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Evaluation of Free Radical Scavenging, Anti-inflammatory and Analgesic Potential of *Benincasa hispida* Seed Extract

N.S. Gill, K. Dhiman, J. Bajwa, P. Sharma and S. Sood
Rayat Institute of Pharmacy, SBS Nagar, Punjab Technical University, Ropar-144533, India

Abstract: The present study was designed to investigate the free radical scavenging, anti-inflammatory and analgesic potential of methanolic extract of *Benincasa hispida* seeds (MEBH). Free radical scavenging potential of MEBH was evaluated by DPPH (1,1-diphenyl-2-picryl-hydrazyl) and hydrogen peroxide (H_2O_2) method. The extract showed significant free radical scavenging activity in a dose dependent manner when compared with ascorbic acid. The highest radical scavenging activity of MEBH was found to be 79.8% at concentration of 300 µg mL^{-1}. The H_2O_2 scavenging effect of MEBH was 63.7% at a concentration of 200 (µg mL^{-1}). Further, the extract was studied for its anti-inflammatory and analgesic activities at the dose level of 100, 200 and 300 mg kg^{-1}. Anti-inflammatory activity was evaluated using carrageenan-induced paw edema in rats. Analgesic activity was evaluated by tail immersion and tail flick methods in mice. The extract showed significant decrease in paw volume (59.7% reduction) and pain at the dose level of 300 mg kg^{-1} when compared with reference drug diclofenac and morphine, respectively. The MEBH may be useful as a natural antioxidant in the treatment of inflammation and pain.

Key words: *Benincasa hispida,* DPPH, methanolic extract, pain, inflammation

INTRODUCTION

The natural products today symbolize safety in contrast to the synthetic drugs that are regarded as unsafe to humans and environment. So, people are returning to the natural products with the hope of safety and security. Numerous evidences have shown that increased consumption of fruits and vegetables reduce the risk of various pathological events such as cancer, cardiovascular diseases and cerebrovascular diseases (Goodwin and Brodwick, 1995; Rimm *et al*., 1996). This is often attributed to the antioxidants in the fruits and vegetables such as vitamin C, E, carotenoids, lycopenes and flavonoids that prevent damages caused by free radicals (Stahelin *et al*., 1991). Free radicals play an important role in the development of tissue damage and pathological events in living organisms (Kehrer, 1993). Natural medicines with free radical scavenging properties have been used for various purposes and epidemiological data also points at widespread acceptance and use of these agents. Presently, the active constituents from the natural sources are extracted, purified and tested. Results have shown their benefits in prevention of the free radical mediated diseases (Venkat Ratnam *et al*., 2006).

Many researchers have paid attention towards the Cucurbitaceae family. The seeds and fruits of various plants of the family, such as *Momordica chirantia*, *Citrullus colocynthis* have been evaluated for their antioxidant, anti inflammatory and analgesic activities (Semiz and Sen, 2007; Kumar *et al*., 2008; Marzouka *et al*., 2010). The seeds of *Cucumis sativum* are reported to possess antioxidant activity (Gill *et al*., 2009). *Benincasa hispida* is an important plant of the family. It is a large climbing or trailing herb with stout, angular and hispid stems widely cultivated in tropical Asia. It is called Petha in Hindi and White Gourd, Wax Gourd or Ash Gourd in English. In Ayurveda, *Benincasa hispida* is recommended for management of peptic ulcer, diabetes mellitus, urinary infection, hemorrhages from internal organs, epilepsy and other nervous disorders (Warrier *et al*., 1994). The extract of the fruit is reported to possess anti-ulcer (Grover *et al*., 2001), anti-angiogenic (Lee *et al*., 2005) and antihistaminic activities (Kumar and Ramu, 2002). The fruit pulp is used in confectionary industry for the preparation of sweets and million tones of seeds are discarded. These seeds have been used traditionally in Ayurveda for treatment of various disorders such as peptic ulcer and as vermifuge (Warrier *et al*., 1994). The seeds can be used for their therapeutic potential. So our present study was carried out to evaluate the free radical scavenging, anti inflammatory and analgesic potential of *Benincasa hispida* seeds.

