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Analytical Study of Terpenoids Present in the Medicinal Extracts of *Tagetes erectes* L. and *Tridax procumbens* L. of Family Compositae

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ABSTRACT

Plant used for traditional medicine contains a wide range of substances that can be helpful to treat chronic as well as infectious diseases. *Tagetes erecta* L. is an annual herb with family Compositae with natural pigments from its yellow/orange flowers. *Tridax procumbens* L. commonaly known as coat buttons or *Tridax daisy* belongs to flowering plant species of family asteraceac. The objective of this work is to isolate the terpenoids from the extracts of these plants, and to ascertain the curative properties of terpenoids. A subclass of prenyllipids termed as terpenoids were obtained from these dried plants by extracting essential oil by following the steam distillation method. Thereafter, the terpenoids were isolated from essential oils using organic solvents. The detection was carried out using UV-Vis spectroscopy as peaks were identified at certain wavelengths. The study revealed the presence of tannins, alkaloids, saponins, flavonoids, terpenoids, glycosides, and other bioactive molecules. These phytochemicals may be non nutritive chemical compounds but have many health benefits as well as important properties to prevent or to fight many common diseases. They defend the plants against illness and injuries and supply to the plants quality in terms of color, odor and taste.

Keywords Essential oil, Phytochemicals, Terpenoids, *Tagetes erecta*, *Tridax procumbens.*

INTRODUCTION

Nature and products obtained from the nature are boon to all forms of life, including humans. Even present day government is taking keen interest in the same and Ayurveda, Unani, Siddhasha, Unani and Homeopathy have been made integral part of therapy. Asia, including Indian subcontinent have been depending upon herbal extracts for obtaining medicines (Archana and Bose 2022).

In this context, medicinal plants are of great importance as most of the population in developing countries is dependent on them as source of medicines for different ailments (Hashim *et al*. 2010). Anti microbial agents are loaded in such plants and due to this, the plants are used medicinally in many countries (Nandagoapalan *et al.* 2016).

Plant used for traditional medicine contains a wide range of substances that can be helpful to treat chronic as well as infectious diseases. Ample amount of empirical evidences have established the curative

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potency of herbal medicines. The most important of these bioactive compounds of plants are terpenoids, flavonoids, tannins, and phenolic compounds (Ullah *et al*. 2018, Umaru *et al*. 2018, Nandagoapalan *et al*. 2016). Highly concentrated substances that are obtained as extract are termed as essential oils, which may reach the blood stream due to inhalation and subsequently be absorbed by blood. Steam distillation is the process through which the essential oils can be extracted. In this process, the plant material is dried, mashed and is then subjected to distillation either hydro distillation or steam distillation. Thereafter, the essential oil reach the distillate after which they are subjected to pure organic volatile solvent, like light petroleum ether, n-hexane, where they are further purified.

A subclass of prenyllipids termed as terpenoids are obtained from plants and is among the most commonly found natural products described as organic compounds formed by living cells and have been categorized as metabolites. These organic compounds may be called as prenylquinones, terpenes and sterols. Being combination of mixture of isomeric hydrocarbons, these were known as terpenes, which is a major component in essential oils.

Basically the name terpenes was given to a combination of isomeric hydrocarbons having molecular formula $C_{10}H_{16}$ happening in turpentine and many other essential oils. Terpenoids, derived metabolites in plants with multiple structures are abundant in natural products. Plant growth and development cannot take place without terpenoids. These compounds are also useful in behavior of the plant to the environment and other physiological processes. Terpenoids were extensively employed as building blocks in the food, cosmetic, and pharmaceutical industries. They have anti-inflammatory, antibacterial, antiviral, antimalarial, anticancer, pro-trans dermal absorption, cardiovascular disease prevention and treatment, and hypoglycemic properties. Terpenoids also offer a wide range of potential uses, including insect resistance, immune regulation, against oxidation and aging, and for neuroprotection, according to earlier studies. Terpenoids have a complicated structure, a wide range of effects, and several modes of action. This research reviewed the terpenoids' functions and mechanisms. Additionally, the development and application prospects related to such compounds were explored, serving as a useful resource for new drug discovery and terpenoid-based drug design. Terpenoids, a group of chemicals with multiple isoprene units, have been categorized into various further sub groups with respect to number of carbon atoms present such as monoterpene, sesquiterpene, diterpene, triterpene, tetraterpene and polyterpene, among others, based on the number of isoprene units they contain.

