JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2015. Vol. **9**(3), p. 2547-2550

Anti-inflammatory Effect of Bioactive Compounds of *Tagetes erecta (Linn.)* Flower Extract

R. Devika* and Justin Koilpillai

*Department of Biotechnology, Sathyabama University, Chennai- 600 119, India.
Department of Botany, St. Joseph's College, Trichirappalli, India.

(Received: 02 April 2015; accepted: 09 June 2015)

Today, plant based drugs has taken its great attention in modern pharmaceutical industries for its less cost and side effects. Phytomedicines utilization has exhibited effective treatment in curing many aliments in Human. The present investigation emphasize on the compounds (Flavonoid and salicylic acid) extracted from *Tagetes erecta Linn*. flower sample. *Tagetes erecta Linn*. belong to the Asterceae family and it is proved to exhibit potent anti-inflammatory effect. Flavonoid showed significant effect than the salicylic acid during the period of study.

Key words: Therapeutic, bioactive compounds, phytomedicine, anti-inflammatory, macrophage, vero cells.

Inflammation in the tissue is known for its complexicity and pathophysiological processes[1] conducted by a variety of response actions by leucocytes, macrophytes, mast cells etc.[2] at the point of target site with symptoms of redness, swelling, pain etc.[3,4]. The introduction of inflammation was started by the Greek Physician, Hippocrates as the beginning of a healing process with edema and erysiplelas[5]. A comprehensive report on inflammation was put forward by Aulus Celsus in his De Medicina which described four symptoms such as rubor, humor, color and dolor which means redness, swelling, heat and pain, respectively [1]. Later, after 100 years, the fifth symptoms was introduced as functiolaesa (impaired function) by Galen of Pergaman[5]. Inflammation can be classified into two types as acute inflammation and chronic inflammation associated with infilteration, activation, proliferation etc., respectively during curing[6].

God's creation forms a complete store-house of therapeutics remedies for many diseases[7]. The plant sources had became a valuable resource for new drug preparation because of its easy availability, low cost and with less side effect [8,9]. Literature indicate that the plants are the source for therapeutic values and it is old as 4000-5000 B.C and appears in Rigveda (1600-3500 B.C) [10]. It was also referred that over 7800 pharmaceutical industries consume 2000 tonnes of herbs annually[11,12] and they have proved that the phenolic compounds of plants have high anti oxidant properties[13,14] and it was revealed that over 1.5 million practitioners use herbal therapeutics as preventive medicine or curative applicable medicine[15] either as raw, crude drugs or as formulation of pharmaceuticals forms[16].

Reasearch evidences proved that the berries of *Solanum nigrum*are highly effective against inflammation, tuberculosis[17] and highly active for acute and chronic inflammations[18]. *Achilllea millefolium L.* of Asterceae family contained three different flavonoids (rutin, aspigenin 7-0-glucoside and luteolin-7-0-glucoside)[19] which inhibited the human neutrophil elastase and proved to be anti-inflammatory during in vitro studies[20]. The target plant (*Tagetes erecta Linn.)* of this investigation, has been thoroughly

* To whom all correspondence should be addressed.
E-mail: vineethdevika@gmail.com

studied in terms of qualitative analysis of phytochemical[21], and the constituents were separated by column chromatographic methods and the evidence showed the presence of flavonoids and salicylic acid[22]. In the present investigation, an attempt has been made to evaluate the anti inflammatory action and the percentage of toxicity of the separated compounds (Flavonoid and salicylic acid).

MATERIALS AND METHODS

Healthy, disease free plants of *Tagetes erecta (Linn.)* was collected, taxonomically identified and authenticated in the Herbal Science, Plant Anatomy Research Centre, Chennai. The plant parts were segregated separately as stem, leaf, flower and roots. The collected plant parts were washed properly in the tap water and rinsed with distilled water carefully (damaged plant parts were removed). The fresh plants parts were separately air dried in a closed room (25-28°C) for 10-15 days. After assuring the full dryness of the plant parts, they were pulverized to obtain as powder form by using sterile electrical blender. The powdered samples (leaf, stem, flower and root) were stored in air tight containers and protected from sunlight for further investigation.

