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FORMULATION AND EVALUATION OF NATURAL ANTI-ACNE SERUM USING *CINNAMOMUM CAMPHORA* (BHIMSENI KAPUR)

Md. Rageeb Md. Usman*, Quresh A. I. Shaikh[1], M. Z. Shaikh[2]

*[1]Department of Pharmacognosy, Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopda, Maharashtra, India

[2]Department of Zology, RFNS Senior Science College, Akkalkuwa, Maharashtra, India

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Abstract:

The moisture content present in human skin makes it look young and the use of moisturizer results in fastening the moisture with a surface film of oil. Acne vulgaris is one of the most commonly seen diseases among the youth. The present study is focused on the use of herbs as moisturizer for acne treatment. The anti-acne moisturizer was formulated from herbal crude extracts and investigated the physico-chemical parameters as well as antibacterial activity of the formulation. The study revealed that extract of Cinnamomum Camphora possessed the potential for inhibiting acne. It was observed that the optimal formula of anti-acne moisturizer was satisfactorily effective to control acne inducing bacteria i.e., Gram negative bacteria. The physico-chemical parameters of the formulation were also optimal with no signs of irritation.

Keywords: *Formulation, Evaluation, Cinnamomum, Camphora, Bhimseni Kapur.*

Corresponding author:

Md. Rageeb Md. Usman,

Department of Pharmacognosy,
Smt. Sharadchandrika Suresh Patil College of Pharmacy,
Chopda, Maharashtra, India

QR code

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INTRODUCTION:

It is a concentrate of active ingredients, which targets specific skincare concerns, and the ingredients are powerful, and made up of smaller molecules. The level of active ingredients is higher than in a usual face cream, since the heavier oils and ingredients have been done away with. So, while the latter could have around ten per cent of active ingredients, the former has a whopping seventy per cent or more! Serum is a skincare product you can apply to your skin after cleansing but before moisturizing with the intent of delivering powerful ingredients directly into the skin [1-3]. Serum is particularly suited to this task because it is made up of smaller molecules that can penetrate deeply into the skin and deliver a very high concentration of active ingredients. This makes them a great tool for targeting specific skincare concerns, like wrinkles. Goodbye, signs of aging [4-5].

The present project is also a humble attempt in the same direction, where *Cinnamomum Camphora* has been evaluated microbiologically to check its anti-microbial property against various infectious problems. *Cinnamomum Camphora* is commonly used in Indian traditional medicine as antibacterial, antifungal, antioxidant, wound healing, anti-inflammation.

It is reported that the *Cinnamomum Camphora*. has antibacterial activity against some Gram-negative bacteria. Therefore, this study aims to evaluate antibacterial properties of Cinnamomum Camphora on the bacteria associated with the skin [6-7].

MATERIAL AND METHOD:

Cinnamomum Camphora Wood is collected in Horticultural Garden and (Bhimsen Kapur) sample was collected from the Market. camphora leaves were later cut into slices to reveal the tighter inner stem until when ready for use. The analysis of Essential oils is generally derived from one or more plant parts, such as flowers, woods. Fresh *Cinnamomum Camphora* (Bhimsen Kapur) sample was collected from the Market and authenticated by Botanical survey of India Pune.

Procurement of Raw Material

Table 1: Procurement of Raw Material

Sr. No.	Ingredients	Suppliers	Role
1.	Glycerine	Dhruvicameru chem. Pvt. Ltd.	Humectant
2.	Propylene glycol	Dhruvicameru chem. Pvt. Ltd.	Humectant
3.	Xanthum gum	Acuroarganicspvt. Ltd.	Gelling agent
4.	DMDM Hydentoin	Unicarelid,Goyalchem	preservative
5.	Sodium EDTA	Inter MESH Ltd	Chelating agent
6.	Allantoin	Hi tech life sciences,kip chemicals	Healing agent

Formulation of Active Herbs

Table 2: Formulation of Active Herbs

Ingredients	Parts Used	Category	Qty%
Cinnamomum Camphora 	Leaves	Antioxidants Anti-inflammatory	3
Cinnamomum Camphora (Pure Bhimseni Kapur) 		Demulcent Anti- Microbial	3

Cinnamomum Camphora 	Wood	Anti- bacterial Aromatheraphy	4

Formulation of Base

Table 3: Formulation of Base

Sr. No.	Ingredients	Quantity for 100%
1.	Glycerine	5%
2.	Propylene glycol	5%
3.	Xanthum gum	0.5%
4.	Sodium EDTA	0.2%
5.	Allantion	0.1%
6.	DMDM Hydentoin	0.15%
7.	Active	10%
8.	Water	79.05%

EXPERIMENTAL WORK

Extraction of *Cinnamomum camphora*

Extraction of Camphor Oil use in Fractional Distillation and Vaccume Distilation (Fig. 1-3).

