENTRATION	ABSORBANCE	
	Gallic acid and korosanai (MCG/ML)	Gallic acid Mean ± SEM	Korosanai Mean ± SEM
1	0.2	1.381± 0.001346	0.1432± 0.001566
2	0.4	2.9268± 0.001456	0.2954± 0.001646
3	0.6	4.1152±0.001334	0.3540±0.002348
4	0.8	5.5016±0.001346	0.3976±0.001346
5	1	7.136 ±0.0015346	0.4231±0.001896
		GAE	17mg/g

***Table 5:* Total Phenolic content in korosanai**

➢ DETERMINATION OF TOTAL FLAVONOID CONTENT:



Graph 3: **Calibration curve of Gallic acid in Total flavonoid content**

S.NO	CONCENTRATION	ABSORBANCE	
	Quercertin & Korosanai (MCG/ML)	Quercertin Mean ± SEM	Korosanai Mean ± SEM
1	0.2	1.0707 ± 0.00256	0.1089 ± 0.00198
2	0.4	2.056 ± 0.00206	0.2241 ± 0.00185
3	0.6	3.0781± 0.00234	0.2919 ± 0.00186
4	0.8	3.943 ± 0.00223	0.3596 ± 0.00186
5	1	4.95 ± 0.00215	0.3998 ± 0.00201
		QE	79.3mg/g

Table 6: **Total flavonoid content in korosanai**

➢ QUANTITATIVE ESTIMATION OF KOROSANAI:

S.NO	ESTIMATEDSECONDARYMETABOLITES	KOROSANAI
	Total Tannin content	**21.89 mg/g**
	Total Phenolic content	**17 mg/g**
	Total Flavonoid content	**79.3 mg/g**

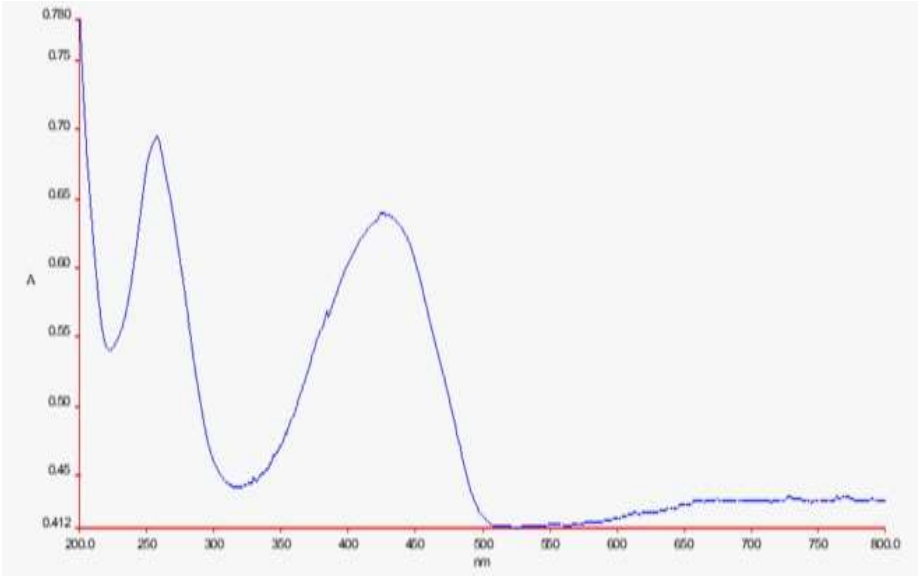
Table 7: **Quantitative analysis of korosanai**

