Pharmacogn J. 2018; 10(2):384-393
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

Isolation, Characterization and *in vitro* Pharmacological Activities of Tagetes Erectus Linn

Resmi S, Divya V Nair, Athulya Subhash, Rose Jose, Vishnu V, Subin Mary Zachariah*

ABSTRACT

Tagetes erectus (family: Asteraceae), commonly known as 'African marigold', is an ornamental plant widely distributed in India. The plant has been used in traditional medicines in India and elsewhere in the treatment of cold, bronchitis, rheumatism and inflammation. The flavonoid quercetin has been isolated from the methanol extract of the plant by column chromatography and identified by co-TLC, comparison of its melting point, UV, IR and NMR spectra. Quercetin content has been estimated as 1.22 (±0.26) % in the methanol extract of *Tageteserectus* whole plant based on HPTLC method using the solvent systemchloroform: methanol (8.5:1.5). The essential oil was isolated by hydrodistillation (oil yield 0.04% v/w) and 25 compounds comprising 93.9% of the constituents were identified by GC-MS analysis. Monoterpenoids Predominated the oil (78.5%), with piperitone as the major compound (40.1%) followed by terpinolene (12.7%) and limonene (12.0%). The *in vitro* antioxidant assays of the methanol extract showed moderate activity with IC50 values 109.45 (±1.22) μg/ml for DPPH radical scavenging assay and 201.53 (±4.48) μg/ml for superoxide scavenging assay and the marker compound quercetin showed IC50 values 26.40(±1.75) and 13.87 (±0.54) μg/ml respectively. Reducing power assay also showed moderate activity. Antibacterial activity of the methanol extract and the essential oil against gram positive and gram-negative bacteria were done using disc diffusion method. The essential oil showed remarkable activity against the tested organisms, while the extract showed only moderate activity.

Key words: Antibacterial activity, Disc diffusion method, DPPH radical scavenging assay, Flavonoids, Quercetin, Terpinolene.

Resmi S, Divya V Nair, Athulya Subhash, Rose Jose, Vishnu V, Subin Mary Zachariah*

Department of Pharmaceutical Chemistry and Analysis, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, Kochi-682041, Kerala, INDIA.

Correspondence

Dr. Subin Mary Zachariah

Department of Pharmaceutical Chemistry and Analysis, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, Kochi-682041, Kerala, INDIA.

Phone No: 0484-2857900,7903

E mail Id: subinmaryzachariah@aims.amrita.edu

History

- Submission Date: 12-10-2017;
- Review completed: 18-11-2017;
- Accepted Date: 27-12-2017

DOI : 10.5530/pj.2018.2.64

Article Available online
http://www.phcogj.com/v10/i2

INTRODUCTION

Phytochemistry deals with the biosynthesis, natural distribution, isolation, structural elucidation and biological functions of the variety of chemical compound found in the plant kingdom.It has application in food, dye, flavour, cosmetic, nutraceuticals and pharmaceutical industries.[1]

Flavonoids

It is the largest group of naturally occurring phenolic compound with a basic skeleton of 2- phenyl benzo-γ-pyrons. The various types of flavanoids differ in the degree of oxidation and in the pattern of substitution.

Essential Oil

Essential oil is the steam volatile component of plants responsible for the aroma of the plant and constitute mainly of terpenoids and some simple phenolics and hydrocarbons. essential oils are formed in glands or special cells. They may have some antibacterial or antiseptic action. These aromatic oils are used in the preparation of perfumes, cosmetics, and as medicine and as food adjuncts. Chemically essential oils are mixtures aliphatic hydrocarbons, monoterpens, sesquiterpene and their oxygenated derivatives. These terpenoids are composed of isoprene units. Monoterpenes are made of two isoprene units and sesquiterpenes by three isoprene units.

MATERIALS AND METHODS

Collection of plant

The plant was collected from Tirunelveli, Tamilnadu during February 2011 and authenticated by Dr. V. J. Dominic, Head, Dept. of Botany, S.H. College, Thevera, and a voucher specimen was deposited at Herbarium of Tropical Botanic Garden and Research Institute (Herbarium No. TBGT 66412).

Preparation of the Extraction

Plants were cut into small pieces, shade dried and powdered. The powder (50g) was subjected to soxhlet extraction for 72 h using petroleum ether, chloroform, and methanol successively. The extracts were concentrated under reduced pressure using a rotary evaporator to yield 0.402 g of pet-ether extract, 0.2348 g of chloroform extract and 0.1929 g of methanol extract respectively.[2]

Cite this article: Resmi S, Nair DV, Subhash A, Jose R, Vishnu V, Zachariah SM. Isolation, Characterization and *In vitro* Pharmacological Activities of Tagetes Erectus Linn. Pharmacog J. 2017;9(1):73-82.

Qualitative Chemical Evaluation

Chemical Test for the Detection of Chemical Constituents

A small quantity of petroleum ether, chloroform and methanol extract were taken for the following chemical test

Carbohydrates (Molish's test)

About 1 g of the extract is dissolved in 1 ml water. Two drops of 1% alcoholic solution of α-naphthol is added. Then, 1 ml conc. H2SO4 is carefully added along the sides of the test tube so that it forms a heavy layer at the bottom. Deep violet colour at the junction of two liquids indicates the presence of carbohydrates.

Steroids and Terpenoids (Leibermann-Burchard Test)

10 mg of extract is dissolved in dry CHCl3, into this acetic anhydride is added followed by a few drops of con. H^2SO^4. Green colour shows the presence of steroids and pink colour shows the presence of terpenoids.

3) Alkaloids

a) Dragendorff's test

10 mg of extract is dissolved in MeOH and few drops of Dragendorff's

Reagent are added. Orange red precipitate shows the presence of alkaloids.

b) Mayer's test

10 mg of the extract is dissolved in conc. HCl and filtered. A few drops of the solution are poured into the centre of a watch glass. Mayer's reagent is added in drops to the sides of the watch glass with the help of a glass rod. Formation of gelatinous white precipitate at the junction of two liquids shows the presence of alkaloids.

c) Wagner's test

To the extract add few drops Wagner's reagent (dissolve 2g of iodine 6g of potassium iodide in 100ml of water) gives reddish brown precipitate.

d) Hager's test

To the extract add few drops of Hager's reagent (saturated solution picric acid) gives yellow precipitate.