Corresponding Author: Naresh Singh Gill, Rayat Institute of Pharmacy, SBS Nagar, Punjab Technical University, Ropar-144533, India Tel: +91-9888611679

MATERIALS AND METHODS

Plant material: The seeds were purchased from the Khari Baoli (spice market) Delhi (India) in August 2009. The healthy looking seeds were selected for authentication and voucher specimen number 0389 has been deposited in the Botanical and Environmental Science Department, Guru Nanak Dev University, Amritsar (India). The seeds were cleaned, washed, dried at low temperature and powered.

Drugs and chemicals: Ascorbic acid and carrageenan were obtained Central Drug House Pvt. Ltd., Mumbai, India. Hydrogen peroxide was obtained from E-Merck Ltd., Mumbai. 1,1-Diphenyl-2-picrylhyderazyl (DPPH) was obtained from Sigma Chemical Co., USA. Physician sample of morphine and diclofenac sodium was procured from Government Medical College and Hospital, Patiala. All other chemical reagents used were of analytical grade which were procured from different companies (Loba Chem, Mumbai and Merck Limited, Mumbai).

Animals: The wistar albino rats (200-250 g) and swiss albino mice (25-30 g) of either sex were obtained from NIPER Mohali. They were kept at standard laboratory diet, environmental temperature and humidity. A 12 h light and dark cycle was maintained throughout the experimental protocol. The experimental protocol was duly approved by Institutional Animal Ethics Committee (IAEC) and care of the animals was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg No. 874/ac/05/CPCSEA).

Extraction: The powdered seeds were extracted for 72 h with methanol at room temperature. The solvent was filtered off and residue macerated again with the fresh solvent. Both solvents were combined and concentrated under reduced pressure on a rotary evaporator (Hedolph) at 40°C. The concentrated filtrate was suspended in distilled water and partitioned successively with hexane. The aqueous layer was separated and concentrated on water bath. The crude extracts were used for further investigation.

Phytochemical screening: The crude extract was studied for the presence of phytochemicals such as alkaloids, tannins, saponins, flavonoids, steroids, triterpenoids, carbohydrates, proteins and amino acids using standard procedures (Harborne, 1973).

The alkaloids were tested using various reagents such as Dragendroff's reagent (potassium bismuth iodide solution), Hagers reagent (picric acid solution), Mayer's reagent (potassium iodide solution) and Wagner's reagent (solution of iodine in potassium iodide). The sterols were tested by Liebermann's test (blue green color with acetic anhydride plus sulphuric acid). The triterpenoids were tested by Liebermann-Burchard's test. In this test a few mg of the extract was dissolved in chloroform, a few drops of acetic anhydride were added followed by sulphuric acid. Red/pink/purple/violet color confirms the presence of triterpenoids. The ferric chloride test was used for tannins (blue green color with ferric chloride), Molish test for carbohydrates, Ninhydrin reagent test for proteins and amino acids. The flavonoids were tested by concentrated nitric acid (crimson or magenta color with concentrated nitric acid).

Free radical scavenging activity

DPPH radical scavenging activity: The free radical scavenging activity of MEBH was determined by using 1,1-diphenyl-2 picryl-hydrazyl (DPPH) method (Sreejavan and Rao, 1997). Briefly, a 0.05 mM solution of DPPH in methanol was prepared and 1.5 mL of this solution was added to 0.5 mL of extract solution in methanol at different concentrations (100-300 μg mL^{-1}). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a spectrophotometer (Shimadzu UV- 1700 Pharma spec). A blank without DPPH was used to remove the influence of the color of the samples. A methanolic solution of DPPH was used as negative control. Ascorbic acid was used as a reference drug. All measures were carried out in triplicate. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The DPPH radical scavenging activity was calculated using the Eq. 1:

$$\text{Percentage scavenging of DPPH radical} = 100\times\left(\frac{A_o - A_s}{A_o}\right) \quad (1)$$

where, A_o is absorbance of the negative control A_s is the absorbance of the sample.

