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Pharmacognosy, Physicochemical, Toxicity Quality evolution studies of Marigold - *Tagetes erecta* L. flower of plant

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Abstract
Introduction: Quality control of herbal products remains a big challenging task. There needs to be more than the Pharmacognosy, physicochemical and screening parameters to validation, authenticate and differentiate adulterants in medicinal plants. Genda phool / Gul-e-Sad Barg (Marigold), *Tagetes erecta* L. is one of the herbs used to treat various health wellness and therapeutic illness of public mankind. This study aims to evaluate the QC, Botanical identification, Pharmacognosy, physicochemical studies of the flower part of plant of Marigold.
Methods: The Botanical identification, Pharmacognosy physicochemical and quality control research analysis of the flower part of plant of T E. coarse powder were carried out using standard methods. The Quality, safety and toxicity effects of the tested drug samples were also investigated.
Results: The Botanical identification, Pharmacognosy, physicochemical and QC. Properties of T E. have shown that all the parameters were within the permissible limits. The tested drug samples showed significant Quality, safety and toxicity studies against certain pathogens organisms and promising anti-pathogenic activity.
Conclusions: The Botanical identification, Pharmacognosy, quality control research findings revealed that the test drug was free from adulterations. This investigated herb's research data confirmed to drug standardization and therapeutically may treat that the drug is safe for internal use and cures in Scabies, Fever, Skin diseases and eyes diseases.

Keywords: Genda phool / Gul-e-Sad Barg, *Tagetes erecta* L. (Marigold), Drug Standardization Research, QC. and QA, HPTLC fingerprint, and GC-MS profiles, Quality, Safety and Toxicity studies

Introduction

Many people consider herbal remedies as an alternative choice for treating a variety of illnesses, particularly chronic lifestyle issues (Chattopadhyay and Maurya, 2015) [12]. WHO formulates suitable guidelines and methodologies for research and evaluation of herbal medicines. This includes a literature review, botanical verification, quality considerations, research and evaluation of safety and efficacy. (Anonymous, 2000) [5] Studies have shown that substandard herbal medicines may have negative side effects. Substitution, adulteration, spoilage, contamination with heavy metals, microorganisms, pesticides, residues, and other variables are the main causes of the poor quality of herbal drugs (Mukherjee *et al*., 2015) [25]. The major portion of the population of India uses a variety of medicinal plants and their products for health care purposes. (Mukherjee, 2003) [26] According to World Health Organization (WHO), in much of the developing world, especially in Asia, Africa, Latin America and the Middle East, 70-95% of the population rely on these traditional medicines for primary health care. (Robinson *et al*., 2011) [33] To address this problem and provide primary healthcare to everyone, worldwide is seeking affordable, conveniently accessible, and more biologically compatible traditional medical systems (Jon, 2022; WHO, 2013) [17]. The extensive use of this traditional medicine by the public gives rise to the need to evaluate the health claims of these medicinal agents and to develop standards for their manufacturing and quality. (Patra *et al*., 2010) [20]. Where the significance of integrating conventional and alternative medical approaches was emphasised in order to advance global health and ensure the efficacy, safety, and quality of such medications (Anonymous, 1991; Philipsion and Linda, 1989) [4, 32].

In recent decades, the traditional medical systems used in many nations have become incredibly significant (Bruneton, 1999; Eunhye *et al*., 2022) [9, 13]. Countries like India, Egypt, South America, and China still use plant-based preparations for treatment (Mohd, 2019) [21]. Quality control of herbal drugs is challenging (Klein-Junior et al., 2021) [21], aiming to confirm their quality, purity, efficacy, and safety (Mukherjee *et al*., 2015) [25]. Establishing precise and concise quality control procedures through the fusion of traditional and modern analytical techniques is required to ensure acceptable reciprocity within the quality of herbal pharmaceuticals (Yadav *et al*., 2011) [38].

The Standardization and Validation of ASU herbal Drugs is not an easy challenge as various factors influence the bio efficacy and reproducible therapeutic effects. Validation of pharmacopoeial standards by experimentation and observations provides a set of characteristics to a particular herbal medicine. Therefore, Scientific Validation of Unani Formulations is an important tool used in the standardization process. (Kunle, 2012) [22].

The leaves of *T.erecta* has been used empirically by Indonesian to treat various illnesses. Some diseases that are believed to be cured by T.erecta including respiratory tract infections, cough, scabies, boils and open sores on the skin. Chemical constituents of this plant include saponins, flavonoids, tagetiin, polyphenols, quercetin and quercetagetin. Several scientific studies that have been reported are antibacterial and antioxidant properties of extract *T.erecta*. Extract of *T.erecta* have also been investigated and were able to heal the wounds of the rat kidney and wounds on the skin of white rats, investigated and reported Antibacterial and Antioxidant activities in *T.erecta* root extracts samples. (Chatterjee *et al*.,2011; Jain *et al*., 2012 and Edy *et al*.,2017) [11, 18, 15] *Tagetes erecta* L is an ornamental plant with flowers that are yellow or orange lit. *T. erecta* is very easy to grow fast and bloom even without special treatment. This plant is commonly used as a natural insecticide because it produces a distinctive odor. *T. erecta* in Indonesia began with difficulties due to the low economic value of this plant. The flower of *T. erecta* have a scent unfavorable and only part interest only commonly used as ornamental flowers (Priyanka *et al*.,2013) [31].

