IJPSR (2012), Vol. 3, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 August, 2012; received in revised form 30 September, 2012; accepted 24 November, 2012

ASSESSMENT OF PHYTOCHEMICAL & PHARMACOLOGICAL ACTIVITIES OF THE ETHANOL EXTRACTS OF *XANTHIUM INDICUM*

Ahmed Ullah Mishuk, Md. Anisur Rahman*, Shazia Afrin, Md. Iqbal Ahmed, Samir Kumar Sadhu and Faroque Hossain

Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh

Keywords:
Xanthium indicum,
Antioxidant activity,
Analgesic activity,
DPPH,
Ascorbic acid,
Diclofenac sodium

Correspondence to Author:

Md. Anisur Rahman

Lecturer, Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh

E-mail- ar_anis06@yahoo.com

QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Website:
www.ijpsr.com

ABSTRACT

The objective of the present study was to investigate the antioxidant and analgesic activity of ethanol extract of *Xanthium indicum* Koen. Family: Compositae. Ethanol extract of *X. indicum* plant was prepared and tested for its phytochemical and pharmacological investigation. Preliminary phytochemical investigation showed that extracts possessed the active principles-alkaloid, carbohydrate, glycoside, flavonoid & tannin. The ethanol extract of *X. indicum* showed significant antioxidant property in the qualitative assay. The extract showed the antioxidant property by the presence of strong yellow spot on a purple background on the TLC plate & it also in the quantitative assay, *X. indicum extract* displayed free radical scavenging activity in the DPPH assay (IC_{50} ~141.25 µg/mL) which is comparable to standard (ascorbic acid, IC_{50} ~14.12 µg/mL). The extract showed dose-dependent reduction ability (Fe^{3+} to Fe^{2+} transformation) in reducing power assay; showing a maximum absorbance of 0.492 at a concentration of 100 µg/mL of the ethanol extract comparable to that of ascorbic acid which were used as positive control and gave maximum absorbance of 0.659 at a concentration of 100 µg/mL. The extract inhibited the acetic acid induced writhing in mice (47.5% P<0.001 and 24.55% P<0.05) at the dose of 500 and 250 mg/kg body weight respectively and it is comparable to the activity of the standard analgesic drug Diclofenac sodium (67.96% P<0.001) at the dose of 25 mg/kg body weight. In summary, the ethanol extract of *X. indicum* possesses antioxidant constituent(s) & the ethanol extract has had beneficial effects as a pain reliever.

INTRODUCTION: *X. indicum* locally known as Ghagra, Banokra, Bichaphal is a coarse annual about a meter or more in height. Leaves are numerous, 5-7.5 cm long and almost as broad as long, broadly triangular-ovate or suborbicular, acute, often 3-lobed, rough with appressed hairs, irregularly inciso-serrate. Heads in terminal and axillary racemes. Fruits are ovoid; about 1.6 cm long, with 2 erect mucronate beaks, thickly clothed with usually hooked prickles.

It grows as a gregarious weed in fallow paddy fields and by the canal or ditch banks in all areas of

Bangladesh. The plant is reported to have diaphoretic, diuretic, sudorific, CNS depressant and styptic properties. Decoction of the plant is used in urinary and renal complaints, gleet, leucorrhoea and menorrhagia. Seeds are used to resolve inflammatory swellings while the root being useful against scrofulous tumours and cancer.

The root extract of the plant is reported to be employed in cancer and scrofula, the fruits are rich in vitamin B and are utilized as demulcent and said to be effective in treating small pox, herpes and bladder affections The plant is reported to contain alpha and gamma tocopherols, polyphenols, glucoside, xanthostrumarin and xanthonolides as the principal constituents [1].

Medicinal plants are still valuable source of safe, less toxic, lower price, available and reliable natural resources of drugs all over the world. Plants have been used as remedies and still they play an important role in health care for about 80% of the world's population from the beginning of civilization. The therapeutic basis of herbal medication has formed by the presence of diverse bioactive compounds like steroids, terpenoids, flavonoids, alkaloids, glycosides etc. in plants. Also for the treatment of diseases which are still incurable, medicinal plants can serve as a source of novel therapeutic agent.

Considering the importance of this area and as a part of our ongoing investigation on local medicinal plants of Bangladesh [2], in this paper, we reported a study of the antioxidant and analgesic activity of the leaves of *X. indicum*.

