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Phytochemical Screening for Petals Extract of *Tagetes Erecta* for Natural Mosquito Repellents

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ABSTRACT

Plants were collected from herbal garden of Shrinathji institute of pharmacy, during the month of September 2022. Taxonomic and ethno medicinal identification of the collected petals of flower by the plant species has been identified and authentified by Dr. Raju Lal Bhardwaj, associate professor (horticulture) OSD College of agriculture, Sumerpur Pali Rajasthan. The petals of flower were shade dried, reduced to coarse powder with the help of grinder and stored in airtight container till further use. Extraction of marigold petals of flower a Soxhlet extraction method is used as methanol as solvent (90% v/v). The Physiochemical analysis of leaves powder was carried out. In this study ash values (moisture content, total ash, acid insoluble ash, water soluble ash and water-soluble ash) were determined. The total ash value, acid insoluble, water soluble and moisture content were found.

Key Words: *Tagetes erecta,* mosquito repllents, extractive value, ash value.

Introduction

Mosquito borne diseases are major human and animal health problem in all tropical and subtropical countries. The diseases transmitted include malaria, filariasis, yellow fever, Japanese encephalitis, and dengue fever. There has been exploration of various methods over the centuries to combat threats from mosquito borne diseases. With the beginning of the 20th century there grew an interest for use of biological control agents but this was declined with the discovery of insecticidal properties of DDT in 1939. However, its deleterious impact on non-target population and the development of resistance prompted for the search of alternative, simple and sustainable methods of mosquito control [1-3].

Mosquito borne diseases:

Since ancient times, mosquitoes have been reported as the source of various ailments affecting human. Comprising approximately 3500 species, mosquitoes are found beyond the tropical and subtropical regions of the world. The chief genera which vector human disease-causing pathogens are Anopheles (malaria, filariasis), Aedes (yellow fever, dengue, chikungunya) and Culex (West Nile, Japanese encephalitis, filariasis). Over its life span a female mosquito repeatedly takes a blood meal as protein source to complete egg development.

By injecting the saliva which may contain pathogens into the host animal, the pathogens thus complete an obligatory life cycle phase and multiply in the mosquito's salivary glands. This thereby makes female mosquito an ideal transmitter of diverse blood borne pathogens and agents of devastating human diseases [4,5]. Over the years malaria have been considered as one of the leading causes of death in India (Table: 1). History reveals that the highest incidence of malaria in India occurred in the 1950s with an estimated 75 million cases and 0.8 million deaths per year [6]. Malaria is caused by the protozoal parasites Plasmodium vivax Grassi and Feletti, Plasmodium malariae Feletti and Grassi, Plasmodium ovale Stephens, and Plasmodium falciparum Welch, which are transmitted by Anopheles mosquitoes. Studies suggest that for any form of malaria to be endemic in a certain area several requirements must be fulfilled, it needs the presence of a large number of competent anopheline mosquitoes with a sufficient preference for human blood and an exposed human population with enough number of malaria carriers and susceptible individuals for the chain of infection to persist [7].

Table 1: Countrywide malaria surveillance data (1995- 2008)

Year	Total malaria cases (million)	Death due to malaria (thousand)
1995	2.93	1151
2000	2.03	932
2008	1.52	924

Control of Mosquitoes:

are assumed due to population growth, uncontrolled urbanization in tropical and subtropical countries, proliferation of breeding sites for Aedes mosquitoes and the lack of effective mosquito control [12]. One of the leading causes of acute encephalopathy is encephalitis affecting children and adolescents particularly in the tropics. Mosquitoes proliferate in close association with pigs & other animal reservoirs and are found to spread virus of Japanese encephalitis basically in malnourished children of poor families from rural area [8].

Table 2: The efficient way to control these diseases is to control mosquito vector populations

Chemical methods	Non-chemical methods	Biological methods
Synthetic repellents: DEET, Permethrin	**Physical method**: Medicated net, Nonmedicated net, Mosquito traps	By growing some fish species that feeds on mosquito larvae in water bodies
Natural repellents: Neem oil, Citronella oil	**Mechanical methods:** Electric mosquito zapper, Mosquito magnet	

The efficient way to control these diseases is to control mosquito vector populations and prevent mosquito bites. Studies reflect that insect repellents play an important role in preventing the mosquito vector, deterring an insect from flying to, landing on or biting human and animal skin. Generally, the widely used compounds as insect repellents are synthetic chemical repellents but they bear the disadvantage of being not safe for human, especially children, domestic animals because they may cause skin irritation, hot sensation, rashes, or allergy [9].