4) Flavonoids

a) Shinoda's test

10 mg of extract is dissolved in MeOH. Mg turnings are added into this,and then conc. HCl is added in drops. A pink color shows the presence of flavanoids.

b) One drop of above extract placed on a filter paper is exposed to ammonia vapour. A yellow colour shows the presence of flavonoids.

5. Saponins

Extract is dissolved in water and shaken well. Froth formation, which lasts for a long time, shows the presence of saponins.

6. Terpenoids

0.5 g of crude powder was dissolved in 5 ml of methanol. 2 ml of the extract was treated with 1 ml of 2, 4-dinitrophenyl hydrazine dissolved in 100 ml of 2M HCl. A yellow-orange coloration was observed as an indication of terpenoids.[3]

Standardisation

The standardisation of plant or plant parts was done. This is to check the whether the plant parts correspond to the various parameters mentioned in the WHO guidelines. The various parameters evaluated include Ash value, Bitterness value, Swelling and Foaming index etc.

High performance Thin Layer Chromatography

Test solution

15 mg of the methanol extract was dissolved in 1.5 ml of methanol.

Solvent system

Chloroform: methanol (8.5:1.5, v/v)

Procedure

Methanolic solution of the extract and standard compound of quercetin were applied on the Pre- coated TLC plate. 15µl and 20µl of the test solution and 1µl-4µl 0f standard quercetin were applied on the pre-coated silica gel F254 TLC plate (E. Merck) of uniform thickness of 0.2 mm. The plate was developed in the solvent system to 8 cm. The solvent system is Chloroform: methanol. After the development the plate was observed under 254nm.

Total phenolic content

Modified Folinciocalteu's reagent (FCR) colorimetric method was used for the determination of total phenolics.0.5ml of methanol extract was mixed with 5ml of Folinciocalteu's reagent. After 5min, 4ml of 20% of sodium carbonate solution was added. The mixture was allowed stand for 15 min and absorbance was measured at 765nm. Total phenolic were estimated as Gallic acid equivalent/g dry weight from the calibration curve using standard Gallic acid (20μg-100μg) in methanol.[4]

GC-MS Analysis of essential oil

Essential Oil isolation

Whole plant (fresh: 400g) were cut into small pieces and hydro distilled using a Clevenger-type apparatus for 3h. The oil obtained was dried over anhydrous sodium sulphate and stored in a refrigerator till further analysis.

Gas chromatography

The GC-FID analysis was carried out on a Varian CP-3800 gas chromatograph operated with a splitless injector, fitted with CP Sil 8CB fused silica capillary column (30 m x 0.32 mm, 0.25 µm film thickness) with FID detector using nitrogen as carrier gas at flow rate of 1mL/min. Temperature programme: injector temperature 220oC, oven temperature 50-230oC at 3oC/min, detector temperature 250oC. Relative percentages of the components were obtained from the peak area percent reports of volatiles from GC-FID.

Gas chromatography- Mass spectrometry

The GC-MS analysis was done on a Hewlett Packard 6890 gas chromatograph fitted with a cross-linked 5% PH ME siloxane HP-5 MS capillary column (30 m x 0.32 mm, film thickness 0.25 µm) coupled with a 5973-series mass selective detector. Splitless injection of 1.0 µl of essential oil with helium as the carrier gas at 1.4 ml/ min constant flow mode was carried out. The temperature programme for the analysis was; injector temperature 220oC, oven temperature 60oC to 246oC (3oC/min), detector temperature 250oC. Mass spectra: Electron Impact (EI+) mode, 70 eV, ion source temperature 250oC and with mass range 40-400µ.

Identification of the components

The oil components were identified by MS library search, relative retention indices (RRI) calculated with respect to homologues of n- alkanes and compared with literature data and by comparison with published mass spectra.

In vitro Antioxidant Assays

DPPH Radical Scavenging Activity

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) reducing activity is widely used method for the screening of phytochemicals for their antioxidant activities. DPPH is characterized as a stable free radical due to delocalization of the spare electron over the molecule. This delocalization also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centred at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of the violet colour. Representing

the DPPH radical by Z. And the donor molecule by AH, the primary reaction is,

$Z + AH \leftrightarrow ZH + A.$

Where ZH is the reduced form and A. is free radical produced in this first step. This A. radical will then undergo further reactions which control the overall stoichiometry. The absorbance of the resultant solution is measured at 517 nm. The important parameter in this method is the IC50 value. IC50 is defined as the concentration of substrate that causes 50% loss of the DPPH activity.[4,5,6]

$$\% \text{ of inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Superoxide Scavenging Activity

Superoxide scavenging activity was determined by the method of Mc-Cord and Fridovich (Mc-Cord and Fridovich, 1969). This method involves the light induced super oxide generation by riboflavin and the corresponding reduction of nitro blue tetrazolium (NBT). Super oxide anions (O2-) act as an electron reducing agent in several reactions including reduction of quinines, tetra nitro methane, cytochrome C and nitro substituted aromatics such as NBT. The reduction of NBT to blue formazan has been widely used as a probe of O2- generation in chemical and biological systems. NBT in oxidized form is a yellow compound soluble in aqueous mixture. Its reduction to formazan is accompanied by disappearance of positive charges resulting in substantial decrease in solubility, disruption of the tetrazole ring with marked modification of the absorption spectrum in the visible region and the appearance of intense blue colour.

