Formulation and evaluation of anti-acne gel containing natural agent.

Ganesh Sambhaji Pawale, Vishwavijay Sanjay Patil, Prathmesh Kiran Powar, Sairaj Sattapa Powar, Dakshata Navnath Pinjari, Ms.Ankita A. karande

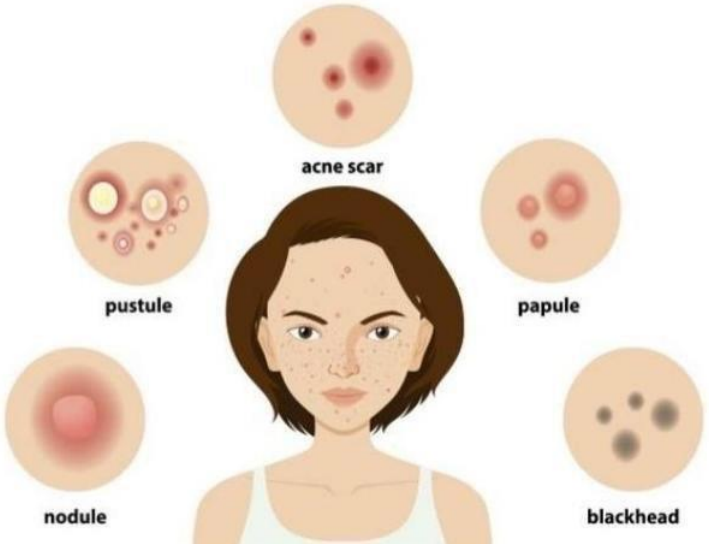
R. L. Tawde Foundations Sarojini college of pharmacy kolhapur ,
Maharashtra, India 416008.

Abstract: Acne is an inflammatory disease of sebaceous follicles of skin. The present study was conducted to formulate and evaluate the topicality-acne formulation of Murraya Koenigii extract. Murraya koenigii(Curry leaf) belongs to the Rutaceaefamily, which is commonly used as a medicinally important thereof Indian origin in the Ayurvedic system of medicine. Curry leaves are natural flavoring agents with numerous health benefits. They contain several medicinal properties that include it benignant-diabetic, antioxidant, antimicrobial, anti- inflammatory, anti-carcinogenic and with hepatic-protective properties. The aim of this study was to overcome the antibiotic resistance and side effects of synthetic drugs by avoiding use of synthetic agents. Hence, extract of Murraya Koenigii was used in this work because natural remedies are more acceptable with the belief that they are safe and having less side effects. The result of the photochemical screening revealed the presence of the bioactive constituent comprises flavonoids, alkaloids, carbohydrates, glycosides, terpenoids and amino acids. The topical anti-acne formulation was developed and tested for physical parameters, drug content, spread ability. This study work is essential for understanding of all aspects of herbal anti-acne gel.

Keywords-Anti- acne, Murray Koenigii, Herbal, curry leaves, Extraction, Gel formulation, evaluation etc.

INTRODUCTION:

Acne is a skin disease with the highest prevalence among other skin disorders. Almost everyone has experienced acne prone skin, especially in an adolescent. Although it is considered not as a dangerous disease, but infact, almost all Acne sufferers feel disturbed appearance that often leads to lower levels of Confidence and interfere with the daily activities.[1]The infection of acne vulgaris exhibits wide distribution and its prevalence Increase over time. Acne vulgaris is characterized by various clinical Conditions such as scaly red skin (seborrhea), ery thematous papules and Pustules, come dones, nodules, deep pustules, and sometimes piles.[2]The Pathogen city mechanism of acne was the production of sebum's, follicular Hyperkeratinisation, bacterial colonization, and inflammation. S. epidermidis Within sebaceousunit is responsible for superficial infection. When bacteria Colonize into the comedons, then the in flammatory factors are released by Those bacteria. This made the comedons transformed into pustules and Pimples. The inflamed acne becomes rupture and forms nodulus,also probably Forms scars after healing.[3]