Merigold consists of dried flowers of *T. erecta* L., The plant is an annual or perennial, tall, erect herbaceous flowering plant bearing large bright colour flowers (Fam. - Asteraceae). The plant is native to Mexico and other warmer parts of America and naturalized in tropical and subtropical regions. Inflorescence mostly heterogamous or homogamous. Three different types of florets present in Asteraceae namely ray floret (female), disc floret (bisexual) and neutral florets (rudimentary).

Other binocular name

English- Merigold, Hindi- Genda Phool, Urdu- Gul-e-Sad Berg, Arabian - Hajai, Hamahama, Gujrati-Guljharo, Makhamala, Persian - Kejekharusa, Sadabarg, Bengla - Genda, Marathi - Makhamla, Rojiachaphul, Zendu, Punjabi- Genda, Mentok, Sadbargi, Tangle, Sanshkrit - Sthuapushpa, Zandu, Zanduga, Tamil - Thurukasamanthi, Chendu malli, Temil- Banti etc.

Materials and Methods

Source of Data Collection

All the data for the present study were collected from the Regional Research Institute of Unani Medicine, Chennai (NABH and NABL accredited), Central Council for Research in Unani Medicine, Ministry of AYUSH, Government of India, Tamil Nadu, India. Collection and Authentication of the Plant Material The dried plant of *T. erecta* L. was procured from an authorised drug supplier in Chennai, Tamil Nadu. The local market of Chennai, India, and authenticated by Dr. Subbiah Mangeswari, Consultant - Botany, Drug Testing Laboratory, Drug Standardization Research Unit, and Dr K. Venkatesan, Assistant Research Officer (Botany), Survey of Medicinal Plants Unit, Regional Research Institute of Unani Medicine, Chennai, vide reference ID. No.-7544. (Seen in Figer-1) Dr. Sonali Sajwan, Astt. Research Officer (Botany), Drug Standardization Research Institute, PCIM&H Campus, IInd floor, Kamla nehru nagar, Ghaziabad UP., Central Council for Research in Unani Medicine, Ministry of AYUSH, Government of India. The voucher specimen has been deposited and verified at the Herbarium of the SMPU, Botany Department, Regional Research Institute of Unani Medicine, Chennai, and botanically identification and cross confirmation by Mr. Jitendar, Research Assistant (Botany), Pharmacognosy Department, PCIM&H, Ministry of AYUSH, Govt. of India, Ghaziabad UP, India, vide reference ID. No.-1066.

Pharmacopoeial standard parameters

Pharmacopoeial research studies such as organoleptic characters, microscopical, macroscopical and physicochemical, TLC/HPLC., quality control and quality assurance parameters were carried out

Organoleptic Evaluation: Organoleptic evaluation refers to evaluation of formulation by colour, odour, taste, texture etc., using the sensory organs of our body. The organoleptic characters of the drugs samples were carried out based on the method described by Siddique *et al*. (1995) [34].

Pharmacognosy and Botanical identification

Description

Powder Microscopy: Take 3-5g powder drug sample was weighed, mixed with 50ml of distill water in a beaker and warmed gently in order to make complete dispersion in water. Then mixture was centrifuged and decanted supernatant. The sediment were washed several times with distilled water, centrifuged again and decanted the supernatant. Small quantity of the sediment was taken and mounted in 58eculariz, out of which another small quantity was taken in watch glass and a few drops of phloroglucinol and concentrated hydrochloric acid were added, mounted in 58ecullariz to locate lignified cells. The following characters in different mounts were observed (Wallis, 1987; Johansen, 1940).

Microscopic

Peduncle

T. S. and LS. peduncle from young flower shows almost circular in outline; epidermis consisting of single layer of thick walled parenchyma cells with numerous trichomes; cortical parenchyma consisting of few layers of collenchymas, chlorenchyma and parenchyma cells; vascular bundles arranged in the form of ring shaped with numerous collateral vascular bundles with xylem towards inner side and phloem towards outside; sclerenchyma fibres

present above the phloem; pith present in the centre. (Sowed in Figer-1 & 2, a. & b. respectively.)

Involucre - Young flower

T. S. shows an outline deeply convex on the abaxial and slightly concave on the adaxial side; epidermis consisting of single layer of thin walled parenchyma cells on the upper side and thick walled cells on the lower side; mesophyll consisting of few layers of parenchyma cells; vascular bundle present in the centre with sclerenchyma cap towards the outer side. (Sowed in Figer-1 & 2, a. & b. respectively.)

Powder

Yellowish green; thin walled elongated parenchyma cells with tapering ends from the pappus hairs of calyx, rectangular thin walled parenchyma cells with fibro-vascular bundles from calyx and corolla, thin walled elongated parenchyma cells from the corolla and some of the cells filled with yellowish brown contents, thick walled epidermal cells in surface view, papillose epidermal from the corolla and spiral vessels upto 15µ. (clearly sowed and mentioned in Figer-4 a. b. c. d. & f. respectively.)

Involucre - Flower

T. S. of lower side of the involucres shows an almost straight in outline; epidermis consisting of single layer of thick walled parenchyma cells on the upper side and thin walled cells on the lower side; mesophyll consisting of several layers of very thick walled parenchyma cells on the upper side; vascular bundle present with sclerenchyma cap; numerous parenchyma cells of circular to oval shaped cells arranged with large intercellular spaces present in the lower side.