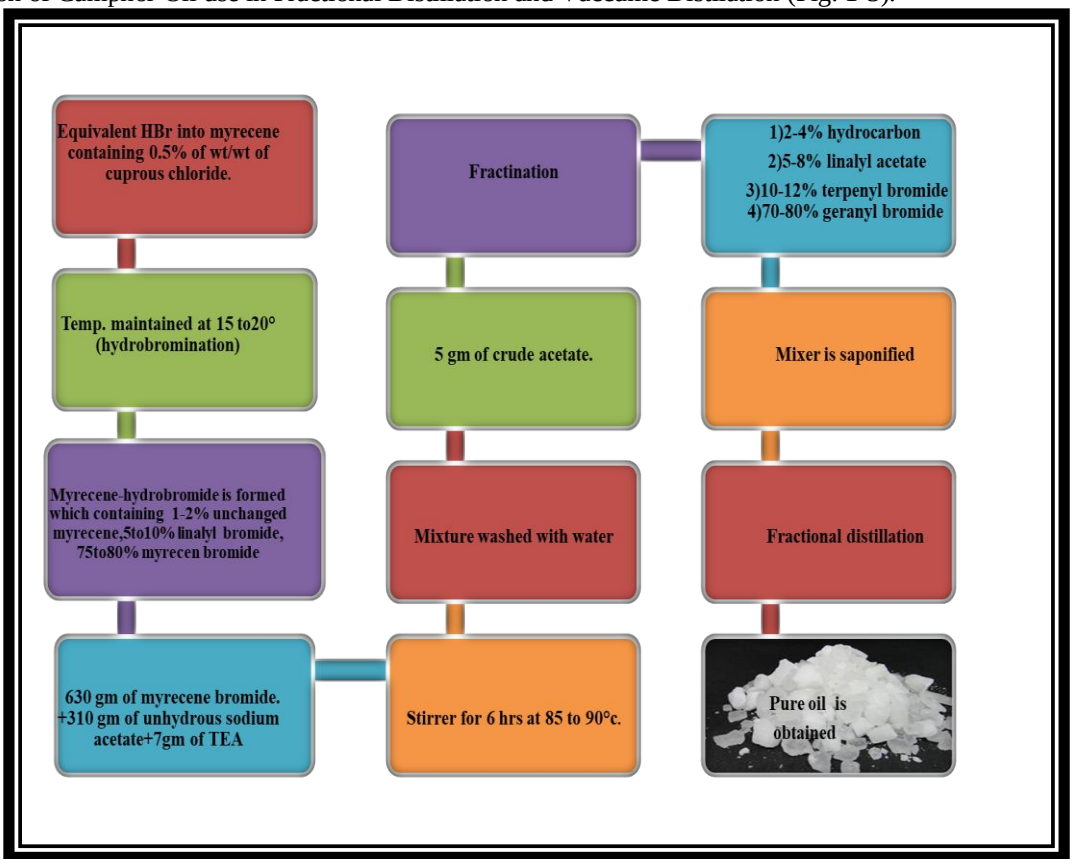

Fig. 2: Extraction of Camphor oil

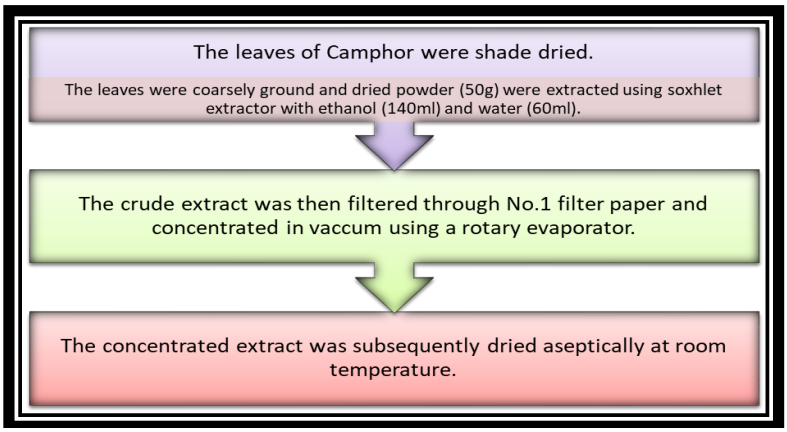

Fig. 3: Extraction of Camphor oil From Leaves

Extraction of Camphor oil Use in Hydrodistillation

Hydrodistillation Method

Hydrodistillation is a traditional method for the extraction of bioactive compounds from plants. In this method, plant materials are packed in a still compartment then water is added in sufficient amount and brought to a boil. Alternatively, direct steam may also be injected into the plant sample [8].

Preparation of Serum

All the ingredients were weighed according to the different percentage listed (Table). The net weightof all formulated serum was 100g. Add water and EDTA into a disinfected glass beaker and stir, until EDTA has dissolved. Add hyaluronic acid and mix thoroughly with a stick blender or homogenizer until phase A is free of lumps. Add phase B to phase A, stir well after each ingredient has been added. Mix with stick blender. Add phase C to phase A/B, again, stirring well after each ingredient. Especially after sodium acrylate uses the stick blender. Serum should be free of any lumps. Viscosity can be adjusted by adding, 2.5% of the sodium acrylate, if needed [9-12].

Phytochemical Screening

Table 4: Preliminary Phytochemical Analysis of Leaves of Camphor

Phytochemical Constituents	Leaves Extract		
	Ethanol	Chloroform	Hexane
Alkaloids	+	+	-
Flavonoids	+	+	+
Terpenoids	+	+	-
Tannins	-	+	-
Saponins	+	-	-
Carbohydrate	+	+	-

+ = indicates presence of phytochemicals, - = indicates absence of phytochemicals

Table 5: Preliminary Phytochemical Analysis of Wood of Camphor

Sr. No.	Chemical Constituents	*Cinnamomum camphora* (Wood Extract)
1	Resin	-
2	Tannins	+
3	Anthraquinones	-
4	Trepenoids	+
5	Flavonoids	+
6	Alkaloids	-
7	Carbohydrates	+
8	Saponin	-

+ = indicates presence of phytochemicals, - = indicates absence of phytochemicals

Antioxidant Activity of Extracts

1) Reducing Power Method