Herbal creams: -

Herbal creams are defined as semisolid preparations consists of emulsion system, prepared usually for application to the skin, herbal creams are semisolid emulsion of oil and water, classified in two types O/W (oil in water) and W/O (water in oil). Herbal extracts are used in herbal cream preparation to protect skin, to enhance beauty and to cure diseases. Herbal

creams are formulated different purposes like mosquito repellent cream, vanishing cream, cleansing cream and cold cream etc.

Mosquito repellent cream Mosquitoes are one of the most disturbing insects affecting human being by sucking blood often causes itching, redness and even leaves bumps sometimes, happens due to the saliva of mosquito and spreading various major diseases like, Dengue, Yellow fever, Chikungunya and Malaria etc. Mosquito repellent creams are formulated from the extractions of active constituent having mosquito repelling action from the biological sources of plants.

The herbal mosquito repellent cream is formulated with the formula to get long duration of action of cream as it need to retain on skin for long time to provide maximum repellent action. Mosquitoes detect its prey by the odours that body emits [10].

Skin Physiology

Skin is the single largest organ of the human body, which combines the different many organs like mucosal linings of respiratory organ, digestive organs and urinogenital tract to form compact internal structure which separates it from external environment. Skin is the heaviest organ in the body which is multi-layered organ that is composed of many histological layers. Generally described in the form of major three layersepidermis layer, dermis layer and hypodermis layer. Epidermis is the most important layer for topical drug delivery as it composed of five parts of epidermis microscopic sections- stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and the stratum germinativm.

Epidermis: Epidermis is classified as the upper most part of the skin which is consist of epithelial cells and doesn't contain of blood vessels, the main function of the epidermis cells is to provide protection, haemostasis and absorption of nutrients to the skin. Keratinocytes are the major cell found in epidermis which produces keratin a fibrous protein that provides protection to skin; other cells present in epidermis are melanocytes, merkel cells, and Langerhans cells.

Dermis: Dermis is the intermediate layer of skin between epidermis and hypodermis which composed of dense irregular connective tissues of collegen and elastin. Dermis layer provides elasticity to the integuments allowing flexibility and stretching.

Hypodermis: Beneath dermis which is mainly helps in fat storage, it is the layer just after the dermis layer helps in connecting the skin to fibrous tissue, it is consists of loose, areolar connective tissues and adipose tissue functions as insulator and as cushions to the integument. Hypodermis helps in managing mostly the fat-storing cells called adipocytes that help as stored fat which helps in provides energy reserve [11-15].

Disease profile: - Bite of an infected mosquito can transmit number of disease-causing viruses to people, not all mosquitos' vectors transmit the viral diseases, generally female mosquitoes transmit the disease-causing agents that infects peoples and causes many diseases such as Chikungunya, Dengue, Zika and Yellow fever. Most effective way to get rid of theses dangerous diseases is to control the mosquitoes in nearby surroundings by understanding their life cycle, by identifying the type of mosquitoes present in our surroundings and by using best methods to control and to keep them away. Identifying mosquitoes Generally the adult mosquitoes small, long-legged flies and have two wings, they are different from other pests by three characteristics.

They have long and many segmented antennae [16].

They have a piercing and sucking mouthpart system

Life cycle of Mosquitoes

Life cycle of mosquitoes is divided into four distinct stages: egg, larva, pupa and adult.¬ Mosquito-borne diseases. The human and animals can be affected by mosquitoes directly or indirectly, mosquitoes feed themselves with blood from human, animals and other vertebrates that can be annoying and disturbs peoples in outdoors, with bites of mosquito species often causes mild allergic reactions [17].