FigureNo.1.1:Types of Acene

The treatment of acne can be given by topical or systemic therapy. The topical Therapies include antibiotics, anti-inflammatory and come dolytic agent.[4]For Systemic therapy, oral antibiotics such as tetracyclines and its derivatives were The first choice. It is indicated mainly for moderate-to-severe inflammatory Acne.[5] But long-term therapy of oral antibiotic, not only can induce bacterial Resistance but also associated with the incidence of upper respiratory tract Infection. The progress of antibiotic resistance is multi-factorial, including the Specific nature of the relationship of the bacteria to antibiotics, how the Antibacterial is used, host characteristics and environmental factors. The Presence of bacterial resistance and unexpected side effects opens the Opportunities for traditional medicine to replace the effectiveness of synthetic Drugs in overcoming acne vulgaris.[6]Leaves of Murraya koeinigii, a tropical tree, belonging to the family Rutaceae.[7]The plant leaves are widely used in Indian culture for cooking Purpose (used as a flavoring agent in curry, chutney ,pulse etc.), useful for its Aromatic property. The plant parts (Leaves, root, seed)from centuries year ago Beneficial for traditional medicinal use such as useful for bite of poisonous Animal, leaves and roots useful to cure external. Eruption/wound, blood purifier, dysentery. According to various research reports Murraya koenigii roasted leaves are used as a anti emesis, the paste of the leaves mixed with buttermilk and daily consumed orally for stomach-ache.The plant parts is also beneficial for the treatment of deficiency of calcium and vitamins due to the presence of vitamins(vitaminA, vitamin B & C), Calcium source, Iron in plant. The leaves paste is apply on the boils/furuncle (painful pus filled bump in skin) for reducing pain and Inflammation, Prevent cataract, curry leaves is also useful for retained black colour in hair. Juice of roots is useful to cure Renal/Kidney pain.[14]

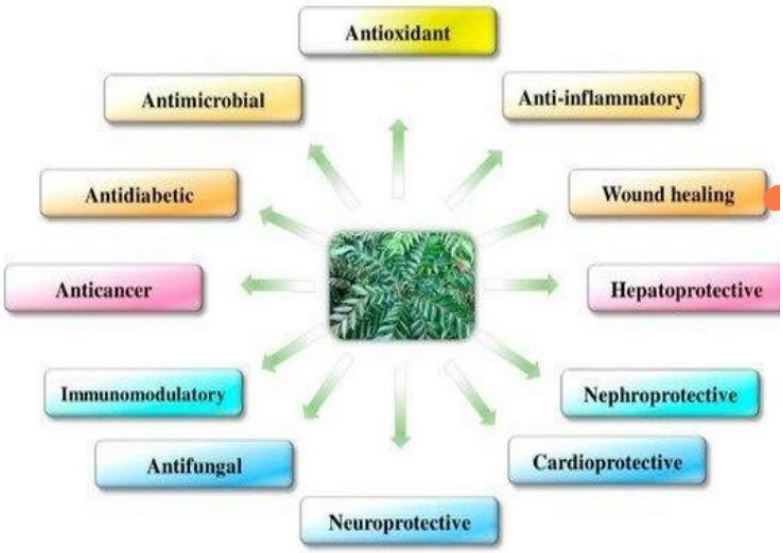
The leaves of Murraya koenigii contain proteins, carbohydrate, fiber, minerals, carotene, nicotinic acid, Vitamin C, Vitamin A, calcium and oxalic acid. It also contains crystalline glycosides, carbazole alkaloids, koenigin, girinimbin,iso-mahanimbin,koenine,koenidine and koenimbine. Triterpenoid alkaloids cyclomahanimbine, tetrahydromahanimbine are also present in the leaves. Murrayastine,murrayaline,pyrayafoline carbazole alkaloids and many other chemicals have been isolated from Murraya koenigii leaves.[8]

Figure no.1.2: Plant of Murraya Koenigii

TableNo.1.1: Taxonomic Classification

Category	BotanicalDescription
Kingdom	Plantae
Subkingdom	Tracheobionta(VascularPlant)
Superdivision	Spermatophyta(SeedPlant)
Division	Magnoliophyta(FloweringPlant)
Class	Magnoliospida
Subclass	Rosidae
Order	Sapindales
Family	Rutaceae

