Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 524-527
Received: 11-05-2017
Accepted: 12-06-2017

Sandeep Kumar Rajvanshi
Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, (A Central University) Vidya-Vihar Rae Bareli Road, Lucknow, Uttar Pradesh India

Deepa H. Dwivedi
Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, (A Central University) Vidya-Vihar Rae Bareli Road, Lucknow, Uttar Pradesh India

Correspondence
Sandeep Kumar Rajvanshi
Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, (A Central University) Vidya-Vihar Rae Bareli Road, Lucknow, Uttar Pradesh India

Phytochemical screening studies of bioactive compounds of African marigold (*Tagetes erecta* L.)

Sandeep Kumar Rajvanshi and Deepa H Dwivedi

Abstract
Ever since Rig-Veda, various parts of plant sources were used as nutraceutical, food supplements, traditional medicines, the major constituent in modern medicines and Ayurveda India is known for its vast diversity of plants species and currently about 2500 medicinal plants are used in various sectors of manufacturing industries African marigold (*Tagetes erecta)* has been used for medicinal purposes since ancient times. The medicinal values of the bioactive constituents of such as alkaloids, tannins, flavonoids and phenolic compounds plants have a definite physiological action on the human body The oil extracted from African marigold plant parts such as fresh flower, dry flower, fresh leaves and dry leaves by two methods, hydro-distillation and solvent extraction Total thirteen phytochemical tests were carried out. Out of which 6 phytochemicals were identified in all the samples, which was tested.

Keywords: Phytochemical, bioactive compounds, African marigold, *Tagetes erecta*

1. Introduction
Tagetes is a genus (family Asteraceae) containing about 50 species of annual or perennial herbaceous plant. The plant *Tagetes erecta* Linn. locally known as Genda Phul (Marigold). It is stout, branching herb, native to Mexico and other warmer parts of America and neutralized elsewhere in the tropics and subtropics including India and Bangladesh. These are rapid-growing annual flowering plants in height ranging from dwarfs of 6-8 inch, to medium and taller and erect-growing plants with heights from 10 in to 3ft, bearing large pompon-like double flower up to 5 in across and has a shorter flowering period from midsummer to frost. It is very popular as a garden plant and yields a strongly aromatic essential oil (*tagetes* oil), which is mainly used for the compounding of high-grade perfumes (Khulbe 2015) [8].

Ever since Rig-Veda, various parts of plant sources were used as nutraceutical, food supplements, traditional medicines, the major constituent in modern medicines and Ayurveda. The medicinal values of the bioactive constituents of such as alkaloids, tannins, flavonoids and phenolic compounds plants have a definite physiological action on the human body (Tariq *et al.*, 2013 and Sangeetha *et al.*,2014) [14, 13]. Attention on the use and research of medicinal plant constituents has increased all over the world from traditional to modern and even in the industrial sectors (Ncube *et al*., 2008, Abu-Rabia 2005 and Raveen *et al*., 2010) [9, 1, 12]. India is known for its vast diversity of plants species and currently about 2500 medicinal plants are used in various sectors of manufacturing industries (Raja *et al*., 2010) [11].

The medicinal values of the bioactive constituents of such as alkaloids, tannins, flavonoids and phenolic compounds plants have a definite physiological action on the human body.

Studies of its different parts have resulted in the isolation of various chemical constituents such as thiophenes, flavonoids, carotenoids and triterpenoids. The plant *Tagetes erecta* has been shown to contain quercetagetin, a glucoside of quercetagetin, phenolics, syringic acid, methyl-3,5-dihydroxy-4- methoxy benzoate, quercetin, thienyl and ethyl gallate. Lutein is an oxycarotenoid, or xanthophyll, containing 2 cyclic end groups (one beta and one alpha-ionone ring) and the basic C-40 isoprenoid structure common to all carotenoids. It is one of the major constituents and the main pigment of *Tagetes erecta* (Gopi, 2012, Tereschuck, 1997 and Perich 1995) [5, 15, 10].

Medicinal plants are not only a major resource base for the traditional medicine & herbal industry but also provide livelihood and health security to a large portion of Indian Population. With this background of investigations, an attempt has been made to investigate the phytochemicals present in *Tagetes erecta* known as African marigold.