Many plant parts exhibit aromatic qualities that aid in the preliminary identification of different natural constituents. These, after going through different separation processes, aid in the thorough identification of elements. Various methods have to be developed and validated for the target compound to be identified (Rani and Sud 2015). The plant *Tagetes erectes* has proven to have significant insecticidal activity and antimutagenic activity. The plant is also rich source of antioxidants; carotenoids, luteolin and zeaxanthin, which are extensively applied in food industry (Nandagoapalan *et al.* 2016).

The other plant *Tridax procumbens* is again a flowering plant having great medicinal values. In India, it is commonly used as insect repellent, wound healing agent and anti-coagulant. The extracts of this plant are used gastritis and heartburn (Shrivastav *et al*. 2020). This plant is successfully used to reduce high blood pressure, in dysentery and diarrhea.

Keeping in mind the importance of these plants, the present study deals with the extraction of essential oil from *Tagetes erectes* and *Tridax procumbens* of Family Compositae using steam distillation and organic solvents. The terpenoids present in the extract were detected using UV-Vis spectroscopy and NMR spectroscopy techniques.

MATERIALS AND METHODS

Plant material

Tagetes erecta Linn.

Tagetes erecta L. is an annual herb that has been commercialized globally as a decorative plant and as

a big source of natural yellow/orange color. This plant can be grown in diverse climates and lots of seeds are produced. The flower is commonly known as Genda in Hindi and belongs to the family Compositae. It is also otherwise called Asteracae. Flower part of this plant to be used for research work was collected from Government College Nohar (Rajasthan) premises in the month of January and February during the winter session.

***Tridax porcumbens* Linn.**

Tridax procumbens L. commonaly known as coat buttons or *Tridax daisy* belongs to flowering plant species of the family asteraceac. It bears daisy like yellow centered white flowers and is propagated by seed and from cuttings. Conventionally, this plant is effective against number of ailments such as wound healing and acts against coagulation, fungal diseases and helpful in insect repellent applications and for growth of hair. The juice extracted from the leaves can be directly applied on wounds and also used for skin diseases. The material was collected from the area of Ganga Farm,Village Bhukarka , Nohar in the month of November-December during winter session and whole plant was used for research work.

Extraction, isolation and purification methods

First of all, the collected samples were washed under tap water. Thereafter drying under shade took place. The material was then powdered by mashing manually.

Steam distillation

Steam distillation is the most general method for production of essential oil. Steam distillation is used to obtain oils and extracts and, involves the inflow of steam into distillation chamber containing raw plant material. Efforts have been made to get the essential oil using steam generated from solar energy (Tiwari *et al*. 2022 a,b). Oil, in naturally occurring sacs, is released and is carried out of the chamber. This is followed by condensation in chilled chamber, where steam as well as oil vapors convert to liquid. This whole process continued for about 3-4 hrs to ensure that all the oil has been extracted. As essential oil and water are non mixing liquid, the oil being lighter makes the upper layer and water (hydrosol) being heavier makes the lower layer. Oil is separated from this mixture by use of separating funnel. The oil thus obtained may still contain some water in it. These traces of water were removed by using rotary vacuum evaporator.

Preliminary detection of presence of terpenoides in the extracts of both species were done byLibermann- Burchar test and Salkowski test. After preliminary test of crude extract, furtheri dentification wascarried out by the following techniques:-

UV-Visible Spectroscopy
NMR Spectroscopy

UV-visible spectroscopy

There is a need to make exact measurements of absorption of light at varying wave lengths to have a good understanding related to colorful ness of different compounds. The optical spectrometers do this job by investigating visible as well as ultra violet spectrum. The energy level of the photons in the visible spectrum is low, varying from 36 to 72 kcal/mole whereas in the near UV region (200 nm), the energy level extends upto 143 kcal/mole. Below 200 nm, the process is very difficult and no such device is used for making analysis. The UV visible spectra were determined using Perkin Elmer Larnbda EZ 201UV-VIS Spectrophotometer.

NMR spectroscopy

In NMR, the structural and chemical composition of various substances can be found out by their nuclei, which have their distinctive magnetic field. Using the magnetic field, the NMR analyzes and a special detector assesses the changes. Because of the magnetic field, the nuclei are electronically charged and move from lower energy level to higher energy level and the difference of these energy levels is dependent on power of magnetic field and size of nuclear field moment. The electromagnetic radiation pulse attains the NMR signal having some frequency. When electromagnetic radiation is stopped, it results in the nuclei to relax and achieves the thermal equilibrium.

