A Study of Phytochemistry in Medicinal Extract of Terpenoids

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Abstract **- Terpenes are basic hydrocarbons, while terpenoids are adjusted class of terpenes with various practical gatherings and oxidized methyl bunch moved or eliminated at different positions. • Plant species is used in various applications especially for medicinal purposes. They are significant element of the world cultural heritage; they resort for treating health problems. The objective of this exploration work is to disconnect the terpenoids from Tridax daisy or Coatbuttons and Tagetes erecta of family Compositae, then again known as Asteraceae . Scientific study shows that thiophenes, natural phytochemical that include sulfur containing rings may be the active ingredients they have been shown to kill gram negative and gram positive bacteria in vitro. This marigold may help protect certain crop plants from nematode pests when planted in field. It is most effective against the nematodes species penetrans. The phytochemical studies of its different parts have resulted in the isolation of various chemical constituent such flavonoids , carotenoids and triterpenoids. It has been shown to contain methyl-3,5-dihydroxy-4-methoxy benzoate, quercetin, thienyl and ethyl gallate.**

Index Terms **- Terpenes, tissues, Tridax daisy, enzymatic, terpenoids etc.**

I.INTRODUCTION

The term “medicinal plant” include various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses. The word “herb” has been derived from the Latin word, “herba” and an old French word “herbe”. Now days, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a nonwoody plant. Earlier, the term “herb” was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities. Plants have been used for medicinal purposes long before prehistoric period. Ancient Unani manuscripts Egyptian papyrus and Chinese writings described the use of herbs. Evidence exist that Unani Hakims, Indian Vaids and European and Mediterranean cultures were using herbs for over 4000 years as medicine. Indigenous cultures such as Rome, Egypt, Iran, Africa and America used herbs in their healing rituals, while other developed traditional medical systems such as Unani, Ayurveda and Chinese Medicine in which herbal therapies were used systematically. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in AYUSH systems in INDIA. Ayurveda, Unani, Siddha and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda and Unani Medicine are most developed and widely practiced in India. Recently, WHO (World Health Organization) estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants. As per data available over three-quarters of the world population relies

mainly on plants and plant extracts for their health care needs. More than 30% of the entire plant species, at one time or other were used for medicinal purposes. It has been estimated, that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as India and China, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes. The ancient scholars only believed that herbs are only solutions to cure a number of health related problems and diseases. They conducted thorough study about the same, experimented to arrive at accurate conclusions about the efficacy of different herbs that have medicinal value. Most of the drugs, thus formulated, are free of side effects or reactions. This is the reason why herbal treatment is growing in popularity across the globe. These herbs that have medicinal quality provide rational means for the treatment of many internal diseases, which are otherwise considered difficult to cure. Medicinal plants such as Aloe, Tulsi, Neem, Turmeric and Ginger cure several common ailments. These are considered as home remedies in many parts of the country. It is known fact that lots of consumers are using Basil (Tulsi) for making medicines, black tea, in Pooja and other activities in their day to day life. In several parts of the world many herbs are used to honour their kings showing it as a symbol of luck. Now, after finding the role of herbs in medicine, lots of consumers started the plantation of tulsi and other medicinal plants in their home gardens. Medicinal plants are considered as a rich resources of ingredients which can be used in drug development either pharmacopoeial, non- pharmacopoeial or synthetic drugs. A part from that, these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values. Some of these plants include ginger, green tea, walnuts, aloe, pepper and turmeric etc. Some plants and their derivatives are considered as important source for active ingredients which are used in aspirin and toothpaste etc. Apart from the medicinal uses, herbs are also used in natural dye, pest control, food, perfume, tea and so on. In many countries different kinds of medicinal plants/ herbs are used to keep ants, flies, mice and flee away from homes and offices. Now a day's medicinal herbs are important sources for pharmaceutical manufacturing. Recipes for the treatment of common ailments such as diarrhoea, constipation, hypertension, low sperm count, dysentery and weak penile erection, piles, coated tongue, menstrual disorders, bronchial asthma, leucorrhoea and fevers are given by the traditional medicine practitioners very effectively. Over the past two decades, there has been a tremendous increase in the use of herbal medicine; however, there is still a significant lack of research data in this field. Therefore, since 1999, WHO has published three volumes of the WHO monographs on selected medicinal plants.