The reaction mixture(s) contained EDTA (6 mM), NaCN (3 μg), riboflavin (2 μg), nitro blue tetrazolium (50 μg), KH2PO4 buffer (67 mM, pH 7.8) and various concentrations of the extract in a final volume of 3 ml. The tubes were illuminated in bright sunlight for 15 min. The optical density at 560 nm was measured before and after illumination. The inhibition of superoxide radical generation was determined by comparing the absorbance values of the control with those of treatments.[4,6]

$$\% \text{ of inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Reducing Power assay method

In this assay the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of the extract. Presence of reducers causes the conversion of Fe3+ / ferricyanide used in this method to ferrous form. By measuring the formation of persion blue at 700nm, it is possible to determine the Fe2+ concentration, a higher absorption at 700nm indicate a higher reducing power.

Various concentrations of extraction (1 ml) were mixed with 1 ml of 200 mmol/l sodium phosphate buffers (pH 6.6) and 1 ml of 1% potassium ferricyanide. The mixture was incubated at 50oC for 20min. After 1 ml of 10% trichloroacetic acid (w/v) was added. From that 1 ml was mixed with 1 ml of deionised water and 250μl of fresh ferric chloride (0.1%). The absorbance was measured at 700 nm: a higher absorbance indicates a higher reducing power.[4]

Anti bacterial assay

Bacteria grown in nutrient broth for 24 h (pH 7.2-7.4) were used as inoculum. Mueller-Hinton agar medium were poured into Petri plates to a uniform depth 5 mm and allowed to solidify. Microbial suspensions at 5 x 106 cfu/ml were spread over the surface of the media using a sterile cotton swab to ensure the confluent growth of the organism. Aliquots (10 μl) of each plant extract at 4mg/10μl dilutions in the inert solvent Dimethyl sulphoxide were impregnated on Whatman No.1 filter paper discs (6 mm diameter). These discs were then aseptically applied to the surface of the agar plates at well-spaced intervals. These plates were incubated at 37°C for 24 h and observed inhibition zones including the diameter of the discs were measured. Control discs impregnated with 10 μl of the solvent (DMSO) and streptomycin (2 μg/disc, reference for bacteria) were used alongside the test discs in each experiment.[7,8]

Anti fungal assay

Fungus grown in nutrient broth for 24 h (pH 7.2-7.4) was used as inoculum. Mueller-Hinton agar medium were poured into petri plates to a uniform depth of 5 mm and allowed to solidify. Microbial suspensions at 5 x 106 cfu/ml were spread over the surface of the media using a sterile cotton swab to ensure the confluent growth of the organism. Aliquots (10 μl) of each plant extract at 4mg/10μl dilutions in the inert solvent Dimethyl sulphoxide were impregnated on Whatman No.1 filter paper discs (6 mm diameter). These discs were then aseptically applied to the surface of the agar plates at well-spaced intervals. These plates were incubated at 37°C for 24 h and observed inhibition zones including the diameter of the discs were measured. Control discs impregnated with 10 μl of the solvent (DMSO) and Nystatin (2 μg/disc, reference for fungus) were used alongside the test discs in each experiment.[7,8]

RESULTS

Extraction

Tagetes erectus plant powder (350g) was packed in a soxhlet apparatus. 3 litters of solvent were used for extraction. The plant powder was extracted with Petroleum ether (60-80oc), chloroform, and methanol for 72 h each. These extracts were concentrated in rotary vacuum evaporator to get 2.8, 1.6 and 1.4 g of petroleum ether, chloroform, and methanol extracts respectively The extractive values are given in Table 1.

Phytochemical Screening

Petroleum ether extract of the plants of *Tagetes erectus* gave positive results for the presence of Terpenoids. Chloroform extract showed positive tests for Terpenoids, Steroids. Methanol extract showed positive test for

Table 1: Extractive value.

Solvent	1	2	3	Average
Pet.ether	0.39	0.398	0.42	0.402
Chloroform	0.22	0.25	0.23	0.23
Methanol	0.19	0.18	0.20	0.19

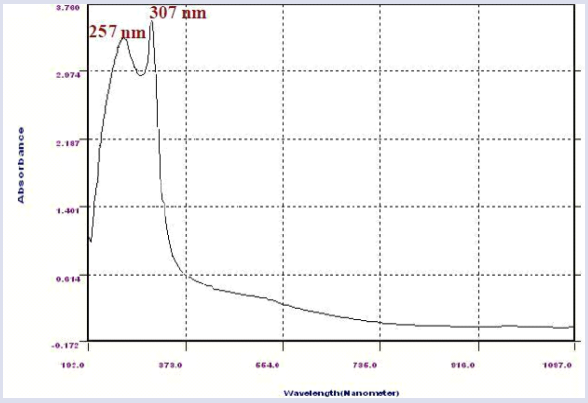

Figure 1: UV spectrum.

Table 2: **Phytochemical screening of Tagetes erectus extracts.**

Class of compound	Pet Ether Extract	Chloroform Extract	Methanol Extract
Steroids			
a) Liebermann-burchard Test	-	+	-
Flavonoids			
a)Shinoda test	-	-	+
Alkaloids			
a)Dragendorff Test	-	-	-
b) Mayer's Test	-	-	+
c) Hager's test	-	-	+
d)Wagner's test	-	-	+
Terpenoids			
a) Test for terpenoids	+	+	+
Tannins and Phenolic compound	-	-	
a) Lead acetate solution	-	-	
b) 5%Fecl3 solution	-	-	
c) Dilute HNO3	-	-	
d) Potassium dichromate	-	-	

Table 3: **Standardisation Methods Observation.**

Methods	Observation
Total Ash	12.81% w/w
Acid Insoluble Ash	0.49% w/w
Water Soluble Ash	3.65% w/w
Bitterness Value	3.44
Swelling Index	3cm
Foaming Index	<100
Iodine Value	8.95

alkaloids, carbohydrate, phenolic compound, terpenoids and flavonoids and ash values given in Table 2 and Table3.

Compound isolated from methanol extract of Tagetes erectus

TE-01 has been obtained as yellow crystals from ethanol, yield 500 mg, gave positive test for Flavonoid. UV, IR and NMR of the compound were taken. Identity of the compound has been done with co-TLC.