Genus	Murraya
Species	MurrayaKoenigii



FigureNo.1.3: Pharmacological activities of Murraya Koenigii

Uses of Murraya Koenigii:

- **As Antidiabetic Agent:** The Petroleum ether extract of Murraya Koenigii contains mahanimbine Chemical compound. In study the mechanism of mahanimbine shown it is Useful for reduces the blood sugar level and potentiate/increase the level of Insulin by improving the pancreatic secretion of insulin from β-cells.[7]

- **As a Wound Healing agent:** Wound healing is a complex and multifactor process involving numerous Biochemical and cellular processes which helps in the restoration of functional And anatomical continuity. Murraya Koenigii possesses wound healing activity Due to presence of mahanine, mahanimbicine, mahanimbineand essential oil.[11]

- **As an Antifungal agent:** The antifungal activity of the leaves of M. koenigii is due to the presence of Photochemical constituents of complex molecular structures and diverse Action mechanisms,viz. alkaloids, terpenoids, flavonoids, phenolics,tannins, And saponins,which are known for their anti microbial properties.[12]

- **As a Food Preservative:** Murraya koenigii plant berry extract contains many bioactive compounds(carotene, flavonoids, phenolic compounds) which possess anti-oxidative Property and due to the presence of mahanimbine, koenigine compounds the Plant prevent the oxidation process in food and also useful for maintain the Quality for food includes flavor (used as a flavouring agent in meats, curries), Texture of food, prevent rancidity,exhibitsantimicrobial property.Hence, its Proved the plant is useful for prevent food deterioration.[14]

- **As an Anti-cancer Agent:**This activity is duet presence of mahanimbine,girinimbine,mahanine and Murraya foline. They act by increasing death of canceroud cells and by Inhibitingproteasome.[8]

1.0 Benefits of Murraya Koenigii

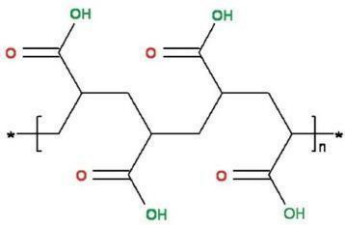
- Beneficial of eye sight
- Lowers cholesterol level
- Cures gas
- Tro intestinal issues
- Improves blood circulation
- Promotes weight loss
- Hastens hair growth
- Stops diarrhea
- Prevent diabetes
- Reduce stress
- Great for skincare
- Fights cancer
- Protect against pathogen attack

Figure N0.1.4: Curry Leaves

Experimental work:

Drug and excipient profile.

Carbopol934: Carbopol934 is cross-linked with allyl sucrose and polymerized in benzene.Carbopol 71, 971, and 974 are cross-linked with allylpentaerythritol and polymerized in ethyl acetate. Carbomer polymers contain 56%–68% of carboxylic acid (–COOH)groups and 0.75%–2% of cross-linking agents.



FigureNo.6.1: StructureoftheCarbopol934

Triethanolamine: Triethanolamine (TEA or TEOA) is an oily, viscous organic chemical compound that is a tertiary amine and a triol(a molecule with three alcohol groups).TEA is a bi functional compound that exhibits both properties of alcohols and amines. The compound is used to make surfactants in industrial and cosmetics as a pH adjuster for skin and hair conditioning products as well as sun screen lotions, liquid laundry detergents, polishes, and paints

Methyl Parabean: Methylparaben, also methyl paraben, one of the parabens, is a preservative with the chemical formula CH_3. It is the methyle sterof p-hydroxybenzoic acid.

Polyethylene glycol: PEG400 is a low-molecular-weight grade of poly ethylene glycol. It is a clear, colorless, viscous liquid.