MATERIALS AND METHODS

Sample collection and identification: For this present investigation, *X. indicum* was collected from Khulna University, Bangladesh in janury 2011 and was identified by Bangladesh National Herbarium, Mirpur, Dhaka (Accession number DACB-35522).

Preparation of Extract: The collected plants were separated from undesirable plant parts, washed with water and shade-dried for 3 weeks. After grinding of plant parts by suitable grinder the powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 150gm of powered was taken in a clean, flat-bottomed glass container and soaked in 500 ml of 96% ethanol for 7 days accompanying occasional shaking and stirring. The mixture was then filtrated by a piece of clean, white cotton and finally by filter paper. 7 gm (yield 5.83%) of gummy concentrate of greenish black color crude ethanol extracts of *X. indicum* was obtained by evaporation of filtrate by rotary evaporator.

Animals: Mice of random sex (*Swiss-webstar* strain, 19-40 gm body weight) collected from animal resources branch of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) were used for the experiments. The animals were kept at animal house (Pharmacy Discipline, Khulna University, Khulna) for adaptation after collection under standard laboratory conditions (relative humidity 55-65%, room temperature 25 ± 2^{o}C and 12 hour light: dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water. According to the animal ethics provided by ICDDR, Bangladesh.

Chemicals: Diclofenac-Na, DPPH, Ascorbic acid, Molisch's, Mayer's, Dragendroff's, Fehling's and Benedict's reagent, Sodium dihydrogen phosphate, Disodium hydrogen phosphate, Potassium ferric cyanide, Trichloroacetic acid, Ferric chloride.

Phytochemical tests: Small amount of dried extract was appropriately treated to prepare sample solution and then subjected to the specific phytochemical tests [3]. Libermann-Burchard test was performed to identify steroids. Mayer's reagent and Dragendroff's reagent test was performed to identify alkaloids. Ferric chloride test was performed to identify tannin. Molisch's test, Fehling's test and Benedict's test were performed to investigate the presence of reducing sugar. For saponin, flavonoid and glycosides general identifying test were performed.

Determination of Analgesic Activity: The acetic acid induced writhing method [4-6] was adopted with minor modification. The experimental animals were randomly divided into four groups, each consisting of five animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 in water at the dose of 10 mL/kg of body weight; group II was treated as 'positive control' and was given the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight; group

III and group IV were test groups and were treated with the extract of *X. indicum*at the doses of 250 and 500 mg/kg of body weight respectively. Control vehicle, standard drug and extracts were administered orally, 30 minutes prior to the intraperitoneal injection of 0.7% acetic acid; after an interval of 15 minutes, the number of writhes was counted for 5 minutes. The number of writhings in the control was taken as 100% and percent inhibition was calculated as follow:

% Inhibition of writhing = 100 – (treated mean / control mean) × 100

Determination of *In-vitro* Antioxidant Activity (DPPH Free Radical Scavenging Activity):

Qualitative assay: A suitably diluted stock solutions were spotted on pre-coated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract. The plates were dried at room temperature and were sprayed with 0.02% 1, 1-diphenyl-2-picryl hydrazyl (DPPH) in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 minutes and the color changes (yellow on purple background) were noted [7]. DPPH forms deep pink color when it is dissolved in ethanol. When it is sprayed on the chromatogram of the extract, it forms pale yellow or yellow color which indicates the presence of antioxidants.

Quantitative assay: The antioxidant potential of the ethanol extract was determined on the basis of their scavenging activity of the stable 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. The aliquots of the different concentrations (1-500 µg/mL) of the extract were added to 3 mL of a 0.004% w/v solution of DPPH. Absorbance at 517 nm was determined after 30 min, and IC_{50} (Inhibitory conc. 50%) was determined. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals [8].

The formula used for % inhibition ratio is;

% inhibition = (Blank OD-Sample OD/ Blank OD) × 100

Determination of *In-vitro* Antioxidant activity (Reducing Power Assay): The reducing power of extract can be determined according to the method followed by the antioxidant potential of the ethanol extract was determined on the basis of their reducing power on $FeCl_3$ [9]. The absorbance is measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power.

RESULTS AND DISCUSSION: The ethanol extract of *X. indicum* was subjected to different qualitative phytochemical tests for detection of different classes of biologically active chemical compounds and the results are summarized in the (**Table 1**). It shows that the ethanol extract of *X. indicum* contains alkaloids, reducing sugars, tannins, flavonoids, glycosides. These compounds are supposed to be responsible for biological activities of *X. indicum.*

Analgesic activity of the extract of *X. indicum* was tested by acetic acid induced writhing model in mice. Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings [10].