FORMULATION AND EVALUATION STUDIES

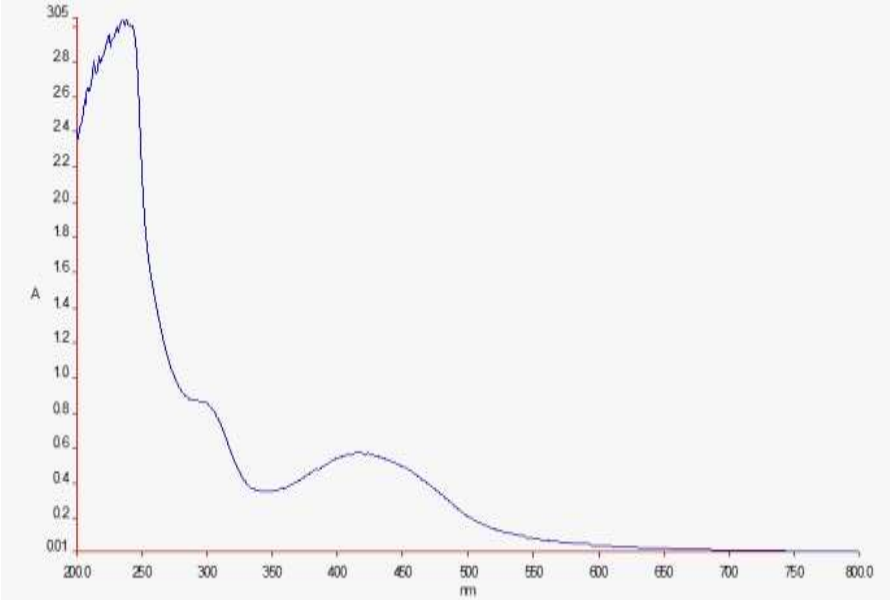
❖ Synthesis of Silver Nanoparticles

UV-visible –spectroscopy analysis:

The formation of silver nanoparticles in aqueous solution was confirmed by the SPR band in the UV-visible spectral analysis. **Graph 4** depicts the UV-visible absorption spectra of aqueous solution of *Calculus Bovis* , silver nitrate and biosynthesized silver nanoparticles. Size ,shape and distribution of nanoparticles were depending upon the formation of one or more SPR bands. Formation of a single SPR band at short wavelength revealed the presence of small sized spherical nanoparticles in the reaction mixture ,whereas two or more SPR bands shown at longer wavelengths indicated the presence of nanoparticles. The symmetric SPR bands indicates that the solution does not have much aggregated particles.400 to 420nm in the UV-visible spectrum corresponds to spherical shaped metallic nanoparticles. Small absorption peak at 200 to 210nm were developed in both sample and biosynthesized silver nanoparticle due to further formation of silver nanoparticles. The **Graph 5** Depicts the UV-visible absorption spectra of aqueous solution of Calculus Bovis has peak at 250 nm. The wavelength of silver nanoparticles in aqueous solution of *Calculus Bovis*(416nm) is more than that of wavelength of aqueous solution of *Calculus bovis*(250nm) So we confirmed that the nanoparticle was developed.



***Graph 4*: UV-visible spectra of *Calculus bovis* solution ¸1mM silver nitrate and biosynthesized silver nanoparticles**



Graph 5*: UV –visible spectra of *calculus bovis

DISCUSSION:

SEM was used to determine the size and morphology of synthesized silver nano particle. SEM images and its particular magnified image was shown in **Fig no. 6**. The magnified SEM image of synthesized silver nanoparticle showed the agglomeration of silver nanoparticles. Observation showed that most of the particle are in porous layered structure and particle size distribution in the range of 200-300nm.