The amount of energy released from the nuclei is recorded in the form of spectra on some output device such as computer. These spectra are unique for each nucleus and are linked to the levels of energy between two conditions (Chichester 2011). The NMR spectra were –recorded at 399.951 MHz for ^{1}H- and 100.578 MHz for ^{13}C-NMR, using standard varian pulse sequence programs.

RESULTS AND DISCUSSION

Drying of plant material and extraction of essential oil

Drying of both the plant materials took place in shade and continued till constant weight was obtained. The initial weight of 2680 g of *Tagetes erectes* was reduced to 2145 g resulting in nearly 82 % loss in weight. Similarly, *Tridax procumbens.* Material was reduced from 2550 g to 2090 g causing a loss of 80 % in weight. The drying results indicated that most of the material was having water which was removed during drying. Even the final moisture content of dried material was found to be 5.1 % and 5.4 % for *Tagetes erectes* and *Tridax procumbens* material. The dried material was powdered manually and was used for release of volatile oil using the steam distillation technique. The essential oil yield was 0.42 % in case of *Tagetes erectes* and 0.48 % in case of *Tridax procumbens*. As oil content in these plant materials was quite less, it had to be extracted for a long time of about 5 hrs so that almost all the oil is extracted. As steam distillation involves use of water, the essential oil obtained might have contained traces of water, which simply cannot be removed in the condensation and physically separation process. For removal of these traces of water, rotary vacuum evaporator was used which works on the principle of negative pressure i.e., vacuum. The removal of water traces under vacuum helps in maintaining the quality of essential oil. This purified oil was further used in UV-Visible and NMR spectroscopy for the identification of phytochemicals.

UV–Visible absorption spectra

The function of any optical spectrometer is to get the data of wavelengths at which absorption occurs along with the level of absorption at each and every wavelength. The spectrum so obtained is presented as a chart showing relationship between wavelength and absorption level. No absorbance means zero optical density and 99 % absorbance means optical density of 2.0. This density is directly linked with the number of absorbing molecules in the light beam of selected wavelength. The necessary correction in the value should be made to include other operational parameters. Only then we can evaluate the spectra of different compounds.

The molar absorptivity represents the true absorption value and quite beneficial when evaluating the spectra of different compounds and finding the comparative strength of chromophore which is nothing but light absorbing function. The expression for molar absorptivity (ε) is

$$\varepsilon = \frac{A}{cl}$$

Where:

A= Absorbance,
c= Concentration of sample (moles/liter),
l=Path length of light through the sample (cm).

In UV spectroscopy, for the presence of α, β unsaturated carbonyl groups in terpenoids, an intense absorption band was obtained at 210 to 330 nm and isolated carbonyl group showed aband at 270 to 300 nm. The species *Tagetes erecta* L. is having a UV-Visible spectra with maxima found 254nm, 280nm, 470nm and 510nm (Table 1). This table also shows that in the UV-Visible spectra of *Tridax procumbens*, maxima were found at 210nm and 280nm.

The UV-Visible profile of both species showed that different absorption bands with the range of 200 nm to 750 nm demonstrating the presence of tannins,

Table 1. UV spectra of selected plant species.

S.No.	Plant name	Observation
1	*Tagetes erectes* L.	Maxima found at 254nm, 280nm, 470nm and 510nm
2	*Tridax procumbens* L.	Maxima found at 210nm and 280nm

saponins, terpenoids and flavonoids. The presence of phytochemicals by recognizing compounds having π-bonds, lone pair of elections, π-bonds, aromatic ring and chromophores can be detected by this technique.

The peaks at 470nm and 510nm noticed in the present work showed the peaks occurring at 400nm to 550nm which in turn pointed towards the presence of Terpenoids (Saxena and Saxena 2012, Renuka *et al.* 2016).

NMR spectroscopy

NMR spectroscopy is a type of spectroscopy by which one can determine the quality and purity of a sample and the molecular structure of a compound. This technique is based on the nuclear magnetic resonance of atoms of the sample being examined. The reality that the nuclei of the large number of the atoms shows spin and all nuclei electrically charged has been based on this technique. The NMR spectroscopy depends upon the fact that most isotopes of the elements possess gyromagnetic properties, as they behave like tiny spinning bar magnet. In the radio frequency region, the absorption of electromagnetic radiation is determined by NMR spectroscopy.

