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COLUMN CHROMATOGRAPHIC SEPARATION OF BIOACTIVE COMPOUNDS FROM *TAGETES ERECTA LINN.*

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ABSTRACT: Traditional medicines in many developing countries rely on the plant phytochemicals as a source of drug in curing many diseases. *Tagetes erecta Linn.* is an ornamental plant containing valuable therapeutic phytochemicals. In the current investigation, ethanol extraction of the dried flower sample was subjected to column chromatographic separation. Around 185 fractions were separated and recorded with different R_f values. The fractions were analyzed qualitatively and it was proved that the flower sample was rich in tannins, saponins, carbohydrates, alkaloids, quinines, terpenoids, phenols and coumarins.

INTRODUCTION: Since time immemorial, Man utilized the potent biochemicals from plants and converted them into marvelous assortments of industrial use in various sectors like pharmacy, chemical, dye, pesticides etc¹. The plant based secondary metabolites are taxonomically distinct² and are highly potential and beneficial in the field of medicine³. Indian ayurvedic system (basically plant origin) has been proved to be predominantly successful in curing many type of diseases⁴ and the plant extract has been used as anti – inflammatory agent, astringent, aphrodisiac, liver tonic etc. and used to treat asthma, ulcers, emaciation, insomnia, senile dementia and bronchitis⁵. Plant phytochemicals can be derived from any part such as bark, leaves, flowers, roots, fruits, seeds etc.³ in combination with secondary metabolites which are taxonomically distinct for any particular plant².

The naturally occurring phytochemicals includes alkaloids, flavonoids, tannins, steroids, phenolics, terpenes, volatile oils etc., which are used in medicines, dyes, cosmetics, flavors and foods⁶. Many research studies proved that natural antioxidants from plant sources can effectively inhibit oxidation of food and reduce the risk of age dependent diseases^{7, 9}. Methanolic extracts of *Polyathia longifolia* have yielded 20 known and two new organic compounds which showed highly cytotoxic properties⁹ and the ethanolic extraction separation possessed a potent nitric oxide radical scavenging activities^{10, 11}.

The genus *Tagetes* consists of 56 species¹² which was originated in North and South America and widely cultivated in other Asian countries like Bhutan, China, Nepal, India etc.¹³ which has a strong historic evidence for its religious and therapeutic value¹⁴, in the treatment of hiccups, dermatitis, athlete's foot, colitis, wound burns etc¹⁵. These plants are rapid growing annuals with the height ranging from 6 inches (dwarf plants) to 3 ft. Bearing large pompon- like¹⁶ floral heads of 4-6 cm diameter having both ray and disc florets (Blooms are golden, orange, yellow etc.). Since

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ancient era, various parts of *Tagetes* has been used as skin wash dye¹⁷, food additives¹⁸, fodders¹⁹, pesticides²⁰. The flower part of the plant are efficient therapeutic in the treatment of epileptic fits (Ayurveda), astringent, carminative, scabies etc^{21,22}.

MATERIALS AND METHODS:

The ornamental plant, *Tagetes erecta* Linn. Belongs to Asteraceae family. The whole plant has been proved to have high therapeutic value²³. The flower Samples were segregated separately from the plant. The dried powdered flower sample was subjected for ethanol extraction process. The extracted sample used for separation of phytochemicals by standard column chromatographic method.

Isolation methods with Column Chromatography:

Ethanol extract (20g) was subjected to column chromatography on silica gel (100 – 200 mesh – Merck) packed and eluted with mixture of n-Hexane, chloroform, ethyl acetate, ethanol, methanol and water of increasing polarity to obtain fractions.

Twenty grams of the method extract was chromatographed over silica gel column (100-200 mesh). The admixture was packed on a silica gel column (Merck, India) and eluted started with 100% hexane and increased with solvent polarity chloroform, ethyl acetate, ethanol, methanol, and water in the ratio of 90:10, 80:20, 70:30, 50:50, 30:70, 20:80, 10:90. Ethanol (70:30) gave a colorless compound and on further purification with acetone and methanol the isolated compound yield was 200mg.