***Figure 5*: Final Anti-acne cream**

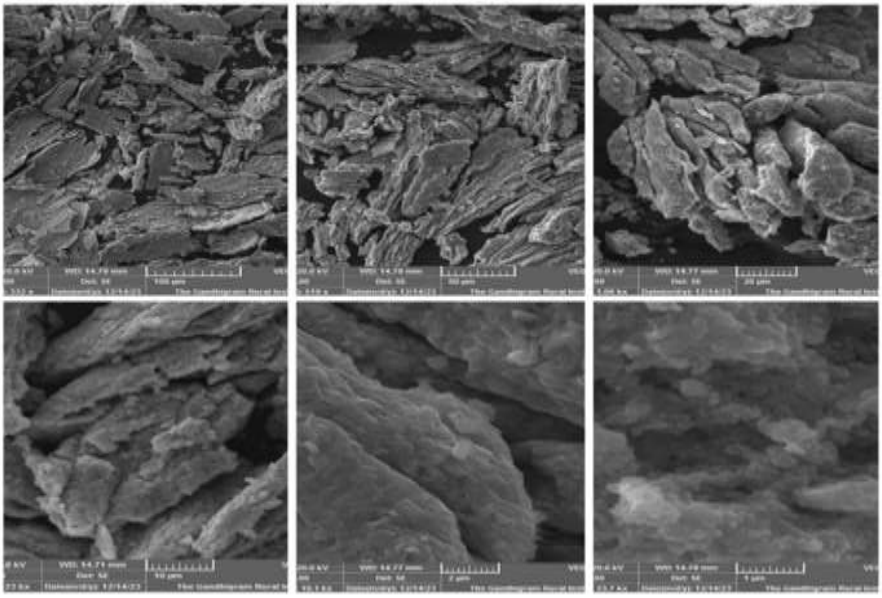


***Figure 6*: SEM image of silver nano particles sample in different magnification**

EVALUATION OF ANTI-ACNE CREAM

S.NO	PARAMETER	OBSERVATION
1.	Colour	Puff colour
2.	Odour	Pleasant
3.	Consistency	Smooth
4.	PH	7.4
5.	Irritancy effect	Nil
6.	Erythema	Nil
7.	Edema	Nil
8.	Wash-ablity	Easily wash
9.	Viscosity	2700-27089cp