T. S. of upper side of the involucres shows an almost straight in outline with slight raised portion on the upper side and concave on the lower side; epidermis consisting of single layer of thick walled parenchyma cells on the upper side and thin walled cells on the lower side; mesophyll consisting of few layers of very thick walled parenchyma cells on the upper side; vascular bundle present with sclerenchyma cap; numerous polygonal parenchyma cells present in the mesocarpic region and some of the cells filled with chloroplast. (Sowed in Figer-5 a. b. c. d. e. f. & g. respectively.).

Genda phool / Gul-e-Sad Barg

***Tagetes erecta* Linn.**

(Ref.: Siddique *et al*. 1995; Sagar *et al*., 2022;2021;2020;2017;2015).

Fig 1: Herbarium Sheet for Botanical Identification and Confirmation

T.S. of flower

Ray Floret

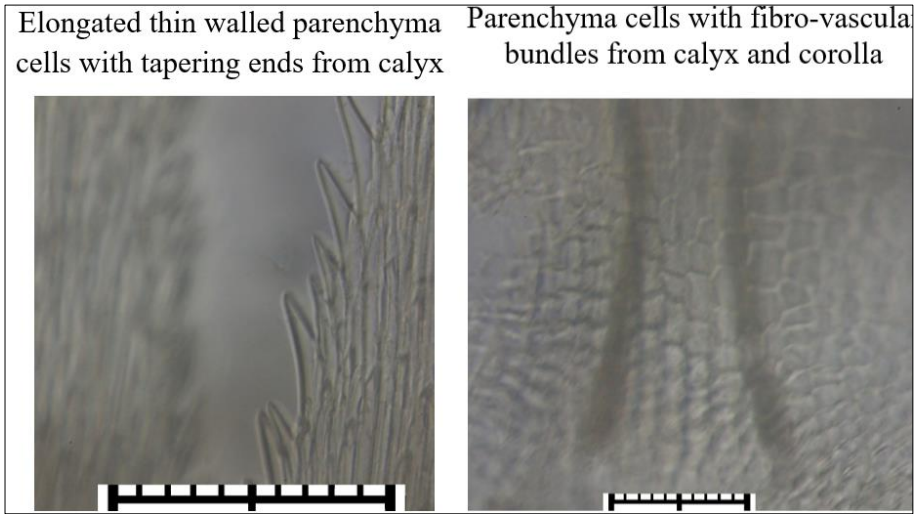
Fig 2: a. & b.

L. S. of the flower

Fig 3: a. & b.

Powder

(Ref.: Wallis, 1987; Johansen, 1940; Sagar *et al*., 2022;2021;2020;2017;2015)