Increased levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid [11].

In acetic acid induced writhing test, the ethanol extract of *X. indicum* significantly and dose dependently suppressed the frequency of acetic acid induced writhing in mice. It showed 47.5 ($P<0.001$) and 24.55% ($P<0.05$) writhing inhibition at the dose of 500 and 250 mg/kg body weight respectively, while the standard drug diclofenac-Na showed 67.55% writhing inhibition (**Table 2**). These results showed that the analgesic effect of *X. indicum*was significant at 500 mg/kg body weight. Several flavonoids isolated from medicinal plants have been discovered to possess significant anti-nociceptive and/or anti-inflammatory effects [12].

Systemic (i.p. or p.o.) administration of the flavonoid myricitrin, at doses that did not produce any important motor dysfunction, alterations in basal temperature, or any other obvious side effects induced a dose-

dependent inhibition of acetic acid-induced visceral nociceptive response in mice [13].

The Gi/o protein dependent mechanism is involved on antinociception caused by flavonoid myricitrin. The opening of voltage and small conductance calcium-gated K^+ channels and the reduction of calcium influx led to the antinociceptive of flavonoid myricitrin.

It is therefore, possible that the anti-nociceptive effects observed with this extract may be attributable to its flavonoid component, shown to be present during phytochemical analysis. On the basis of the result of acetic acid induced writhing test, it can be concluded that the dried ethanol extract of whole plant of *X. indicum* possess analgesic activity and the mechanism to suppress the nociception is probably the opening of voltage and small conductance calcium-gated K^+ channels and the reduction of calcium influx.

The DPPH radical contains an odd electron which is responsible for visible purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized & observed as light yellow spot. *X. indicum* extract may contain some anti-oxidative compounds which give an electron & produce light yellow color.

Therefore quantitative determination is necessary to find out the extent of antioxidant activity. 0.2 % DPPH solution in ethanol is sprayed onto a TLC plate, the compounds having antioxidant properties are seen as yellow zones on a purple background [14]. After applying DPPH on the TLC plate, yellow color on purple background was observed which indicated the presence of antioxidant components in the ethanol extract of *X. indicum*.

In the quantitative assay, *X. indicum* displayed a very free radical scavenging activity in the DPPH assay (IC_{50} ~141.25 µg/mL) which is comparable to that of ascorbic acid (IC_{50} ~14.12 µg/mL), (**Table 3, Table 4 & Fig. 1**), a well-known standard antioxidant. Plant antioxidants have generally phenolic moiety. Phenolic compounds can easily donate electrons to reactive radicals because of the resonance stability of phenoxy radical and thus retard radical chain reactions [15]**.**

The flavonoids from plant extracts have been found to possess antioxidants, antimicrobial and anti-inflammatory properties in various studies [16-17]. Several plant components like tannins are responsible for showing antioxidant property [18].

As in the phytochemical test, *X. indicum* showed the presence of flavonoids and tannins, its antioxidant and anti-inflammatory properties may be observed due to the presence of these chemical components.

The DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2 picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants [19].

The antioxidant potential of the ethanol extract was determined on the basis of their reducing power on $FeCl_3$. The absorbance is measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. It is observed that the absorbance of both extract & ascorbic acid is increasing which shows the reducing power of both extract & ascorbic acid, which ultimately gives us the idea about the antioxidant activity of both extract & ascorbic acid (**Table 5 & Fig. 2**).

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing properties are generally associated with the presence of reductones. The antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom. The reduction of ferrous ion (Fe^{3+}) to ferric ion (Fe^{2+}) is measured by the intensity of the resultant blue-green solution which absorbs at 700 nm. When substances exhibiting high reducing tendencies donate electrons which can react with free radicals converting them to more stable products in the process, radical chain reactions could be terminated.

The extract showed dose- dependent reduction ability (Fe^{3+} to Fe^{2+} transformation) in reducing power assay; showing a maximum absorbance of 0.492 at a concentration of 100 µg/mL of the ethanol extract comparable to that of ascorbic acid which were used as positive control and gave maximum absorbance of 0.659 at a concentration of 100 µg/mL [20].