UV spectrum (Figure 1) exhibit two major absorption peak. Peak 1 is at 257nm and another peak is at 307nm. UV spectra were recorded from 220 to 450 nm. Flavonols basically shows two peaks at 250nm and 370nm (Figure 4). Which are related to the structure of each two moieties in their skeleton: phenyl propane (Band I) and chromanone (Band II). Flavonols are a class of flavanoids that have the 3-hydroxyflavone back-bone (Figure 3). The most common flavonols are quercetin, myricetin, kaempferol, rutin and azaleatin. For each compound, peak areas were determined at the wavelength providing maximal UV absorbance.[9]

IR spectrum of isolated compounds

IR spectrum exhibited absorption bands at 3411cm^{-1} which indicating the presence of OH stretching vibration of phenols, 1383.1cm^{-1} band indicating for the presence of OH bending of phenols, and shows a band at 1203cm^{-1} for CO stretch of phenol. It also shows a band at 1663.8cm^{-1} that indicates C=O aryl ketonic stretch and a band at 1167cm^{-1} which shows the presence of C-CO-C stretch and bending in ketone. It also

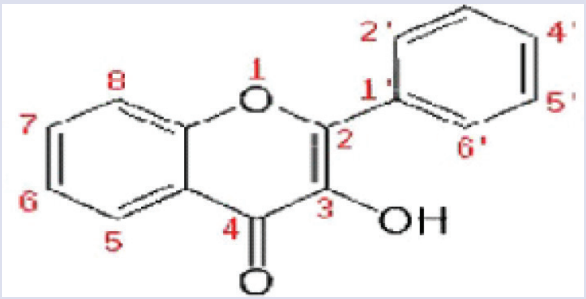

Figure 2: Skeletal structure of flavonol.

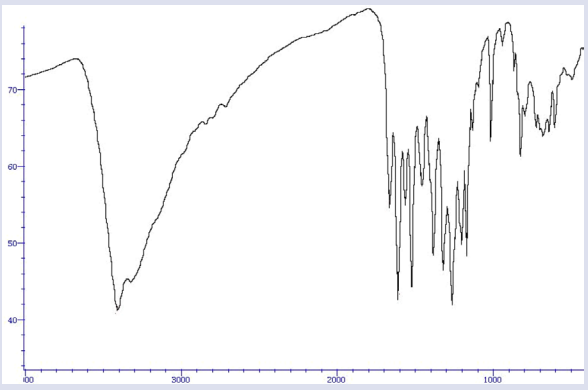

Figure 3: IR spectrum.

shows a peak at 1318.9cm^{-1} for the presence of bending of CH in aromatic hydrocarbon.

NMR spectrum of isolated compound

The 1H-NMR (400MHz, CD3OD): 6.274 (9, 1H, d, J=2.269), 6.439 (11, 1H, d, J=2.269) 6.813 (7, 1H, dd, J=8.430, J=2.776), 7.398 (10, 1H, dd, J=2.776, J=1.774), 7.633 (8, 1H, dd, J=8.430, J=1.774).

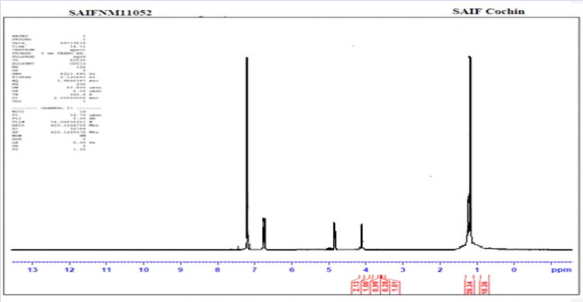

Figure 4: NMR spectrum.

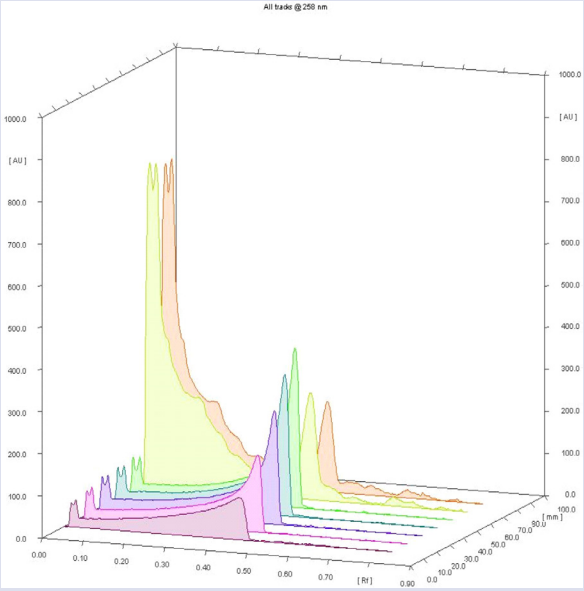

Figure 5: Plate developed under 254nm.

From the results of UV, IR and NMR spectra, as well as co-TLC with authentic compound, the isolated compound was considered as quercetin.

Estimation of methanol extract of Tagetes erectus using HPTLC Calculation

Methanol extract was estimated for the presence of quercetin. 15 mg of methanol extract dissolved in 1.5 ml of methanol. 15μl and 20μl of the test solution and 1μl-4μl of stdquercetin was added. Concentration of quercetin in the extract was calculated on the area of the graph. In 15μl (150μg) - contains 1.77μg. 100μg - 1.18μg i.e.; 1.18%. In 20μl (200μg) –contains 1.97μg quercetin i.e.; 100μg - contains 0.985μg i.e.; 0.985%.