Electrical charged nuclei of atoms having spin generate magnetic field. The return of electrons from excited to the ground state causes the transfer of energy from ground to excited state in the presence of external magnetic field. This further causes the emittance of radio frequency. The NMR spectrum is the result of this emitted radio frequency. The strength of the magnetic field is directly related with emitted radio frequency.

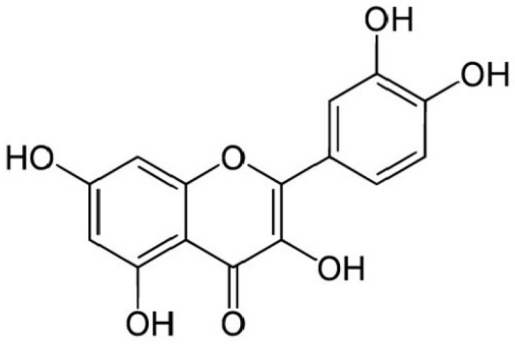

Fig. 1. Structure of Quercetin.

Table 2. 1H-NMR spectroscopic data of compounds extract of *Tagetes erecta* ((500MHz for 1H) recorded in CD3OD).

Position	δH	δH
1	5.48 (1H,t,8.5)	5.27 (1H,d, 5.0)
2	3.12 (1H,dd, 8.0), 3.38 (1H,dd, 8.0)	5.33 (1H,d, 5.0)
3	7.68 (1H,s)	8.18 (1H,s)
4	6.27 (1H,s)	6.32 (1H,s)
5	2.54 (3H,s)	2.61 (3H,s)
6	5.32 (1H,s), 5.28 (1H, s)	5.36 (1H,s),5.41 (1H,s)
7	4.49 (1H,d,10.0), 4.22 (1H,d)	4.16 (1H,d), 4.23 (1H,d)
8	---	4.54 (1H,d,H-10)

The 1H-NMR spectrum (Table 2) displayed the occurrence of the signals of two singlet aromatic protons at δH 8.18 (1H, s, H-4) and 6.30 (s, H-7), an AB type of methylenic signals at δH 4.23, 4.16 (2H, d, J = 10.0 Hz and J = 15.0 Hz, H-14), one singlet acetyl group at δH2.61(3H,s,H-11),two coupled heterocyclic protons at δH5.33 (1H,d,J =5.0Hz, H-2) and 5.17 (1H,d,J=5.0Hz,H-3), two germinal olefinic protons at δH 5.41 and 5.36 (2H, eachs, H-13), and one anomeric proton at δH4.54 (1H,d,J=5.0 Hz,H-10).

The method discussed in this work provides an easy and simple way to simultaneously test Quercetin. UV spectra of Quercetin shows λmax 369nm (Wang *et al.* 2019, Sumbe *et al.* 2021) (Fig. 1).

CONCLUSION

The major forms of terpenes occurring in nature are hydrocarbons, alcohols and their glycosides, ethers, aldehydes, ketones, carboxylic acids and esters.

Substituted terpene hydrocarbons, are known as terpenoids. These compounds are broadly used in pharmaceuticals,food and cosmetic industries. The purpose of the research work was to isolate the terpenoids from *Tridax daisy* or coat buttons and *Tagetes erecta* plants of family Compositae, alternatively known as Asteraceae, to explain the nature of functional group and phytochemical properties, and

to ascertain the curative properties of terpenoids. Phytochemicals are not necessary nutrients and may not be essential for the human body for supporting life, but they have some very vital characteristics to stop or to fight some common diseases. Most of these benefits of phytochemicals suggest a feasible role in the prevention and treatment of disease. Various applications especially for medicinal require the use of plant species. Ever since ancient times, the essential oils/fractions from herbal medicinal plants played a vital role in food, drug formulations and perfumery. Most essential oils are reported to possess antioxidant, anti-inflammatory and antimicrobial activities. The group of natural products which have been derived from five- carbon isoprene units is known as terpenoids. The UV-Visible profile of both species under study showed different absorption bands with wave lengths from 200nm to 750nm indicating the presence of tannins, saponins, flavonoids and terpenoids. Because of their functional groups and basic carbon skeletons, multicyclic structures of most terpenoids change from one another. Such types of natural lipids are available in every class of living things, and therefore considered as the largest group of natural products. Terpenoids have anti-inflammatory, antibacterial, antiviral, antmalarial, anticancer, pro-trans dermal absorption, cardiovascular disease prevention and treatment, and hypoglycemic properties. Terpenoids also offer a wide range of potential uses, including insect resistance, immune regulation, antioxidation, antiaging, and neuro protection.

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