Due in part to its low toxicity, PEG 400 is widely used in a variety of pharmaceutical formulations

Experimental Work

A. Analysis of Raw Material

1. Moisture Content: Dried Curry leaves were powdered using laboratory mixer.About10gof powder sample was Taken in a small aluminum pan and kept for drying in hot air oven at 130+58C for 17+2 hr. (AOAC, 1990). After that the pan was covered and cooled in a desiccators and weighed. Moisture content was Presented in percent wet Basis.

B. Extraction of Dried Curry leaves:

- The methanol extract was made by, adding ten grams (10gms) of curry powder To 100 ml of 70% aqueous methanol solution (w/v) covered with filter paper Kept on rotary shaker for 24hours, and then kept in a dark area at room Temperature for 2 to 3 days. The filtered supernatant was collected and the Solvent was evaporated to make the final volume of the curry leaf methanol Extract for the experiment.

2. Extraction Procedure

A lab scale Sox hlet apparatus was used to extract from Curry leaves. About 300 gm. Of size reduced leave particles were fed into a Sox helt extractor fitted with a 1-Lround-bottomflask and a condenser. The extraction was executed on a water bath for56 hr. with 0.6 Lof n-hexane. After extraction, the Solvent was distilled off under vacuum in a rotary evaporator and stored under refrigeration until used For further analysis.

FigureNo.6.3: Soxhletapparatus.

FigureNo.6.4:Water BathThermostatic

C. Phytochemical studies: Phyto chemical screening of the methanol extract has been performed to Determine the presence of the various phyto constituents by using following Standard procedures:

Testfor alkaloid:

Dragendorff'stest

2mlof aqueous extract was taken, a few drops of Drag end orff'sreagent added In it. Presence of reddish brown precipitate showed the presence of alkaloid.[17]

Test for Carbohydrate:

Molisch'stest

In 2mlof aqueous extract a small amount of Molisch's reagent(α-naphthol Dissolve in ethanol) was added in a test tube. After mixing ,a small amount of Concentrated sulfuric acid was slowly added down the slides of the slopping Test-tube.[17]

Test for Glycosides:

Kedde test

Aqueos extract was treated with small amount of Kedde reagent. Development Of a blue or violet colour that was faded out in 1 to 1 hrs showed the presence of Glycoside.[18]

Test for Flavonoids:

Shinoda test

A few fragments of magnesium and concentrated hydrochloric acid were added To the ethanolic extract. The appearance of red to pink color indicated the Presence off lavonide.[18]

Test for Terpenoid:

Salkowskitest

2mlof chloroform was added to the extract solution. Concentrated sulphuric Acid was added to it to form a layer. A reddish brown coloration of the Interface indicated the presence of tepenoid.[18]

Test for Tannins:

0.5g of sample was stirred with 10ml of boiling distilled water. This was Filtered and a few milliliters of 6% ferric chloride added to the filtrate. Appearance of deep green coloration indicated the presence of tannins. The Second portion of the filtrate was treated with a few milliliters of iodine Solution. Appearance of a faintbluish coloration confirmed the presence of tannins [18]

Test for Amino acid:

Ninhrdrine test

Three drops of 1% solution.Ninhydrinein ethanol was added to 1mlextractSolution and heated for 5 minutes in a boiling water bath. The formation of Blue, purple color indicated the positive test.[18]Photochemical

TableNo.6.1: PhytochemicalScreeningofMurrayaKoenigii

Phytochemicals	Methanol extract
Alkaloid	+
Carbohydrate	+
Glycoside	+
Flavonoid	+
Terpenoid	+
Tannin	-
Aminoacids	+

TableNo.6.2: Composition of Gel

Composition of Gel	Formula(%w/w)	Function
Carbopol940	0.8	Gelling agent
Triethanolamine	Q.S	Alkalizingagent
MethylParabean	0.2	Preservative
PropylParabean	0.1	Preservative
Polyethyleneglycol200	20ml	Solvent

PropyleneGlycol400	3ml	Emollient
MethonoicExtract	0.5	Antibacterialactivity
Distilledwater	100ml	Vehicle

Procedure:

Preparation of Gel:

1] 0.8 gm.Of Carbopol934 was suspended in 50 ml of distilled water in beakerno.1 With continuous stirring.
2] The required quantities of methyl and propyl parabens were dissolved into 5 ml of Distilled water in beaker2 by heating on a water bath.
3] Propyleneglycol400andPolyethyleneglycol200werethenaddedtothecooledsolution.