Thin Layer Chromatography:

The Thin Layer Chromatography was developed in Twin through chamber with silica gel 60 F₂₅₄ pre coated aluminium plate of 0.2 mm thickness using ethyl acetate: methanol (1:1) as the developing solvent system and Rf value was calculated. Distance moved by the molecule the spot)

$$\frac{\text{Distance moved by the molecule}}{\text{(Location by the spot)}}$$

$$\text{Rf Value} = \frac{\text{Distance moved by the molecule}}{\text{Distance moved by the mobile phase (Solvent Front)}}$$

Visualization was carried out by dipping the plate in vanillin sulfuric acid (1%) and heat on 105°C when the colour of the spot appeared distinctly.

RESULTS AND DISCUSSION: The dried powdered flower sample of *Tagetes erecta* linn. was subjected to ethanol extraction. About 20g of the extract was subjected to column chromatography on silica gel (100-200 mesh - Merck) pack as shown in **Fig 1**. The selection of solvents in a systematic order proves the effect of polarity on the extraction and the extracted phytochemicals²⁴. About 185 fractions with different Rf values were separated during column chromatography procedure. (**Table 1**).

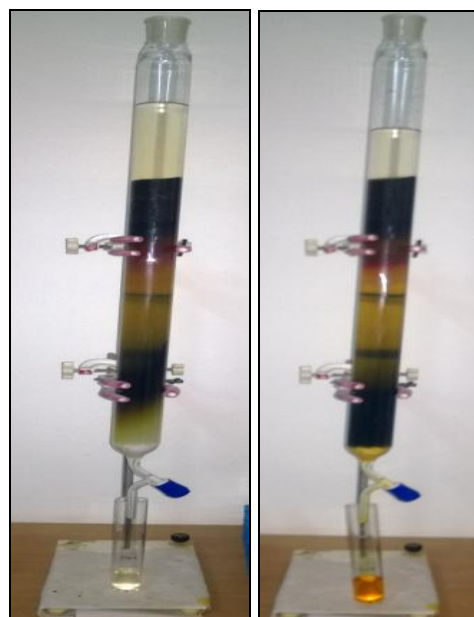


FIG 1: COLUMN CHROMATOGRAPHY OF TAGETES ERECTA LINN. FLOWER EXTRACT

The column fractions of *Tagetes erecta* Linn. flower sample was subjected to various qualitative phytochemical tests like carbohydrates, tannin, saponin, alkaloid, quinines, glycosides, terpenoids, phenols and coumarin as per standard procedure²³. It was evident that the fractions from 1 -58 showed positive result for alkaloid and terpenoid (**Table 2**) and the fractions from 59 – 80, 81-90 showed positive result for alkaloid, terpenoid and coumarin. It was also evident that the fractions form 91-165 showed positive result for alkaloids, terpenoids, phenols and coumarin but the fractions from 126 to 165 had tannins in addition. The fractions from 166 to 185 registered terpenoids and coumarin in their fractions (**Table 2**).

TABLE 1: R_f VALUES OF FLOWER EXTRACTION FRACTIONS OF TAGETES ERECTA LINN.

Fractions	R _f values
1-25	-
26-45	0.32, 0.46
46-58	0.30, 0.45
59-68	0.42, 0.56
69-72	0.41, 0.62
73-80	0.22, 0.25, 0.31
81-84	0.18, 0.25, 0.32
85-90	0.44, 0.45,
91-95	0.44, 0.45,
96-102	0.14, 0.33, 0.45,
103-108	0.56, 0.57, 0.58, 0.59, 0.66
109-113	0.57, 0.58, 0.60, 0.71,
114-117	0.32, 0.44, 0.50, 0.54,
118-121	0.19, 0.25, 0.44
122-125	0.25, 0.31, 0.38, 0.42
126-130	0.22, 0.32, 0.40, 0.44
131-134	0.20, 0.25, 0.31, 0.76
135-139	0.21, 0.25, 0.32
140-143	0.25, 0.32, 0.36
144-153	0.20, 0.24, 0.35
154-159	0.46, 0.51
160-165	0.44, 0.56
166-170	0.42, 0.69
171-175	0.40, 0.72
176-186	-