The data obtained from the experiment for determining the reducing power of plant extract in

which increasing absorbance values implied increased conversion of Fe^{3+} to Fe^{2+}, hence increasing reducing ability of plant extract, showed that the extract exhibited concentration–dependent ferric reducing ability within the range of plant extract concentrations used for the experiment. This agrees with the report from other works done in this direction [21]. The antioxidants contained in the extracts may be partly responsible for the underlying mechanism.

TABLE 1: RESULTS OF PHYTOCHEMICAL GROUP TESTS

Extract	Reducing sugar	Steroid	Glycosides	Gum	Tannins	Alkaloid	Saponin	Flavonoid
Ethanol extract of *X. indicum*	+	-	+	-	+	+	-	+

"+" = Presence "-" = Absence

TABLE 2: EFFECT OF *X. INDICUM* EXTRACT ON ACETIC ACID INDUCED WRITHING IN MICE

	Dose (mg/kg, p.o.)	No of writhing (% writhing)	Inhibition (%)
Control (1% tween-80 solution in water, 10 mL/kg, p.o.)	-	44±3.312 (100)	0
Positive Control (Diclofenac sodium)	25	14.10±1.923 (32.04)	67.96**
Ethanol extract of *X . indicum*	250	33.20±1.475 75.45	24.55*
Ethanol extract of *X . indicum*	500	13.201±2.050 52.50	47.50**

X. indicum; values are expressed as mean ± SEM (Standard Error of Mean); (n=5) n= number of mice; **P<0.001, *P<0.05, p.o. = per oral.

TABLE 3: DPPH SCAVENGING ASSAY OF *X. INDICUM*.

Conc. (extract) (µg / mL)	log conc.	Abs1	Abs2	avg	% inhibition
0		0.672	0.672	0.672	0
1.57	0.196	0.596	0.600	0.598	11.01
3.13	0.496	0.576	0.579	0.578	14.062
6.25	0.796	0.563	0.568	0.567	15.69
12.5	1.097	0.548	0.549	0.549	18.42
25	1.398	0.513	0.515	0.514	23.51
50	1.699	0.498	0.499	0.499	25.81
100	2	0.367	0.371	0.369	45.36
200	2.301	0.288	0.285	0.287	57.36
400	2.602	0.213	0.217	0.215	68.3

TABLE 4: DPPH SCAVENGING ASSAY OF ASCORBIC ACID

Conc.(extract) (µg / mL)	log conc.	Abs1	Abs2	avg	% inhibition
0		0.672	0.672	0.672	0
1.57	0.196	0.554	0.562	0.558	16.96
3.13	0.496	0.565	0.565	0.565	17.55
6.25	0.796	0.538	0.539	0.5385	19.86
12.5	1.097	0.439	0.438	0.4385	34.74
25	1.398	0.132	0.137	0.1345	80.35
50	1.699	0.129	0.124	0.1265	80.8
100	2	0.073	0.075	0.074	89.12

TABLE 5: ABSORBANCE OF EXTRACT OF *X. INDICUM* AND ASCORBIC ACID ON DIFFERENT CONCENTRATION

Concentration	Absorbance (*X. indicum*)	Absorbance (Ascorbic acid)
0	0.401	0.401
1	0.425	0.451

3.13	0.431	0.469
6.25	0.438	0.486
12.5	0.451	0.492
25	0.473	0.560
50	0.492	0.618
100	0.492	0.659