Hydrogen peroxide radical scavenging activity: Hydrogen peroxide radical scavenging activity was determined according to the method of Wettasinghe and Shahidi (2000). The extract solution (1.0 mL) in various concentrations (25-200 μg mL^{-1}) was mixed with 2.4 mL of 0.1 M phosphate buffer (pH 7.4) and then 0.6 mL of a 43 mM solution of H_2O_2 in the same buffer were added.

After 10 min the absorbance values of the reaction mixtures were recorded against a blank solution containing phosphate buffer without H_2O_2 at 230 nm using a spectrophotometer (Shimadzu UV- 1700 Pharma spec). For each concentration, a separate blank sample was used for background subtraction. Ascorbic acid was used as a standard and mixture without sample was taken as a control. The percentage scavenging of H_2O_2 was calculated as:

$$\text{Percentage scavenging of } H_2O_2 = \left(\frac{A_o\text{-}A_1}{A_o}\right)\times 100 \quad (2)$$

where, A_o is the absorbance of the control and A_1 is the absorbance of the extract/standard. All tests were done in triplicate (Sood *et al*., 2009).

Anti-inflammatory activity

Carrageenan-induced rat paw edema: The carrageenan-induced rat paw edema assay was carried out according to Winter *et al.* (1962). Wistar rats were divided into 5 groups each consisting of 6 animals.

Group I : (Disease Control): Carrageenan (1%) was administered in the plantar surface of rat
Group II : (Standard): Diclofenac sodium (12.5 mg kg^{-1}, p.o.)
Group III: (MEBH 100): Methanolic extract (100 mg kg^{-1}, p.o.)
Group IV: (MEBH 200): Methanolic extract (200 mg kg^{-1}, p.o)
GroupV : (MEBH 300): Methanolic extract (300 mg kg^{-1}, p.o.)

Edema was induced on the left hind paw of the rats by subplantar injection of 0.1 mL of a solution of 1% (w/v) carrageenin in a 0.9% NaCl (w/v). The paw volume was measured at intervals of 60, 120, 180 min by the mercury displacement method using a plethysmograph after administration of the extract/drug orally. The percentage inhibition of paw edema in drug treated group was compared with the carrageenan control group and calculated according to the following in Eq. 3.

$$\text{Percentage inhibition} = \left(\frac{V_t\text{-}V_c}{V_c}\right)\times 100 \quad (3)$$

where, V_c is the inflammatory increase in paw volume of control group of animals and V_t is the inflammatory increase in paw volume of drug-treated animals.

Analgesic activity: Swiss albino mice of either sex were divided into 5 groups each consisting of 6 animals.

Group I : (Control): Vehicle (1% CMC, p.o.)
Group II : (Standard): Morphine (10 mg kg^{-1} p.o.)
Group III: (MEBH 100): Methanolic extract (100 mg kg^{-1} p.o.)
Group IV: (MEBH 200): Methanolic extract (200 mg kg^{-1} p.o.)
Group V : (MEBH 300): Methanolic extract (300 mg kg^{-1} p.o.)

Tail immersion test: The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice (Toma *et al*., 2003). The tail of mice was immersed in warm water kept constant at 52.5"0.5°C. The reaction time of the tail-withdrawal response was determined at 0, 30, 60, 90 and 120 min after the administration of drugs. A cut off time of 15 sec was maintained to prevent tissue damage (Grotto and Sulman, 1967).

Tail flick test: The tail of mice was placed on the radiant heat source (1 cm distance from the nichrome wire) of an analgesiometer and time taken by the animals to withdraw its tail from the radiant heat source was taken as the reaction time. The reaction time was recorded at 0, 30, 60, 90 and 120 min after the drug administration. A cut off time of 15 sec was maintained to prevent tissue damage. The temperature was maintained at 52"0.5°C (Davies *et al*., 1946).