II.MATERIAL AND METHODS

a. Extraction, Isolation and Purification Methods

The species being studied were collected followed by washing and drying at room temperature. After thorough drying the material was meshed and powdered.

This was followed by extraction using n-Hexane , petroleum ether and chloroform using soxhlet apparatus. The crude extract was evaporated to dryness at low temperature and pressure using vacuum evaporator.

Steam distillation

Steam distillation is used to obtain oils and extracts and, involves the inflow of steam into distillation chamber containing raw plant material. Oil, in nature sacs , is released and is carried out of the chamber.

This is followed by condensation in chilled chamber, wherein steam gas converted in to water. The non mixing of oil and water (Hydrosol) makes then separated of extract easier and feasible.

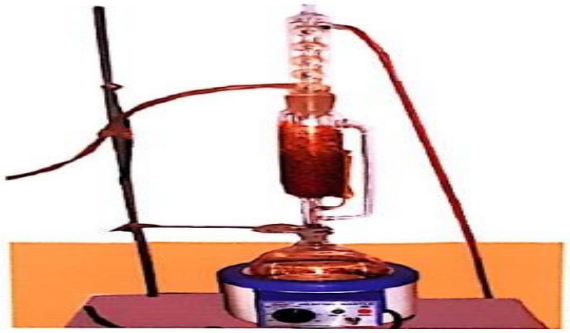


Figure 1. Showing Soxhlet apparatus

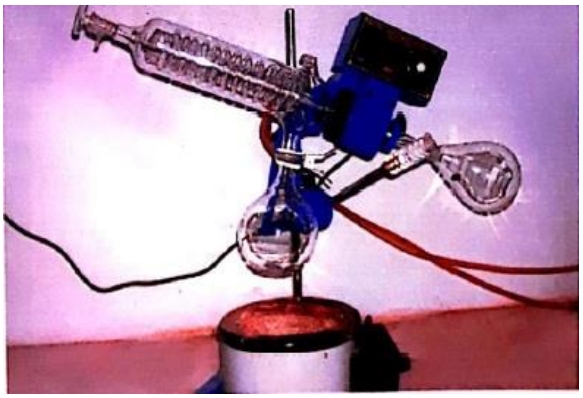


Figure 2: Showing Vaccum evaporator

Plate II

Table 1: Showing Percentage Loss in weight on drying and % of ash contents.

Name of plant	Weight of plant material	Weight of plant after drying	Loss in weight on drying	Percentage loss in weight	Ash contents
Tagetes erectes Linn.	2680 gram	535 gram	2145 gram	81.9% or 82%	0.064 %
Tridax procubens Linn.	2550 gram	460 gram	2090 gram	80.04%	0.072 %

Table 2: Showing Percentage yield of crude extract.

Name of plant	Solvent	Weight of powdered material	Volume of Solvent	Weight of extract	Percentage yield
Tagectes erectes Linn.	n-Hexane-Petroleum ether/Chloroform	590 gram	750 ml	2.5 gram	0.42%
Tridax procumbens Linn.	n-Hexane/Petroleum ether Chloroform	600 gram	750 ml	2.9 gram	0.48 %

Preliminary Test: The following test provides a Preliminary detection of crude extract.

(A) Libermann- Burchard Test: It involves treating the extract with few drops of acetic anhydride followed by boiling and subsequent cooling. Then add small amount of conc. H_2SO_4 along the sides of the test tube. Formation of brown ring at the junction two layers and deep red colour indicates the presence of terpenoids.