The reducing power was assayed by taking different concentration of extract (1ml) from each other were mixed in different test tubes with 2.5 ml of phosphate buffer (pH-7) and 2.5 ml of 1% potassium ferric cyanide. The mixture was then incubated at 50®C for 20 minutes. Then 2.5 ml of trichloroacetic acid (10%) solution was added to the mixture, which was mixed for 15 minutes. Finally 1.25 ml of distilled water was mixed with 0.50 ml of Fecl3 solution (0.1 w/v). The absorbance was measured at 700nm.

Table 6: Determination of Antioxidant Activity of Extract

Sr. No.	Extract	Concentration (µg/ml) and absorbance	
1	**Cinnamomum Camphora Oil**	**10**	**20**
		0.18	**0.96**

Microbial Assay

Procurement of organisms

The standard bacterial culture used for this study was procured from our college.

Preparation of media

Weighed a medium in required quantity and added to a dry conical flask.

Added required quantity of freshly distilled water, a little at a time with constant agitation to prevent the formation of lumps.

Heated the flask in boiling water bath with intermittent stirring till the contents were completely dissolved. Filled the test tubes using a funnel when medium is hot.

Plugged the test tubs of medium using non-absorbent cotton, covered them with paper and tie them together using a rubber band sterilized for 15 minutes at 1210C i.e. at 15 ibsp.s.i. pressure [13-15].

Method Used for Evaluation of Microbial Activity of *Cinamomum camphora*

1. The Well Diffusion Method

- The Petri dishes prepared in the above step are used in this method.
- After the media solidified small well (3-4mm) bored on the media with the help of sterilized cork borer.
- Different concentration of extract prepared.
- These well were filled with different concentration of extract of Cinamomum Camphora and incubated at 340C for 24hrs.
- Tetracycline solution used as standard.
- After 24 hrs. zone of inhibition was observed10.

Table No.7 concentration of extract

Sr. No.	Concentrations	Extract
1.	1500mg	1.5ml
2.	2000mg	2ml

Observation

Following plate No.1 & 2 showing measurement of zone of inhibition when using well diffusion method for Propionibacterium acnes.