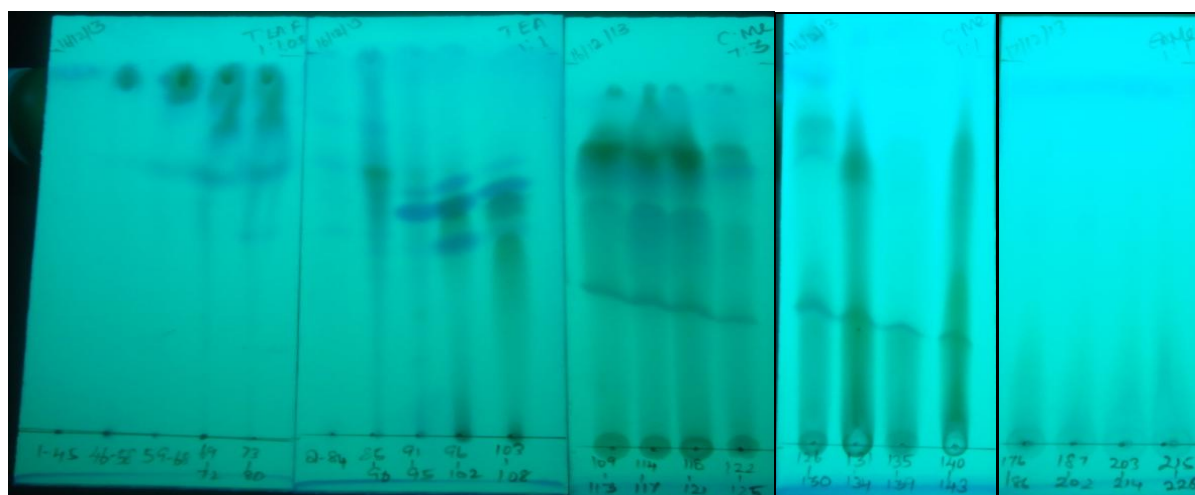


FIG 2: IDENTIFICATION OF CLEAR SPOTS FRACTIONS UNDER UV 254 nm

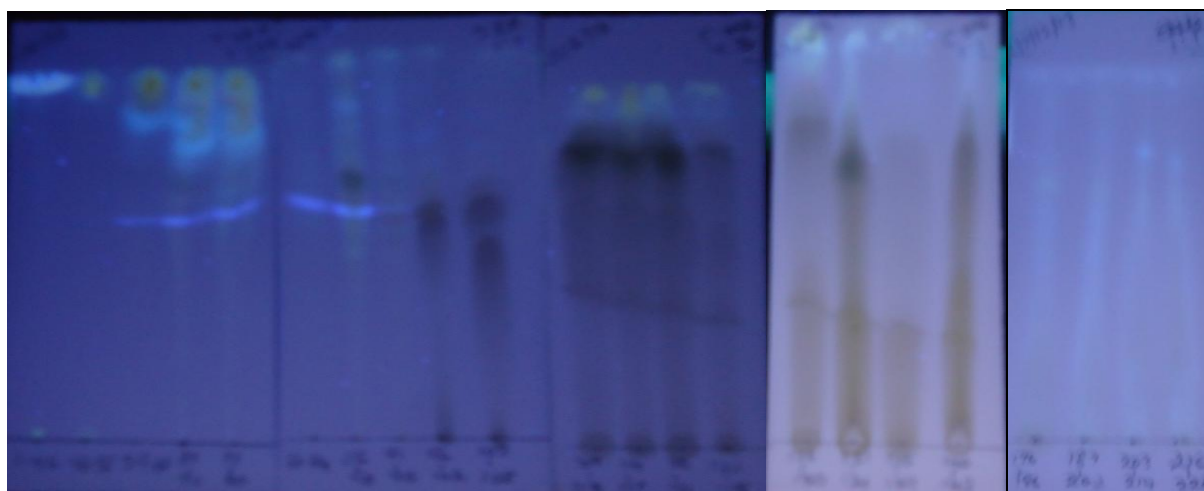


FIG 3: IDENTIFICATION OF SPOTS FRACTION UNDER UV 366nm

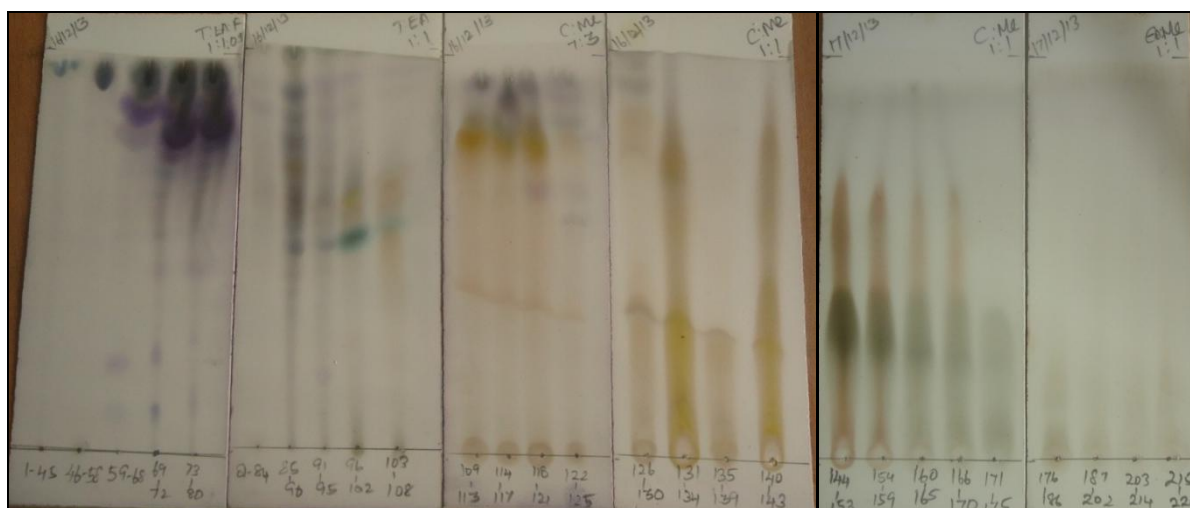


FIG 4: IDENTIFICATION OF SPOTS FRACTION UNDER VISIBLE LIGHT AT 512 nm

TABLE 2: PHYTOCHEMICAL ANALYSIS OF COLUMN FRACTIONS OF *TAGETES ERECTA* LINN.

Phytochemical test	Inference of Column Fractions of <i>Tagetes erecta</i> linn.							
	1-58	59-80	81-90	91-102	103-125	126-139	140-165	166-225
Carbohydrates	-	-	-	-	-	-	-	-
Tannins test	-	-	-	-	-	+	+	-
Saponin test	-	-	-	-	-	-	-	-
Alkaloid test	+	+	+	+	+	+	+	-
Quinones	-	-	-	-	-	-	-	-
Glycosides test	-	-	-	-	-	-	-	-
Cardiac glycosides test	-	-	-	-	-	-	-	-
Terpenoids test	+	+	+	+	+	+	+	+
Triterpenoids	-	-	-	-	-	-	-	-
Phenols	-	-	-	+	+	+	+	-
Coumarins	-	+	+	+	+	+	+	+

CONCLUSION: From the above investigation it is evident that *Tagetes erecta* Linn. Flower sample is rich in phytochemicals having high therapeutic value. On further isolation and purification of these bioactive compounds will pave way for discovery of new effective drug for curing various diseases in the future.

REFERENCES:

- Mahesh Chand Meena and Vidya Patni. Isolation and identification of flavonoid " Quercetin" from *Citrullus colocynthis* (Linn.) Schrad. Asian Journal Experimental Science. 2008; 22(1): 137-142.