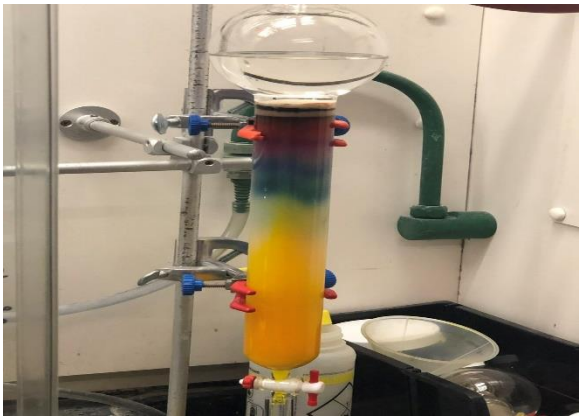
(B) Salkowski test

In test small amount of crude extract is treated with few drops of conc. H2SO4 . formation of yellow colour at lower layer indicates presence of terpenoids. The aforesaid test are followed by following purification steps :

1. Column Chromatography
2. Thin Layer Chromatography
3. High Performance Liquid Chromatography

1. Column Chromatography:

Column Chromatography was used the crude extracts using glass column Chromatography as shown in the fig.



The small sized glass column was thoroughly washed with detergent and water so as to make grease free and was dried.

silica Gel was used as packing material and the slurry was prepared using ethyl acetate . This was followed by frequent stirring so as to obtain a homogenous sol. The crude extract was pipette so as to apply on the column wall. A cotton plug soaked in the solvent was put of the top of the column so as that the concentrated

extract could easily drained from the pipette. It was ensured that it did not touch the silica gel or the walls of the container. After whole of the crude extract has been absorbed upon the column top , the vacant space present it was filled using solvent and the column was allowed to run. At regular of time the supply of the solvent and combinations of solvent was replenished from a separating funnel. The various fractions thus obtained were collected in small glass vials.

Table 3: Showing Column fractions of Tagetes erectes Linn.

Name of Plant	Solvent system	Fractions	Colour of fractions	Wt. Of fractions
Tagetes erectes Linn.	Chloroform :Benzene (1:1)	Fr. – I Fr. –II Fr. – III Fr. –IV Fr. – V	Brown Light brown Yellow Light yellow Light green	0.13 mg 0.11 mg 0.234 mg 0.008 mg 0.062 mg

Table 4: Showing Column Fractions of name of species of flower

Name of Plant	Solvent system	Fractions	Colour of fractions	Wt. Of fractions
Tridax procumbens Linn.	n-Hexane: Chloroform (3:2)	Fr. – I Fr. –II Fr. – III Fr. –IV Fr. – V	Light blue Dark Blue Bluish Light brown Light blue	0.15 mg 0.19 mg 0.16 mg 0.10 mg 0.007 mg

Figure 3. Showing Column Chromatography

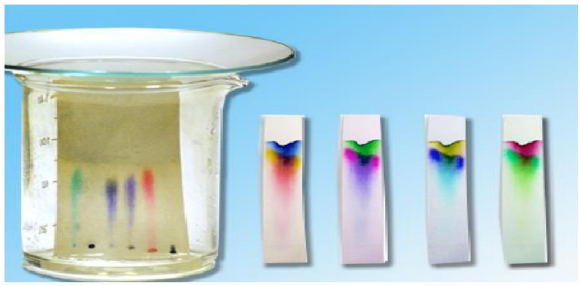


Figure 4. Showing Thin layer Chromatography

Plate III

Thin Layer Chromatography

The fractions purified using column were assessed using Thin Layer Chromatography. Glass plates (20 x5 cm) were used . They washed using soap and water followed by cleaning with acetone. It should be ensured that the plats were not touched with necked hands.

20g of silica gel was mixed in 20 x20cm of water so as to prepare slica gel slurry . The slurry was stirred was properly and was stirred in the rectangular hopper followed by passing over the plates.