After various investigation and analysis, it was confirmed that the flower extract of *Tagetes erecta (Linn.)* had higher therapeutic value compounds such as flavonoids and salicylic acid. These compounds were extracted by column chromatography[22] and the compounds were separated by Thin Layer Chromatography (TLC) and the purified compounds was authenticated by High Performance Liquid Chromatography (HPLC) with their respective standards. The extracted compounds (Flavonoids and salicylic acid) were subjected to anti inflammatory analysis with RAW 264.7 macrophage cells and the toxicity study (MTT Assay) was conducted as per standard procedures.

Macrophage scavanging assay

The effect of the column fractions on the phagocytic activity of RAW 264.7 macrophages was studied according to the standard method[23]. Fresh heparinised blood from healthy volunteers was used for the isolation of Polymorphonuclear cells (PMNs). PMNs were isolated and suspended in RPMI-1640 medium supplemented with 10% Fetal Bovine Serum (FBS). From the suspension about 20 µL of cells (1 x 10^8 cells/ mL) were added to 20 µL of the floral bioactive fraction sample (each isolated compound; flavonoid and salicylic acid) along with 40 µL of RPMI1640. The plates were incubated at 37ºC in 5% CO_2 atmosphere. After 24 hours, 20 µL of *Candida albicans* (5 x 10^7 particles/ mL), 20 µL of Nitroblue tetrazolium (NBT) and 1.5 mg/mL in Phosphate Buffer Solution (PBS) were added and incubated for 10 minutes. The medium was removed and the cells were rinsed with the fresh medium. Then the cells were washed with 200 µL of methanol to remove unreduced NBT. Finally, 120 µL of 2M KOH and 140 µL of DMSO were added to each well and the absorbance was read at 570 nm. The percentage of NBT reduction was calculated by the following formula.

$$\text{NBT reduction} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

Ttoxicity studyvero Cell Preparation

MTT assay[24] is a calorimetric technique which is based on the ability of the live cells to reduce yellow tetrazolium dye to a purple formazon. Vero cells were maintained in DMEM medium supplemented with 10% Fetal Bovine Serum at 37ºC in humidified atmosphere with 5% CO_2.

MTT Assay

The vero cells (1.2 x 10^4 cell/well) were plated in 96 well microtiter plates and was placed undisturbed at 37°C overnight (For cell attachment). Later, the developed cells were incubated with different concentrations (100µg, 200µg, 300µg) of flavonoid and salicylic acid for 24 hours. After incubation, fresh 10 µl of MTT (5mg/ ml) was added. After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals. Then the absorbance was read at 570nm in a microtitre plate reader. Cyclophosphamide was used as a positive control.

Cell viability % = (Test OD/control OD) x 100

Cytotoxicity % = 100 - viability%

RESULTS AND DISCUSSION

The effect of isolated bioactive compounds (Flavonoid and salicylic acid) from *Tagetes erecta Linn.* was subjected to macrophage

scavenging assay[23]. Various concentrations (50 µg/ml, 100 µg/ml, 150 µg/ml) of flavonoid and salicylic acid were prepared and they were analysed for NBT reduction at 570 nm. Duplicate analysis were made for all the concentrations and the average readings were used for calculation of percentage NBT reduction. The results showed an increase of NBT reduction as the concentration of bioactive compounds increased. The maximum reduction was observed at 150 µg/ml and the minimum reduction was observed at 50 µg/ml with flavonoid sample were 25.26% and 19.6%, respectively. Interestingly, salicylic acid also showed equivalent percentage reduction of NBT during the period of study (Table 1).Macrophages are scavengers which are responsible to remove the body of worn out cells, debris and create a vital immune response. Hot water extract of *Actinidia arguta* stem showed inhibitory effect against a-glucosidase enzyme with RAW 264.7 cells activated with lipopolysaccharide [25].

