ISSN–2230-7346
Journal of Global Trends in Pharmaceutical Sciences

SYNTHESIS AND ANTIOXIDANT ACTIVITY OF N-(4-ACETAMIDOPHENYL)-2-SUBSTITUTED PHENOXY ACETAMIDE AND ACETATE DERIVATIVES; EFFECTS ON PROTEINASE ENZYME TRYPSIN AND ALBUMIN DENATURATION

Shaheen Begum[a]*, Arifa Begum Sk[b], K. Sushmaratna[a] Poojitha Harisree[a]

[a]Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India,
[b]Bharath Institute of Technology, Jawaharlal Nehru Technological University Hyderabad, Telangana
*Corresponding Author E-mail: shaheen.pharmchem@gmail.com

ARTICLE INFO

Key Words
phenoxy acetamide, phenoxy acetate, paracetamol, 4-aminoacetanilide, antioxidant, antidenaturant effect, antiproteolytic activity

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

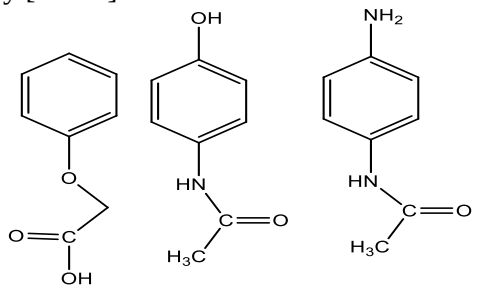
ABSTRACT

Antioxidant ability of a molecule offers protective effects against life threatening disease such as cancer, neurodegenerative disorders and obesity. The antioxidant property when combined with antiniflammatory effects, proved beneficial effects in Alzheimer's and other diseases. Considering these findings, a novel series of N-(4-acetamidophenyl)-2-substituted phenoxy acetamide and acetate derivatives were synthesized and tested for antioxidant and in vitro antiinflammatory activities. Paracetamol exhibits promising antioxidant and antiniflammatory activities. Paracetamol or its bioisostere 4-aminoacetanilide and the phenoxy (substituted with 3,4-$(CH_3)_2$) acid and phenoxy propionic acid were reacted to obtain a series of amide and ester derivatives. Compound IVa, 4-acetamidophenyl 2-phenoxyacetate displayed marked free radical scavenging activity in DPPH assay with IC_{50} value of 11.97±0.48µM, comparable to the standard ascorbic acid ($IC_{50,}$ 12.50±0.5µM). Out of the six synthesized compounds, IVa exhibited significant proteinase i.e. trypsin inhibition activity and albumin denaturant activity.

INTRODUCTION

Antioxidants are gaining significant importance in curing diseases associated with oxidative-stress such as atherosclerosis, cancer, skin aging, arthritis and neurodegenerative disorders [1-2]. Antioxidants such as vitamin A& E, docosahexaenoic acid (DHA) have shown promising results in neurodegenerative disorders including Alzheimer's and Parkinson's diseases [3-4]. In the presence of free radicals cell components, lipids, proteins, and DNA undergo oxidation to release peroxides leading to oxidative stress. Oxidative stress may lead to structural and functional abnormalities in proteins.Oxidative stress and protein aggregation combined leads to cell death causing neurodegeneration [5].In this context, NSAIDs like ibuprofen, mefenamic acid and diclofenac were tested for their efficacy in Alzheimer's diseases. Ibuprofen and diclofenac showed promising results and the mechanism of action involves protective effects against neuroinflammation probably by exhibiting anti-amyloid properties [6]. Neuroinflammation involves complex mechanisms involving release of free radicals, cytokines (IL-1β, IL-18 & IL-33) chemokines (CCL2 &CCL5) and the activation of microglia [7-10].These findings evidences that an agent

with combined properties of antioxidant and antiinflammatoy agent might be beneficial in neurodegenerative disorders. Phenoxy acid derivatives are bioactive compounds with several interesting activities viz., anti-inflammatory, antioxidant, antibacterial, analgesic, antiplatelet, antimycobacterial, and diuretic activities [11-17].Prashanth et al reported potent antioxidant molecules, containing phenoxy acid moiety (1) in their structures. Phenoxy acetic or propionic acid moiety is also present as a pharmacophoric element in important therapeutic agents [18-19]. 2-Phenoxy-N-phenylacetamide scaffold is associated antimicrobial, anticancer, anti-inflammatory activity and ability to inhibit Pgp efflux transporters. Paracetamol (2) a potent antipyretic and antinociceptive agent exhibits promising antioxidant activity. Several paracetamol derivatives found to possess antitubercular, antifungal and antibacterial activity [20-23].