The hopper has a trailing face that can be adjusted to provide a layer of 0.25 mm thickness. The slurry was spread uniformly and the plates were allowed to dried at 100 C in oven.

These dried plates were used for further experimental, wherein the purified samples were applied using capillary tube in the form of a spot marked at 1 cm from the edge of the plate. After applying the spot it was allowed to dry and was kept in glass bottles containing approporiate solvent . Type of solvent varied according to the nature of material being analysed . In general Harborne method is used for determining type of solvent. Location of spot was done using iodine / UV chamber and this solvent was allowed to run position of spot is measured using centimeter scale. R_f is calculated using formula

$$Rf = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvant front}}$$

Chromatographic analysis of crude extract

This was achieved by dissolving the extract of targets erects Linn. in solvent system comprising chloroform and benzene in ratio(1:1).

The table VI shows the number of spots along with their R_f values. Similarly the crude extract of Tridax procumbers Linn. was dissolved in solvent system comprising of n- Hexane and chloroform in 3:2 ratio. Table VII shows the number of spots and obtained R_f Values.

Table 5: Showing Rf value of Name of species of flower

Solvent System	Spot	Colour characterization			Rf Value
		In visible light	In UV light	In Iodine Chamber	

Chloroform :Benzene (1:1)	1 2 3 4 5 6	Brown Light brown Yellow Light Yellow Light green Dark green	Green Light green Brown Purple Dark Green Green	Green Yellow Light yellow Brownish green Dark green Green	0.23 0.60 0.78 0.88 0.90 0.96

Table 6: Showing Rf value of Name of Species of flower

Solvent System	Spot	Colour characterization			Rf Value
		In visible light	In UV light	In Iodine Chamber	
n-Hexane: Chloroform (3:2)	1 2 3 4 5	Light blue Dark blue Bluish Light Brown Light blue	Greenish blue Blue Florescent Green Reddish Blue	Dark blue Dark blue Bluish Dark brown Dark blue	0.18 0.24 0.28 0.33 0.40

Figure 5 : showing TLC inidoine chamber

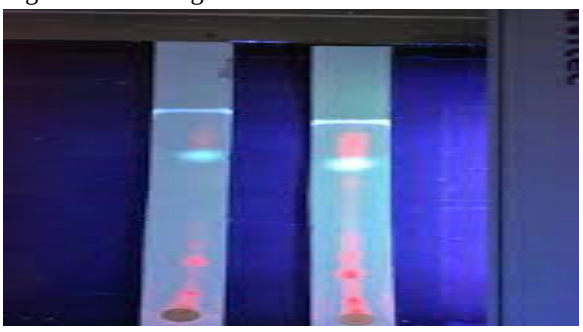


Figure 6 : showing TLC in UV chamber

Purified by High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is a Chemistry-based tool for quantifying and analyzing mixtures of chemical compounds.

High Performance Liquid Chromatography (HPLC) is an analytical technique for the separation and determination of organic and inorganic solutes in any samples especially biological, pharmaceutical, food, environmental, industrial, etc. In a liquid chromatographic process a liquid permeates through a porous solid stationary phase and elutes the solutes into a flow-through detector.

The stationary phase is usually in the form of small-diameter (5-10 mm) uniform particles, packed into a cylindrical column.

The typical column is constructed form a rigid material (such as stainless stell or plastic) and is generally 5-30 cm long and the internal diameter is in the range of 1-9 mm.