2. Materials and Methods

2.1 Collection Of Sample

Sample (fresh flower and leaves) were collected at full bloom stage from the Horticulture Research Farm of the Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, Lucknow, U.P. All fresh samples (flower and leaves) were subjected to Hydro-distillation and for Solvent extraction and the rest fresh sample were subjected to air drying for a week in shade, protected from direct sunlight and subsequently stored in air tight containers for phytochemical analysis.

2.2 Hydro-distillation (extraction of essential oil)

A known quantity of all the samples of marigold (*Tagetes erecta* L.) was subjected to hydro-distillation for 4 hours using a Clevenger apparatus. The oil extracted was subjected to phytochemical screening.

2.3 Solvent extraction of essential oil

All the samples such as fresh flower, dry flower, fresh leaves and dry leaves were subjected to solvent extraction by using soxhlet apparatus with the help of solvent n-hexane. For dry samples screening, the fresh samples were dried for a week and powdered using a grinder. The extracted oil was further used for phytochemical screening.

2.4 Phytochemical Tests

The samples were tested for several phytochemicals using standard procedures as quoted by Mostafavi and Pezhhanfar (2015) to identify the phytochemical.

2.4.1 Test for Terpenoids (Chloroform test)

To 0.5ml of sample, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown colour at the interface indicated the presence of terpenoids.

2.4.2 Test for Protein (Biuret test)

To 0.5 ml of sample, 4% NaOH solution and few drops of 1% CuSO4 solution were added. Violet colour appears, indicated the presence of protein.

2.4.3 Test for Steroids

To 0.5ml of oil sample equal volume of chloroform was added and subjected with few drops of concentrated sulphuric acid where the appearance of brown ring indicated the presence of steroids

2.4.4 Test for Flavonoids (Alkaline reagent test)

0.5ml of sample, 1ml of 2N sodium hydroxide was added. Presence of yellow colour indicated the presence of flavonoids.

2.4.5 Test for Alkaloids

To 0.5ml of sample, 0.5ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green colour or white precipitate indicated the presence of alkaloids.

2.4.6 Test for Quinones

To.0.5ml of sample, 0.5ml of concentrated sulphuric acid was added. Formation of red colour indicated the presence of quinines.

2.4.7 Test for Glycosides

To 1ml of oil sample, 2ml of chloroform and 10% ammonia solution was added. Formation of pink colour indicated the presence of glycosides.

2.4.8 Test for Phenols

To 1ml of the sample, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green colour indicated the presence of phenols.

2.4.9 Test for Triterpenoids

To 0.5ml of sample, 0.5ml of Libemann – Buchard Reagent (aectic anhydride+concentrated sulphuric acid) was added. Formation of blue green colour indicated the presence of triterpenoids.

2.4.10 Test for Coumarins

To 0.5 ml of sample, 0.5ml of 10% NaOH was added. Formation of yellow colour indicated the presence of coumarins

2.4.11 Test for Carbohydrates

To 0.5ml of sample, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish colour indicated the presence of Carbohydrates

2.4.12 Test for Tannin

To 0.5ml of sample, 1ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicated the presence of tannins.

2.4.13 Test for Saponins (Froth test)

To 0.5ml of oil sample, 1ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicated the presence of saponins.

3. Results and Discussion

The results concerning the qualitative screening of the sample are presented in Table 1 and results of observation of phytochemical analysis are presented in Table 2. The qualitative analysis carried out revealed the presence of the phytonutrients namely terpenoids, alkaloids, flavonoids, quinones carbohydrates, tannins and coumarins, The identification of the above compounds supports the use of these oils in traditional medicine as these compounds have valuable antifungal, antibacterial and anti-inflammatory properties (Hassanshshian *et al.*, 2014; Gilani *et al.*, 2005).

Terpenoids was identified by the formation of red colour after adding chloroform and sulphuric acid in all the samples of flowers and leafs of *Tagetes* erecta L. essential oil extracted by both Hydro-distillation and Solvent extraction method. The bioactive compound flavonoid was identified by presence of yellow colour after addition of 2N sodium hydroxide in all the samples (Table 1 and 2). Test for alkaloid showed a positive result for almost all the samples of flowers and leaf where green colour developed after the addition of conc. hydrochloric acid which ensure that *Tagetes erecta* L. can be used to cure anthelminthic, ear inflammation and carminative. It was also observed in contrast to study of Burkil, 1984 [2] and Gills, 1992 [4]. There is no colour change in case of protein and steroids test which clearly indicates that it was completely absent in all the samples of leaf and flowers of *Tagetes erecta* L.(Table 1 and 2).Presence of carbohydrates was clearly observe in all the samples after adding Molisch's

reagent and few drops of concentrated sulphuric in the sample.