Visualization**:** After developing, the plate was observed under UV 254nm

Image of the chromatographic plate is visualized under 254 nm as well as 366 nm. This visual aspect parameter is unique to HPTLC. In 254 nm, the background i.e. plate appears green or blue, due to the fluorescence indicator incorporated in the adsorbent while the fractions appear as dark spots due to fluorescence quenching. This image is non-specific as only black spots are seen on a bright fluorescent background**.**

Common requirement of 3D images Figure 5 and 7 is to take a series of images at a fixed position, but at different focal depths. Since most microscopic samples are essentially transparent, and the depth of field of the focused sample is exceptionally narrow, it is possible to capture images "through" a three-dimensional object using 2D equipment like confocal microscopes. Software is then able to reconstruct a 3D model of

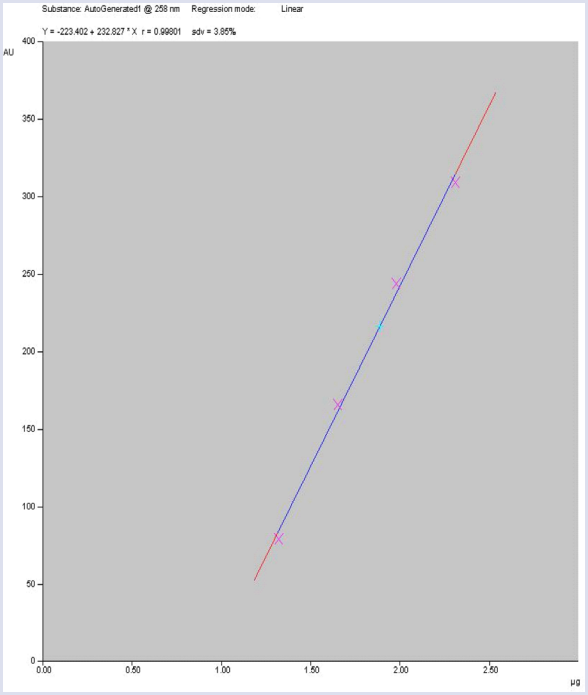

Figure 6: 3D image of HPTLC.

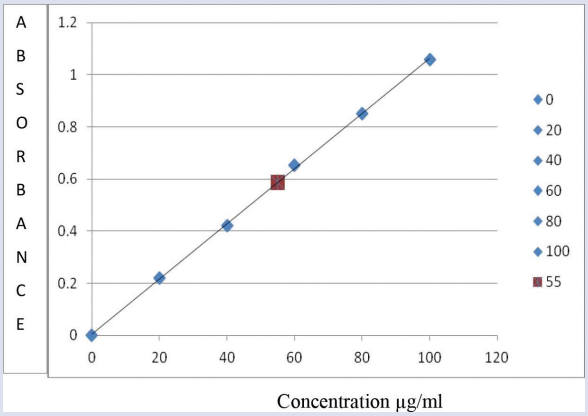

Figure 7: standard curve based on peak height.

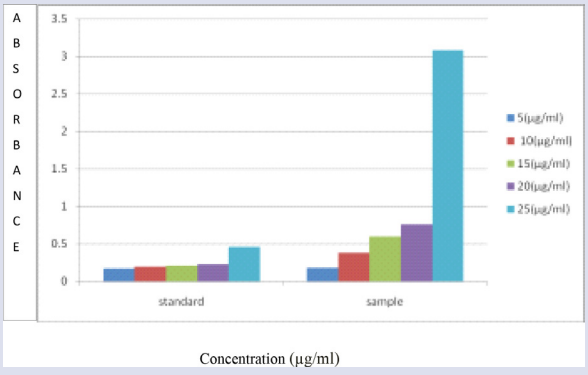

Figure 8: Total phenolic content.

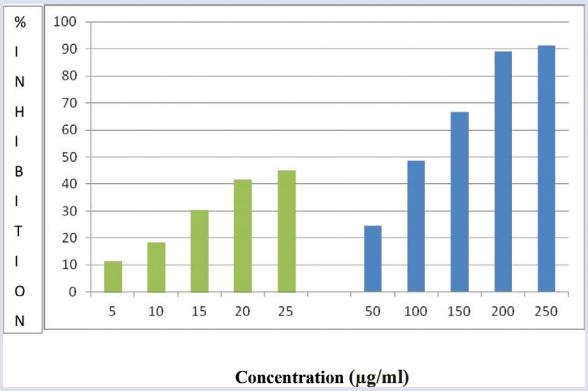

Figure 9: Reducing power assay method of Tagetes erectus and Standard.

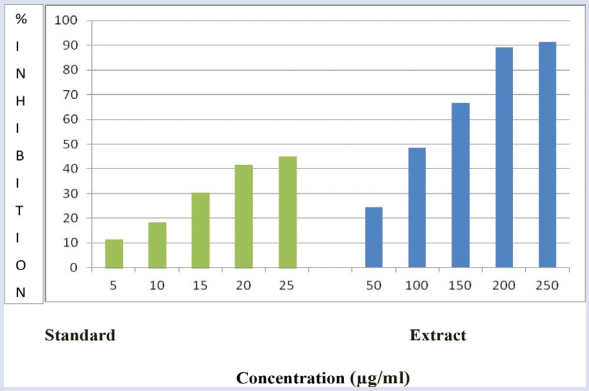

Figure 10: DPPH Free radical scavenging Activity of Tagetes erectus and Standard.

Table 4: Total phenolic content.

Gallic acid (µg/ml)	Absorbance of Gallic acid	Methanol Extract (µg/ml)	Absorbance of Extract
20	0.222	100	0.087
40	0.422	200	0.201
60	0.652	400	0.588
80	0.852		
100	1.057		

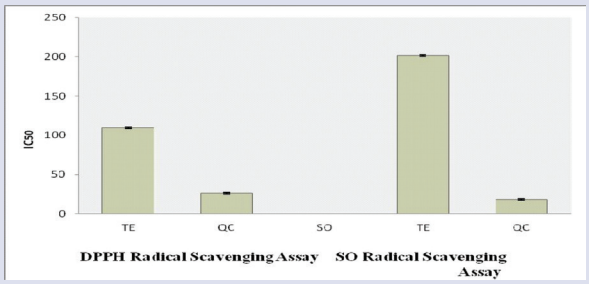

Figure 11: DPPH Free radical scavenging Activity of Tagetes erectus and Standard.