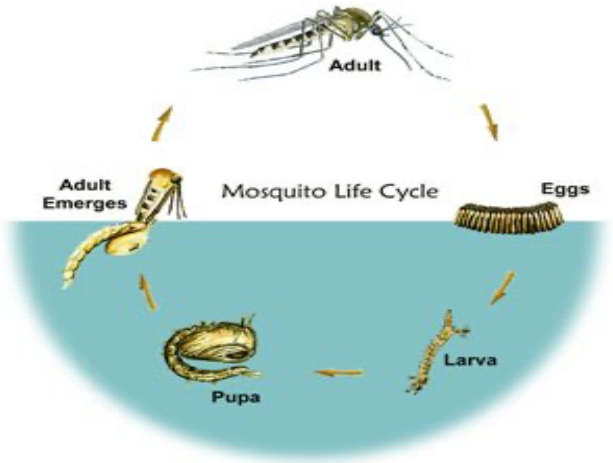

Fig:1 Mosquitoes Life cycle

The indirect effects can be more dangerous as their bite transmits disease causing pathogens, even in animals like dogs and cats it transmits heart-worm particularly in humid areas, it is a paracytic round worm courses serious symptoms like cough, fainting, weight loss and congestive heart failure. Worldwide mosquitoes affect the health of human and animals more than any other insects as their bite transmits numerous infectious agents which causes diseases such as [18].

Encephalitis Encephalitis is defined as inflammation of brain generally caused by certain viruses, some of which transmitted by mosquitoes, the virus is transmitted from bird to mosquito to human.

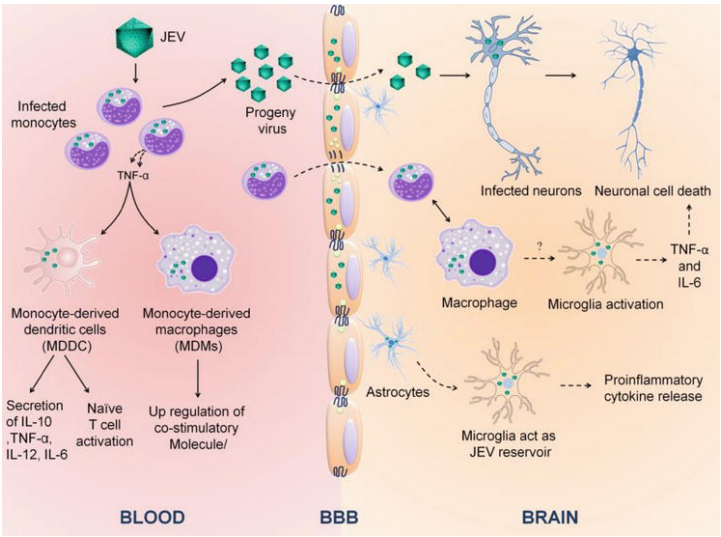

Fig:2 Encephalitis Pathophysiology

Symptoms: Encephalitis symptoms includes, High fever, Convulsions, Delirium And other central nervous system problems. A person with these symptoms seeks medical check-ups immediately [19].

Malaria: - Malaria in human is life threatening, transmitted by female Anopheles mosquitoes, malaria in human can be acute or chronic disease caused by parasites belongs to Plasmodium genus. The infected mosquito transmits the parasite to human body which multiplies into the host's liver and later destroys the red blood cells of host. Symptoms: the symptoms of malaria resemble those of Flu, symptoms of malaria are very common so generally peoples misunderstand that may cause delay in screening of malaria and its treatment, symptoms of malaria include [20];

