



Received on 23 January, 2015; received in revised form, 19 March, 2015; accepted, 29 April, 2015; published 01 May, 2015

ANTIULCEROGENIC ACTIVITY OF STEM EXTRACT AND FRACTIONS OF *HOMALIMUM LETESTUI*

Louis U. Amazu¹, Jude E. Okokon^{2*} and Lucky L. Nwidu³

Department of Pharmacology and Therapeutics, College of Medicine, Evans Enwerem University, Orlu Campus, Imo State

Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State

Keywords:

Homalium letestui,
gastric protective, antiulcer

Correspondence to Author:

Jude E. Okokon

Department of Pharmacology and
Toxicology, Faculty of Pharmacy,
University of Uyo, Uyo, Nigeria

E-mail: judeefiom@yahoo.com

ABSTRACT: *Homalium letestui* Pellegr (Flacourtiaceae) used traditionally by the Ibibios of Southern Nigeria to treat stomach ulcer, malaria and other inflammatory diseases was evaluated for antiulcer activities of the stem extract and its fractions. The effects of ethanol stem extract of *H. letestui* (200 – 600 mg/kg) and fractions (Aqueous and Dichloromethane, 400 mg/kg) on experimentally induced ulcer were studied in rats using ethanol, indomethacin, reserpine and histamine –induced ulcer models. The extract (200 – 600 mg/kg) inhibited ethanol, indomethacin, reserpine and histamine –induced ulcer models in a dose dependent fashion. The various degrees of inhibitions were statistically significant ($p < 0.05, 0.01, 0.001$). The effects of the extract/fraction were comparable to that of the standard drugs used in indomethacin and ethanol-induced ulcer models with the dichloromethane fraction having the highest activity. Thus, *H. letestui* extract demonstrated a good antiulcer activity which supports the use of this plant in the traditional medicine.

INTRODUCTION: *Homalium letestui* Pellegr (Flacourtiaceae) is a forest tree growing up to 80–100 ft and found in the rainforest of West Africa^{1, 2}. The plant parts; particularly stem bark and root are used in various decoctions traditionally by the Ibibios of the Niger Delta of Nigeria to treat stomach ulcer, malaria and other inflammatory diseases as well as an aphrodisiac³.

Reports of antiplasmodial³, antidiabetic⁴, anti-inflammatory and analgesic⁵, cellular antioxidant, anticancer, and antileishmanial⁶, depressant and anticonvulsant⁷ activities of the plant have been published.

We report in this study the antiulcerogenic activity of this plant in order to provide scientific basis for its use in traditional medicine in the treatment of stomach ulcer.

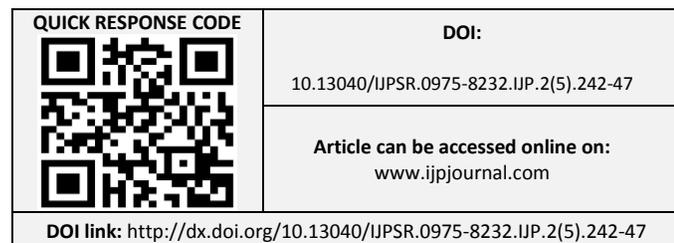
MATERIALS AND METHODS:

Plants collection:

The plant material *Homalium letestui* (stem) was collected in a forest in Uruan area, Akwa Ibom State, Nigeria in July, 2014. The plant was identified and authenticated by Dr. Margaret Basse of Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Herbarium specimen (FPUU 382) was deposited at Department of Pharmacognosy and Natural Medicine Herbarium.

Extraction:

The stem was washed and shade-dried for two weeks. The dried plant material was further chopped into small pieces and reduced to powder. The powdered material was macerated in 70%



ethanol. The liquid filtrates were concentrated and evaporated to dryness *in vacuo* 40°C using rotary evaporator. The crude ethanol extract (10 g) was partitioned with a 50:50 mixture of distilled water and dichloromethane. The aqueous fraction was evaporated to dryness in a water bath at 60°C and the dichloromethane fraction air-dried. The ethanol extract, the aqueous and dichloromethane fractions were stored at -4°C until used.

