ISSN 1996-0700

Asian Journal of Biotechnology

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Asian Journal of Biotechnology 6 (1): 21-24, 2014
ISSN 1996-0700 / DOI: 10.3923/ajbkr.2014.21.24

In vitro Quantification Study of Flavonoids from *Tagetes erecta*

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ABSTRACT

Various plants are used in herbalism because of its phytochemical constituents which have been proven to have medicinal effects by rigorous science and approved by regulatory agencies. In the present investigation an attempt has been made on qualitative and quantitative analysis of flavoniods from the leaf and flower extract of *Tagetes erecta*. After the confirmation for the presence of flavonoids in both the samples (Leaf and Flower) by qualitative analysis, quantitative analysis (Ammonium chloride method) was carried out. In the present investigation the recorded flavonoids ranged from 9.2 to 24.5 μg mg^{-1} in the leaf sample and from 7.3 to 30.4 μg mg^{-1} in the flower sample, respectively.

Key words: Flavonoids, qualitative, quantitative, calorimetric, therapeutic

INTRODUCTION

Widely distributed important plant pigment "Flavonoids" is known for its multifunction (colouration of petals, UV filteration etc) among the plant kingdom. The "Flavonoids" (secondary metabolite of plants) has derived from the Latin word "Flavus" meaning "Yellow". Flavonoids were classified as "Vitamin P" for its easy permeability of vascular capillaries from mid 1930's to early 50's (Galeotti *et al*., 2008). Yamamoto and Gaynor (2001) illustrated that the derivatives of flavonoids as catechins and as quercetin (Flavonols) are ubiquitous in lesser quantities among the plants. Recent researches indicate that flavonoids modify allergens, viruses, carcinogens etc., and have a biological significant with a title as "Response Modifier". *In vitro* studies indicate that flavonoids are anti allergic, anti-inflammatory (Cushnie and Lamb, 2005), anti-microbial (Cushnie and Lamb, 2011; De Sousa *et al*., 2007). Pekkarine *et al*. (1999) demonstrated that flavonoids are highly effective antioxidants with lower toxicity than BHA and BHT (Synthetic antioxidants).

Many research evidences have proved that flavonoids acts as antibacterial, antiprotozenic agents, inhibits important viral enzymes like reverse transcriptase, protease etc., and also in evaluating the taxonomical classification of plants. Even modern authorized physicians are increasing their use of pure flavonoids to treat many important common diseases, due to their proven ability to inhibit specific enzymes, to stimulate some hormones and neuro transmitters and to scavenge free radicals (Havsteen, 2002).

Table 1: Classification of flavones

Group	Description	Functional group		Examples
Flavone	2-phenyl chromen-4-one	×	×	Luteolin, apigenin, tangeritin
Flavonol	3-hydroxy-2-phenylchromen-4-one	✓	×	Quercetin, kaempferol, myricetin,
or 3-hydroxyl flavone				Fisetin, isorhamnetin, pachypodol rhamnazin
Flavanone	2-3-dihydro-2-phenylchromen-4-one	×	✓	Hesperetin, naringenin, eridictyol, homoeriodiclyol
Flavanonol or	3-hydroxyl-2,3-dihy	✓	✓	Taxifolin or dihydroquercetin dihydrokaem pferol
3-hydroxy flavanone or	dro-2-phenylchromen-4-one			
2,3-dihydro flavonol				

According to IUPAC nomenclature (Galeotti *et al*., 2008) flavonoids are classified into:

Flavonoids: Derived from 2-phenylchromen-4-one (2-phenyl-1, 4-benzopyrome structure)
Isoflavonoids: Derived from 3-phenyl chromen-4-one (3-phenyl-1, 4-benzopgrone) structure
Neoflavonoids: Derived from 4-phenyl coumarine (4-phenyl-1, 2-benzophrone structure) (Table 1)

Quercetin, a flavonoid is the agly cone form of other flavonoid glycosides, such as rutin and quercitin (Knekt *et al*., 2002) and are abundant in citrus fruit, buck wheat and onions. Plants belonging to Cactaceae family reported high content of flavonol 3-0-glycosides in the form of quercetin, kaempferol, isorhamnetin, dihydroflavonols, flavonones and flavanonols (Burret *et al*., 1982; Miller and Bohmt, 1982). The citrus bioflavonoids include hesperidin (a glycoside of the flavanone hesperetin) quercitin, rutin (two glycosides of the flavonol quercetin) and tangeritin (flavones) (Slimestad *et al*., 2007; Marotti and Piccaglia, 2002). In spite of its antioxidant activity and increase intracellular levels of vitamin C, rutin and hesperidin have beneficial effect on capillary permeability and blood flow (Spedding *et al*., 1989).