(1) **(2)** **(3)**

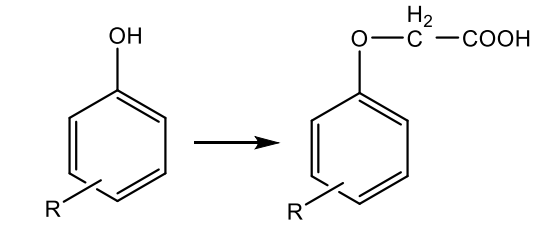
In view of the potentiality of phenoxy acid derivatives, it was planned to synthesize a series of phenoxy ester derivatives using paracetamol. Considering 4-amino acetanilide (3) as boisosteric replacement for paracetamol, a series of phenoxy amide derivatives were synthesized.

MATERIALS AND METHOD:

Phenoxy acetic acid, phenoxy propionic acid and curcumin were purchased from Himedia and paracetamol from SD fine chemicals Mumbai. Sigma melting point apparatus and Systronic UV-VIS Spectrophotometer-117 were used to determine melting points and absorbance. A mixture of methanol and chloroform was used as mobile phase to trace the completion of the reaction.IR spectra of the compounds were obtained using Bruker FTIR spectrophotometer (cm^{-1})and ^1H NMR was recorded on Bruker-400.130 MHz and Jeol-400MHz.Mass (*m*/*z*) spectra were obtained using Apex Mass spectrum (300800.D).

General method for the synthesis of N-(4-acetamidophenyl)-2- substituted phenoxy acetamide derivatives (IIIa-IIIc)

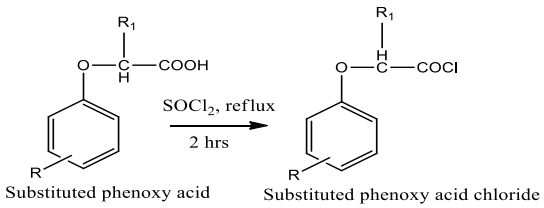
Step 1: Synthesis of 3,4-dimethyl substituted phenoxy acetic acid: Chloroacetic acid (6g) was weighed accurately and dissolved in 10 ml of water to get a clear solution. 3,4-dimethylphenol (5g) was dissolved in 10 ml of 25% w/vsodium hydroxide separately in round bottomed flask.The acid solution was slowly added to the phenolic solution and the obtained mixture was heated for 6 hr at 75-80°C. Upon cooling, a solid product was obtained. After drying, it was used in the next step without further purification [18].



Substituted Phenol Substituted phenoxy acid

R= 3,4-dimethyl

Step 2: Synthesis of various phenoxy acid chlorides: Various phenoxy acid chlorides were obtained by refluxing equimolar amounts of phenoxy acetic acid/ 3,4-dimethoxy phenoxy acetic acid/ 2-phenoxy propionic acid (0.01 mol) and thionyl chloride (0.01mol) for around two hours.



Substituted phenoxy acid Substituted phenoxy acid chloride

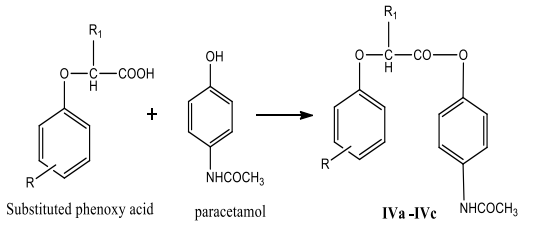
R = H;3,4-dimethyl R_1= H, CH_3

Step 3: Synthesis of N-(4-acetamidophenyl)-2-substituted phenoxy acetamide derivatives (IIIa-IIIc):

Green synthetic approach, a neat phase trituration was employed to obtain phenoxy acetamide derivatives. Equimolar amounts4-amino acetanilide (0.01 mol) and phenoxy acid chloride/3, 4-dimethoxy phenoxy acetic acid chloride/2-phenoxy propionic acid chloride (0.01 mol) were triturated in a mortar for at least 1 hr. Completion of the reaction was monitored by TLC and then ice was added.