Animals:

Albino wistar rats (168 – 175 g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo.

Indomethacin induced ulcer

Male adult albino rats were used for the experiment. They were randomized into seven groups of six rats each. Food was withdrawn 24 hours and water 2h before the commencement of experiment^{8,9,10,11}. Group 1(control) received only indomethacin (Sigma, 60 mg/kg p.o. dissolved in 5% Na₂CO₃); Groups 2- 4 were pretreated with *H. letestui* stem extract (200, 400 and 600 mg/kg p.o. respectively); Group 5 received aqueous fraction (400 mg/kg); Group 6 received dichloromethane fraction (400 mg/kg), and Group 7, cimetidine (100 mg/kg p.o. dissolved in 5% Tween 80). One hour later, groups 2 - 7 were administered with indomethacin. Four hour after indomethacin administration, animals were killed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored¹². Ulcer index (UI), preventive ratio (PR) and degree of ulceration (DU) of each of the groups pretreated with extract were calculated using standard methods^{9,10}.

Ethanol induced gastric ulceration:

The procedure was similar to that used in indomethacin induced ulceration. The rats randomly assigned into were randomized into eight groups of six rats each. Food was withdrawn 24

hours and water 2h before the commencement of experiment⁸. Group 1(control) received only ethanol (2.5 ml/kg p.o), Groups 2- 4 were pretreated with *H. letestui* stem extract (200, 400 and 600 mg/kg p.o. respectively); Group 5 received aqueous fraction (400 mg/kg); Group 6 received dichloromethane fraction (400 mg/kg) and Group 7, received propranolol (40 mg/kg p.o. dissolved in distilled water). One hour later, groups 2 -6 were administered with ethanol. Four hour after ethanol administration, animals were killed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored¹⁰.

Histamine-induced gastric ulceration in rats:

The procedures were similar to that used in indomethacin-induced ulceration except that the negative control group (group 1) received only histamine acid phosphate (Sigma, 100 mg/kg i.p. dissolved in distilled water)¹¹, Groups 2 - 4 were pretreated with *H. letestui* extract (200, 400 and 600 mg/kg p.o. respectively); Group 5 received aqueous fraction (400 mg/kg), Group 6 received dichloromethane fraction (400 mg/kg) and Group 7 received cimetidine (100 mg/kg p.o. dissolved in 50% Tween 80), One hour later, groups 2 - 7 were administered with histamine acid phosphate, 100 mg/kg i.p). Eighteen (18) hours after histamine administration, animals were killed by cervical dislocation.

The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored¹², stomach processing and examination as well as ulcer scoring were similar to that used in indomethacin-induced ulceration.

Reserpine induced gastric ulceration in rats:

Male adult albino rats weighing 120 – 170 g were used for the experiment. They were randomized into six groups of six rats each. Food was withdrawn 24 hours and water 2h before the commencement of experiment⁸. Group 1(control)

received only reserpine (Sigma, 8 mg/kg p.o. dissolved in Tween 80); Groups 2 - 4 were pretreated with *H. letestui* extract (200, 400 and 600 mg/kg p.o. respectively); Group 5 received aqueous fraction (400 mg/kg), Group 6 received dichloromethane fraction (400 mg/kg) and Group 7 received cimetidine (100 mg/kg p.o. dissolved in 50% Tween 80), One hour later, groups 2 - 5 were administered with reserpine, 8 mg/kg i.p dissolved in 10% Tween 80¹¹. Eighteen hours (18 h) after reserpine administration, animals were killed by cervical dislocation.

The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored¹². Ulcer index (UI), preventive ratio (PR) and degree of ulceration (DU) of each of the groups pretreated with extract were calculated using standard methods^{9,10}.

Statistical analysis:

Data are reported as mean \pm standard error of the mean (SEM) and were analyzed statistically using

One way ANOVA followed by Tukey-kramer multiple comparison test and values of $p < 0.01$ were considered significant.