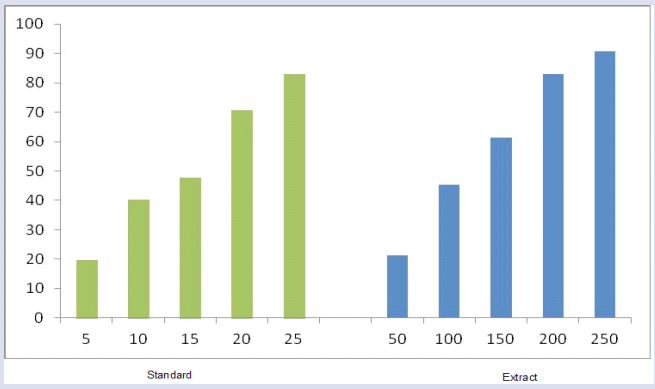

Figure 12: Super Oxide Scavenging Activity of *Tagetes erectus* and Standard.

the original sample which may be manipulated appropriately. The processing turns a 2D instrument into a 3D instrument, which would not otherwise exist.

The peak areas of methanolic extract (15µland 20µl) and Quercetin (1µl to 4µl) concentration were recorded. The results showed linearity and the best fitting linear equation was y = 223.402+232.827x. r=0.998 and stdev is 3.85%. There was a good correlation between peak area and the corresponding concentration of methanolic extract and quercetin as shown in Figure of standard curve (Figure 8). Stationary phase silica gel and TLC plate and mobile phase chloroform: methanol (8.5: 1.5) had given good separation. The standard curve was found to be linear dependent on the concentration against area.

In vitro antioxidant property

Total phenolic content

The Folin-Ciocalteau method has the advantage of a equivalent response to different phenols, making it suitable for measuring accurate mass levels of total phenolic substances. This method depends on the reduction of FCR by phenols to a mixture of blue oxides which have a maximal absorption in the region of 760 nm. The intensity of blue staining produced is proportional to the total quantity of phenolic compounds present in the testing samples. Here gallic acid was taken as the standard.

From the graph it is calculated that 400µg/ml of methanol extract contains 55µg of phenolic content which is equalent to 55µl of gallic acid contains phenolics is given in Table 4.

Reducing power assay method

In this method the concentration of methanolic extract of *Tagetes erectus* and quercetin taken was 5-25(µg/ml) is showed in Table 5. The graph (Figure 9) is plotted against absorbance vs concentration. When the concentration increases absorbance also increases because when the concentration increased reduction of ferri form to ferrous form of ferricyanide also increased

In this method the concentration of methanolic extract of *Tagetes erectus* and quercetin taken was 5-25(µg/ml). The graph is plotted against absorbance vs concentration. When the concentration increases absorbance also increases because when the concentration increased reduction of ferri form to ferrous form of ferri cyanide also increased.

Free Radical Scavenging Assay

DPPH Radical Scavenging activity:

Table 5: Reducing power assay method.

Concentration of standard (μg/ml)	Absorbance	Concentration of extract (μg/ml)	Absorbance of methanol extract
5	0.169	50	0.18
10	0.190	100	0.38
15	0.212	150	0.6
20	0.228	200	0.76
25	0.461	250	3.08

Table 6: DPPH radical scavenging activity.

Concentration of standard (μg/ml)	% Inhibition of Standard	Concentration of methanol extract (μg/ml)	% Inhibition of methanol extract
5	11.441± 0.316	50	24.53± 0.2491
10	18.414± 0.504	100	48.40± 0.7057
15	30.490±0.391	150	66.62± 1.6604
20	41.686 ±0.630	200	89.01± 0.1371
25	45.073± 0.140	250	91.17± 1.2040

Table 7: Super oxide scavenging activity.

Concentration of standard (μg/ml)	% Inhibition of Standard	Concentration of methanol extract	% Inhibition of methanol extract
5	19.8± 0.21	50	21.32± 0.4918
10	40.2± 0.70	100	45.31±0.2801
15	47.8± 0.78	150	61.40± 0.6135
20	70.8± 0.49	200	83.03± 0.7938
25	82.9± 0.37	250	90.66± 0.9950

DPPH is a stable free radical at room temperature often used to evaluate the anti oxidant activity showed in Figure 10. The reduction capacity of DPPH radical was determined by the decrease in its absorbance at 517nm, which induced by antioxidants is showed in Table 6. Radical scavenging activity increased with increasing percentage of free radical inhibition.

Super oxide scavenging Activity

In this method nitro blue tetrazolium (NBT) is used as one of reagent. The percentage inhibition of NBT reduction can be used to quantify super oxide-scavenging. Percentage inhibition of super oxide can be increased with concentration is given in Table 7.

In this method nitro blue tetrazolium (NBT) is used as one of reagent. The percentage inhibition of NBT reduction can be used to quantify super oxide-scavenging. Percentage inhibition of super oxide can be increased with concentration.

IC^{50} value of DPPH and super oxide scavenging activity is showed in Figure 11

GC-MS analysis of essential oil

Hydro distillation of the fresh whole plant *Tagetes erectus* gave light yellow oil at a yield of 0.04% (v/w). The composition of the oil is given in Table 8. A total of 25 components comprising 93.9% of the oil were identified. The oil was found to be rich in monoterpenoids (78.5%), with 26.5% monoterpene hydrocarbons and 52.0% oxygenated monoterpenoids. Piperitone (40.1%) was the major compound followed by limonene (12.0%) and terpinolene (12.7%). The present study shows indole, the nitrogenous volatile compound at low level (1.3%). The oil contained 11.4% sesquiterpenoids, with 9.9% sesquiterpene hydrocarbons and 1.5% oxygenated sesquiterpenoids.