Table 7:Showing HPL of Name of species of flower

pK No.	Time	Area	MK	Conc.
1.	8.292	32813	V	2.8955
2.	10.817	15166	V	1.3718
3.	11.858	67785	V	6.1311
4.	13.86	212954	V	19.2615
5.	16.233	55737	V	5.8414
6.	17.625	112998	V	18.1934
7.	22.888	28618	V	2.5884
8.	25.825	92166	V	8.3363
9.	31.875	49763	V	4.581
10.	34.808	146221	V	13.2255
11.	39.3	122439	V	11.0745
12.	44.342	62485	V	5.6517
13.	50.008	23718	V	2.1452
14.	68.05	83261	S	7.3309
15.	76.908	570	T	0.0516
Total		1105594		100

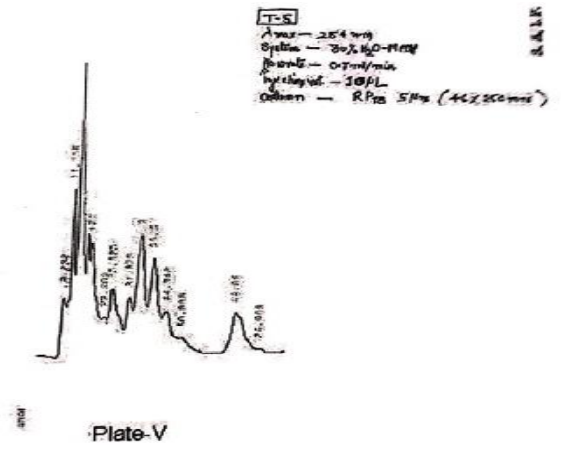
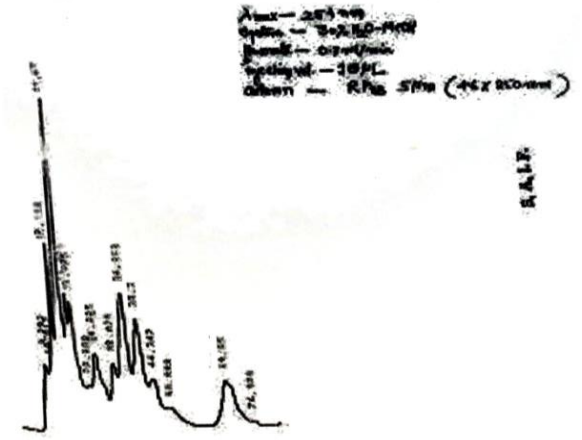


Table 8: Showing HPLC of Tridax procumbens Linn.

pK No.	Time	Area	MK	Conc.
1.	9.292	32813	V	2.8955
2.	10.817	11166	V	1.3718
3.	12.858	67785	V	6.1311
4.	15.457	202954	V	19.2615
5.	16.233	58737	V	5.8414
6.	19.725	82698	V	18.1934
7.	22.888	30618	V	2.5884
8.	24.899	90166	V	8.3363
9.	32.875	65783	V	4.581
10.	34.808	116221	V	13.2255
11.	38.345	152439	V	11.0745
12.	44.342	32485	V	5.6517
13.	50.008	53718	V	2.1452
14.	69.052	53261	S	7.5309
15.	76.908	670	T	0.0516
Total		1105594		100



III.CONCLUSION

Natural products are the compounds which isolate from different natural sources such as plants, animals, microbes, insects, plant pathogens, and endophytes and marine. These are known as secondary metabolites since they are formed due to the enzymatic resections of primary metabolites (amino acids, sugars, vitamins, etc.). Terpenes belong to the biggest class of secondary metabolites and basically consist of five carbon isoprene units which are assembled to each other (many isoprene units) by thousands of ways. An optical spectrometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength. The resulting spectrum is presented as a graph of absorption at each wavelength. Absorbance usually ranges from (no absorption) to 2 (99% absorption), and is precisely defined in context with spectrometer operation. Because to absorbance of a sample will be proportional to the number of absorbing molecules in the spectrometer light beam (e.g. their molar concentration in the sample tube), it is necessary to correct the absorbance value for this and other operational factors if the spectra of different compounds are to be compared in a meaningful way. Plant species is used in various applications especially for medicinal purposes. They are significant element of the world cultural heritage; they resort for treating health problems. It is to isolate the terpenoids from coatbuttons or toidax daisy and tagetes erect a maxican marigold and provide the detail phytochemistry of two types of terpenoids and their functional group present.

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