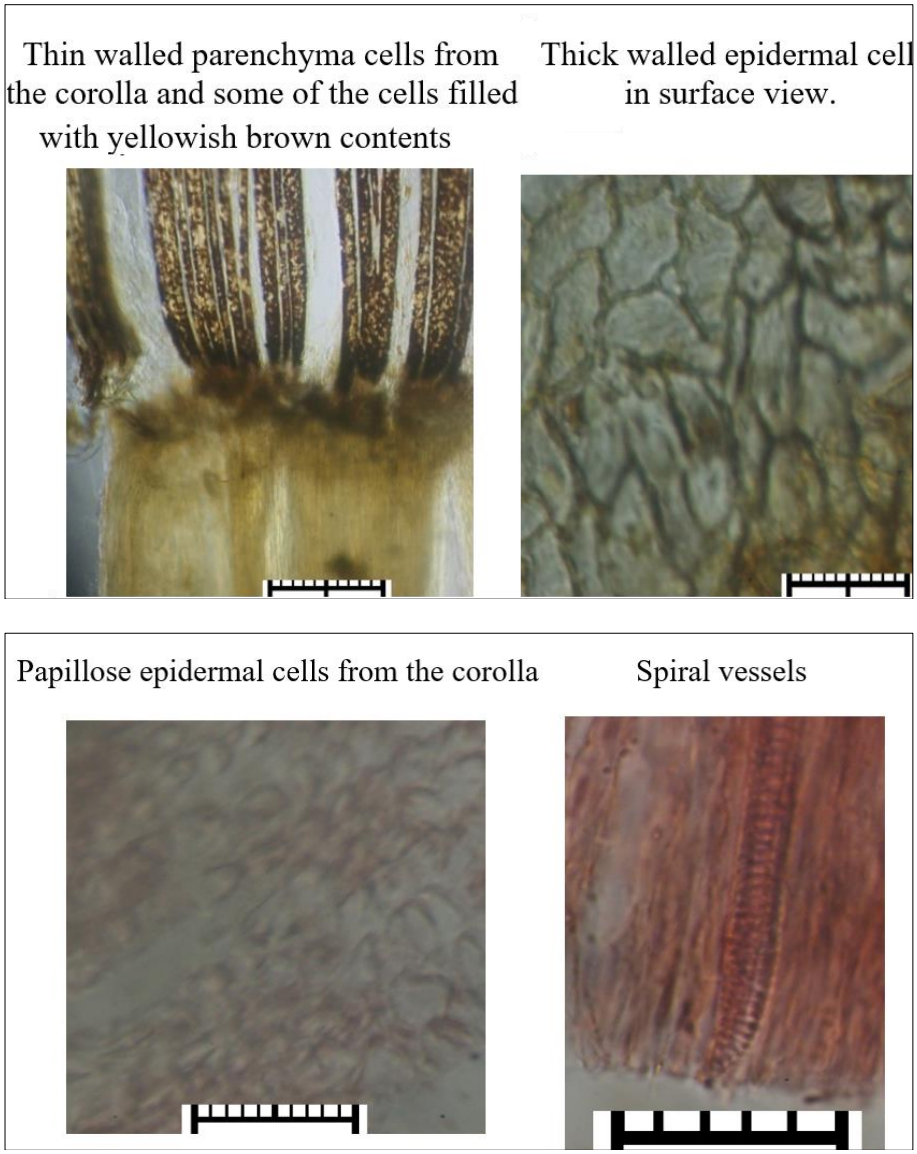
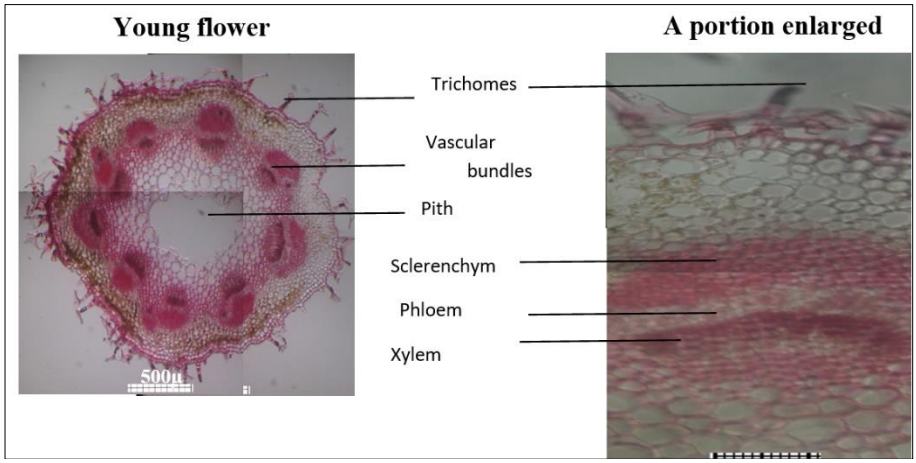
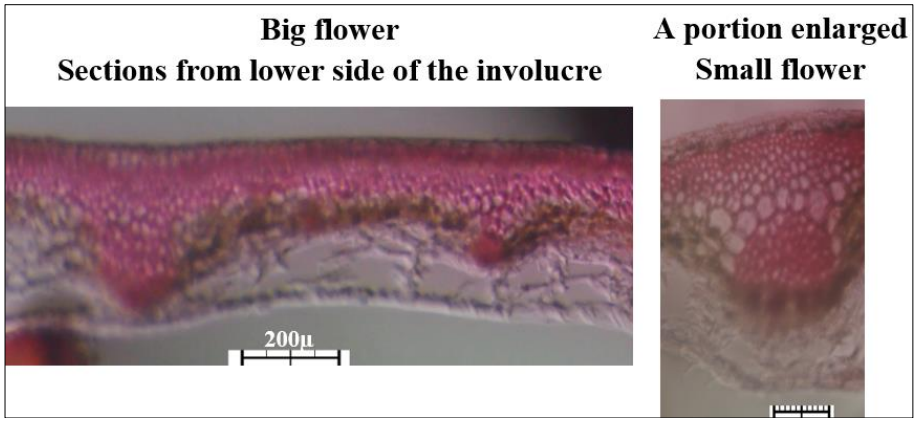


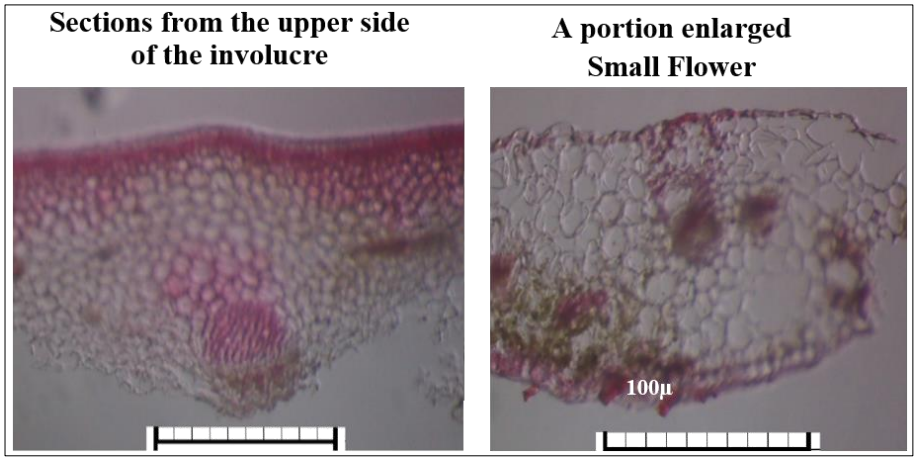
Fig 4: a., b., c., d., e. & f.