Table 8: Diameter of Zone of Inhibition of *Cinamomum camphora* Extract

Microorganism	Diameter of zone of inhibition in(mm)	
Propionibacterium acnes	**Ethanol extract**	
	1500mg/ml	2000mg/ml
	14mm	15mm

Evaluation Tests for Product

Physical Appearance

- **Colour-** White to Yellowish
- **Odour-** Aromatic/Pleasant
- **Consistency-** Liquid
- **Homogeneity-** The formulation produces uniform distribution of extract.

1. Thermal Stability

Formulation and development of a pharmaceutical product is not complete without proper stability analysis carried out on it to determine physical and chemical stability and thus safety of the product. A general methodology for predicting the stability is accelerated stability analysis which subjects the material to elevated temperatures. The stability studies were carried out as per ICH guidelines. Short term accelerated stability study was carried out for the period of 3 months for the formulation. The samples were stored at different storage conditions of temperatures such as 3-5oC, 250C RH=60% and 40oC±2% RH=75%. Samples Were withdrawn on monthly interval and analysed

Stability	1st week	2nd week	3rd week	4th week
A	Stable	Instable	Instable	Instable
B	Stable	Stable	Instable	Instable
C	Stable	Stable	Stable	Stable
D	Stable	Stable	Stable	Instable

CONCLUSION

From the above test it was observed that formulation C was stable throughout stability period, formulation A, B, and D shown instability at week 3 week 4.

2. pH Test

The pH meter was calibrated using standard buffer solution. About 1 ml of the serum was weighed and dissolved in 50.0 ml of distilled water and its pH was measured. pH of prepared serum is 5.2.

3. Spreadability

Spread ability denotes the extent of area to which the serum readily spreads on application to skin or the affected part. To simulate human skin, Fisher brand filter Paper was chosen. Each filter paper weighs within milligrams of any other sheet of that size and type. A Becton Dickinson &Co. 5ml latex syringe without the needle attached was used. Liquid pushed out of the needle attachment end of the B-D syringe formed very uniform drops. Each drop is approximately 0.03 gram in weight. Standard aluminium foil is used as a base to lay the filter paper on for testing. The prepared serum has excellent spreading property.

Organoleptic, Physicochemical and Stability Testing of Serum

1. Homogeneity

This will be confirmed by spread some of the serum formulation on the transparent glass and observe it. The formulation should produce uniform distribution of serum.

2. Rheological Study

Viscosity of the formulation is determined by Brookfield® Viscometer at 100rpm, using spindle type model S64.5 ml of the serum. The serum will placed in a big mouth container with the spindle dipped in it for about 5 minutes before the measurement.

In vivo studies

Patch test

Patch test was performed on sensitive part of skin, e.g. blend of elbow, popliteal space of skin behind ears. The cosmetic was tested by applying to an area of 1 sq.cm of the skin. Central patches were also applied. The site of the patch was inspected after 24 hours. There was no reactions and then test was repeated once more on the same side. Since there was no reaction as the person was considered as not hyper sensitive and product pass the test [16-18].

Table 9 : Patch Test of Serum

Sr. No.	Parameter	M1	M2	M3
1	Immediately after removal of product	N.R.	N.R.	N.R
2	After 24 hrs	N.R.	N.R.	N.R
3	After 48 hrs	N.R.	N.R	N.R

EVALUATION PARAMETERS

Table 10: Evaluation Parameter of Essential oil

Sr. No	Parameters	Observation
1	Color	Yellowish
2	Odor	Aromatic
3	Consistency	Good
4	pH	pH 5.2
5	Viscosity	293.15-343.15*
6	Irritability	Non-irritant

RESULTS AND DISCUSSION:

The experiment was run at different parameters condition in order to indentify the most optimize condition to obtain a maximum yield of essential oil.

Optimization of Oil Extraction

In order to optimize the extraction operating conditions for achieving maximum oil yield, the distillation was conducted at three unlike parameters conditions which were at different water to Raw material ratio (v/w)(8:1, 10:1, 12:1),different extraction period (30min, 60min, 90min, 120min, 150min) and different operating power (200W, 250W).