GC-MS spectrum of compounds obtained from TE oil

In GC-MS analysis the relative retention index (RRI) calculated by using the formula.

$$RRI = \frac{n\times 100 + 100\,R\,compound - R_{cn}}{R_{cn} + 1 - R_{cn}} \times 100$$

$$n = C^{n}\,(C^{5}H^{8})$$

Anti microbial assay

In this method different gram positive and gram negative bacteria like *Bacillus cereus, Staphyllococcus aureus, Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, p.aureogenosa, Bacillus subtilis, Proteus vulgaris*. Antibacterial activity of methanolic extract of TE and oil were tested. Streptomycin used as the standard. Methanolic extract shows activity against *Bacillus cereus, Serratia marcescens, Pseudomonas aeroginosa, and Bacillus subtilis* is given in Table 9. Oil shows more activity than standard streptomycin.

The concentration of the methanolic extract taken for disc diffusion method was 40mg dissolved in 100μl DMSO. From this stock solution 10μl/disc taken. Stock solution of essential oil (40mg/100μl) of essential oil from *Tagetes erectus* made in DMSO. The results expressed along with the disc diameter i.e.; 6mm. Disc diameter-6mm including disc diffusion.

40mg → 100μL → 4mg (10 μL/disc)

Antifungal assay carried out with fungus like *Candida albicans* and *Candida glabrata*. Both methanolic extract and oil of TE shows more active than standard is given in Table 10. The concentration of the methanolic extract taken for disc diffusion method was 40mg dissolved in 100μl

Table 8: Essential oil constituents of Tagetes erectus whole plant.

RT	Compound	RRI(cal c)	RRI(lit)	%
4.60	α-Phellandrene	994	1002	0.4
6.04	Limonene	1014	1024	12.0
6.54	E-β-Ocimene	1030	1044	1.4
7.88	Terpinolene	1074	1086	12.7
8.27	Linalool	1087	1095	2.2
9.72	E-Epoxy ocimene	1128	1137	4.1
10.88	Terpinen-4-ol	1159	1174	0.5
11.43	P-cymen-8-ol	1173	1179	2.4
11.65	Ui	1179	-	1.3
11.95	-	1187	-	1.6
14.58	Piperitone	12.53	1249	40.1
14.74	-	1257	-	0.2
15.23	-	1269	-	0.8
15.39	-	1273	-	0.3
15.69	Indole	1280	1290	1.3
16.06	Terpinen 4-ol acetate	1289	1299	0.7
16.17	-	1292	-	0.4
17.01	--	1312	--	0.2
17.46	Piperitenone	1323	1340	1.8
17.60	-	1327	-	0.4
19.23	Geranyl acetate	1367	1379	0.2
19.47	Cyperene	1373	1398	0.9
20.40	β-Caryophyllene	1395	1417	3.6
20.88	-	1407	-	0.1
21.04	α-trans-Bergamotene	1411	1432	0.3
22.12	E-β-Farnesene	1439	1454	3.0
22.80	γ-Muurolene	1456	1478	1.5
22.99	-	1461	-	0.8
23.36	Bicyclogermacrene	1469	1500	0.6
26.13	E-nerolidol	1541	1561	0.4
26.43	Spanthulenol	1549	1577	0.6
26.57	Caryophyllene oxide	1553	1582	0.5
28.65	-	1608	-	0.2
29.98	-	1646	-	0.1
34.39	n-Octadecane	1773	1800	0.2
35.95	Phytol isomer	1817	-	2.3
44.41	Phytol isomer	2090	2105	0.2
TOTAL		93.9		
Monoterpene hydrocarbon		26.5		
Monoterpene oxygenated		52.0		
Sesquiterpene hydrocarbon		9.9		
Sesquiterpene oxygenated		1.5		
Diterpene		2.5		
Aliphatic hydrocarbon		0.2		
Nitrogenous volatiles		1.3		

Table 9: Anti-bacterial activity.

Micro-organism	Methanol extract	*Tagetes erectus* oil	Standard(Streptomycin)
430 *Bacillus cereus*	8mm	20mm	17mm
96 *Staphyllococcus Aureus*	Nil	22mm	15mm
443 Escherichia coli	Nil	16mm	10mm
109 *Klebsiellapneumoniae*	Nil	28mm	16mm
97 *Serratiamarcescens*	14mm	18mm	17mm
741 *Pseudomona Aureogenosa*	7mm	16mm	15mm
441 *Bacillus subtilis*	12mm	20mm	21mm
426 *Proteus vulgaris*	Nil	8mm	20mm

Table 10: Anti-fungal assay.

Micro organism	Methanol extract	Tagetes erectus oil	Standard (Nystatin)
Candida albicans (3017)	10mm	14mm	8mm
Candida glabrata (3049)	12mm	14mm	8mm

DMSO. From this stock solution 10μl/disc were taken. Stock solution of essential oil (40mg/100μl) of essential oil from *Tagetes erectus* made in DMSO. The results expressed along with the disc diameter i.e.; 6mm.

DISCUSSIONS AND CONCLUSION

Tagetes erectus is used from the ancient time for its great medicinal value. It hasbeen used in Indian traditional medicine for rheumatism, cold and bronchitis. Preliminary phytochemical evaluation discovered the existence of alkaloids, flavonoids, polyphenols like phenolic compounds and tannins.[10] Petroleum ether, chloroform and methanol extract of plant were prepared by soxhlet extraction. Pet. Ether extract shows positive result for terpenoids, chloroform shows positive results for steroid, and methanol shows positive result for flavanoids, alkaloid, terpenoids, tannins and phenolic compound. A flavonoidal structure was isolated from the methanolic extract of the plant.[11] HPTLC finger printing of methanol extract has been also carried out. Invitro antioxidant study has been performed using the methanolic extract as it shows positive result for Flavonoid. Reducing power assay showed moderate activity. DPPH radical scavenging assay.[12] and super oxide scavenging assay shows moderate activity with values 109.45 (±1.22) μg/ml and 201.53 (±4.48) μg/ml respectively. The antioxidant activity in all the antioxidant models were comparable to the standard.[13]

IC^{50}

Essential oil of the plant was distilled and its gc-ms analysis is also carried out. Monoterpenoids predominated the oil (78.5%), with piperitone as the major compound (40.1%) followed by terpinolene (12.7%), limonene (12.0%) and linalool. Further, Methanolic extract and oil has been analysed for antibacterial and antifungal study. Antibacterial assay carried out with Gram positive and Gram-negative bacteria were done using disc diffusion method. In which oil is more active towards *S. aureus, K. pneumonia, E. coli* than standard and methanol extract. Thus, it can be concluded that while screening of methanol extract of *Tageteserectus* and its oil against various Gram positive and Gram-negative bacteria andfungi oil exhibited very satisfactory inhibition. *Tagetes erectus* shows better antioxidant activity. These activities may be due to the presence of active constituent present in the plant and the exact constituent responsible for the activity.