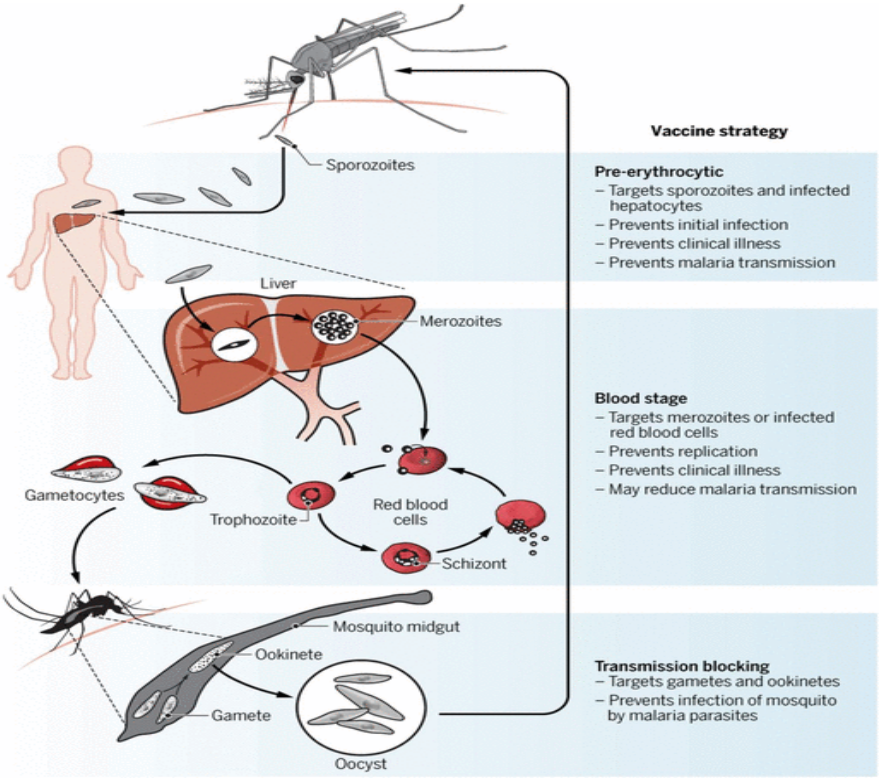

Fig:2 Life cycle of malaria

- A sensation of cold and shivering¬
- Headache, fever and vomiting.
- Sometimes seizures occur in younger peoples.
- Sweat, tiredness and thirstiness.
- Difficulties in breathing and multi
- ple convulsions

Dengue:- Dengue is a mosquito transmitted life threatening disease caused by dengue-virus; aedes mosquito transmits the dengue virus to the people when it bites with virus presents in their blood. The dengue virus cannot spread from one person to another directly. The serious condition of dengue is called dengue hemorrhagic fever [21].

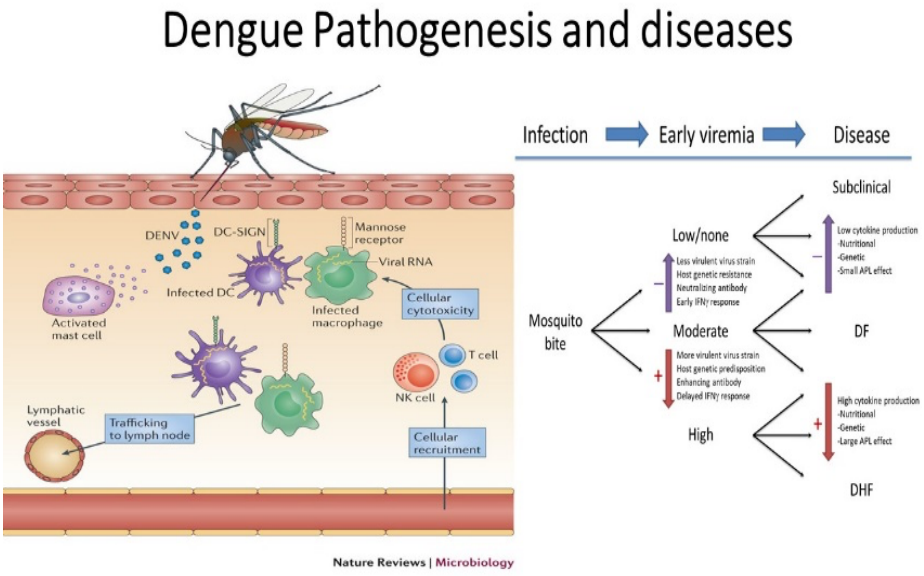

Fig:4 Dengue Pathogenesis

Symptoms: The dengue symptoms are painful as it causes sever fever and other serious problems.

- Severe headache.
- Severe join pain and muscle pain.
- Nausea
- Vomiting Pain behind the eyes.
- Sudden high Fever.
- Fatigue
- After the onset of fever skin rashes are appears.
- Mild nose bleeding, gums bleeding or easy bruising.