Then the reaction mixture was filtered and

General method for the synthesis of 4-acetamido phenyl 2- substituted-phenoxyacetate (IVa- IVc): Ethylchloroformate was added drop wise to a cooled solution (0-5^{0}C) of phenoxy acetic acid and triethylamine (1.02g, 0.01mol) in dichloromethane (50ml). The mixture was stirred for 30 min and then paracetamol (1.51g, 0.01mol) was added over a period of] % inhibition = (Absorbance of control-Absorbance of test) / Absorbance of control× 100



Nitric oxide scavenging assay: The test samples (5, 10, 20, 40, 80µm) were prepared using methanol. 2 ml Sodium nitroprusside (10µm) in phosphate buffer pH 7.4 was incubated with 2ml of test sample at 25ºC for 120 min. Curcumin was considered as positive control. The absorbance of test samples and standard drug was read at 546nm using methanol as blank [27]

2, 2'- Azino- bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) reduction assay

Test samples of 100µm concentration were prepared using ethanol. To 1ml of test sample 1ml of ABTS solution was added and mixed thoroughly. After 15 min, the absorbance values were measured at 734 nm. Similarly 5, 10, 20, 40, 80µM concentrated test solutions were prepared and the absorbance values were noted to determine IC_{50} values [28].

***IN VITRO* ANTIINFLAMMATORY STUDIES**

Protein Inhibitory Action: Proteinases play an important role in the tissue damage during inflammation. 0.06 mg Trypsin and 1 ml of 20mM Tris-HCl buffer (pH 7.4) were added with 1 ml test sample of different concentrations (1µM, 2µM, 4µM, 8µM, 16µM).The reaction mixture was incubated at 37°C for 5 min and then 1ml of 0.8% (W/V) dried [24].

casein was added. The mixture was incubated for an additional 20 min then 2 ml of 70% perchloric acid was added to terminate the reaction. A suspension was obtained which was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage of inhibition of proteinase inhibitory activity was calculated [29]. % inhibition = (Absorbance of control-Absorbance of test) / Absorbance of control× 100

Protein Denaturation Activity

Denaturation of protein is considered as one of the reasons of inflammation. The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of test samples (1 - 16µm). Distilled water served as control. The mixtures were incubated at 37ºC for 15 min and then heated at 70ºC for 5 min. After cooling, absorbance was measured at 660 nm using methanol as blank. Aspirin was used as reference drug. The percentage inhibition of protein denaturation was calculated [30].

% inhibition = (Absorbance of control-Absorbance of test) / Absorbance of control× 100

RESULTS AND DISCUSSION

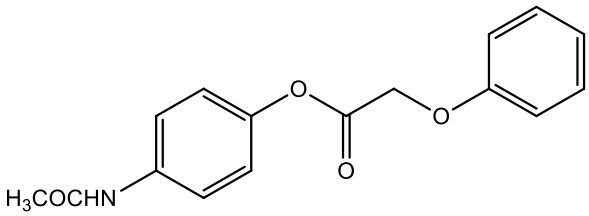
Chemistry

N-(4-acetamidophenyl)-2-substituted phenoxy acetamide derivatives (IIIa-IIIc) were synthesized by treating various substituted phenoxy acid chlorides with 4-aminoacetanilide. Different phenoxy acids were coupled with paracetamol usingethylchloroformate andtriethylamine to obtain N-(4-acetamidophenyl)-2-substituted phenoxy acetate derivatives. Phenoxy acetic acid / 3, 4-dimethylphenoxy acetic acidwas reacted with chloro acetic acid to give substituted phenoxy acids while 2-phenoxy propionic acid was purchased from Sigma Chemicals, India. For the conversion of acids to acid chlorides, thionyl chloride was used. After synthesizing the acid chlorides, they were triturated in solvent free conditions with 4-amino acetanilide to form IIIa-IIIc. The amide analogues were obtained in good yields (63-81%) when compared to ester analogues (33-

75%). In the IR spectra, absorption bands due to N-H stretch and C=Ostretch were observed around 3300 cm^{-1} and 1660 -1700 cm^{-1} in amides, whereas in ester derivatives, C=O and C-O-C stretch around 1700 cm^{-1} , 1010-1096 cm^{-1} respectively. In the NMR spectra, aromatic protons were observed at around 7-8ppm and phenoxy methylene protons were observed at around 4.8 to 5.0 ppm. The titled compounds showed characteristic M^+ or [M+1] peaks.