The present investigation was aimed to extract flavonoids from *Tagets erecta* commonly known as African marigold which is known for medicinal purpose since prehispanic times.

MATERIALS AND METHODS

In the present investigation, an attempt has been made on the qualitative and quantitative analysis of flavonoids from *Tagetes erecta*. The whole plant was air dried after segregation as leaf, stem, flower and root and made into powder and stored in air tight containers for analysis. A known quantity of the powdered samples were taken and subjected to qualitative analysis (Kapoor *et al*., 1969) and quantitative analysis of flavonoids as per standard methods. The extraction procedures were carried out with two solvents such as ethanol and petroleum ether.

Qualitative analysis: To 2 mL of the plant extracts (Stem, leaf, flower and root), 1 mL of 2N sodium hydroxide was added. Presence of yellow colour indicated the presence of flavonoids.

Quantitative analysis

Aluminium chloride colorimetric method (Chang *et al*., 2002): Leaf and flower extracts (1 mg mL^{-1}) were prepared and 100, 250 and 500 µL of each sample were taken in separate tubes

and made upto 2 mL with methanol, 0.1 mL of 10% aluminium chloride and 0.1 mL at room temperature for 30 min. The absorbance of the reaction mixture was mearsured at 415 nm. A standard solution was maintained by quercitin.

RESULTS AND DISCUSSION

The various parts (stem, leaf, flower and root) of *Tagetes erecta* were air dried and powdered for qualitative and quantitative analysis. All the various parts of the plant were subjected to qualitative analysis for flavonoids with two solvents such as ethanol and petroleum ether and the results are tabulated in the Table 2. From the above result it was confirmed that the leaf and flower showed positive results for flavonoids in both the solvents (ethanol and petroleum ether). Under this assumption further quantitative analysis were carried out only with the leaf and flower sample of *Tagetes erecta* with ethanol as the solvent.

The extraction procedure of both leaf and flower were done carefully with ethanol and the extract was filtered using Whatman No. 1 filter paper and the quantitative analysis of flavonoids were carried out with above purified extracts.

Aluminium chloride colorimetric method (Chang *et al*., 2002) was followed to estimate the total flavonoid content in both the extracted samples (Leaf and flower). A standard graph was obtained with various concentrations of quercitin (10 mg 100 mL^{-1}). The plant leaf and flower extracts were prepared at various concentrations (100, 200 and 500 µL) and subjected to calorimetric analysis at 415 nm. The total content of flavonoids was expressed in µg mg^{-1} and results are tabulated in the Table 3. Cai *et al*. (2010) had also reported that the *Opuntia milpa alta* showed positive flavonoid content by calorimetric method at 415 nm.

From the present quantitative results it was proved that the total flavonoid was found to be more in flower sample than the leaf sample (Table 3). The absorbance at 415 nm of flavonoids in both the cases increased with the increase in the concentration of sample (0.227 to 0.594 in the leaf sample and 0.165 to 0.721 in flower sample). The total flavonoids recorded during the period of study ranged from 9.2 to 24.5 µg mg^{-1} in the leaf sample and from 7.3 to 30.4 µg mg^{-1} in the flower sample, respectively (Table 3).

Table 2: Qualitative analysis of flavonoids from *Tagetes erecta*

	Leaf		Stem		Flower		Root	
Solvents	Presence	Colour	Presence	Colour	Presence	Colour	Presence	Colour
Ethanol	+	Y	-	-	++	Y	+	Y
Petroleum ether	+	Y	+	Y	+	Y	-	-

Y: Yellow

Table 3: Quantitative analysis of flavonoids from *Tagets erecta*

Sample	Concentration of the sample (µg)	Absorbance at 415 nm	Total flavonoid (µg mg^{-1})
Leaf	100	0.227	90.2
	250	0.360	15.0
	500	0.594	24.5
Flower	100	0.165	70.3
	250	0.500	20.8
	500	0.721	30.4

CONCLUSION

From the above investigation it is proved that *Tagetes erecta* plant has a high therapeutic value in terms of high yield of flavonoids which is of effective medicinal value. Future investigation on the flavonoids of *Tagetes erecta* may prove to be highly anti-allergic, anti-inflammatory and antioxidant. Further investigation on the various types of flavonoids will pave a confident way towards specific therapeutic approaches.

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