Chikungunya: - Chikungunya is also transmitted from mosquitoes, mainly Aedes aegypti and albopictus mosquitoes transmit the virus to people. Still there is no vaccine present to prevent it so we should protect ourselves from bite of mosquito by taking prevention such as applying mosquito repellent cream while moving outside, by covering full body wearing full sleeves and people travelling to countries with chikungunya virus should use insect repellents and stay in air conditioning [22].

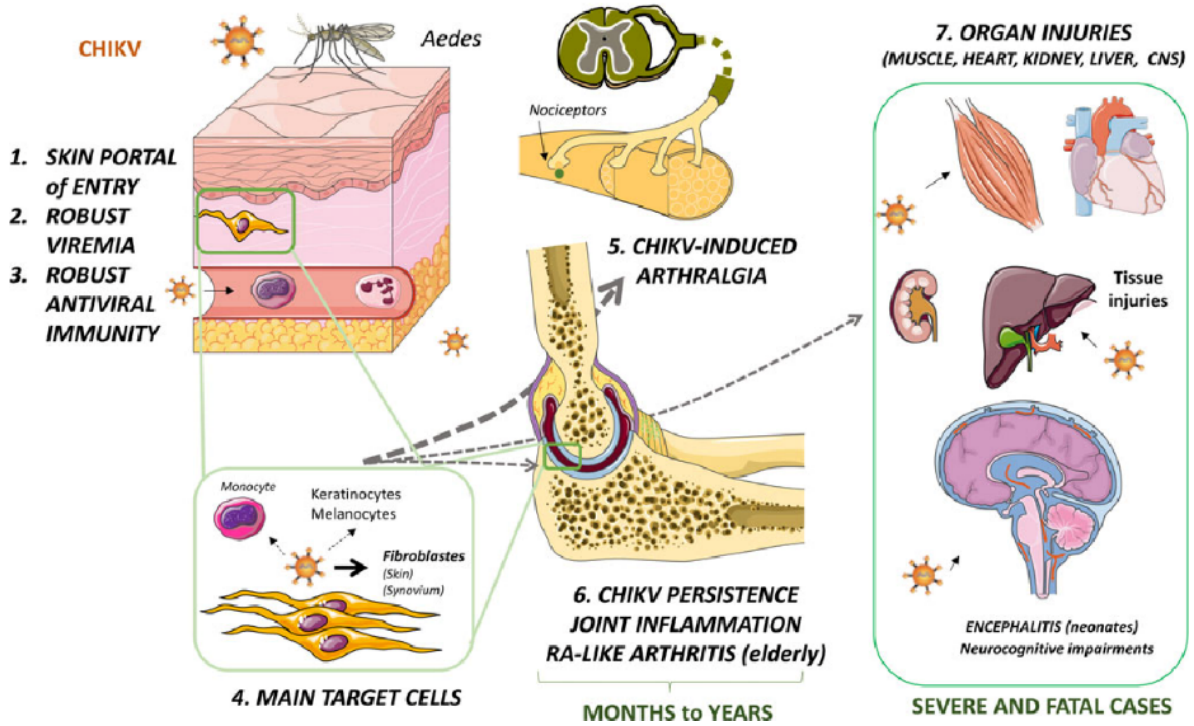

Fig:5 Chikungunya pathophysiology

Symptoms;

- Fever and joint pain
- Headache
- Muscle pain
- Joint swelling and rashes

Zika;

Zika is viral disease spread mostly by the bite of mosquitoes (Aedes species) infected with zika virus. These species of mosquitoes can bite in night and even during day time. Zika is life-threatening for human and it even passed to fetus during pregnancy from a pregnant woman it even causes certain defects in time of child birth. It is considered as one of the dangerous mosquitos related disease as its treatment or vaccines are still not available. Symptoms: Generally, the symptoms of Zika are mild; several people do not develop symptoms as zika virus disease can take one to two weeks to observe symptoms [23].