***In vitro* antioxidant studies**

Among all the compounds, compound IVa displayed marked radical scavenging activity with IC_{50}value of 11.97µM in DPPH assay while ascorbic acid displayed high ability ($IC_{50,}$ 12.50 µM)to inhibit the reduction of DPPH radical. Activity profile of the title compounds in nitric oxide scavenging assay is similar to that of DPPH assay. Compound III c, which displayed moderate activity in DPPH assay, also displayed moderate activity in NO scavenging assay. A perusal of data in ABTS assay indicates that IIIa, IIIc and IVa exhibited less activity when compared to the standard compound BHT. Antioxidant profile of title compounds highlighted IVa which possess paracetamol moiety in its structure. Paracetamol has strong antioxidant properties due to its ability to transfer hydrogen or electron to DPPH free radical. Free radical scavenging ability of paracetamol was reported to be increased with the introduction of indazole or N-methylmophonium group on acyl moiety [21].



4-Acetamidophenyl 2-phenoxyacetate

RESULTS OF ANTI-INFLAMMATORY STUDIES

Proteinase inhibition

In this method, trypsin inhibits leucocytes proteinase which plays an important role in the development of tissue damage during antiinflammatory activity. Compounds IVa, IVc and IIIb showed promising inhibition in this method.

Protein denaturation

NSAIDs have ability to protect albumin like proteins against denaturation. The antidenaturant effect plays important role in chronic inflammatory conditions such as rheumatoid arthritis and neurodegenerative diseases. Aspirin and majority of the NSAIDs (Ibuprofen, mefenamic acid) reported to exhibit neuroprotective activity in conditions like Alzheimer's disease possibly due to their protective action on proteins [31]. These experimental evidences suggested this model as a reliable screening model for new anti-inflammatory drugs [32-34]. Compound IVb exhibited good protecting ability compared to other compounds.

Table. 1 *In vitro* antioxidant studies (IIIa – IIIc & IVa-IVc)

Compound No	IC_{50} (DPPH scavenging assay) µM	IC_{50} (NO scavenging assay) µM	IC_{50}(ABTS assay) µM
IIIa	43.61±2.12	63.08±2.01	66.21±2.42
IIIb	67.32±2.28	-	-
IIIc	22.70±1.98	26.45±1.02	34.25±1.54
IVa	11.97±2.24	41.70±2.04	58.95±1.62
IVb	67.96±3.01	-	-
IVc	54.61±1.86	-	-
Standard	Ascorbic acid 12.5±0.42	Curcumin 16.54±0.88	Butylated Hydroxy Toluene (BHT) 21.32±0.62

Table.2 *In vitro* anti-inflammatory studies (IIIa - IIIc& IVa-IVc)

Compound No	IC_{50} (proteinase inhibition) µM	IC_{50} (protein denaturation) µM
IIIa	45.25±1.86	36.32±1.62
IIIb	36.25±1.20	41.03±0.94
IIIc	42.32±2.04	34.52±2.02
IVa	32.28±1.48	37.15±1.55
IVb	41.28±1.62	31.25±1.24
IVc	37.14±1.88	46.24±1.86
Standard (Positive control)	Aspirin (5µM) 63.14±2.68	Ibuprofen (5µM) 12.14±1.80

CONCLUSION

The synthesized compounds showed promising antioxidant and *in vitro* antiinflammatory activity. Among all Compound IVa, 4-acetamidophenyl 2-phenoxyacetate displayed marked free radical scavenging activity in DPPH assay with IC_{50} value of 11.97±0.48µM, comparable to the standard ascorbic acid ($IC_{50,}$ 12.50±0.5µM). The title compounds also displayed remarkable *in vitro* antiniflammatory activity suggesting their potential in treating neurodegenerative disorders.