RESULTS:

Indomethacin induced gastric ulceration:

The extract and fractions (p.o.) pretreatment on indomethacin induced gastric ulceration showed a dose dependent reduction in ulcer indices in pretreated groups relative to control. The reductions were statistically significant ($P < 0.05$, 0.001) compared to control. The dichloromethane fraction exerted the highest antiulcerogenic effects which was comparable to that of the standard drug used, cimetidine. **Table 1**

Ethanol induced gastric ulceration:

The extract and fractions significantly protected rats from ethanol – induced ulcer (**Table 2**). There was a significant ($p < 0.001$) dose-dependent reduction in the ulcer indices relative to control. The effect of the extract and fractions were less than that of the standard drug, propranolol.

TABLE 1: EFFECT OF HOMALIUM LETESTUI EXTRACT ON INDOMETHACIN - INDUCED ULCERATION IN RATS

Treatment	Dose(mg/kg)	Ulcer Indices	Preventive Ratio
Control(indomethacin)	60	18.34 \pm 2.72	-
<i>Homalium letestui</i> extract p.o.	200	10.05 \pm 1.22 ^a	45.20
	400	2.10 \pm 0.60 ^c	88.54
	600	2.00 \pm 0.76 ^c	89.09
	Dichloromethane fraction	400	1.60 \pm 0.22 ^c
Aqueous fraction	400	3.83 \pm 0.25 ^c	79.11
Cimetidine	100	1.52 \pm 0.88 ^c	91.71

Data were expressed as mean \pm SEM. significant at $ap < 0.05$, $bp < 0.01$, $cp < 0.001$ when compared to control $n = 6$.

TABLE 2: EFFECT OF HOMALIUM LETESTUI EXTRACT ON ETHANOL - INDUCED ULCERATION IN RATS

Treatment	Dose(mg/kg)	Ulcer Indices	Preventive Ratio(%)
Control(ethanol)	-	4.63 \pm 0.33	-
<i>Homalium letestui</i> extract p.o.	200	2.66 \pm 0.33 ^a	42.54
	400	2.00 \pm 0.61 ^b	56.80
	600	1.34 \pm 0.57 ^c	71.05
Dichloromethane fraction	400	1.20 \pm 0.25 ^c	74.08
Aqueous fraction	400	2.66 \pm 0.22 ^a	42.54
Propranolol	40	0.82 \pm 0.15 ^c	88.76

Data were expressed as mean \pm SEM. significant at $ap < 0.05$, $bp < 0.01$, $cp < 0.001$ when compared to control $n = 6$.

Histamine – induced ulceration:

Administration of the extract and fractions significantly ($p < 0.001$) reduced histamine-induced gastric ulceration in a dose dependent fashion compared to control (**Table3**). The dichloromethane exhibited a higher antiulcer potential than the

aqueous fraction but less than that of the standard drug, cimetidine.

Reserpine – induced ulceration:

Administration of the extract and fractions significantly ($P < 0.001$) reduced reserpine induced

gastric ulceration in a dose dependent fashion compared to control (**Table 3**). These effects were incomparable to that of the standard drug, cimetidine.

TABLE 3: EFFECT OF HOMALIUM LETESTUI EXTRACT ON HISTAMINE - INDUCED ULCERATION IN RATS

Treatment	Dose(mg/kg)	Ulcer Indices	Preventive Ratio
Control(Histamine)	100	16.01 ± 0.81	-
<i>Homalium letestui</i> extract p.o.	200	12.62 ± 1.40 ^a	21.17
	400	9.25 ± 0.82 ^b	42.22
	600	3.12 ± 0.12 ^c	80.51
	Dichloromethane fraction	400	2.25 ± 0.14 ^c
Aqueous fraction	400	10.25 ± 0.66	35.97
Cimetidine	100	1.00 ± 0.11 [*]	94.12

Data were expressed as mean ± SEM. significant at *P < 0.001 when compared to control n = 6.