Blue green colour formation was observed only in the dry flower oil sample extracted by soxhlet apparatus which showed the presence of Triterpenoids in the Tagetes oil. The presence of coumarins in the all oil samples was tested by adding 0.5ml of 10% NaOH in sample, formation of yellow colour clearly showed the presence of coumarins in the all samples. Phenols was absent in all leaf samples whether extracted by hydro-distillation or by soxhlet apparatus. Presence of green colour indicating the presence of phenol in fresh and dry flowers essential oil extracted by both methods, while tannins were noticed only in fresh and dry flowers oil extracted by hydro-distillation method and dry flower oil extracted by soxhlet method. Negative results were obtained for phytochemicals such as glycosides and saponins in all oil samples extracted by both methods

Table 1: Qualitative Phytochemical screening of oil of Tagetes erecta L.

Phytochemical Tests	Hydro-Distilled Oil				Soxhlet Extracted Oil			
	Fresh Flower	Dry Flower	Fresh Leaf	Dry Leaf	Fresh Flower	Dry Flower	Fresh Leaf	Dry Leaf
Terpenoids	+	+	+	+	+	+	+	+
Protien	-	-	-	-	-	-	-	-
Steroids	-	-	-	-	-	-	-	-
Flavonoid	+	+	+	+	+	+	+	+
Alkaloid	+	+	+	+	+	+	+	+
Quinones	+	+	-	+	+	+	-	+
Glycosides	-	-	-	-	-	-	-	-
Phenols	+	+	-	-	+	+	-	-
Triterpenoids	-	-	-	-	-	+	-	-
Coumanins	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+
Tannin	+	+	-	-	-	+	-	-
Saponin	-	-	-	-	--	-	-	-

Table 2: Results of observations of phytochemical analysis of *Tagetes erecta* L.

Phytochemical Tests	Hydro-Distilled Oil				Soxhlet Extracted Oil			
	Fresh Flower	Dry Flower	Fresh Leaf	Dry Leaf	Fresh Flower	Dry Flower	Fresh Leaf	Dry Leaf
Terpenoids	Red brown color	Red brown color	Red brown color	Red brown color	Red brown color	Red brown color	Red brown color	Red brown color
Protien	No Change	No Change	No Change	No Change	No Change	No Change	No Change	No Change
Steroids	No Change	No Change	No Change	No Change	No Change	No Change	No Change	No Change
Flavonoid	Yellow colour	Yellow colour	Yellow colour	Yellow colour	Yellow colour	Yellow colour	Yellow colour	Yellow colour
Alkaloid	Green colour	Green colour	Green colour	Green colour	Green colour	Green colour	Green colour	Green colour
Quinones	Red colour	Red colour	No Change	Red colour	Red colour	Red colour	No Change	Red colour
Glycosides	No Change	No Change	No Change	No Change	No Change	No Change	No Change	No Change
Phenols	Green colour	Green colour	No Change	No Change	Green colour	Green colour	No Change	No Change
Triterpenoids	No Change	No Change	No Change	No Change	No Change	Blue green colour	No Change	No Change
Coumanins	Yellow colour	Yellow colour	Yellow colour	Yellow colour	Yellow colour	Yellow colour	Yellow colour	Yellow colour
Carbohydrates	Red colour	Red colour	Red colour	Red colour	Red colour	Red colour	Red colour	Red colour
Tannin	Blue colour	Blue colour	No Change	No Change	No Change	Blue colour	No Change	No Change
Saponin	No Change	No Change	No Change	No Change	No Change	No Change	No Change	No Change

4. Conclusion

The present phytochemical screening of the *Tagetes erecta* proved to contain flavonoid, terpenoids, alkaloid, quinones, phenols, and coumarins bioactive compounds which are of medicinal value and have a definite physiological action on the human body. Further investigation like quantification, extraction, purification and bioassay with these compounds will pave a way for confident approach towards therapeutic applications.

5. Acknowledgment

Authors are grateful to University Science Instrumentation Centre (USIC) Babasaheb Bhimrao Ambedkar University, Lucknow for providing Fourier Transform Infrared Spectroscopy (FT-IR) facility during the experimental work.

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