Statistical analysis: All the results were expressed as Mean"Standard Error of Means (SEM). The data was statistically analyzed by one way Analysis of Variance (ANOVA) followed by Tukey=s multiple range tests by using Sigmastat Version-2.0 Software. The p-value<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening: The results of the phytochemical screening of the methanolic extract are shown in Table 1; the results reveal the presence of chemical constituents such as proteins and amino acids, triterpenoids, carbohydrates, sterols and tannins.

DPPH radical scavenging activity: DPPH, reacts with antioxidants and gets converted into 1,1-diphenyl-2-picrylhydrazine by accepting a hydrogen atom and hence shows decrease in absorbance. The MEBH showed concentration-dependent DPPH radical scavenging activity. The highest radical scavenging activity of MEBH was found to be 79.8% at concentration of 300 μg mL^{-1}; the results are shown in Table 2.

Table 1: Phytochemical chemical screening of MEBH

Chemical constituents	Results
Alkaloids	-
Flavonoids	-
Protein and amino acid	+
Triterpenoids	+
Saponin	-
Carbohydrates	+
Phytosterols	+
Tannins	+

+: Presence of chemical constituent, -: Absence of chemical constituent

Table 2: Percentage scavenging of DPPH radical

Conc. of extract ($\mu g\ mL^{-1}$)	Percentage scavenging of DPPH radical	
	Methanol extract	Ascorbic acid
100	54.4±0.37	62.07±0.79
200	66.5±0.52	71.20±0.64
300	79.8±0.49	85.30±0.67

Values are the average of triplicate experiments and represented as Mean±SEM

Table 3: Percentage scavenging of H_2O_2

Conc. of extract ($\mu g\ mL^{-1}$)	Percentage scavenging of H_2O_2	
	Methanol extract	Ascorbic acid
25	36.2±0.26	41.1±0.37
50	48.1±0.52	57.2±0.26
100	56.3±0.37	63.5±0.34
200	63.7±0.23	74.0±0.26

Values are the average of triplicate experiments and represented as Mean±SEM

Hydrogen peroxide radical scavenging activity: The free radical scavenging activity of the MEBH was evaluated by H_2O_2 scavenging method. The results are shown in Table 3, the MEBH showed concentration dependent activity. The H_2O_2 scavenging effect of MEBH was 63.7% at a concentration of 200 ($\mu g\ mL^{-1}$) which was comparable to the scavenging effect of ascorbic acid.

Anti-inflammatory activity: Table 4 shows the results of the anti-edematous effect of orally administered MEBH on carrageenin induced paw edema in rats; the MEBH showed dose dependent anti-inflammatory activity in carrageenan induced paw edema in rats. At dose of 100 mg kg^{-1}, MEBH caused a reduction in paw edema (19.4%) 3 h after the subplantar injection of carrageenin. Doses of 200 and 300 mg kg^{-1} caused a significant reduction of paw edema (41.7 and 59.7%, respectively) from the first till the third hour compared with the disease control group.

Analgesic activity

Tail immersion test: Table 5 depicts the analgesic activity shown by MEBH by tail immersion method. MEBH showed dose dependent analgesic activity against conduction of heat-induced algesia in mice. Medium (200 mg kg^{-1}) and high (300 mg kg^{-1}) doses showed significant difference in the analgesic activity when compared with control group. Maximum analgesic effect was observed at 90 min interval.

Tail flick test: Table 6 depicts the analgesic activity shown by MEBH by tail flick method. The MEBH showed dose dependent analgesic activity against radiant heat-induced algesia. Medium (200 mg kg^{-1}) and high (300 mg kg^{-1}) doses showed significant difference in the analgesic activity when compared with control group. Maximum analgesic effect was observed at 90 min interval. Hence, it was observed that the MEBH significantly reduced the pain sensation in mice at medium and higher dose.