TABLE 4: EFFECT OF HOMALIUM LETESTUI EXTRACT ON RESERPINE - INDUCED ULCERATION IN RATS

Treatment	Dose (mg/kg)	Ulcer Indices	Preventive Ratio
Control(Reserpine)	8	16.22 ± 0.65	-
<i>H.letestui</i> extract p.o.	200	12.56 ± 0.94 ^a	22.56
	400	8.21 ± 0.53 ^b	49.38
	600	3.44 ± 0.67 ^b	78.79
	Dichloromethane fraction	400	2.75 ± 0.34 ^b
Aqueous fraction	400	7.56 ± 0.52 ^b	65.72
Cimetidine	100	1.10 ± 0.84 ^b	93.21

Data were expressed as mean ± SEM. significant at ^aP < 0.01; ^bP < 0.001 when compared to control n = 6.

DISCUSSION: *Homalium letestui* stem has been reported to be used traditionally in the treatment of ulcer³. For this reason, the antiulcer activity of the stem bark extract and fractions was evaluated using indomethacin, ethanol, reserpine and histamine-induced ulcer models. Indomethacin, a known ulcerogen especially in an empty stomach¹³ causes ulcer mostly on the glandular (mucosal) part of the stomach^{12,13} by inhibiting prostaglandin synthetase through the cyclooxygenase pathway¹⁴. Prostaglandins function to protect the stomach from injury by stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal turn over and repair^{15,16}.

Suppression of prostaglandin synthesis by indomethacin results in increased susceptibility of stomach to mucosal injury and gastroduodenal ulceration. The extract was observed to significantly reduce mucosal damage in the indomethacin – induced ulcer model, suggesting the possible extract mobilization and involvement of prostaglandin in the anti-ulcer effect of the extract. Administration of ethanol has been reported to cause disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production¹⁷. This is attributed to the release of superoxide anion and hydroperoxy free

radicals during metabolism of ethanol as oxygen derived free radicals has been found to be involved in the mechanism of acute and chronic ulceration in the gastric mucosa¹⁸.

It was observed in this study that the extract reduced significantly ethanol- induced ulcer. This may be due to cytoprotective effect of the extract via antioxidant effects which the stem extract has been reported to exhibit⁶.

Okokon *et al.*,⁵ reported the presence of α -terpineol, Vanillin, 4-phenyl isocoumarin, 3,4,5-trimethoxy phenol, 2-Coumaranone, and xanthenes in the stem bark extract of *H. letestui*. Vanillin, a phenolic aldehyde has been reported to possess antioxidant and free radical scavenging ability^{19,20},²¹ which could possibly account for the anti-ulcer property of this plant.

α -terpineol present in this extract, is an isomer of the monoterpene, terpinen-4-ol. α -terpineol and terpinen-4-ol have been reported to possess anti-ulcer activity^{22,23}. The antiulcer activity of this extract could also be due to the present of α -terpineol. Similarly, xanthenes have been reported to demonstrate antiulcer activity²⁴. These compounds present in the stem extract maybe responsible for the observed antiulcer activity.

Ethanol is also reported to cause gastric mucosal damage by stimulating the formation of leukotriene C4 (LTC4)²⁵. The gastroprotective effect of the extract may in part be due to the suppression, by the extract of lipoxygenase activity¹². Histamine-induced ulceration is known to be mediated by enhanced gastric acid secretion as well as by vasospastic action of histamine²⁶. The inhibition of ulcer due to histamine by the extract may be due to its suppression of histamine-induced vasospastic effect and gastric secretion.

Okokon et al.,⁴ had reported the presence of flavonoids, saponins, terpenes and tannins in the stem extract of *H. letestui*. Flavonoids such as quercetin has been reported to prevent gastric mucosal lesions in various experimental models^{27, 28} by increasing the amount of neutral glycoproteins²⁷. Flavonoids have been reported to protect the gastric mucosa from damage by increasing the mucosal prostaglandin content and by inhibiting histamine secretion from mast cells by inhibition of histidine decarboxylase. Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion²⁹. Saponins, especially triterpenes type have been implicated in antiulcer activity mediated by formation of protective mucus on the gastric mucosa and also protect the mucosa from acid effects by selectively inhibiting PGF2 α ^{30, 31}.