REFERENCES

1. Hajhashemi V, Vaseghi G, Pourfarzam M, Abdollahi A. Are antioxidants helpful for disease prevention? Research in pharmaceutical sciences, 5(2010):1
2. Rajendran P, Nandakumar N, Rengarajan T, Palaniswami R, Gnanadhas EN, Lakshminarasaiah U, Gopas J, Nishigaki I. Antioxidants and human diseases. Clinicachimicaacta, 436(2014) 332-47.
3. Chang KH, Cheng ML, Chiang MC, Chen CM. Lipophilic antioxidants in neurodegenerative diseases. ClinicaChimicaActa, 485(2018)79-87.
4. Cacciatore I, Marinelli L, Fornasari E, Cerasa LS, Eusepi P, Türkez H, Pomilio C, Reale M, D'Angelo C, Costantini E, Di Stefano A. Novel NSAID-derived drugs for the potential treatment of Alzheimer's disease. International journal of molecular sciences, 17(2016):1035.
5. Chen X, Guo C, Kong J. Oxidative stress in neurodegenerative diseases. Neural regeneration research,7(2012):376-385.
6. Rivers-Auty J, Mather AE, Peters R, Lawrence CB, Brough D. Anti-inflammatories in Alzheimer's disease—potential therapy or spurious correlate? Brain communications, 2(2020):fcaa109.
7. Spagnuolo C, Moccia S, Russo GL. Anti-inflammatory effects of flavonoids in neurodegenerative disorders. European journal of medicinal chemistry, 153(2018) 105-15.
8. Benito-Leon J, Contador I, Vega S, Villarejo-Galende A, Bermejo-Pareja F. Non-steroidal anti-inflammatory drugs use in older adults decreases risk of Alzheimer's disease mortality. Plos one, 14(2019):e0222505.
9. Esposito E, Di Matteo V, Benigno A, Pierucci M, Crescimanno G, Di Giovanni G. Non-steroidal anti-inflammatory drugs in Parkinson's disease. Experimental neurology, 205(2007):295-312.
10. Zuena AR, Casolini P, Lattanzi R, Maftei D. Chemokines in Alzheimer's disease: new insights into prokineticins, chemokine-like proteins. Frontiers in pharmacology, 10(2019): 622.
11. Gasparini L, Ongini E, Wenk G. Non-steroidal anti-inflammatory drugs (NSAIDs) in Alzheimer's disease: old and new mechanisms of action. Journal of neurochemistry, 91(2004):521-36.
12. Pattan SR, Hullolikar RL, Pattan JS, Kapadnis BP, Dighe NS, Dengale SS, Nikalje A, Nirmal SA. Synthesis and evaluation of some new pyrazolophenoxy acetic acid derivatves