In the present study, the methanolic extract of *Benincasa hispida* seeds was evaluated for its free radical scavenging, anti-inflammatory and analgesic potential. Results have revealed that MEBH possess *in vitro* free radical scavenging activity. Hence, the extract was further evaluated for its *in vivo* anti-inflammatory and analgesic potential. Carrageenan induced rat paw edema test has frequently been used to assess the anti-edematous effect of natural products. Carrageenan brings about inflammation by the release of mediators of inflammation (prostaglandins, leukotrienes, histamine, bradykinin etc.) (Crunkhorn and Meacock, 1971); also, several free radicals are released during such inflammation (Koblyakov, 2001). The MEBH showed significant free radical scavenging activity, so this can be responsible for the reduction of inflammation in the carrageenan-induced paw edema in rats (Cuzzocrea *et al*., 2001). Tail immersion and tail methods were carried out to evaluate the analgesic potential of seed extract. Significant decrease in the tail withdrawal latency indicated that the methanolic extract of seeds possess analgesic property. This analgesic activity may be due to its free radical scavenging activity as these free radicals are involved during pain stimulation and antioxidants show reduction in such pain (Kim *et al*., 2004). The literature also reveals the antioxidant capacity of the pumpkin seed extract against DPPH free radical formation (Xanthopoulou *et al*., 2009). The seeds of *Citrullus lanatus* are found to possess antioxidant, anti-inflammatory and analgesic activities (Gill *et al*., 2010). The seed extract of *Momordica Charantia* normalizes the impaired antioxidant status in streptozotocin induced-diabetes by scavenging of free radicals (Sathishsekar and Subramanian, 2005).

The triterpenoids isolated from various species of cucurbita family have been reported to possess anti-inflammatory activities (Miro, 1995). The cucurbitacins from *Cucurbita andreana* were evaluated for their anti inflammatory and inhibitory effects on the

Table 4: Effect of MEBH on carrageenin induced paw edema in rats

Treatment groups	Dose (mg kg^{-1}) orally	Mean paw volume (mL) 60 min	120 min	180 min	Percentage inhibition of edema
Disease control	1% CMC	0.54±0.009	0.62±0.006	0.67±0.005	-
Diclofenac	12.5	0.38±0.002[a]	0.35±0.009[a]	0.20±0.005[a]	70.1
MEBH	100	0.48±0.002	0.58±0.005	0.54±0.015	19.4
MEBH	200	0.45±0.009[a,b]	0.42±0.006[a,b]	0.39±0.013[a,b]	41.7
MEBH	300	0.40±0.008[a,b]	0.35±0.015[a,b]	0.27±0.007[a,b]	59.7

The values are Mean±SEM of 6 animals. [a]$p<0.05$ compared with disease control group, [b]$p<0.05$ compared with diclofenac treated group

Table 5: Analgesic effect of the MEBH by tail immersion test

Group	Dose (mg kg^{-1}) orally	Tail withdrawal latency (time in sec) 0 min	30 min	60 min	90 min	120 min
Control	1% CMC	3.32±0.04	3.64±0.01	3.51±0.05	3.48±0.28	3.53±0.03
Morphine	10	3.23±0.02	7.48±0.02[a]	12.93±0.18[a]	14.81±0.01[a]	14.43±0.01[a]
MEBH	100	3.45±0.16	4.32±0.08	6.76±0.05	7.86±0.05	6.57±0.06
MEBH	200	3.69±0.05	4.95±0.01[a,b]	8.33±0.01[a,b]	11.28±0.22[a,b]	9.84±0.34[a,b]
MEBH	300	3.54±0.01	5.47±0.05[a,b]	10.25 ±0.05[a,b]	13.76± 0.01[a,b]	13.41±0.05[a,b]

Values are Mean±SEM of 6 animals in each group. [a]$p<0.05$ compared with control group, [b]$p<0.05$ compared with Morphine treated group