Toxicity study was conducted in a 96 well microtitre plate with vero cells. After incubation period, the absorbance was observed at 570 nm and the viability percentage was calculated and recorded in the Table 2.Interestingly, the lower concentration of both flavonoid and salicylic acid (50 µg) recorded the maximum viability percentage with 92.59 and 88.81, respectively. Percentage of toxicity was maximum at 150 µg flavonoid and salicylic acid with 18% and 21%, respectively. Cancer cell lines MTT assay proved to have an 30-50% inhibition on the mitochondrial activity[26]. Evidences also proved that the patients with hematological malignancies showed significant viability with multidrug resistant malignancies [27].

Table 1. Percentage NBT reduction by flavonoid and salicylic acid of *Tagetes erecta (Linn.)*

Parameter	Control	Flavonoid (µg/ml)			Salicylic acid (µg/ml)		
		50	100	150	50	100	150
Absorbance at 570 nm	0.927	0.751	0.724	0.698	0.742	0.726	0.684
	0.914	0.749	0.717	0.701	0.738	0.712	0.692
Average absorbance	0.920	0.750	0.720	0.699	0.740	0.719	0.688
% NBT reduction	0	18.52	21.73	24.00	19.61	21.89	25.26

Table 2. Toxicity studies of flavonoid and salicylic acid against vero cells

Parameter	Control	Vero cell line with Flavonoid			Vero cell line with Salicylic Acid		
		50µg	100µg	150µg	50µg	100µg	150µg
% of Viability	100	92.58	86.15	81.81	88.81	83.07	78.74
% of Toxicity	0	7.41	13.84	18.18	11.18	16.92	21.25

CONCLUSION

The present investigation has proved that the bioactive compounds extracted from the target plant has high therapeutic value and the flavonoid compound is found to be more significant than the salicylic acid. The evidences of the present investigation also proved that the flavonoid would be an alternate compound as anti inflammatory active drug in near future.

REFERENCES

1. Mona S.Mohammed, Wadah, J.A., Osman, Elrashied, A.E., Garelnabi, Zuheir Osman, Bashier Osman, Hassan S. Khalid, Magdi A.Mohamed, Secdary metabolites as anti-inflammatory agents. *J. of Phytopharmacology*, 2014; **3**(4): 275-285.
2. White, M. Mediators of inflammation and inflammatory process. *J Allegy and Clin. Immu*., 1999; **103**: 378-381.