4] Further, the required quantity of methanolic extract of curry leaves was added in the Beaker no.2.

5] thenthesolutionpresentinbeaker2was addedinbeakerno.1andvolumewasmadeupto100 mlbydistilledwater.

6] After mixing all the ingredients, drop wise addition Triethanolamine was made to the formulation for obtaining the desired Consistency of the gel and to adjust the desired skin pH.

Evaluation of gel
To evaluate the prepared formulation different tests were Performed including visual assessment and physicochemical controls Such as pH, spread ability etc.

- **Physical Evaluation of gel:**

Organoleptic characteristics:

The physical appearance of the formulation was checked visually which Comprised of:
1] Color: The color of the formulation was checked out against white Background.
2] Odour: Theodourof the gel was checked by mixing the gel in Water and taking the smell.
3] Consistency: The consistency was checked by applying on skin.
4] Greasiness:The greasiness was assessed by the application on to the skin

5] Grittiness:The formulation was evaluated microscopically under40x magnifications for the presence of any particulate matter OR aggregates.

6] Homogeneity :Homogeneity was tested by visual inspection after allowing them To set in a container. They were evaluated for their appearance And presence of aggregates.

7] Skin irritancy test : This test was performed on 10 healthy human volunteers of Either sex after obtaining consent for the same. About0.5 gm. Of gel was applied to an area of about6cm2 on skin of hand Covered with a gauze patch. The patch was held in contact with The skin for period of 1hr,the gauze was removed and residual Test substance was scrapped, without altering the existing Response or integrity of the epidermis. The skin was observed at 1 hr, 3hrs, 6hrs, 12hrs.,24hrs., 48hrs.and 72hrs. for any visible Response on the skin.

- **P^H Determination:** About 20mg of the formulation was taken in a beaker and was Subjected to the pH measurement using adigital pHmeter Within 24hrs. of manufacture.

- **Spreadability**: Spreadability denotes the extent of area to which a gel readily Spreads on the application to the skin or affected part. The Therapeutic potency of the gel also depends on spreadability value. Spreadability is defined in terms of time in seconds required taken By the upper slide to slip of f from gel which is placed in between the two slides,under certain load. The lesser the time taken for the separation of two slides, the better the spreadability. About500mgOf the formulation was sand witched between the two slides, each With dimensions of 6x2cm. A weight of 100g was placed upon The upper slide so that the formulation between the two slides get Pressured uniformly to form a thin layer. The weight was removed And the excess of the formulation adhering to the slides was Scrapped off. The lower slide was fixed on the board of apparatus And the upper slide was held to the non-flexible string to which 20gLoad was applied with the help of a simple pulley which was in Horizontal level with the fixed slide.The time taken by the upper Slide to slip off the lower slide was noted.

Spreadability=mxl/t

Where,m=weight tied to Upper slide

l=length of the Glass slide(6cm) T= time in seconds

- **Viscosity**: The viscosity of the prepared gel will be measure with a Brook field viscometer at a setting of 100 rpm at25ºC.

- **Anti-acne property of gel:**

Modified agar well diffusion method will be used to detect the Anti bacterial activities of different extract and formulation. In this Method, each nutrient agar plates will be plantwith0.2 ml of 24h broth culture of Saureus, soybean casein digest media Plates will be seeded with 0.2 ml each of 24 h broth culture of S.epidermidis and plates of brain heart in fusion media will be seeded With 48 h broth culture of P. acnes. The plates will be dried for 1 h. In Each of the plates, four equidistant wells will be excavated with a sterile 8 mm borer. Into each plate,0.5 ml of solutions. of extracts, prepared polyHerbal gels, Clindamyc in, marketed herbal formulation and allopathic Clarithromycin gel will be introduced. The plates of S. epidermidis and S. aureus will be incubated at37 °Cfor24h, andP. acnes will be incubated for48h. The diameter of the zones of Inhibition (in mm) will be measured for evaluating the anti bacterial Activity. The experiment will be repeated four times and the mean was Recorded.

