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RESEARCH ARTICLE

IN VITRO ANTI- OXIDANT ACTIVITY, LIPOXYGENASE INHIBITION AND SECRETORY PHOSPHOLIPASE A_2 INHIBITION PROPERTIES OF GYMNOSPORA MONTANA

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ABSTRACT

The enzyme secretory phospholipase A_2 is responsible for the hydrolysis of membrane phospholipids that release arachidonic acid, which serves as a substrate for pro-inflammatory mediators, such as prostaglandins and leucotriens. The plant bio active compounds inhibiting PLA_2 possibly implicate as potential therapeutic agents in the treatment of inflammation related diseases. In the present study *Gymnospora montana* subjected to inhibition of $sPLA_2$ along with *in vitro* anti-oxidant (DPPH scavenging), Anti-lipid peroxidation activity & lipoxygenase inhibition. Among different solvent extracts *G. montana* methanol extract shows grater phytochemical contents along with anti- oxidant activity. The *G. montana* methanol extract exhibit Greater DPPH free radical scavenging activity of 82.03%. The reducing power and anti-lipid peroxidation activity was found to be 80.34% and 78.44% respectively. Further methanol extract of *G.montana* shows 63.24% of HPF-PLA_2 inhibition at 75µg concentration and inhibited 15-LOX enzyme in concentration dependent manner. IC_{50} value of methanol extract of *G. montana* was found to be 64.86 µg/µL. The present study may lead to the better understanding of PLA_2 inhibition by phytochemicals. This may help to develop better anti-inflammatory drugs.

INTRODUCTION

Inflammation is a localized reaction initiated by hydrolytic breakdown of phospholipids at the C_2 position with production of respective lysophospholipids. Because these lipids are principal mediators of inflammatory reactions, PLA_2 has been implicated in the pathogenesis of several inflammatory diseases [1-2]. In various inflammatory cells, activated PLA_2 leads to the aggregation of platelet activating factor (PAF) [3]. PLA_2 also plays a key role in the initial step of cascade mechanism by cleaving ester bonds at sn-2 position of phospholipids and releasing free arachidonic acids. Cyclooxygenases further digest this and leads to synthesis of eicosanoids. The pro-inflammatory mediators involve in deleterious cascade of several chronic inflammatory diseases such as asthma, bronchitis, sepsis, trauma, cholitis, perkinson disease, arthritis and several cancerous types [4].

Injection of $sPLA_2$ purified from synovial fluid and from snake venoms into animal joints confirmed the formation of an acute inflammatory response to oedema, hyperplasia, and swelling of synovial cells [5-8]. Inhibition of such PLA_2 enzymes by xenobiotics is of potential therapeutic importance. Several endogenous and exogenous agents such as lipocortins, gangliosides, cis-unsaturated fatty acids, manoalide, retinoids, flavonoids, aristolochic acid and synthetic lipids have been shown to inhibit PLA_2 enzymes [5, 7-13].

The biotransformation of arachidonic acid was catalyzed by cyclooxygenases (COX-1 and COX-2) and produce prostaglandins and thromboxanes, ultimately responsible for several physiological and pathophysiological responses [14-15]. The COX-1 isozyme facilitates homeostatic functions including regulation of renal blood flow, platelet aggregation, and cyto-protection of the gastric mucosa. Recently, experimental results reveal involvement of COX-1 in angiogenesis, therefore providing the basis for the development of COX-1 inhibitors [16-17]. On the other hand, COX-2 is mainly responsible for the production of inflammatory prostaglandins that induce swelling, pain, and fever [18-20]. Apart from its peripheral inflammation inducing ability, the COX-2 expression is up regulated in numerous human cancers such as colon, lung, breast, gastric, prostate, esophageal, and hepatocellular carcinomas [20].