3. Deepa Murugesan and Renuka Deviponnuswamy, Potential anti-inflammatory medicinal plants-A review, *Int. J. Pharma ansd Pharma. Sci*., 2014; **6**(40: 43-49.
4. Palladino, M.A., Bahjat, F.R., Theodoralds, E.A., Moldovel, L.I., Anti-TNF-therapies: the next generation, *Nat.Rev. Drug. Discovery*, 2003; **2**: 736-746.
5. Khosrow Kashfi, Anti inflammatory agents as cancer therapeutics, *Adv. in Pharma*., 2009; **57**: 31-89.
6. Kumar S., Bajwa, B.S., Singh Kuldeep, Kalia, A.N., Anti inflammatory activity of herbal plants: A review, *Int. J. Adv. in Pharma. Bio. and Chem*., 2013; **2**(2): 272-281.
7. Kokate, C.K.. Purohit, A.P., Gokhale, S.B., Textbook of Pharmacognosy, Nirali Prakasan, Pune, 2002; 1-8.
8. Valsaraj, R., Pushpangadam, P., Smitt, V.M., Adersen, A., Nyman, U., Antimicrobial screening of selected medicinal plants from India, *J. Ethno. Pharma*., 1997; **58**: 75-83.
9. Saikia, B., Ethanomedical plants from Gohpur of Sonitpur district, Assam,*Int. J. Tradi*. *Knod*., 2006; **5**(40: 529-530.
10. Maliheh Najari, Beidokhti and Prakash, H.S., Antioxidant and anti-inflammatory potential of selected medicinal plants of Laminaceae family, *Int.J. of Pharma. and Pharma. Sci*., 2013; **5**(1): 100-104.
11. Prakash, P and Neelu Gupta, Therapeutic uses of *Ocimum sanctum Linn*. (Tulsi) with a note on eyenoid and its pharmacological actions: a short reviews, *Int.J. Physiol. Pharmacol*. 2005; **49**(2): 125-131.
12. Prakash Chandra Gupta, Biological and pharmacological properties of *Terminalia khebula Retz.* (Heritaki) ; an overview, *Int. J. of Pharma. and Pharma.Sci*., 2012; **4**(3): 62-68.
13. Cook, N.C., Samman, S., Flavonoids-chemistry, metabolism, cardioprotewctive effects and dietary sources, *Nutri. Biochem*., 1996; **7**: 66-76.
14. Pourmorad, F., Hosseinimehr, S.J., Shahbinald, N., Anti oxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr. J. of Biotech*., 2006; **5**(11): 1142-1145.
15. Dasilva, E.J., Medicinal plants: a reemerging health aid, *E.J. of Biotech*.,1999; **2**: 57-70.
16. Tiwari, S., Plants: a rich source of herbal medicines., *J. of Nat. Products*, 2008; **1**: 27-356.
17. Chopra R.N, Nayar S.L., Chopra I.C., Glossarys of Indian medicinesal plants, CSIR, New Delhi, 1959; pp.229.
18. Ravi, V., Saleem, T.S.M., Patel, S.S., Raamamurthy, J., Gauthaman, K., Anti-inflammatory effect of methanolic extract of Solanum nigrum Linn. berries, *Int. J of Appl. Res. In Nat. Prod*., 2009; **2**(2): 33-36.
19. David, R., Bruck, Zibigniew, A Cichacz and Sasha M Duskulova, Aqueous extract of *Achilla millefolium L*. Asterceae inflorescencesuppresses lipo[polysaccharide induced inflammatoty responses in RAW 264.7 murine macrophages, *J. of Medi. Plants Res*., 2010; **4**: 225-234.
20. Benedek, B., Kopp, B and Meizig, M.F., *Achillea millefolium L* is a anti inflammatory activity mediated by protease inhibition , *J. Ethbno*., 2007; **2**: 312-317.
21. Devika, R and Justin Koilpillai, Phytochemical screening studies of bioactive compounds of *Tagetes erecta,Int. J. of Pharma and Biosci*., 2012; **3**(4) (B): 596-602.
22. Devika, R and Justin Koilpillai, Column chromatographic separation of bioactive compounds from *Tagetes erecta Linn,Int. J. of Pharma. Sci. and Res*., 2015; **6**(2): 762-766.
23. Manosroi, A., Saraphanchotiwitthaya,. A and Manosroi., J., *In vitro* immunomodulatory effect of *Pouteria Cambodiana* (Pierre ex Dubanrd) Baehni extract, *J. of Ethopharma*., 2005; **101**: 90-94.
24. Mossman,. T., Rapid colorimetric assay for cellular growth and survival application to proliferation and cytotoxicity assays, *J. of Immuno. Meth*., 1983; **65**: 55-63.
25. Jaehak, Lee, Kandasamy Sowdharajan, Mihae Kim, Jaehun Kim, Daeho Kim, Sunpyo Kim, Gur-Yoo Kim, Songmun Kim, Jin Woo Jhoo, Antioxidant, inhibition of"-glucosidase and suppression of nitric oxide production in LPS-induced murine macrophages by different fractions of *Actinidia arguta* stem, *J. of Ethopharma*., 2014; **21**(6): 532-538.
26. Jabbar, S.A, Twentyman, P.R and Watson, J.V, The MTT assay underestimates the growth inhibitory effects of interferons. *British J.of Cancer*, 1989, **60**(4): 523-528.
27. Denese C Marks, larissa Belov, Mary W Davey, Ross A Davey, Antony D Kidman, The MTT cell viability assay for cytotoxicity testing in multidrug resistant human leukemic cells, *Leukemia Res*., 1992; **16**(12): 1165-1173.