In conclusion, the results of the present study show that stem extract and fractions of *H. letestui* displays gastroprotective activity as demonstrated by significant inhibition of the formation of ulcers induced through four different ulcer models studied. The antiulcer activity of the extract maybe due to the action of its phytochemical compounds present in the extract. The observation justifies the ethnomedical uses of the plants as antiulcer and antacid in addition to its nutritional values.

CONCLUSION: The present study demonstrates that stem extract of *Homalium letestui* might be useful for the treatment of ulcer.

ACKNOWLEDGEMENTS: The authors are grateful to Ms. Sifon Akpan of Pharmacology and Toxicology Dept for her technical assistance.

AUTHORS' CONTRIBUTIONS: All the authors contributed financially and technically to the preparation of the work.

CONFLICT OF INTERESTS: The authors declared no competing interests.

REFERENCES:

- Hutchinson J, Dalziel JM. Flora of West tropical Africa. 2nd edition. Crown Agents for Overseas Government and Administration. Vol.1. part.2. 1973, p.638.
- Keay RWJ. Trees of Nigeria. A revised version of Nigerian trees (Vol 1 and 2), Keay RWJ, Onoche CFA, Stanfield DP (eds). Clarendon Press: Oxford, 1989.
- Okokon JE, Ita BN, Udokpoh AE. Antimalarial activity of *Homalium letestui*. Phytother Res, 2006; 20(11), 949 - 951.
- Okokon JE, Antia BS, Ita BN. Antidiabetic effects of *Homalium letestui* (Flacourtiaceae) in streptozotocin induced diabetic rats. Res J Med Plants, 2007;1(4):134 - 138.
- Okokon JE, Okokon PJ, Dar A., Choudhary MI. Antiinflammatory and analgesic activities of *Homalium Letestui*. Pharm Biol 2013a; 51(11): 1459 - 1466.
- Okokon JE, Dar A, Choudhary MI. Cellular antioxidative, anticancer and antileishmanial activities of *Homalium letestui*. Avicenna J Phytomed. 2013b; 3(1):35 - 44.
- Okokon JE, Davies K. Psychopharmacological studies of *Homalium letestui* stem extract. J Pharma Biol. 2014; 4(3): 158 - 164.
- Alphin RS, Ward JW. Action of hexopyrionium bromide on gastric secretion in dogs and on gastric secretion and ulceration in rats. Archives Internationales de Pharmacodynamie et de Therapie. 1967; 270: 128 -140.
- Zaidi SH, Mukerji B. Experimental peptic ulceration. Part 1. The significance of mucus barrier. Ind J Med Res.1958; 46:27 - 37.
- Nwafor PA, Okwuasaba FK, Binda IG. Antidiarrhoeal and antiulcerogenic effects of methanolic extracts of *Asparagus pubescens* root in rats. J Ethnopharmacol 2000; 72:421 - 427.
- Maity S, Vedasiromoni JR, Ganguly DK. Anti-ulcer effect of the hot water extract of black tea (*Camellia sinensis*). J Ethnopharmacol 1995; 46: 167 - 174.
- Nwafor PA, Effraim KD, Jacks TW. Gastroprotective effects of aqueous extracts of *Khaya senegalensis* bark on indomethacin - induced ulceration in rats. West Afr J Pharmacol Drug Res.1996; 12: 46 - 50.
- Bhargava KP, Gupta MB, Tangri KK. Mechanism of ulcerogenic activity of indomethacin and oxyphenbutazone. Eur J Pharmacol.1973; 22:191 - 195.
- Rainsford KD. The effects of 5- lipoxygenase inhibitors and leukotriene antagonists on the development of gastric lesions induced by nonsteroidal anti-inflammatory drugs in mice. Agents and Action.1987; 21:316 - 319.
- Hayllar J, Bjarnason I. NSAIDS, COX-2 inhibitor and the gut. Lancet 1995; 346 - 522.
- Hiruma-Lima CA, Calvo TR, Rodriguez CM, Andrade FDP, Vilegas W, Brito ARM. Antiulcerogenic activity of *Alchornea castaneafolia* effects on somatostatin, gastrin and prostaglandin. J Ethnopharmacol. 2006; 104: 215 - 224.