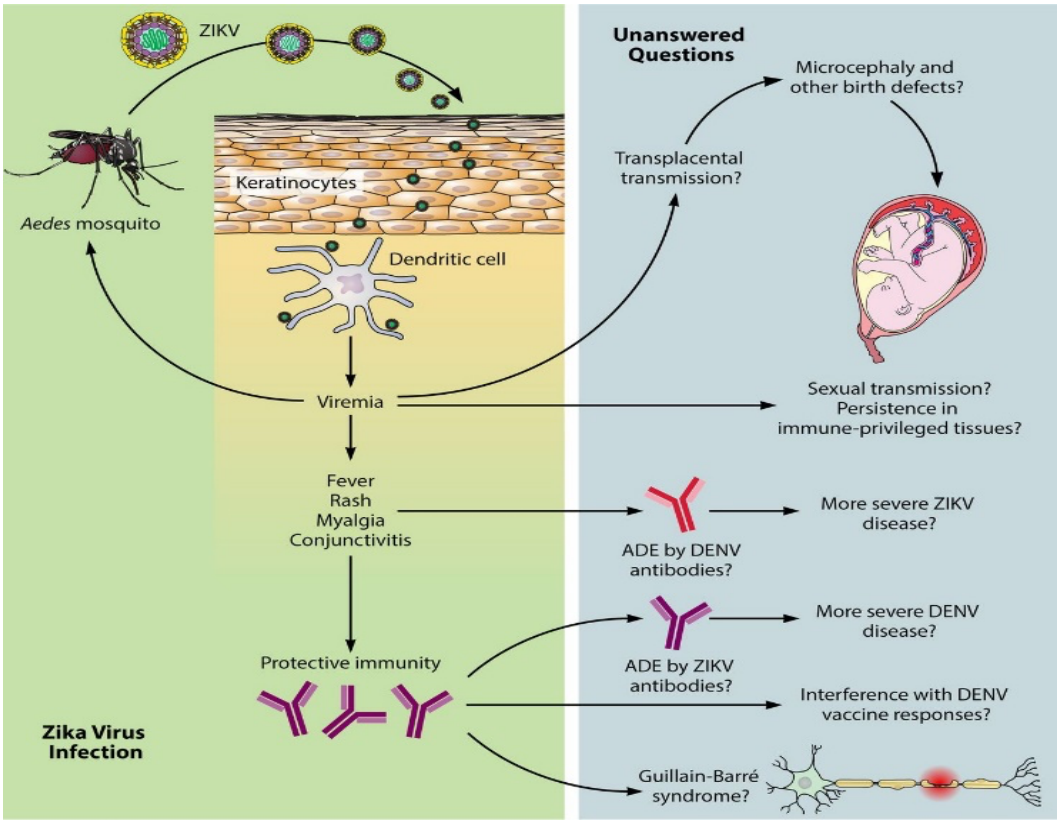

Fig:6 pathophysiology of zika Virus

Generally, symptoms include, Fever, Rashes, Conjunctivitis, Muscle, and joint pains.

MATERIALS AND METHODS:

Identification of drug:

For the best result of any drug formulation, correct identify of plant is first step towards quality control botanical herbal preparation. Identification of accurate medicinal herbal plant is important for getting true extraction and safeguard of patient [24].

Marigold flower (*Tagetes erecta)*;

Large numbers of varieties of marigold flower are present. The marigold flower (*Tagetes erecta*) can be identified by its morphological appearance as thus plant generally reaches height of between 40 and 110 centimetres. Marigold plant is herbaceous annual whose root ispivoting, cylindrical with shallow branching and fibrous. Leaves are alternate at the top and opposite the bottom, composed of 10 to 18 leaflets, lanceolate, 3 to 5 cm long and 1 to 1.5 cm wide, acute to acuminate. Main characteristics of the marigold flower are that they are grouped in small heads. Flower is yellow to yellow red in colour flowering period in summer and can easily be grown by seeds [25-28].

Kingdom:	Plantae
Order:	Asterales
Family:	Asteraceae
Subfamily:	Asteroideae
Class:	Magnoliopsida
Division:	Magnoliophyta
Genus:	Tagetes
Species:	erecta

Collection and identification:

Plants were collected from herbal garden of Shrinathji institute of pharmacy, during the month of September 2022. Taxonomic and ethno medicinal identification of the collected petals of flower by the plant speices has been identified and authentified by Dr. Raju Lal Bhardwaj, associate professor (horticulture) and officer on special duty , OSD College of agriculture, Sujmerpur Pali Rajasthan-306902, and certificate issued no. F./Essst./COA/Sum/2021/1652 on dated 26 Sep 2022.