- **Drug Content:**

The drug content of the formulation will be determined by taking An accurately weigh quantity of gel1gm in a 100 mlvolumetricFlask.70 ml of methanol will be added to it and shake. The volume Will be made up to 100 ml. A suitable filter paper will be used to Filter the contents effectively. 1ml filtrate will be taken and dilute And the drug content (extract) will be estimated using UV/Visible Spectrophotometer at250 nm.

- **Stability study:**

Stability study Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 2^0C, 25^0C and 37^0C. The herbal ointment was found to be physically stable at different temperature i.e. 2^0C, 25^0C, 37^0 C within four weeks.

FiguureNo.6.5:Stabilitystudyfor2^0C

FiguureNo.6.6:Stabilitystudyfor25^0C

FiguureNo.6.7:Stabilitystudyfor37^0C

RESULTS AND DISCUSSION: The formulation was developed with Murraya Koenigiiextractusingcarbopol934as a gelling agent. The prepared herbal gel was subjected to physical and microbial evaluation parameters. The formulation was olive green in colour and had characteristic odor of Murraya Koenigii. The formulation was glossy and translucent, and had good consistency. The pH of the formulation ranged from 5.7to6 .0 which was relevant with human skin pH. Hence it may be suitable for topical application without discomfort. Spredability test was performed for evaluation of gel. The viscosity of formulation increased with increase in the concentration of carbopol content. The spreadabiity of the formulation was found to be good. The increase in viscosity was observed with decrease in spreadability and vice versa. The skin irritation test was performed to evaluate the skin irritation of the formulated gel on the skin of human volunteers. The results of these tests were shown that the formulated herbal anti-acne gel was safe to use.

1. Physical appearance:

On the basis of organoleptic properties,the following observations were observed.

Tableno.7.1: Data of Organoleptic Properties

Sr. no.	Tests	Observations
1	Colour	Olivegreen
2	Odour	Characteristicsodourofcurryleaves
3	Consistancy	Smooth
4	Greasiness	Nongreasy
5	Grittiness	Absenceofanyparticulatematter
6	Homogenicity	Homogenous
7	Skinirritancytest	Nonirritant
8	Stabilitystudy(2^0C,25^0C,37^0C)	Stable

2. P^H Determination

The pH of the prepared formulation of anti-acne gel was studied by using the digital pH meter. The observations of pH are mentioned in the below table.

Tableno.7.2: Data of pHdetermination

Sr.no.	Observationtime	pHobserved
1	After12hrs.	5.7
2	After24hrs.	6.0

3. Spreadability:

Table no.7.3: Data of Spreadability

Sr.no.	Test	Observation
1	Spreadability	Easily Spreadable

Conclusion:

Acne vulgar is is a general skin disease which affects common people at least once during his or her total life. Mainly in the teenage time this disease affects to the human being but many people in higher age (between 20-40y)are also became affected by the disease. From many research, a confidence has been grown in people's mind on herbal medications as they are safe than synthetic one. The side effects like contact allergy, local irritation,scaling, photo sensitivity, itching, pruritus, redness, skin peeling,xeros is of the skin etc. are the major reasons to avoid synthetic drugs. The present study was done with formulation and evaluation of herbal anti-acnegels. The main reason behind this investigation was to formulate as table and safe anti-acnegel without using any type of synthetic additive i.e. herbal anti-acnegel. Use of synthetic additive scauses various side effects of skin, to overcome this problem use anti- acnegel from extract of Murraya koenigii is the best solution. Different evaluation test were performed to check the performance of gel. From the result of performed test we conclude that gel formulation of Murraya koenigii extract is safe to use.

9.0 REFERENCES:

1] Lynn DD,UmariT,Dunnick CA,Dellavalle RP.The epidemiology of acne vulgar is in late adolescence. Adolesc Health Med Ther2016;7:13–25.