for their antitubercular activity. Journal of Pharmaceutical Sciences and Research, 1(2009):63.
13. Prashanth T, Ranganatha VL, Naveen P, Gurupadaswamy HD, Begum AB, Al-Ghorbani M, Khanum SA. Synthesis of (4-benzoyl-phenoxy)-acetic acid derivatives and their efficacy as antioxidant agents. Free Radicals and Antioxidants, 3(2013): S50-4.
14. Panczyk K, Zelaszczyk D, Koczurkiewicz P, Słoczynska K, Pękala E, Zesławska E, Nitek W, Zmudzki P, Marona H, Waszkielewicz A. Correction: Synthesis and anticonvulsant activity of phenoxyacetyl derivatives of amines, including aminoalkanols and amino acids.Medicinal Chemistry Communications, 9(2018):2121.
15. Li Z, Wang X, Xu X, Yang J, Xia W, Zhou X, Huang W, Qian H. Design, synthesis and biological activity of phenoxyacetic acid derivatives as novel free fatty acid receptor 1 agonists. Bioorganic & medicinal chemistry, 23(2015):7158-64.
16. Daidone G, Plescia S, Bajardi ML, Schillaci D. Synthesis of New 2-{[(Phenoxy or Phenyl) acetyl] amino} benzoic Acid Derivatives as 3α-Hydroxysteroid Dehydrogenase inhibitors and potential antiinflammatory agents. Archiv der Pharmazie 1995, 328(10):705-8.
17. Rani P, Pal D, Hegde RR, Hashim SR. Anticancer, anti-inflammatory, and analgesic activities of synthesized 2-(substituted phenoxy) acetamide derivatives. BioMed research international, (2014 Jan);2014.
18. Mokale SN, Sanap PT, Shinde DB. Synthesis and hypolipidemic activity of novel 2-(4-(2-substituted aminothiazole-4-yl) phenoxy) acetic acid derivatives. European journal of medicinal chemistry 2010: 45(7):3096-100.
19. Mokale SN, Elgire RD, Sakle N, Shinde DB. Synthesis, hypolipidemic and hypoglycemic activity of some novel 2-(4-(2-substituted aminothiazole-4-yl) phenoxy)-2-methyl propanoic acid derivatives. Bioorganic & medicinal chemistry letters, 21(2011):682-5.
20. Trettin A, Bohmer A, Suchy MT, ProbstI, Staerk U, Stichtenoth DO, Frolich JC, Tsikas D. Effects of paracetamol on NOS, COX, and CYP activity and on oxidative stress in healthy male subjects, rat hepatocytes, and recombinant NOS. Oxidative medicine and cellular longevity,2014:(2014).
21. Alisi MA, Brufani M, Cazzolla N, Ceccacci F, Dragone P, Felici M, Furlotti G, Garofalo B, La Bella A, Lanzalunga O, Leonelli F. DPPH radical scavenging activity of paracetamol analogues. Tetrahedron, 68(2012):10180-7.
22. Kumar R, Jain S, Jain N. Synthesis and evaluation of acetaminophen derivatives as analgesic, antipyretic and anti-inflammatory agents. Der PharmaChemica, 5(2013):73-8.
23. Jozwiak-Bebenista M, Nowak JZ. Paracetamol: mechanism of action, applications and safety concern. Actapoloniaepharmaceutica, 71(2014):11-23.
24. Ghosh S, Das J.Benzoylation of Amines sans Alkali: A Green Protocol in Neat Phase. Organic Chemistry International, 2010(2010.):1-3.
25. T. A. Fadl, and F. A. Omar. Paracetamol (Acetaminophen) esters of some non - steroidal antiinflammatory carboxylic acids as mutual prodrugs with improved therapeutic index. Inflammo pharmacology, 6(1998) 143-157.
26. E. J Gracia, TatianeLuizacadorinOldoni, SeverinoMatias de Alencar, Alessandra reis, Alessandro D. Loguercio, Rosa Helena Miranda Grande. Antioxidant activity of DPPH assay of potential solutions to be applied on bleached teeth. Brazillion dental journal, 23(2012) 22 - 27.

27. Marcocci L, Maguire J. J, Droylefaix M. T, Packer L. The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. 1994. Biochemical and biophysical research communications,201(1994); 748–755.
28. SE Celik, EOzyurek M, Guclu K, Apak R. Solvent effects on the antioxidant capacity of lipophilic and hydrophilic antioxidants measured by CUPRAC, ABTS/persulphate and FRAP methods. Talanta, 81(2010):1300-1309.
29. Oyedepo O.O and Femurewa AJ. Anti-protease and membrane stabilizing activities of extracts of Fagrazanthoxiloides, Olaxsubscorpioid es and Tetrapleuratetraptera. International Journal of Pharmacognosy 33(1995):65-9.
30. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata*Linn. International journal of pharmaceutical sciences, 2(2010) 146-55.
31. Saso L, Valentini G, Casini ML, Grippa E, Gatto MT, Leone MG, Silvestrini B. Inhibition of heat-induced denaturation of albumin by nonsteroidalantiinflammatorydrugs (NSAIDs): Pharmacological implications. Archives of pharmacal research, 24(2001):150-8.
32. Sen S, Chakraborty R, Maramsa N, Basak M, Deka S, Dey BK. In vitro anti-inflammatory activity of Amaranthuscaudatus L. leaves. Indian Journal of Natural Products and Resources (IJNPR)[Formerly Natural Product Radiance (NPR)]. 6(2015):326-9.
33. Banerjee M, Sundeepp Kumar HK, SahuSK , Das A and Parasar P. Synthesis and in-vitro protein denaturation screening of novel substituted isoxazole/pyrazole derivatives, Rasayan Journal of Chemistry4(2011):413-417.
34. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein, Asian Pacific Journal of Tropical Biomedicine, 2(2012):S178-80.