Preparation of plant material:

The petals of flower were shade dried, reduced to coarse powder with the help of grinder and stored in airtight container till further use.

Macroscopical evaluation of plant materials *tagetes erecta*: The petals of flower of *tagetes erecta* were characterized by its morphological features like colour, shape, size and surface characteristics has been studies [29].

Preliminary test

The leaves powder was characterized by its morphological features like green and yellow colour, presence of specific odour and taste.

Analytical Parameter:

Ash Values:

The residues remaining after incineration is the ash content of the petals of flower of *tagetes erecta)*. Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration. Hence, an ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information regarding its adulteration with inorganic matter. Pro cedure given in Indian Pharmacopoeia will use to determine the different ash values such as total ash, acid insoluble ash, and water-soluble ash [30-33].

Determination of total ash value:

Accurately weighed about 5 gm of air dried powdered drugs of *tagetes erecta* was taken in a tared silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air-dried drugs [34].

Determination of water-soluble ash value:

The total ash obtained will boiled with 25 ml of water for 5 minutes. The insoluble matter will collect on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450ºC. The weight of insoluble matter will subtract from the weight of total ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drugs [35].

Determination of acid insoluble ash value:

The ash obtained as directed under total ash value will boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air-dried drugs. [36].

Determination of Alcohol Soluble Extractive Value:

10gms of the air-dried coarse flower petals powders of *tagetes erect* were separately macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105ºC and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air-dried drugs [37].

Determination of Water-Soluble Extractive Value:

Weigh accurately the 10 gm of coarsely powdered *tagetes erecta* and macerate it with 100 ml of water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it is filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish, dried at 105ºC and weighed. The percentage of water-soluble extractive was

calculated with reference to the air-dried drugs [38].

Loss on Drying:

Loss on drying is the loss in weight in % w/w determined by means of the procedure given below. It determines the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (Dessicator or hot air oven). If the sample in the form of large crystals, then reduce the size by quickly crushing to a powder [39].

Procedure:

About 1.5 gm, of powdered drug were weighed accurately in a porcelein dish which was previously dried at 105ºC in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air-dried substances were calculated [40].

EXTRACTION OF DRUGS

Extraction of marigold petals of flower a Soxhlet extraction method is used as follow.

Chemical used: - methanol (90% v/v)

Equipment used: - Soxhlet extractor, Distillation apparatus, round bottom flask

Extraction procedure

- Dried marigold flower; The dried marigold flowers corals are then separated from petals and other part remaining are also removed
- Then the dried corals are grinded in grinder and passed through sieve no. 40
- The grinded material of marigold flower is now put in the soxlet extractor applying a filter bed in beneath to avoid chocking the flow of solvent.
- The soxlet extractor was composed of round bottom flask (1000ml), extractor and condenser, with proper water supply for condensation.
- For 45 gm of plant material 450 ml of hexane is used for the isolation of marigold extract.
- The assembly is allowed to run until the extraction was stopped and the running solvent appears to be clear.
- The extract was transferred to the beaker and allowed the excess solvent to evaporate on water bath at 75ºC When solvent is evaporated, the viscous extract was transferred to china dish allowed to cool [41].

Qualitative examination of phytoconstituents:

Test for Alkaloids:

Dragendorff's Test: To 1 gm of the extract, add 1 ml of Dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Test for Carbohydrates:

Molisch's Test: To 2gm of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet color at the junction of the two liquids reveals the presence of Carbohydrates.

Test for protein:

Biuret Test: To 1 gm of the extract, add 1 ml of Biuret reagent. A pink color indicates the presence of proteins.

Test for phenol:

To 1gm of extract, add ferric chloride and water. A blue green color indicates the presence of phenolic group.

Test For Tannins and Phenolics Compounds:

To 1gm of the extract, add ferric chloride solution, formation of a dark blue or greenish black color product shows the presence of tannins.

Test for Triterpenoids:

Test for Flavonoid:

Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow color solution formed, disappears on addition of an acid indicates the presence of Flavonoid.

Test for Salkowski test

Take small quantity of extract solution, chloroform and conc.H_2So_4. A reddish brown colour solution formed, indicates the presence of terpenoids [42-45].