2] PeckGL,OlsenTG, YoderFW, StraussJS,etal. Prolongedremissions of cystic and conglobate acne with 13-cis-retinoic acid. N Engl J Med 1979;300:329–33.

3] Ebling FJG. Acne vulgaris, Textbook of dermatology, 6thed. Blackwell Scientific,Ox ford;1998;3:552-4.

4] FarhatD, ShubbangiW, Mamta J, etal. Developmentof herbalanti-acnegel and its evaluation againstacne-causingbacteria propionibacterium acne and Staphylococcusepidermidis. Int. J Res Ayurvedha Pharm 2013;4:781-6.

5] CunliffeWJ,GollnickHP.Acne:Diagnosisandmanagement.1sted.MartinDunitz Ltd.London;2001.

6] Margolis DJ,Bowe WP, HoffstadO,Berlin JA.Anti biotic streatment of acne may be associated with upper respiratory tract infection. Arch Dermatol 2005;141:1132–6.

7] YadavS,VatsV,DhunnooY,etal. Hypoglycemic and anti hyperglycemic activity of Murraya koenigii leaves in diabetic rats.J Ethnopharmacol 2002;82:111–6.

8] Samanta SK, Kandimalla R, Gogoi B, et al. Phytochemical portfolioand anticancer activity of murrayakoenigiiand itsprimary active component, mahanine.PharmacolRes2018;129:227–36.

9] Singh Ateendra et al. Anti-inflammatory and analgesic activity of aqueous extracts of dried leaves of Murraya koenigii Linn, National Journal of Physiology, Pharmacy andPharmacology. 2016;6(4):286-290.

10] Ani S. Nushrat et al. The Methanolic Extract from Murraya koenigii L. Inhibits Glutamate-InducedPain and Involves ATP-Sensitive K+Channel as AntinociceptiveMechanism, Advances in Pharmacological Sciences. 2016;1-

11] Nagappan, T.; Segaran, T.C.; Wahid, M.E.A.; Ramasamy, P.; Vairappan, C.S. Efficacy of carbazole alkaloids, essential oil and extract of Murraya koenigii in enhancing subcutaneous wound healing in rats. Molecules. 2012;17:14449– 14463.

12] Maswada,H.F.;Abdallah,S.A.Invitroantifungalactivityofthreegeophytic plant extracts against three post-harvest pathogenic fungi. Pakistan J. Biol. Sci. 2013;16:1698–1705.

13] Iyer, D.;Uma, D.P. Effectof Murraya koenigii(L.)on radiation induced rate of lipid peroxidation in Swiss albino mice. Indian Drugs. 2009;46:160–162.

14] Shinde Jyoti. Advances in disease protecting ingredient of Murraya Koenigii (Curry Leaves)- A textual Herbal Medicine with newer Approach, International Journal of Innovative

Pharmaceutical Sciences andResearch. 2016;4(1):1-6.

15] Shah,P.;Singh,S.P.;Kumar,A.Combined effect of hydroethanolic extracts of Murraya koenigii and Phyllanthus niruri leaves on paracetamol and ethanol- induced toxicity in HepG2 cellline.Curr. Sci. 2015;109:1320.

16] Renga samy Bala krishnan,Dhanraj Vijay raja, Song-Hee Jo .Medicinal Profile ,Phyto chemistry,and Pharmacological Activities of Murraya koenigii and Its Primary Bioactive Compounds. 2020.

17] Mojab Faraz, Kamalinejad Mohammed, Ghaderi Naysaneh, Reza Hamid Validipour, Iranian Journal of Pharmaceutical Research,2(2003)77.

18] Trease, G.E.and Evans, W.C. ,1989, AText book of Pharmacognosy,13th edition., 83,685. Bailliere Tindall Ltd., London, ISBN: 0702013617.

19] Swarnali Roy, Sankhadip Bose,Sanjit Mandaletal. Formulation and evaluation of anti-acne gel containing Murraya koeinigiiextract. 2020;12:4.