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Present anti-inflammatory therapies include the non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit either Cyclo-oxygenase-1/2 (COX-1/2) or Lipoxygenase (LOX) catalysis. The specific inhibitors of COX-1/2 and LOX cannot regulate the production of leukotrienes or PAF that continue to cause inflammation along with severe side effects such as bleeding , intestinal ulceration, cardiovascular complications [21-22]. It appears rational that effective inhibitors of $sPLA_2$ could deplete the sources of arachidonic acid and therefore its downstream metabolites and PAF would not affect the homeostasis of COX-1/2 and LOX enzymes [23].

The $sPLA_2$ activation in inflammatory diseases has raised the attention that the pharmacologically active substances that can inhibit $sPLA_2$ activity [24-25]. Therefore, the great demand for natural products for PLA_2 inhibition rather than NSAIDs to treat inflammatory disorders. Beside these problems, pathologically $sPLA_2$s also involved in the alzheimer's disease (neurodegenerative diseases) and stroke (cerebrovascular diseases). So, the compounds that will inhibit sPLA2 can be potent anti-inflammatory agents. The literature survey showed that several plant extracts and endogenous and exogenous agents have been reported to inhibit $sPLA_2$ enzymes [26-27]. In addition, several laboratories are synthesizing compounds to inhibit $sPLA_2$ [28]. But none of them are successfully made into the market, which demands researchers to look for new specific $sPLA_2$ inhibitors.

Gymnosporia montana a tropical and sub-tropical plant of India belongs to family celeastraceae. *G. montana* was reported as hepatoprotective, anticancer, antioxidant, antibacterial, analgesic, antispasmodic agent [29]. Apart from this it is claimed to be useful in jaundice, rheumatic pain, corneal opacity, ulcers, gastrointestinal disorders, dysentery and toothache. Although, limited information is available about the anti-inflammatory activity of *G. montana*. In the present communication, various solvent extracts of *G. montana* was subjected for human $sPLA_2$ inhibition as an anti-inflammatory activity.

METHODS

Preparation of extracts and phytochemical analysis

The *G. montana* plant leaves, stem and root were separately washed, shade dried and milled. The powders were subjected to soxhlet extraction with hexane, benzene, chloroform, acetone, ethanol, methanol and water (75g/500ml). The extracts of organic solvents were powdered by flash evaporator and water samples were powdered by lyophilization. The yield was calculated (% w/w) and concentration of total phenolics, tannins and phytochemical analysis of *G. montana* extracts were estimated [27]. TLC for all the extracts was eluted with solvent-n-hexane: ethyl acetate (8:2) and observed under UV light.

Estimation of antioxidant activity

Antioxidant activity of *G. montana* extracts were determined using 2,2-Diphenyl-1-picryl hydrazyl radical ($DPPH^{\bullet}$) as described by Blios [30]. Briefly, 100µg of extracts were mixed with 5 ml of 0.1mM methanolic solution of DPPH and incubated at 20° C for 20 minutes in complete dark. The control was prepared as above without extract and methanol was used for the base line correction. Change in the absorbance of the samples was measured at 517nm. Radical scavenging activity was expressed as percentage activity using the following formula.

% radical scavenging activity = [(Control absorbance-sample absorbance) / (Control absorbance)] X 100

Anti-lipid peroxidation activity

Anti-lipid peroxidation activity of *G. montana* extracts were estimated by TBARS method [31]. 0.5 ml of egg homogenate and 100µg extracts were made up to 1 ml with distilled water. 100µl of 0.07M $FeSO_4$ was added and incubated for 30 min at room temperature. To all test tubes 1.5 ml of acetic acid, 1.5 ml of TBA and 50µl of TCA were added vortexed and kept in boiling water bath for 1 hour. By adding 5 ml of butanol test tubes were centrifuged at 3000 rpm for 10 min. Absorbance of supernatant was measured at 530 nm and percentage was calculated using the formula,