***Table 8:* Physical evaluation of Anti-acne cream**

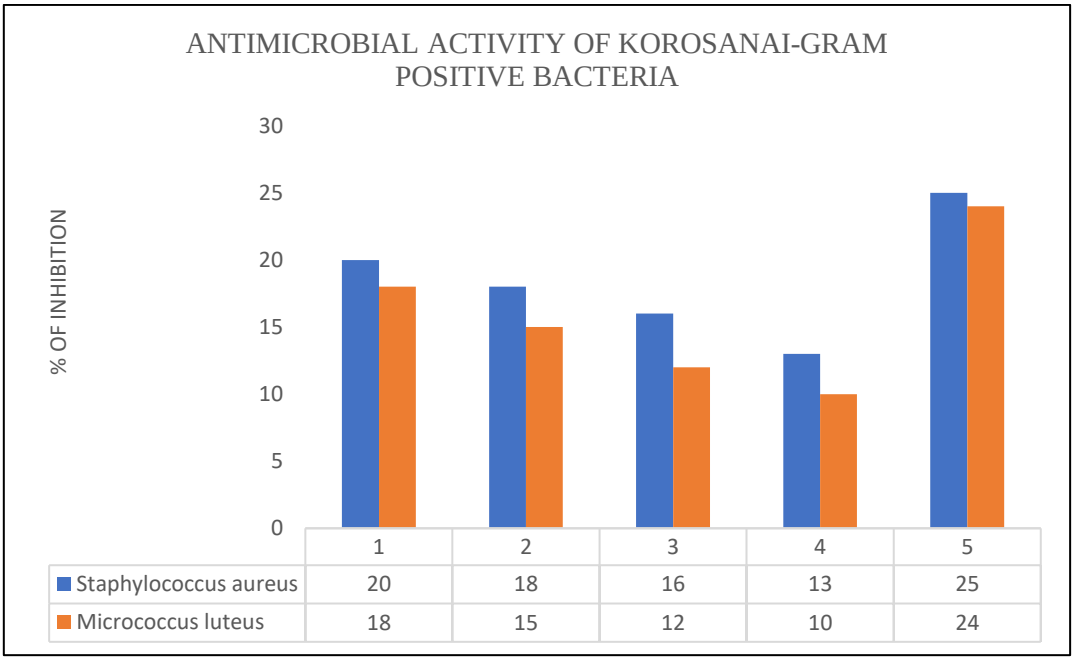
PHARMACOLOGICAL STUDIES

ANTI-MICROBIAL ACTIVITY OF KOROSANAI FOR ACNE

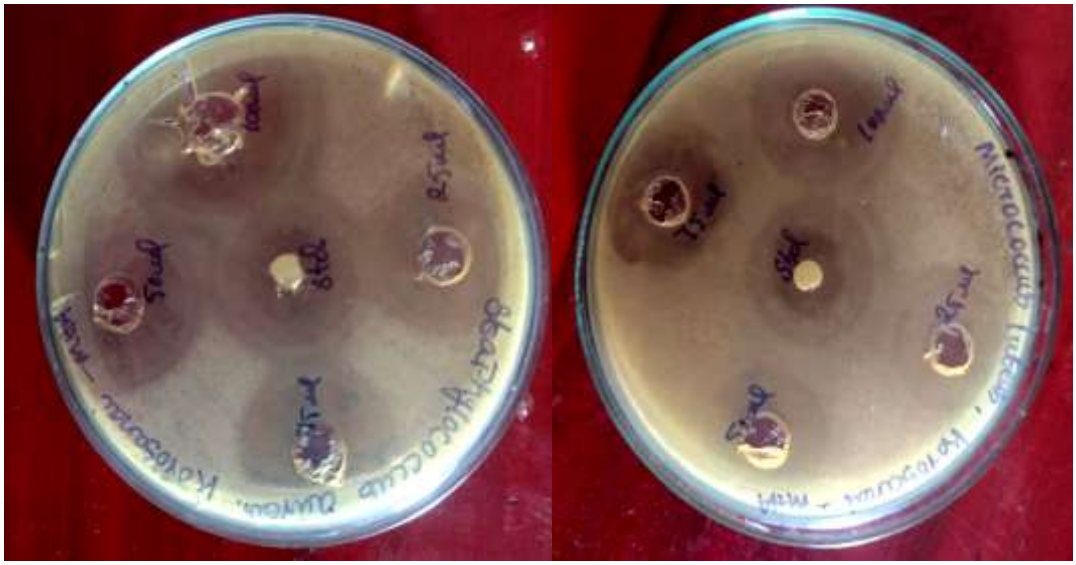
In-vitro systems are easier, faster and more cost-effective compared to traditional bioassays *In-vivo.* Plant based antibacterial have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antibacterials. Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens (Kumaraswamy et al., 2008).

S.NO	MICROORGANISMS	ZONE OF INHIBITION (MM)				
		100	75	50	25	+ve control
GRAM POSITIVE BACTERIA						
1	*Staphylococcus aureus*	20	18	16	13	25
2	*Micrococcus luteus*	18	15	12	10	24

***Table 9*: *In-vitro* anti bacterial study for Calculus bovis**



***Graph 7*: Graphical Representation of Anti-Bacterial activity of Korosanai**



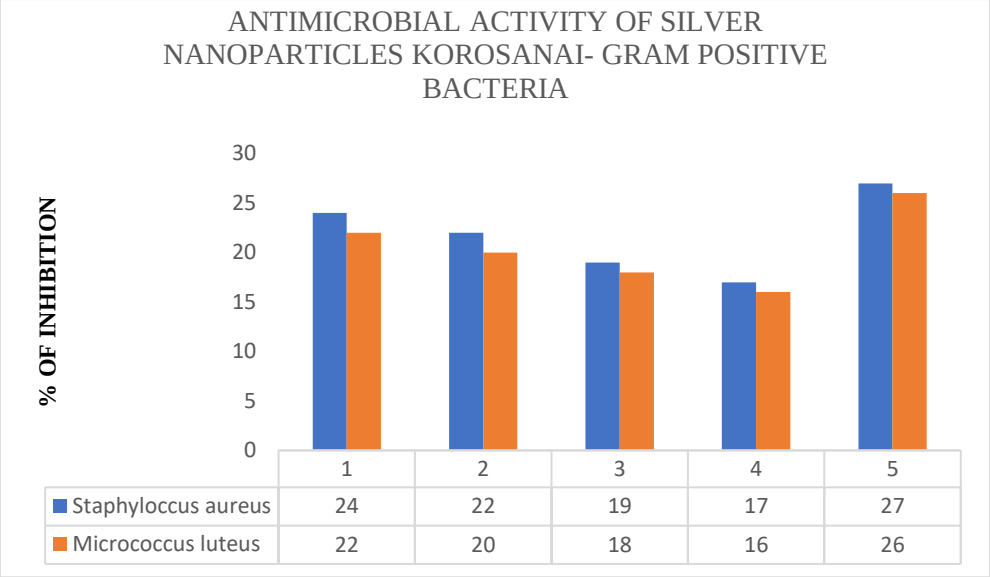
Staphylococcus aureus *Micrococcus luteus*

***Figure 7*: Diagrammatic Representation of antimicrobial activity of Korosanai**

***In-vitro* anti-acne study for formulated silver nano particles**

S.NO	MICROORGANISMS	ZONE OF INHIBITION (MM)				
		100 75 50 25 CONTROL	75	50	25	+VE control
GRAM POSITIVE BACTERIA						
1	*Staphylococcus aureus*	24	22	19	17	27
2	*Micrococcus luteus*	22	20	18	16	26