Analysis of Sample

The extracted essential oils was dried over anhydrous sodium sulfate, weighed and stored in amber vials at 4 0c for the use of analysis. The amount of yield obtained from the extraction was analyzed to evaluate the performance of MAHD in Cinnamomum Camphora extraction. As the result the yield of oil that obtained for every run was calculated by using

Yield of essential oil (%) = Amount of essential oil (c) obtained / Amount of raw materials (c) used x 100%

The extracted essential oil samples were analyzed by using Gas Chromatography Mass Spectrometry (GC-MS) Agilent 6890 gas chromatography instrument coupled to an Agilent 5973 mass spectrometer and an Agilent Chem in order to identify their chemical constituents. This is an essential method to evaluate the quality of the oil samples. The following operating parameters were used for Cinnamomum Camphoraoil sample: capillary GC column HP-5MS 5% phenylmethylsiloxane \ (30 x 0.25 mm i.d. x 0.25 mm film thickness), a carrier gas Helium (flow rate 1.2 mL min-1) and a split-less injection mode. Injector temperature is 250°C, Oven temperature will be set initially at 50°C, and then will be raised to 250°C at a 10°C min-1 rate till the end of analysis. The eluted analytes detected using (5973 network) mass selective detector and Electron Impact ionization (EID) will be carried out at 70 eV. While, Cinnamomum Camphora oil sample, the operating parameters of GC-MS was as followed: system operating in EI mode (70 eV) , equipped with a split/splitness injector (280 ºC, split ratio 1:20) , using DB -5column (30 x 0.2 5 mm i.d x 0 . 2 5 mm). The temperature program was 50ºC (5 min) rising to 300ºC. at rate of 5ºC/min. Injector and detector temperature was 280ºC. Helium was used as carrier gas at a flow rate 1 mL /min

Effect of Extraction Time on Yield

The yield of oil extracted from Cinnamomum Camphora at different extraction time in a fix microwave power of 250W and water to raw material ratio of 8:1. From the graph, the amount of yield does not change significantly after 90 minutes. Most of the oil is extracted within 30 to 90 minutes with the yield of 0.61%, 0.80% and 0.85% respectively. Microwave assited hydro distillation (MAHD) reach the highest yield of 0.85% w/w when extraction time was 90 minutes. However, further increase in extraction time resulted in no improvement in the extraction performance. Similar observations were also reported for MAHD of Saturejahortensis and Satureja Montana by. The extraction was fast at the beginning of the extraction but get slow gradually by time because when the raw material is exposed to the heat, the plant cell started to degrade and as a consequence the essential oil is released to the environment. However prolong the extraction time caused over heat supplied to the plant material and this lead to the evaporation of the volatile component in the oil.

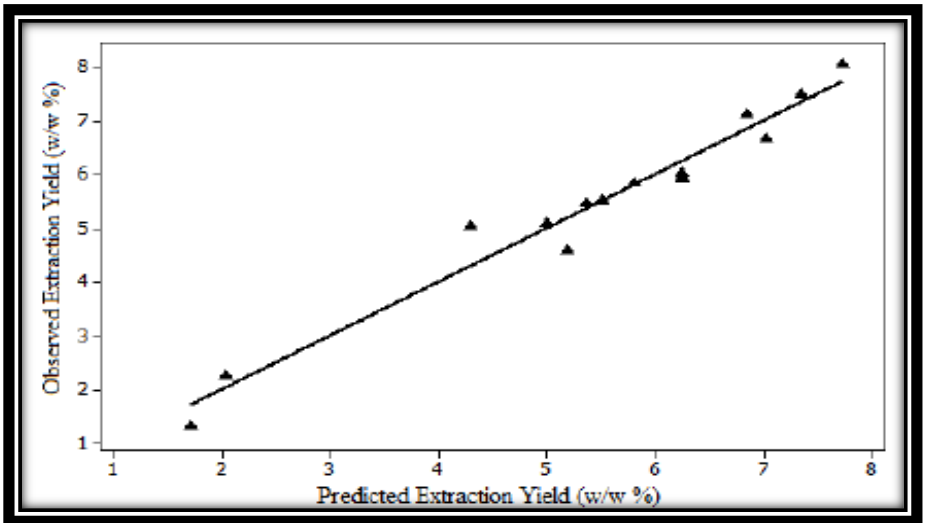

Fig. 6: Extraction Yield of Essential Oil from *Cinnamomum camphora* at Different Extraction Time by 250W in Raw Material to Water Ratio

The yield of oil extracted from Cinnamomum Camphora at different extraction time in a fix microwave power of 250W and water to raw material ratio of 8:1. From the graph, the amount of yield does not change significantly after 90 minutes. Most of the oil was extracted within 30 to 90 minutes with the yield of 0.75%, 1.29% and 1.37% respectively.