AKNOWLEDGEMENT

The authors are grateful for the support and resources provided by Principal , Amrita School of Pharmacy , Kochi and Tropical Botanical Garden and Research Institute for their assistance and support.

CONFLICT OF INTEREST

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

ABBREVIATIONS USED

UV: Ultra violet; **IR:** Infra red; **NMR:** Nuclear magnetic resonance; **DPPH:** 2,2-diphenyl -1-picryl-hydrazyl; **IC$_{50}$:** Inhibitory concentration at 50%; **TLC:** Thin layer chromatography; **GC-FID:** Gas chromatography –Flame ionisation detector; **GC-MS:** Gas chromatography Mass Spectroscopy; **MS:** Mass spectra; **DMSO:** Dimethyl sulfoxide; **TE-01:** Tagetes erectus; **HPTLC:** High perfoemance thin layer chromatography; **FCR:** Folin Ciocalteau Reagent; **NBT:** Nitro blue tetrazolium.

REFERENCE

1. Torssell KBG. Natural Product chemistry, 2nd edition, Swedish Pharmaceutical Press. 1997;15-24.
2. Evans WC, Treas and Evans. Pharmacognosy, 15th edition, WB Saunders company Ltd, London. p.337.
3. Khandelwal KR. A Text book of Practical Pharmacognosy. 16th edition Nirali publication: 2000;79-88.
4. Andre T, Richard WS, Noufou O. Evaluation of Antioxidant Activity, Total Phenolic and Flavonoid Contents of *Entada Africana Guill. Et Perr Organ Extracts* Res J

Med Sc. 2010;4(2):81-7.

5. Asok GC, Dhanaraj SA, Suresh B, Vijayan P, *et al*. Free radical scavenging activity of selected essential oils', Indian drugs. 2010;41(4):244-5.
6. Basav RVC, Karnakumar VB, Rajabhau SS, Kamshetty VM, *et al. In vitro* antioxidant activity studies on the flowers of *Tagetes erecta*. 2011;2(3):223-9.
7. Cappuccino JG, Sherman N, Microbiology. A laboratory manual. California: Benjamin Cumming Science Publishing. 1999.
8. Egwaikhide PA, Gimba CE. Analysis of the Phytochemical Content and Anti-Microbial Activity of *Plectranthus glandulosis* Whole Plant. Middle East J Sci Res. 2007;2(3-4):135-8.
9. Markham KR. Techniques of Flavonoid identification, Academic press New York: 1982;37-9.
10. Subin MZ, Vidya V, Aleykutty NA, Dr Jaykar B, Halima OA *et al*. 'Free radical scavenging and antibacterial activity of *Mirabilis jalapa* Linn using *in vitro* models. Asian J Pharm Clin Res. 2012;5(3):115-20.
11. Leena KP, Annam C. Isolation and Characterization of Flavone *Glycoside Vitexin* from *Peperomia Pellucida Linn*. J Drug Del Ther. 2013;(6):91-2.
12. Rajan J, Krishna S, Menon SV, Pai RG, Surya M, Javahar M *et al. In silico* design, synthesis, characterization, *in vitro* anti-inflammatory, and antioxidant studies of 4-aryl-4H-chromene derivatives. 2017;10:408-13.
13. Padma R, Parvathy NG, Renjith V, Rahate KP. Quantitative estimation of tannins, phenols and antioxidant activity of methanolic extract of *Imperata cylindrica*. Int. J. Pharm. Sci. Drug Res. 2013;4(1):73-7.

SUMMARY

- *Tagetes erectus* is used from the ancient time for its great medicinal value.It has been used in Indian traditional medicine for rheumatism, cold and bronchitis. Petroleum ether, chloroform and methanol extract of the plant were prepared by Soxhlet extraction. Pet.ether extract shows positive result form terpenoids, chloroform showed positive results for steroid and methanolic extract showed positive result for flavanoids, alkaloids, terpenoids, tannins and phenolic compounds. HPTLC fingerprinting of methanolic extract has also been carried out.
- *In vitro* antioxidant studies has been performed using the methanolic extract as it showed positive results for flavanoids.Reducing power assay method showed moderate activity. DPPH radical scavenging and superoxide radical scavenging assay showed moderate activity with IC_{50} values109.45(± 1.22) µg/ml and 201.53 (±4.48) µg/ml respectively. Essential oil of the plant was distilledand its GC-MS analysis is also carried out. Monoterpenoids predominated the oil(78.5%) with piperitone as the major compound(40.1%) followed by terpinolene (12.7%),limonene(12.0 %) and linalool. Further methanolic extract and oil has also been analysed for antibacterial and antifungal activity.Antibacterial activity was carried out with gram +ve and gram-ve bacteria using disc diffusion method in which oil is more active towards *S.aureus, K.pneumoniae, E.coli* than standard and methanolic extract.
- Thus it can be concluded that while screening of methanolic extract of *Tagetes erectus* and its oil against various gram +ve and gram –ve bacteria and fungi oil exhibited very satisfactory inhibition. *Tagetes erectus* showed better antioxidant activity. These activities may be due to the presence of active constituent present in the plant and the exact constituent responsible for the activity.

Cite this article: Resmi S, Nair DV, Subhash A, Jose R, Vishnu V, Zachariah SM. Isolation, Characterization and *In vitro* Pharmacological Activities of Tagetes Erectus Linn. Pharmacog J. 2018;10(2):384-93.