17. Salim AS. Removing oxygen derived free radicals stimulates healing of ethanol induced erosive gastritis in the rats. *Digestion* 1990; 47:24 – 28.
18. Pihan G, Regillo C, Szabo S. Free radicals and lipid peroxidation in ethanol or aspirin – induced gastric mucosa injury. *Digestive Diseases and Sciences*.1987; 32:1395 – 1401.
19. Kamat JP, Ghosh A, Devasagayam TP. Vanillin as an antioxidant in rat liver mitochondria: inhibition of protein oxidation and lipid peroxidation induced by photosensitization. *Mol Cell Biochem*. 2000; 209:47-53.
20. Kumar S, Priyadarsini K, Sainis K. Free radical scavenging activity of vanillin and o-vanillin using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. *Redox Rep*. 2002; 7: 35-40.
21. Lirdpramongkol K, Kramb JP, Suthiphongchai T, Surarit R, Srisomsap C, Dannhardt G, Svasti J. Vanillin suppresses metastatic potential of human cancer cells through PI3K inhibition and decreases angiogenesis in vivo. *J Agr Food Chem*. 2009;57: 3055 – 3063.
22. Matsunaga T, Hasegawa C, Kawasuji T, Suzuki H, Saito H, Sagioka T, Takahashi R, Tsukamoto H, Morikawa T, Akiyama T. Isolation of the anti-ulcer compound in essential oil from the leaves of *Cryptomeria japonica*. *Biol Pharm Bull* 2000; 23: 595-598.
23. Souza RHL, Cardoso MSP, Menezes CT, Silva JP, De Sousa DP, Batista JS. Gastroprotective activity of α -terpineol in two experimental models of gastric ulcer in rats. *DARU* 2011; 19(4): 277 – 281.
24. Ali M, Latif A, Zaman K, Arfan M, Maitland D, Ahmad H, Ahmad M.. Anti-ulcer xanthenes from the roots of *Hypericum oblongifolium* Wall. *Fitoterapia* 2014; 95:258-65.
25. Whittle BJR, Oren-Wolman N, Guth PH. Gastric vasoconstrictor actions of leukotriene C₄ and PGF_{2 α} and thromboxane mimetic (U-4669) on rats submucosal microcirculation in vivo. *Amer J Physiol*. 1985; 248:G580 – G586.
26. Cho CH, Pfeiffer CJ. Gastrointestinal ulceration in the guinea pigs in response to dimaprit, histamine and H₁ and H₂ blocking agents. *Digestive Disease Sci* 1981;26:306 – 311.
27. Di Carlo G, Mascolo N, Izzo AA, Capasso F. Flavonoids: old and new aspects of a class of a natural therapeutic drug. *Life sciences* 1999;64: 337 – 353.
28. Zayachkivska OS, Konturek SJ, Drozdowicz D, Konturek PC, Brzozowski T, Ghegotsky MR. Gastroprotective effects of flavonoids in plants extracts. *J Physiol Pharmacol*. 2005;56: 216 - 231.
29. Borrelli F, Izzo AA. The plant kingdom as source of anti ulcer remedies. *Phytother Res*.2000;14: 581 – 591.
30. Agwu CN, Okunji CO. Gastrointestinal studies of *Pyrenacantha staudii* leaf extracts. *J Ethnopharmacol* 1986;15:45 – 55.
31. Lewis DA, Hanson D. Anti-ulcer drugs of plants origin. *Progr Med Chem*.1991; 28:208 – 210.

How to cite this article:

Amazu LU, Okokon JE, Nwidi LL: Antiulcerogenic Activity of Stem Extract and Fractions of *Homalium Letestui*. *Int J Pharmacognosy* 2015; 2(5): 242-47;.doi link: [http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.2\(5\).242-47](http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.2(5).242-47).

This Journal licensed under a Creative Commons Attribution-Non-commercial-Share Alike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)