Determination of Geraniol and Ginger Solubility and Partition Coefficient (Ko/w)

The solubility of oils in n-hexane was 0.00407±0.25mg/ml, 0.00185±0.45mg/ml in methanol, 0.00084±0.67 mg/ml in PBS. The Partition coefficient (Ko/w) for oils was 3.6. From this value, it was shown that given drug comprised of about sufficient lipophilicity that is beneficial to develop the topical drug.

Fractional Distillation Method
Result Obtained by Fractional Distillation is shown in Table below

Table 11: Weight of Oil With Respect to Time

Weight of oil (g)	Time (mins)
0.2	240
0.3	480
0.7	720
0.72	960
0.78	1200

The amount of pure Camphor oil obtained by extraction method was 2.7g of essential oil per 100g of dry Leaves sample. This gave 2.07% yield of essential oil per 100g of dry Cinnamomum CamphoraThe temperature used was 780C i.e. the boiling point of ethanol. The volume of oil was measured at every 4hr interval to determine the oil yield at varying time. As the time increases the Ethanol solvent reduces thereby leaving the oil in the mixture.

Soxhletion Method
Result Obtained by is Shown in Table below

Table 12: Weight of Oil With Respect to Time

Weight (g)	Time (mins)
0.31	240
0.41	480
0.55	720
0.58	960
0.70	1200

The oil produced by Vaccume Distillation Method is 2.55g weight of oil per 130g of dry Cinnamomum Camphora sample thereby producing 1.96% oil yield at 780C

6.7 Hydro Distillation Method

Table 13: Weight of Oil with Respect to Time

Weight (g)	Time (mins)
0.10	240
0.14	480
0.26	720
0.35	960
0.38	1200

The result of hydro distillation process was 1.23g per 130g of dried Cinnamomum Camphora sample giving 0.95% yield of oil.

Table 14: Result of Essential Oil Extraction

Method of extraction	% Yield
Fractional Distillation Method	2.07
Soxhletion Method	1.96
Hydro distillation	0.95

Physical and Chemical Properties of Oil

The essential oil produced was pale yellow, with an aromatic camphoraceous dour, pungent and cooling taste. Because of its high volatility, it was stored in an air-tight container protected from light in cool place. The essential oil is insoluble in water, miscible in alcohol and in oil.

CONCLUSION:

From above discussion it is concluded that Cinnamomum Camphora Extract had antimicrobial property against Propionibacterium acnes. From the above experimental work, the Cinnamomum Camphora Extract showing good activity against Propionibacterium acnes.

Finally, it was concluded that extract of Cinnamomum Camphora shows antibacterial activity against selected microorganism with increase in concentration the activity is increase therefore it can be incorporated in cosmetics products.

Fractional distillation, Soxhletion and hydrodistillation methods are effective and efficient means of extracting Serum. Extraction is the most common and most economically technique for extracting Natural oil in modern Herbal industry because of its simplicity.

There is high demand for essential oils for various purposes such as medicinal, perfumery, aromatherapy, cosmetic, soap making, insectides to mention but a few. Imported essential oils are very expensive to meet the demand of our local consumer industries, therefore it becomes necessary to source and synthesis these oils from local sources, in particular.

REFERENCES:

1. Anuradha H, Srikumar BN, ShankaranarayanaRao BS, Lakshmana M. *Euphorbia hirta*reverses chronic stress- induced anxiety and mediates its action through the GABAA receptor benzodiazepine receptor – Cl-channel complex. J Neural Transm, 2008;115:35-42.
2. Marie Claire Lanhers, Jacques Fleurentin, Pierre Cabalion, Alain Rolland, Pierre Dorfman, Rene misslin and Jean-Marie Pelt. Behavioral effects of *Euphorbia hirta L.* sedative and anxiolytic properties. J of Ethanopharmacol, 1990; 29:189-198.
3. Sheikh Fayaz Ahmed, Phalisteen Sultan, Abdelkader E, AshourTajdar Husain Khan, Sabry M, Attia, saleh A, Bakheet, Adel RA, Abd-Allah. Modulation of Th1 cytokines and inflammatory mediators by *Euphorbia hirta*in animal model of adjuvant – induced arthritis. Inflammopharmacology, 2012.
4. KanHengLeee, Yu Sui Chen, John Paul Sudson, SrikumarChakravarthi, Yen Misim, HuiMeng ER. The effect of water extract of *Euphorbia hirta*on cartilage degeneration in arthritic rats. Malays J Pathol, 2008; 30(2):95-102.
5. Kamgang R, Zintchem R, Dimoet T, PanjoYewah. Effect des extraitsTotauxAqueux de mallotusoppositifolium ET DE *Euphorbia*