Result and Discussion:

Morphology of plants

marigold is an erect annual herb that are 4.1 cm long petals. The inflorescence is a solitary terminal head, & petals has 1.4 cm in diameter, bright yellow in wild types, lemon-yellow to deep brown-red in cultivated types.

Macroscopical description:

The plant *tagetes erecta* is taller and erect-growing with height from ten in to three feet, bearing massive pompon-like double flowers; the inflorescence is regarding 5cm. in diameter, with various barren disc-florets and one or a lot of rows of fertile ligulate ray-florets. The flower consists virtually entirely of the ligulate corollas of the ray florets, the limb or strap of corollas is one.2-3.7 cm long and three.5-6 cm broad at the widest part; it's unsubdivided and is terminated by acute teeth. The tube of coil is one.5-2 metric linear unit long and bears characteristic trichomes outwardly. The organoleptic analysis of the flower and flower powder discovered that, flower was bright orange and powder was dark-brown orange in color, with aromatic Odour and a clearly bitter style. 15, sixteen the micromorphology of *tagetes erecta* flower area unit shown in figure1 and therefore the results of micromorphology were mentioned in Table one.3

Table 3: micromorphology mentioned

Macroscopic evaluation description	
Characters	**Observation**
Organoleptic Characters	
Colour	Bright Orange
Odour	Aromatic
Taste	Distinctly bitter
Quantitative Macromorphology	
Length	2-3 cm
Thickness	3.5-5 mm

POCESSING OF PLANT MATERIALS:

Physiochemical analysis of crude drug:

The Physiochemical analysis of leaves powder was carried out. In this study ash values (moisture content, total ash, acid insoluble ash, water soluble ash and water-soluble ash) were determined. The total ash value, acid insoluble, water soluble and moisture content were found mentioned in table no. 5

Physicochemical parameters of *Tagetes erecta*

Parameters studied:	Flower powder (values obtained on dry weight basis w/w)
Loss on drying (moisture content):	7.46 %w/w
Total ash:	4.95 % w/w
Acid insoluble ash:	0.2 %w/w
Water soluble ash:	1.65 %w/w
Water soluble extractive value:	72 %w/w
Alcohol soluble extractive value:	16.8 %w/w

Preliminary phytochemical study of *tagetes erecta*:

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. The preliminary phytochemical screening was carried out to assess the qualitative chemical composition of crude extracts and fractions from; *tagetes erecta* by using precipitation and coloration reaction to identify the major natural chemical groups. General reactions in this analysis revealed the presence or absence of these compounds in the crude extracts and fractions tested. Summary of preliminary phytochemical screening of different extracts and fractions is depicted in Table-

Table 4: Phytochemical screening of extracts of plants leaves

Key (+) = Presence, (-) = Absent

S.N.	CHEMICAL TEST	TAGETES ERECTA	CITRATUS LIMON
1.	**ALKALOIDS TEST:** DRAGONDROFF`S TEST	+	+
2.	**CARBOHYDRATE`S TEST:** MOLISCH`S TEST	-	+
3.	**PROTEIN TEST:** BIURET TEST	+	-
4.	PHENOL TEST	+	+
5.	FLAVONOID`S TEST	-	+
6.	TERPENOID`S TEST	-	-
7.	TANNIN TEST	-	+

Extractive yields:

The phytoconstituents were extracted by using solvents like methanol (90% v/v) & ethanol (90%v/v).

Sr. No.	Extracts	Estimated percentage	Colour of extract
1	Extract: Tagetes erecta	4.52 % w/w	Dark brownish, yellow

TLC profile of extracts

Extract	Solvent system	No of Spots	TLC Profile	
			R_f value	Color
Tagetes erecta	Petrolium ether:acetone: dis.water	3	(0.35,0.406,0.949)	Yellow, brownish yellow

Conclusion:

Standardization is essential measure for correct identity, quality, and purity of crude drug. Most of the information on the identity, quality and purity of plant material can be obtained from its macroscopic, microscopic, and physicochemical parameters. The present work is undertaken to produce some pharmacognostical standard and help to proper identification and ensures of quality of drug. This study is useful for further pharmacological and therapeutic evaluation along with standardization.

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