***Table 10*: Antimicrobial activity of Silver nanoparticles Korosanai - gram positive Bacteria**

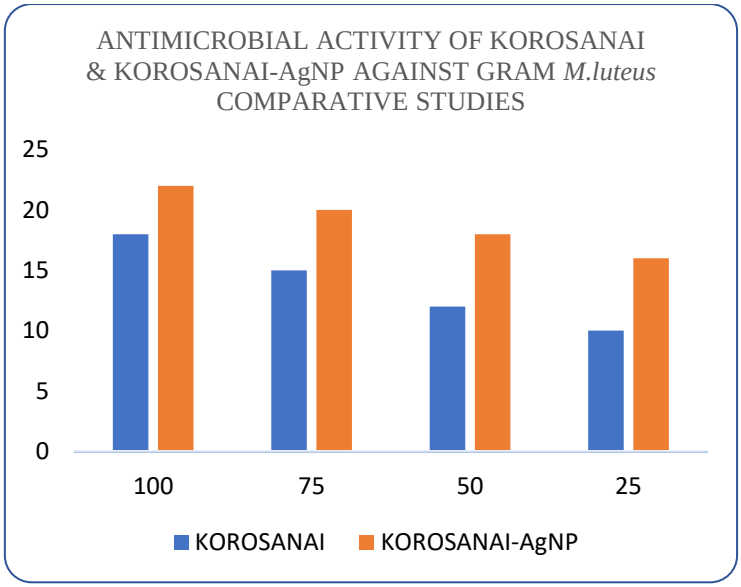


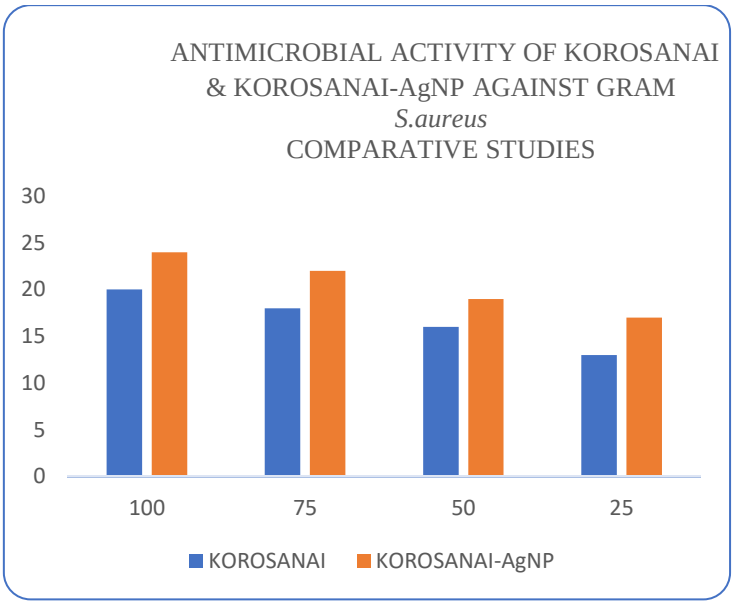
***Graph 8*: Graphical Representation of Antimicrobial activity of silver nanoparticles Korasanai**



Staphylococcus aureus *Micrococcus luteus*

***Figure 8:* Diagrammatic Representation of anti-microbial activity of silver nanoparticle Korosanai**





***Graph 9*: Comparative Study of activity of Korosanai and Korasanai-Ag**

DISCUSSION:

The aqueous extract of korosanai and synthesised silver nano particle of korosanai were proved to be potentially effective against *Staphylococcus aureus* and *Micrococcus luteus* and can be used as natural alternative for the treatment of acne vulgaris and also used to prevent the same. The comparative study of anti-microbial activity of korosanai and korosanai- AgNP against Staphylococcus aureus and Micrococcus luteus revealed that korosanai- AgNP shows better anti-microbial activity against the respective species in dose dependent manner.

CONCLUSION:

This study concluded that aqueous solution of *Calculus bovis* was used to formulate cream for acne infection. The silver nanoparticles were biosynthesized from the aqueous solution of

Calculus bovis and it was characterized by SEM and UV visible analysis. The formulation of anti-acne cream was done and it was evaluated. In *vitro* anti-bacterial studies were completed for Calculus bovis against *staphylococcus aureus*, *Micrococcus luteus* species. The current work provides considerable evidence that aqueous solution of korosanai(*Calculus bovis*) have the potential to cure acne vulgaris[31].

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CONFLICT OF INTEREST:

We declare that we have no conflict of interest.

DECLARATION:

Ethical Approval
Not applicable. This article does not involve any studies with human participants or animals performed by any of the authors.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

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Availability of Data and Materials

Not applicable. No datasets were generated or analyzed during the current study.

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