hirta (Euphorbiaceae) sur L' activite contractile intenstinale Du rat. Afr.J.Sci.Tech, 2001; 2(2) :8-11.

6. Joveneio G, Apostol, Jamesviktor A, Gan, Ryan Justin B, Rayn-es, Anna Andrea S, Sabado, Andrea Q, Carigma, Librado A, Santiago, Mafel C, Ysrael. Platelet – increasing effects of *Euphorbia hirta Linn. (Euphorbiaceae)* in ethanol – induced thrombocytopenic rat models. International journal of Pharmaceutical frontier Research, 2012; 2(2):1-11
7. Johnson PB, Ezzeldin M, Abdurahman, Emmanuel A, Tiam, Ibrahim Abdu-aguye, Hussaini IM. *Euphorbia hirta*leaf extracts increase urine output and electrolytes in rats. J of Ethanaopharmacol, 1999; 65: 63-69.
8. Hore SK, Ahuja V, Mehta G, Pardeeb Kumar, Pandey SK, Ahmad AH. Effect of aqueous *Euphorbia hirta*leaf extract on gastro intestinal motility. Fitoterapia, 2006; 77:35-38.
9. Akinrinmade JF and Oyeleye OA. Antimicrobial efficacy and tissue reaction of *Euphorbia hirta*ethanolic extract on canine wounds. Afr J Biotechnol, 2010; 9(31):5028-5031.
10. Jaiprakash B, Chandramohan D, Narasimha Reddy. Burn wound healing activity of *Euphorbia hirta.* AncSci Life, 2006; 25:3-4.
11. Liu CH, Mishra AK, Tan RX, Tang C, Yang H, Shen YF. (2006) Repellent and insecticidal activities of essential oils from Artemisia princeps and Cinnamomumcamphora and their effect on seed germination of wheat and broad bean. Bioresource Technology, 97, 1969-1973.
12. Amer A, Melhorn H. (2006) Repellency effect of forty-one essential oils against Aedes, Anopheles, and Culex mosquitoes. Parasitology Research,99, 478-490.
13. vanTol RWHM, Swarts HJ, van der Linden A, Visser JH. (2007) Repellence of the red bud borer Resseliellaoculiperda from grafted apple trees by
14. Impregnation of rubber budding strips with essential oils. Pest Management Science, 63, 483-490.
15. Wilkinson PF, Millington R (2009). Skin (Digitally printed version ed.). Cambridge: Cambridge University Press. pp. 49–50. ISBN 978-0-521-10681-8.
16. Bennett H (25 May 2014). "Ever wondered about your skin?". The Washington Post. Retrieved 27 October 2014.
17. Stücker M, Struk A, Altmeyer P, Herde M, Baumgärtl H, Lübbers DW (February 2002). "The cutaneous uptake of atmospheric oxygen contributes significantly to the oxygen supply of human dermis and epidermis". The Journal of Physiology. 538 (Pt3):98594. doi:10.1113/jphysiol.2001.013067. PMC 2290093. PMID 11826181.
18. "Skin care" (analysis), Health-Cares.net, 2007, webpage: HCcare Archived 12 December 2007 at the Wayback MachineDel Rosso JQ, Levin J (September 2011). "The clinical relevance of maintaining the functional integrity of the stratum corneum in both healthy and disease-affected skin". The Journal of Clinical and Aesthetic Dermatology. 4 (9): 22–42. doi:10.1111/j.1365-2133.1990.tb06268.x. PMC 3175800. PMID 21938268.
19. Kligman A (2006). "A brief history of how the dead stratum corneum became alive". Skin Barrier. New York: Taylor & Francis. pp. 35–44. ISBN 9780429163470.
20. "The human proteome in skin – The Human Protein Atlas". www.proteinatlas.org.