T. S. of peduncle involucre



T. S. OF INVOLUCRE





Procurement of Chemicals, Reagents, and Pathogens, Microbes Chloroform, Ethanol, formic acid, hexane, hydrochloric acid, lead acetate trihydrate, sodium hydroxide, Fehling A and B, toluene, and double distilled water or Millipore water were procured from Merck Life Sciences, Pvt. Ltd, India. Ethyl acetate, hydrogen sulphuric acid, ninhydrin, and ferric chloride were obtained from Fisher Scientific, India. Acetonitrile, Methanol, Hexane, HPLC mark grade were used and obtained from the Drug Testing Laboratory, Drug Standardization Research Unit, Regional Research Institute of Unani Medicine, Chennai, TN., India and Sophisticated Instrument Laboratory, Department of Chemistry, Drug Standardization Research Institute, PCIM&H Campus, IInd floor, Kamla nehru nagar, Ghaziabad UP., Central Council for Research in Unani Medicine, Ministry of AYUSH, Government of India. Pathogens, Microbes were purchased and collected from Microbial type culture collection, MTCC (CSIR. Institutional Laboratory), Chandigarh, Punjab, India, National Culture Collection, NCC, (CSIR, Institutional Laboratory), Pune, Maharashtra, India and American type culture collection, ATCC, USA.

Preparation of Chloroform and Ethanolic Extract

Bio-active phyto-chemical constituents

The T E. flower part of plant shows the presence, confirmed and reported Bio-active phyto-chemical constituents earlier in *T.erecta* flower part β - sitosterol, β- daucosterol, 7-

hydroxysitosterol, lupeol, erythrodiol, erythrodiol-3-palmitate, 1-[5-(1-propyn-1-yl)-[2,2-bithiophen]-5-yl]-ethanone,α- terthienyl, quercetagetin, quercetagetin-7-methyl ether, quercetagetin-7-O-glucoside, kaempferol, syringic acid, gallic acid, 3-β-galalctosyl disyringic acid, 3 α galalctosyl disyringic acid, 6-ethoxy-2,4-dimethylquinoline, oplodiol, (3S,6R,7E)-hydroxy-4,7-megastigmadien-9-one, palmitin, ethylene glycol linoleate, n-hexadecane, hexadecanoic acid, 7-tetra decenal (z), vitamin E and norolean - 12-ene, carotenoid - includes all trans and cis isomers of zeaxanthines, all trans and cis isomers of lutein, lutein esters. Volatile oil contains tagetone, dihydrotagetone, cis-tagetone, cis-ocimenone, trans-ocimenone, limonene, valeric acid and ocimene and flower oil (9), were also found in the present study. The compounds trans-sabinene hydrate, α,p-dimethylstyrene, (Z)-myroxide, (E)-myroxide, borneol, p-cymen-8-ol, α-terpineol,myrtenol, (E)-anethol, piperitenone oxide, germacrene D, (E,E)-farnesene, spathulenol, caryophyllene oxide and pentadecanoic acid are being reported for the first time in T. erecta oils. whereas the oil of the flowers had higher concentration of (Z)-β-ocimene, dihydrotagetone, linalool (Z)-myroxide, (Z)-β-ocimene epoxide, piperitenone, piperitenone oxide, β-caryophyllene, germacrene D and (E,E)-α-farnesene. Piperitenone oxide, which was a major constituent of the oil of the flowers. The oil of the flowers contained limonene (6.9%), terpinolene (4.7%), (Z)-myroxide (7.9%), piperitone (28.5%), piperitenone (10.9%), piperitenone oxide (7.2%) and β-caryophyllene (7.0%) (Kancherla *et al*., 2023 Vellingir *et al*., 2018; Edy *et al*., 2017 and Krishna *et al*., 2004) [19, 37, 15, 20]

The chloroform and ethanolic extract of the flower plant of *T. erecta* L. was obtained as per the method described by Yuhao *et al*. (2015) [39] and Kancherla *et al*., (2023) [19]. The plant material was dried in the shade and ground into a coarse powder using an electric grinder. Ten grammes of the powdered drug were extracted in 150 ml of ethanol using the Soxhlet apparatus for 6 hours. The extract was filtered through Whatman filter paper (No. 1). The filtered extract was concentrated under a vacuum evaporator. The dried ethanolic extract was stored at 4 °C until further use. Physicochemical Standardisation The organoleptic properties, including colour, odour, and taste, of the whole plant of T E. were evaluated according to the method described by Siddiqui (1995) [34] and Kancherla *et al*.,(2023) [19]. The physicochemical parameters, such as foreign matter detection, loss of weight on drying at 105 °C, total ash value, acid-insoluble ash value, extractive values, and pH of T E., were evaluated by following standard operating procedures. (Anonymous, 2007 and Kancherla *et al*., 2023) [3, 19].

Results and Discussion

Quality assurance and quality control parameters:

Physicochemical Standardisation The organoleptic evaluation of the whole plant of T E. revealed that it was brown, tasteless, and had a characteristic odour (Table 1, entry 1- 4). The entire T E. flower part of plant organoleptic characteristics were discovered to be the same as those mentioned in botanical literature. Foreign substances including other plants, mould, insects, excrement, sand, stones, chemical residues, etc. are prohibited in herbal medicines. In the present study, the foreign matter in the whole plant of T E. was found to be Nil, which is within the permissible limits with reference to the Ayurvedic Pharmacopoeia of India (The Ayurvedic Pharmacopoeia of India, 2011). The moisture content in any herbal drug is recommended to be up to 10% (Sumbul *et al*., 2012), thus preventing spoilage. The loss of weight on drying at 105 °C in T E. Spl-1, Spl-2 and Spl-3 were found to be 3.04,3.12 and 3.08%. The ash value is an important parameter for identifying adulterants in an herb (Ali *et al*., 2016) [2]. The higher ash value shows the presence of inorganic substances in the tested plant material (Husain *et al*., 2012). The total ash and acid-insoluble ash % values of T E. were found to be 5.86, 5.87, 5.88% and 0.587, 0.588, 0.586% respectively. The extractive values % of ethanol, and water were found to be 23.93, 23.94, 23.93% and 42.12, 42.16 and 42.14% respectively. Such results indicate that most of the phytoconstituents of T E. are soluble in ethanol and water. The pH of the test material were found to be 5.30,5.54 and 5.50 (Table 1, entry 4-10) (Sangeeta *et al*., 2016). The acidic nature of the test drug shows its good absorption through the mucous membrane of the stomach (Hardman *et al*., 2001). Contaminants of heavy toxic metals in plants may cause serious health issues in humans (Sangeeta *et al*., 2016 and Kancherla *et al*., 2023) [19]. The lead, cadmium, arsenic, and mercury were found to be below the permitted limits according to T E. heavy metal analysis findings (Table 2) (Anonymous, 2016 and Kancherla *et al*., 2023) [19]. The entire T E. plant's physicochemical constants were all within acceptable limits according to the Indian Ayurvedic Pharmacopoeia and Indian Unani Pharmacopoeia (Anonymous, 1986; Anonymous, 2007; The Ayurvedic Pharmacopeia of India and Unani Pharmacopeia of India) [8, 3, 8].

Estimation of microbial load: The microbial load *viz*. total bacterial count (TBC), total fungal count (TFC), *Enterobacteriaceae*, *Escherichia coli*, *Salmonella* spp and *Staphylococcus aurous* were estimated as per standard method. microbial load of T E. samples were deducted and found microbial load contamination in to be Table-2 respectively. (Anonymous, WHO: 1991; 1998; Sagar *et al*.,2020;2022) [4, 29, 28]

Analysis of Aflatoxins: Aflatoxins B1, B2, G1 and G2 were analyzed as per Official Analytical Methods of the American Spice Trade Association (ASTA), 1997. Aflatoxins were estimated by Kobra cell techniques using Agilent HPTLC and CAMAG or Anchrom HPTLC instruments as per the method ASTA (Anonymous, 1997; Sagar *et al*.,2020;2022) [29, 28]. samples were deducted and found Aflatoxins B1, B2, G1 and G2 in to be Table-3 respectively.

Estimation of heavy metals: The method used for the analysis estimation of heavy metals like lead, cadmium, mercury and arsenic as per Guidelines of WHO. of T E. Heavy metal samples were deducted and found heavy metals - Pb, Hg, Cd & As in to be Table-4 respectively.

The usage of ASU. herbal drugs and products along with higher safety margins, WHO has taken necessary steps to ensure quality assurance and quality control parameters with the modern techniques and application of suitable standards, (Anonymous, 1998;Sagar *et al*., 2020;2022) [29, 28].

Table 1: Physico-Chemical identification tests: Physico-chemical parameters: (By Physico-chemical instruments and apparatus)

S. No.	Parameters	Resulted Values		
		Spl-1	Spl-2	Spl-3
1.	Colour	Light bright golden yellow	Light bright golden yellow	Light bright golden yellow
2.	Odour	Indistinct	Indistinct	Indistinct
3.	Taste	Characteristic	Characteristic	Characteristic
4.	Foreign Matter (%)	NIL	NIL	NIL
5.	Loss in weight on drying at 105^0 C (%), w/w	3.04	3.12	3.08
6.	Total Ash (%), w/w	5.86	5.87	5.88
7.	Acid insoluble ash (%),w/w	0.587	0.588	0.586
8.	Ethanol Soluble Extractive (%),w/v	23.93	23.94	23.93
9.	Water Soluble Extractive (%),w/v	42.12	42.16	42.14
10.	pH of 10% aqueous solution	5.30	5.54	5.50

Table 2: Analysis of Microbial load: (By microbial culture media)

S.N0.	Parameter Analyzed	Results	WHO Limit
1	Total Bacterial Count	400 cfu/gm	10^5cfu/gm
2	Total Fungal Count	300 cfu/gm	10^3cfu/gm
3	*Escherichia coli*	Absent	Absent
4	*Salmonella typhai Spp.*	Absent	Absent
5	*Staphylococcus aurous*	Absent	Absent

Table 3: Estimation of Heavy Metals: (By AAS.)

S. N0.	Parameter Analyzed	Results	WHO Limit
1	Lead	2.12ppm	10ppm
2	Cadmium	0.02ppb	0.3ppm
3	Mercury	Not detected	1.0ppm
4	Arsenic	0.09 ppm	3.0ppm

Table 4: Estimation of Aflatoxins: (By HPTLC)

S.N0.	Parameter Analyzed	Results	WHO Limit
1	Aflatoxins, B1	Not detected	0.5ppm
2	Aflatoxins, B2	Not detected	0.1ppm
3	Aflatoxine, G1	Not detected	0.5ppm
4	Aflatoxine, G2	Not detected	0.1ppm

Conclusions

The investigated drug Gul-e-sad barg (Merigold) T E. was found to be of standardization of herbal medicine provide an assurance of its genuine quality and free from any impurities or hazardous, toxic contamination according to the drug quality research, botanical identification, Pharmacognosy, physicochemical and quality control results studies data's basis. The ranges of all the botanical identification, Pharmacognosy, physicochemical constants used for the quality analysis of the entire T E. flower plant part are normal. Numerous secondary metabolites have been detected.

As the evaluated of resulted parameters which certainly provides validation that the drug is safe for internal use. As well as its potent quality, safety and toxicity evolution of studies.