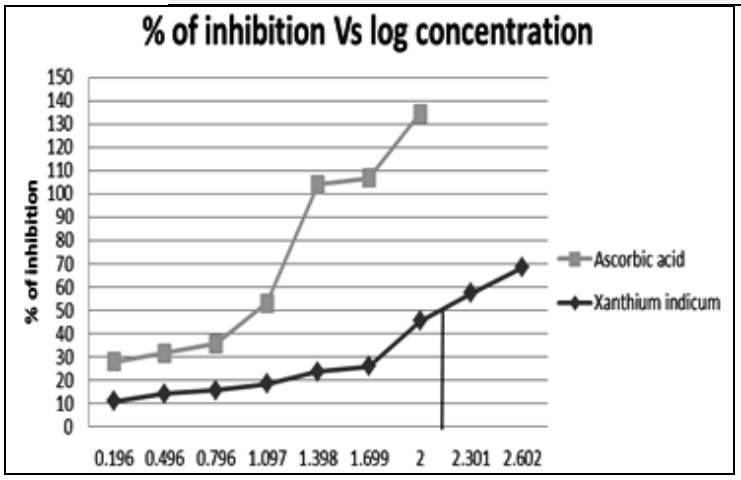

FIG. 1: COMPARISON OF % INHIBITION VS. LOG CONCENTRATION GRAPH FOR STANDARD (ASCORBIC ACID) VS. *X. INDICUM.*

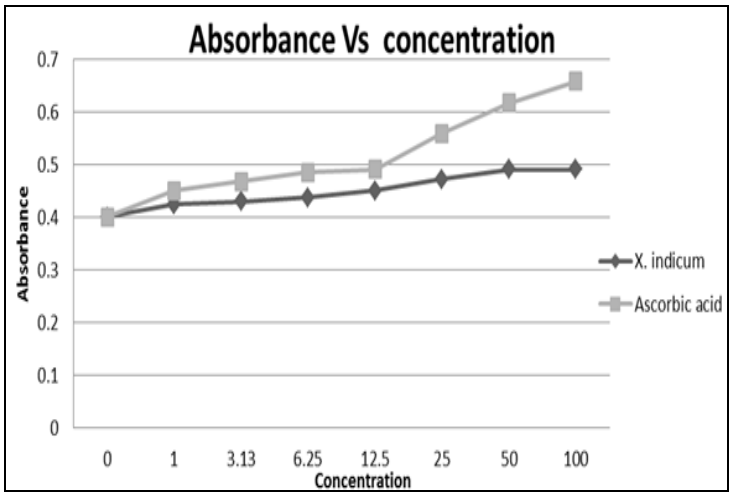

FIG. 2: COMPARISON OF ABSORBANCE VS CONCENTRATION GRAPH FOR STANDARD (ASCORBIC ACID) VS. *X. INDICUM.*

CONCLUSION: The experimental findings from the study showed that the ethanol extract of *X. indicum* possesses organic compounds like- carbohydrate, alkaloid, glycoside, flavonoid & tannin which can show extensively pharmacologic & other activities. The extract showed the free radical scavenging properties indicated by the presence of strong yellow spot on a purple background on the TLC plate. In the quantitative assay, the extract displayed free radical scavenging activity in the DPPH assay which is comparable to that of ascorbic acid a well-known standard antioxidant.

The reducing power assay shows the result of antioxidant activity of plant extract, which is comparable to the ascorbic acid. The observations support that the ethanol extract possesses antioxidant active constituent(s). In the analgesic test the extract produced a moderate writhing inhibition which was comparable to the standard drug Diclofenac sodium.

Based on this, it can be concluded that the ethanol extract of *X. indicum* possess analgesic activity and the mode of action might involve a peripheral mechanism.

ACKNOWLEDGEMENT: Authors are grateful to the Authority of the Khulna University, for giving the opportunity to conduct such experiment and providing necessary chemical, instrument and utility support.

REFERENCES:

1. Ghani, A. 2003. Medicinal Plants of Bangladesh. The Asiatic Society of Bangladesh. Dhaka, Bangladesh, pp. 430, 502-504.
2. Hasan S.M.R., Jamila M., Majumder M.M., Akter R., Hossain M.M., Mazumder M.E.H., Alam M.A., Jahangir R., Rana M.S., Rahman S., 2009. Analgesic and Antioxidant Activity of the Hydromethanolic Extract of *Mikania scandens* (L.) Willd. Leaves. American Journal of Pharmacology and Toxicology. 4(1): 1-7.
3. Kaiser Hamid, Monika Rani Saha, Kaniz Fatima Urmi, Md. Razibul Habib, Muhammad Mukhlesur Rahman, 2010, screening of different parts of the plant *Pandanus odorus* for its antioxidant activity. International Journal of Applied Biology and Pharmaceutical Technology Volume: I: Issue-3: Nov-Dec - 2010.
4. Whittle BA. 1964. The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. *British Journal of Pharmacology and Chemotherapy.* 22: 246-253.
5. Vanu, M.R., Palanivelu, S. and Panchanatham, S. 2006. Immunomodulatory and anti-inflammatory effects of *Semecarpus anacardium* Linn. Nut milk extract in experimental inflammatory conditions. *Biol. Pharm. Bull*. **29**, 693-700.
6. Ahmed, F., M.S.T. Selim, A.K. Das and M.S.K. Choudhuri, 2004. Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn. *Pharmazie*. 59, 329-333.
7. Hai-Feng Gu, Chun-Mei Li, Yu-juan Xu, Wan-feng Hu, Mei-hong Chenand Qiong-hong Wan (2008). Structural features and antioxidant activity of tannin from persimmon pulp. Food Research International, Volume 41, Issue 2, 2008, Pages 208-217.
8. Jain V., S.K. Verma, S.S. Katewa, S. Anandjiwala and B. Singh, 2011. Free radical scavenging property of *Bombax ceiba* Linn. root. Res. J. Med. Plant, 5: 462-470.
9. Hai-Feng Gu, Chun-Mei Li, Yu-juan Xu, Wan-feng Hu, Mei-hong Chenand Qiong-hong Wan (2008). Structural features and antioxidant activity of tannin from persimmon pulp. Food