- Wink M. Introduction Biochemistry, role and Biotechnology of secondary products. In: M Wink, Ed. Biochemistry of secondary product metabolism. CRC Press. Boca Raton, FL. 1999; 1-16.
- Gordon MC and Drauid JN. Natural product drug discovery in the next millennium. Pharmacological Biology. 2001; 39: 8-17.
- Sampath M and Vasanthi M. Isolation, structural elucidation of flavonoids from *Polyathia longifolia* and evaluation of antibacterial, antioxidant and anticancer potential. International Journal of Pharmacy and Pharmaceutical Science. 2013; 5(1): 336-341.
- Heim KE, Tagliaferro Ar, Bobliya DJ. Flavonoids antioxidants: chemistry, metabolism and structure activity relationships. Journal of Nutritional Biochemistry. 2002; 13:572-584.
- Abdul KK, Palwasha A, Ayeesha M, Safdar Ali K and Rasool BT. Response of plant parts and age on the distribution of secondary metabolites on plants found in Quetta. Pakistan Journal of Botany. 2009; 41(5): 2129-2135.
- Pausas JG and Austin M. Patterns of plant of plant species richness in relation to different environments: An appraisal. Journal of Vegetation Science. 2001; 12: 153-166.
- Burda S and Olszek. Antioxidant and antiradical activities of flavonoids. Journal of Agriculture and Food Chemistry. 2001; 49:2774-2779.
- Zou Y, Lu Y and Wei D. Antioxidant activity of flavonoid- rich extract of *Hypericum perforatum* L. *in vitro*. Journal of Agriculture and Food Chemistry. 2004; 52: 5032-5039.
- Chen, Chung-Yi, Fang-Rong, Chang Yao-Ching, Shib Tian-Lye, Hsieh Yi-Chen, Chia Huang-Yi. Cytotoxic