T E. may be therapeutically used as a Astringent, Carminative, Stomachic and Liver tonic and used to cure in Scabies, Fever, Skin diseases and eyes diseases and supported data's of T E. can be helpful to incorporated of pharmacopoeial standard monograph. however, further studies on the isolation and characterisation of these substances may still be conducted and expected to advance comprehend confirmation of the *In-vitro* or *In-vivo* detailed mode of action upon animal trial model of marigold (T E.).

Ethical approval

As the work is purely an in-vitro study, ethical clearance is not required.

Author contributions

Dr Pawan Kumar Sagar (Chemistry): Carried out Instrumental, Chemistry part and Manuscript written. Dr N Zaheer Ahmed (Unani): Unani expert, Work designed and revised manuscript review. Dr Rampratap Meena and Dr. A. S. Khan (Chemistry): Work designed and supervised. S. Kashyap (Chemistry): Analytical data analysis.

Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Anonymous. The Unani Pharmacopoeia of India. Part I. Vol. III. Department of AYUSH, Ministry of Health and Family Welfare, Government of India, New Delhi; 2017;124-136, 148.
2. Ali W, Shaikh H, Ansari A, Khanam S. Standardization of Unani antidiabetic tablet *Qurse Tabasheer*. Journal of Pharmacognosy Research. 2016;8(2):147-152.
3. Anonymous. The Ayurvedic Pharmacopoeia of India. Part 2, 1st ed. Ministry of Health and Family Welfare, Department of AYUSH, Government of India, New Delhi, 2007, 141.
4. Anonymous. Guidelines for the Assessment of Herbal Medicines, Document No. WHO/TRM/91. World Health Organization, Geneva; c1991.
5. Anonymous. Geneva: WHO. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine; c2000, p. 3-4.
6. Anonymous. Geneva: WHO, WHO monographs on selected medicinal plants, World Health Organization (WHO); c2014.
7. Anonymous. Geneva: World Health Organization, WHO traditional medicine strategy. 2013, 2014-2023.
8. Anonymous. The Ayurvedic Pharmacopeia of India, Part-I, Vol-I, published by Govt. of India, Ministry of Health & Family Welfare, Dept. of AYUSH. New Delhi; c1986, p. 55-57.
9. Bruneton J. Pharmacognosy, Phytochemistry, Medicinal Plants, Second edition. Lavoisier Publication, France; c1999.
10. Cahyaningrum PL, Widyantari AAASS. Antibacterial activity of Marigold flower (*Tagetes Erecta* L.) Ethanol Extract Cream against Staphylococcus aureus. Journal of Vocational Health Studies. 2023;06:165-172. DOI: https://doi.org/10.20473/jvhs.V6.I3.2023.165-172
11. Chatterjee S, Prakash T, Kotrsha D, Rao NR, et al. Comparative Efficacy of *Tagetes Erecta* and *Centella Asiatica* Extracts on Wound Healing in Albino Rats. Chinese Medicine. 2011;2:138-142.
12. Chattopadhyay N, Maurya R, *et al*. Herbal Medicine. Reference Module in Biomedical Sciences. Chionis K, Krikorian D, Koukkou AI, Sakarellos-Daitsiotis M, Panou-Pomonis E, editors. Synthesis and biological activity of lipophilic analogs of the cationic antimicrobial active peptide anoplin. Journal of Peptide Science. 2015;22(11-12):731-736.
13. Eunhye S, Lin A, Myeong SL, et al. Increasing trends and impact of integrative medicine research: from 2012 to 2021. Integrative Medicine Research. 2022;11(4):100884.
14. Ehsan TM, Mohsen Y, Elahe T, et al. Current herbal medicine as an alternative treatment in dentistry: *in vitro*, *in vivo* and clinical studies. European Journal of Pharmacology. 2020;889:173665.
15. Edy HJ, Marchaban, Wahyuono S, Nugroho AEE. Characterization and Evaluation of Bioactive Compounds of Extract Ethanol *Tagetes erecta* L. leaves by GC-MS. International Journal of Chemical Technology Research. 2017;10(2):172-175. DOI: https://sphinxsai.com/2017/ch_vol10_no2/1/(172-175)V10N2CT.pdf.
16. Husain SF, Ahmad I, Shamsi S, et al. Standardization of the finished product: Habbe Irqun Nisa - a Unani anti-inflammatory formulation. Ancient Science of Life. 2012;32(1):38-44.
17. Jon W. Balancing promoting the public's right to traditional, complementary and integrative health with evidence-based health care. Advances in Integrative Medicine. 2022;9(3):143-144.
18. Jain R, Katare N, Kumar V, et al. *In Vitro* Anti Bacterial Potential of Different Extracts of *Tagetes Erecta* and *Tagetes Patula*. Journal of Natural Sciences Research. 2012;2(5):84-90.
19. Kancherla M, Arokiarajan MS, Ansari AP, Ahmed NZ, Meena RP, Dar MY, *et al*. Physicochemical, Heavy metal analysis, HPTLC, GC-MS, antibacterial, and antioxidant activity of an ethanol extract of *Viola pilosa* Blume whole plant. Journal of Herbal Medicine. 2023;42:1-5. DOI: https://DOI.org/10.1016/j.hermed.2023.100812
20. Krishna A, Kumar S, Mallavarapu GR, Ramesh S, et al. Composition of the Essential Oils of the Leaves and Flowers of *Tagetes erecta* L. Journal of Essential Oil Research. 2004;16(6):520-522. DOI: http://dx.DOI.org/10.1080/10412905.2004.9698786
21. Klein-Junior LC, De Souza MR, Viaene J, Bresolin TMB, De Gasper AL, Henriques AT, et al. Quality control of herbal medicines: from traditional techniques to state-of-the-art approaches. Planta Medica. 2021;87: 964-988.
22. Kunle OF, Egharevba HO, Ahmadu PO, et al. Standardization of Herbal Medicines: The Review. International Journal of Biodiversity and Conservation. 2012;4:101-112.
23. Latifiane E, Otur C, Abanoz-Secgin B, Arslanoglu SF, Kurt-Kizildogan A, et al. Evaluation of Antimicrobial Activity in Extracts of Different of Three *Tagetes* Species. Turkish Journal of Field Crops. 2021;26(1):117-122. DOI: https://DOI.org/10.17557/tjfc.950272
24. Mekvimol T, Poonthong G, Chayanit C, Pumipuntu N, et al. Antimicrobial activity of marigold (*Tagetes erecta*), mulberry (*Morus indica*), and red shallot (*Allium ascalonicum*) extracts against *Streptococcus agalactiae*. International Journal of One Health. 2020;6(1):56-60. DOI: https://www.onehealthjournal.org/Vol.6/No.1/10.pdf
25. Mukherjee PK, Bahadur S, Chaudhary SK, Kar A, Mukherjee K, et al. Quality-related safety issue-evidence-based validation of herbal medicine farm to pharma. Evidence-Based Validation of Herbal Medicine; c2015, p. 1-28.
26. Mukherjee PK. Exploring botanicals in Indian system of medicine-regulatory perspectives. Journal of Clinical Research & Regulatory Affairs. 2003;20:249-264.
27. Mohd SAK, et al. Herbal Medicine: Current Trends and Future Prospects. New Look to Phytomedicine. Advancements in Herbal Products as Novel Drug Leads; c2019, p. 3-13.
28. Pawan Kumar Sagar, M Alam, S Sajwan, AS Khan, et al. HPTLC finger printing studies and evaluation of Pharmacopoeial Standards for the medicinal plant *Adiantum capillus-veneris* L. International Journal of Scientific Research in Chemistry (IJSRCH). 2022;7(3):01-15. ISSN: 2456-8457, URL: https://ijsrch.com/IJSRCH21657 DOI: https://www.researchgate.net/publication/372251885_H