Table 6: Analgesic effect of the MEBH by tail flick test

Group	Dose (mg kg^{-1}) orally	Tail flick latency (time in sec) 0 min	30 min	60 min	90 min	120 min
Control	1 % CMC	2.65±0.01	2.83±0.27	2.47±0.17	2.54±0.03	2.68±0.15
Morphine	10	2.54±0.14	5.63±0.04[a]	7.35±0.22[a]	9.86±0.04[a]	8.24±0.01[a]
MEBH	100	2.47±0.34	2.92±0.06	3.86±0.05	4.75±0.05	4.17±0.53
MEBH	200	2.73±0.01	3.42±0.15[a,b]	5.28±0.03[a,b]	6.88±0.26[a,b]	5.54±0.32[a,b]
MEBH	300	2.56±0.15	4.31±0.05[a,b]	6.57±0.01[a,b]	8.67±0.47[a,b]	6.79±0.05[a,b]

Values are Mean±SEM of 6 animals in each group. [a]$p<0.05$ compared with control group, [b]$p<0.05$ compared with Morphine treated group

growth of cancer cell lines (Jayaprakasam *et al*., 2003). Cucurbitacin glucosides are reported to possess antioxidant and free radical scavenging activities (Tannin-Spitz *et al*., 2007). Thus, the triterpenoids might be responsible for the free radical scavenging, analgesic and anti-inflammatory activity.

CONCLUSION

The MEBH was found to have free radical scavenging potential and this may be one of the probable reasons for the reduction in carregeenan-induced paw edema in rats along with decrease in algesia in mice models.

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REFERENCES

Crunkhorn, P. and S.C. Meacock, 1971. Mediators of the inflammation induced in the rat paw by carrageenin. Br. J. Pharmacol., 42: 392-402.

Cuzzocrea, S., D.P. Riley, A.P. Caputi and D. Salvemini, 2001. Antioxidant therapy: A new pharmacological approach in shock, inflammation and ischemia/ reperfusion injury. Pharmacol. Rev., 53: 135-159.

Davies, O.L., J. Raventos and A.L. Walpole, 1946. A method for the evaluation of analgesic activity using rats. Br. J. Pharmacol., 1: 255-264.

Gill, N.S., M. Garg, R. Bansal, S. Sood, A. Muthuraman, M. Bali and P.D. Sharma, 2009. Evaluation of antioxidant and antiulcer potential of *Cucumis sativum* L. seed extract in rats. Asian J. Clin. Nutr., 1: 131-138.

Gill, N., R. Bansal, M. Garg, S. Sood, A. Muthuraman and M. Bali, 2010. Evaluation of antioxidant, anti-inflammatory and analgesic potential of *Citrullus lanatus* seed extract in rodent model. Internet J. Nutr. Wellness,

Goodwin, J.S. and M. Brodwick, 1995. Diet, aging and cancer. Clin. Geriatr. Med., 11: 577-589.

Grotto, M. and F.G. Sulman, 1967. Modified receptacle method for animal analgesimetry. Arch. Int. Pharmacodyn. Ther., 165: 152-159.

Grover, J.K., G. Adiga, V. Vats and S.S. Rathi, 2001. Extracts of *Benincasa hispida* prevent development of experimental ulcers. J. Ethnophamacol., 78: 159-164.

Harborne, J.B., 1973. Phytochemical Methods. Chapman and Hall, London, pp: 1-32.

Jayaprakasam, B., N.P. Seeram and M.G. Nair, 2003. Anticancer and antiinflammatory activities of cucurbitacins from *Cucurbita andreana*. Cancer Lett., 189: 11-16.

Kehrer, J.P., 1993. Free radicals as mediators of tissue injury and disease. Crit. Rev. Toxicol., 23: 21-48.

Kim, H.K., S.K. Park, J.L. Zhou, G. Taglialatela, K. Chung, R.E. Coggeshall and J.M. Chung, 2004. Reactive oxygen species (ROS) play an important role in a rat model of neuropathic pain. Pain, 111: 116-124.

Koblyakov, V.A., 2001. Free radicals and inflammation (progress in inflammation research series). Biochemistry, 66: 937-938.

Kumar, A.D. and P. Ramu, 2002. Effects of methanolic extract of *Benincasa hispida* against hitamine and acetylcoline induced bronchospasm in guinea pigs. Indian J. Pharmacol., 34: 365-366.