- constituents of *Polyathia longiflora* var. *pendula*. Journal of Natural Product. 2000; 63: 1475-1478.
11. Mani Rani S Rumana J, Mynol Islam Vauiyen MD, Israt Jahan B. *In vitro* nitric oxide scavenging activity of ethanol leaf extracts of four Bengaldeshi medicinal plants. Stamford Journal of Pharmacy Science. 2008; 1: 57-62.
 12. Nandita Dasgupta, Shivendu Ranjan, Proud Saha, Rahul Jain, Swati Malhotra and Arabi Mohamed Saleb MV. Antibacterial activity of leaf extract of Mexican Marigold *Tagetes erecta* against different Gram positive and Gram Negative bacterial Strains, Journal of Pharmacy Research. 2012; 5(8): 4201 – 4203.
 13. Cruz – Ramirez and Luis Alfredo. Mexican crops of agroalimentary importance, Advances in Agriculture and Food Biotechnology. 2006; 35-53.
 14. Abdul Halim M. The use of plants in traditional health care practice of the shaiji community in Southwestern Bangladesh, Journal of Tropical Forest Science. 2007; 19(3): 168 – 175.
 15. Dormans HJD and Deans SD. Antimicrobial agents from plants; Antibacterial activity of plant volatile oils, Journal of Applied Microbiology. 2000; 88(2): 308-316.
 16. Chowdhury MSH. Use of plants in healthcare: A traditional ethno-medicinal practice in rural areas of Southeastern Bangladesh, International Journal of Biodiversity Science and management. 2009; 5(1): 41-51.
 17. Nikkon F, Habib MR, Saud ZA, Karim MR, Ray AK and Zaman S. Toxicological evaluation of chloroform fraction of flower of *Tagetes erecta* L. on rats. International Journal of Drug Development and Research. 2009; 1(1): 161-162.
 18. Nilani Packianathan and Saravanan Karumbayaram. Formulation and evaluation of herbal hair dye. An ecofriendly process. Journal of Pharmacy Science and Research. 2010; 2(10): 648-656.
 19. Richard Cantrill. Lutein from *Tagetes erecta* L. for use in foods for particular nutrition uses. The EFSA Journal. 2006; 315(1): 1-12.
 20. Rosa Martha Perz and Gutierrez. Antioxidant activity of *Tagetes erecta* essential oil. Journal of the Chilean Chemical Society. 2006; 51(2): 883-886.
 21. Farjana Nikkon. Insecticidal activity of flower of *Tagetes erecta* L. against *Tribolium castaneum* (Herbst.). Res Journal of Agriculture and Biological Science. 2009; 5(5): 748-753.
 22. Roberta Piccaglia, Mauro Marotti and Silvia Grandi. Lutein and lutein ester content in different types of *Tagetes patula* and *T. erecta*. Industrial crops and production. 1998; 8(1): 45-51.
 23. Devika R and Justin Koilpillai. Phytochemical screening studies of bioactive compounds of *Tagetes erecta*. International Journal Pharmacy and Biosciences. 2012; 3(4): (B): 596-602.
 24. Sunil H Ganatra, Shweta P, Durge and Patil SU. Preliminary phytochemicals investigation and TLC analysis of *Ficus racemosa* leaves. Journal of Chemistry and Pharmacology. 2012; 4(5): 2380-2384.

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