PTLC_finger_printing_studies_and_evaluation_of_Pharmacopoeial_Standards_for_the_medicinal_plant_Adiantum_capillus-veneris_L
29. Pawan Kumar Sagar, R Murugeswaran, RP Meena, MW Ahmed, SA Ansari, S Khair, et al. Standardization and HPTLC Finger Printing Studies of Poly Herbal Formulation - Itrifal Haamaan. International Journal of Ayurveda and Pharmaceutical Chemistry. 2020;12(3):220-232. (e-ISSN: 2350-0204) DOI: https://oaji.net/articles/2021/1791-1632827247.pdf
30. Patra KC, Pareta SK, Harwansh RK, et al. Traditional approaches towards standardization of herbal medicines: A review. Journal of Pharmaceutical Sciences and Technology. 2010;2:372-379.
31. Priyanka D, Shalini T, Navneet KV, et al. A Brief Study on Marigold (*Tagetes* Species): A Review. International Research Journal of Pharmacy. 2013;4(1):43-48.
32. Philipsion JD, Linda AA, et al. Ethnopharmacology and western medicine. Journal of Ethnopharmacology. 1989;25:61-72.
33. Robinson MM, Zhang X. Vol. 3. Geneva: World Health Organization. The World Medicines Situation. Traditional Medicines: Global Situation, Issues and Challenges 2011;p. 1. [Google Scholar]
34. Siddiqui HMA. Format for the Pharmacopoeial Analytical Standards of Compound Formulation, Workshop on Standardization of Unani Drugs (Appendix). Central Council for Research in Unani Medicine (CCRUM), New Delhi 1995.
35. Sumbul S, Ahmad MA, Asif M, Akhtar M, et al. Physicochemical and phytochemical standardization of berries of *Myrtus communis* Linn. Journal of Pharmacy and Bioallied Sciences. 2012;4(4):322-326.
36. Sangeeta M, Anindya B, Purnendu P, Rao MM. Pharmacognostic, physicochemical and chromatographic characterization of *Samasharkara Churna*. Journal of Ayurveda and Integrative Medicine. 2016;7(7):88-99.
37. Vellingiri V, Natesan R, Perumal R, Pemaiah B, Aravamudhan G, et al. Microscopic, phytochemical, HPTLC, GC-MS and NIRS methods to differentiate herbal adulterants: pepper and papaya seeds. Journal of Herbal Medicine. 2018;11:36-45.
38. Yadav P, Mahour K, Kumar A, et al. Standardization and evaluation of herbal drug formulations. Journal of Advanced Laboratory Research in Biology. 2011;2(4):161-166.
39. Yuhao Z, Deepak K, Prasad DN, Rajesh KS, et al. Morphoanatomic, physicochemical, and phytochemical standardization with HPTLC fingerprinting of aerial parts of *Aervalanata* (Linn) Juss ex Schult. Journal of Traditional Chinese Medicine. 2015;2:39-44.