Kumar, S., D. Kumar, K. Saroha, N. Singh and B. Vashishta, 2008. Antioxidand and free radical scavenging potential of *Citrullus colocynthis* (L.) Schard. methanolic fruit extract. Acta Pharm., 58: 215-220.

Lee, K.H., H.R. Choi and C.H. Kim, 2005. Antiangiogenic effect of the seed extact of *Benincasa hispida* Cogniaux. J. Ethnopharmacol., 97: 509-513.

Marzouka, B., Z. Marzoukb, E. Halouib, N. Feninab, A. Bouraouic and M. Aouria, 2010. Screening of analgesic and anti-inflammatory activities of *Citrullus colocynthis* from Southern Tunisia. J. Ethnopharmacol., 128: 15-19.

Miro, M., 1995. Cucurbitacins and their pharmacological effects. Phytother. Res., 9: 159-168.

Rimm, E.B., A. Ascherio, E. Giovannucci, D. Spiegelman, M.J. Stampfer and W.C. Willett, 1996. Vegetable, fruit and cereal fiber intake and risk of coronary heart disease among men. J. Am. Med. Assoc., 275: 447-451.

Sathishsekar, D. and S. Subramanian, 2005. Antioxidant properties of *Momordica charantia* (bitter gourd) seeds on Streptozotocin induced diabetic rats. Asia Pac. J. Clin. Nutr., 14: 153-158.

Semiz, A. and A. Sen, 2007. Antioxidant and chemoprotective properties of *Momordica charantia* L. (bitter melon) fruit extract. Afr. J. Biotechnol., 6: 273-277.

Sood, S., S. Bansal, A. Muthuraman, N.S. Gill and M. Bali, 2009. Therapeutic potential of *Citrus medica* L. Peel Extract in carrageenan induced inflammatory pain in rat. Res. J. Med. Plant, 3: 123-133.

Sreejavan, N. and M.N.A. Rao, 1997. Free radical scavenging activity of curcuminoids. Drug Res., 46: 169-171.

Stahelin, H.B., K.F. Gey, M. Eichholzer, E. Ludin, F. Bernasconi, J. Thurneysen and G. Brubacher, 1991. Plasma antioxidant vitamins and subsequent cancer mortality in the 12-year follow-up of the prospective basal study. Am. J. Epidemiol., 133: 766-775.

Tannin-Spitz, T., M. Bergman and S. Grossman, 2007. Cucurbitacin glucosides: Antioxidant and free-radical scavenging activities. Biochem. Biophys. Res. Commun., 364: 181-186.

Toma, W., J.S. Graciosa, C.A. Hiruma-Lima, F.D.P. Andrade, W. Vilegas and A.R.M. Souza-Brita, 2003. Evaluation of the analgesic and antiedematogenic activities of *Quassia amara* bark extract. J. Ethnopharmacol., 85: 19-23.

Venkat Ratnam, D., D.D. Ankola, V. Bhardwaj, D.K. Sahana and M.N.V. Ravi-Kumar, 2006. Role of antioxidants in prophylaxis and therapy:A pharmaceutical perspective. J. Controlled Release, 113: 189-207.

Warrier, P.K., V.P.K. Nambiar and C. Ramankutty, 1994. Indian Medicinal Plants: A Compendium of 500 Species. 1st Edn., Orient Longman Publisher, India, ISBN: 8125003029, pp: 423.

Wettasinghe, M. and F. Shahidi, 2000. Scavenging of reactive oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. Food Chem., 70: 17-26.

Winter, G.A., E.A. Rislfy and G.W. Nuss, 1962. Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drug. Proc. Soc. Exp. Biol. Med., 111: 544-547.

Xanthopoulou, M.N., T. Nomikos, E. Fragopoulou and S. Antonopoulou, 2009. Antioxidant and lipoxygenase inhibitory activities of pumpkin seed extracts. Food Res. Int., 42: 641-646.