Research International, Volume 41, Issue 2, 2008, Pages 208-217.

10. Taesotikul T, Panthong A, Kanjanapothi D, Verpoorte R, Scheffer JJC. (2003). Antiinflammatory, antipyretic and antinociceptive activities of *Tabernaemontana pandacaqui* Poir. J. Ethnopharmacol. 84, 31-35.
11. Deraedt R, Jouquey S, Delevallee F, Flauhaut M (1980). Release of prostaglandins E and F in an algogenic reaction and its inhibition.Eur. J. Pharmacol., 61: 17-24.
12. Amaral S, Mira L, Nogueira JM, da Silva AP, Florencio MH (2009). Plant extracts with anti-inflammatory properties--a new approach for characterization of their bioactive compounds and establishment of structure-antioxidant activity relationships. Bioorg. Med. Chem., 17(5): 1876-1883.
13. Meotti, F.C., A.P. Luiz, M.G Pizzolatti, C.A. Kassuya, J.B Calixto and A.R. Santos, 2006. Analysis of the antinociceptive effect of the flavonoid myricitrin: Evidence for a role of the L-arginine-nitric oxide and protein kinase C pathways. J. Pharmacol. Exp. Ther., 2:789-796.
14. Çiğdem ALTINYAY, Ayşegul GUVENÇ, Mehmet Levent ALTUN (2011). Antioxidant Activities of Oleuropein and the Aqueous Extracts of Olea europaea L. Varieties Growing in Turkey. Turk J. Pharm. Sci. 8 (1), 23-30, 2011.
15. Ufuk Özgen, Ahmet Mavi, Zeynep Terzi, Cavit Kazaz, Ali Asçı, Yusuf Kaya and Hasan Seçen, 2011, Relationship Between Chemical Structure and Antioxidant Activity of Luteolin and Its Glycosides Isolated from *Thymus sipyleus* subsp. sipyleus var. sipyleus. *Rec. Nat. Prod.* 5:1 (2011)12-21.
16. Lin Y, Shi R, Wang X, Shen HM (2008). Luteolin, a flavonoid with potential for cancer prevention and therapy. Curr. Can. Drug Targ., 8: 634- 46.
17. Lopez-Lazaro M (2009). Distribution and biological activities of the flavonoid luteolin. Mini Rev. Med. Chem., 9: 31-59.
18. Liang-liang Zhang and Yi-ming Lin (2008). Tannins from *Canarium album* with potent antioxidant activity J Zhejiang Univ Sci B. 2008 May; 9(5): 407–415.
19. Hasan S.M.R., Jamila M., Majumder M.M., Akter R., Hossain M.M., Mazumder M.E.H., AlamM.A., Jahangir R., Rana M.S., Rahman S., 2009. Analgesic and Antioxidant Activity of the Hydromethanolic Extract of *Mikania scandens* (L.) Willd. Leaves. American Journal of Pharmacology and Toxicology. 4(1): 1-7.
20. Mazumder M.E.H., Rahman S., 2008. Pharmacological Evaluation of Bangladeshi Medicinal Plants for Antioxidant Activity. *Pharmaceutical Biology*. 46(10-11):704-709.
21. Marja PK, Anu IH, *et al.* (1999, activity of plant extract containing phenolic compounds. Journal of Agriculture and Food chemistry, 47: 3954-3962.

How to cite this article:
Prataparedy AJ and Chakravarthi IE: New Spectrophotometric Determination of Ketorolac Tromethamine Bulk and Pharmaceutical Dosage Form. *Int J Pharm Sci Res*. 3(12); 4811-4817.