% Anti lipid peroxidation= (1−Extract absorbance)/(Control absorbance)×100

Purification of $sPLA_2$

Human pleural fluid (HPF) - $sPLA_2$ was purified by the modified method as described by Vishwanath [32]. HPF was centrifuged at 10,000 rpm for 10 min, to the supernatant equal volume of 0.36N H_2SO_4 was added. The sample was strewed overnight; centrifuged at 10,000 rpm for 10 min. Supernatant was dialyzed using 7-8 KD cut off membrane against phosphate buffer (10 mM, pH 4.6). Dialyzed sample was kept on a water bath for 5 min at 60-70^{o}C. The boiled sample was further centrifuged at 10,000 rpm for 10 min and the supernatant will be used as a source of $sPLA_2$

Inhibition of $sPLA_2$

Inhibition of $sPLA_2$ was done by agarose egg yolk plate method as described earlier [27]. 1% agarose in 100 ml of 0.1M Tris HCl (pH 7.4) contains 5 mM $CaCl_2$ was prepared. Add 6 drops of egg yolk to agarose after it reached to room temperature. Stirred and poured into sterile petriplates and allowed to solidify. Make wells using gel puncture and load 25µl HPF-PLA_2. Incubate the plates for overnight at 37^{0}C, measure the zone of clearance using normal scale carefully. Zone of HPF-PLA_2 alone serves as control and the well without HPF-PLA_2 serves as a negative control. HPF-PLA_2 pre-incubated with 50µg *G. montana* extracts of leaf, stem and root were used as test. Percentage of PLA_2 inhibition was calculated using the formula.

$$\left[\frac{(Control\ diameter - Test\ diameter)}{Control\ diameter}\right] X100$$

Inhibition of 15-lipoxygenase

The enzyme assay was performed according to the method of Axelrod [34]. The 1.0 ml standard reaction mixture contained 100µg linoleic acid and soybean 15-lipoxygenase enzyme (5µg) in 200 mM borate buffer pH 9.0. The absorbance was measured continuously for 3 min at 234 nm. The enzyme activity was expressed as µgole of 13-HPODE formed/min/mg protein. For inhibition study, different concentrations (100-500 µg) of *G. montana* extracts were pre-incubated with 15-LOX for 5 min. The reaction was initiated by adding linoleic acid to the respective assay mixtures. Quercetin, a known inhibitor of LOX was used as a positive control for both 5 and 15-LOX inhibition assay.

Statistical analysis

The experimental results were presented as mean ±SD of three determinations. The IC_{50} concentration and the graphs were plotted using Graph pad prism 5.0 USA.

RESULT AND DISCUSSION

The pharmacological value of secondary metabolites from the plants is increasing as these can act as lead chemicals for new drug development. The phytochemicals such as alkaloids, saponins and tannins are used in various antibiotics to treat common pathogenic strains (Kubmarawa *et al*., 2007). They are potential interest in therapeutic intervention for many inflammatory disorders. They act either by inhibiting pro-inflammatory enzymes (PLA_2, COX and LOX) or by inhibiting release of cytokines (IL-1β, TNF-α) and inhibition of mast cell degranulation that are known contributors to chronic inflammatory disorders. The $sPLA_2$ enzyme catalyze rate-limiting step in the production of pro-inflammatory eicosanoids and free radicals. The PLA_2 catalyzed reaction is considered to be a significant pathway for reactive oxygen species (ROS) it turn activates PLA_2 as well as lipid peroxidation and thereby augment the chronic inflammatory diseases to several folds [24]. Hence, the PLA_2 inhibition is legitimate in the neutralization of inflammation. The antioxidants from plants and their potential benefit in the intervention of inflammatory reactions arise if it inhibits the key enzyme PLA_2 along with neutralizing the free radical generation reaction.

The *G. montana* reported as hepatoprotective, anticancer, antioxidant activity, although the mechanism is unclear. In the present study, PLA_2 enzyme is subjected to inhibition as a function of anti-inflammatory activity.

Priliminor phytochemical analysis of *G. montana* showed that the methanol extracts contains higher concentration of phenols, tannins, flavonoids, alkaloids and saponins compared to non-polar solvent extracts (Table. 1) followed by water and ethanol extracts.

The *in vitro* anti-oxidant activity by DPPH free radical scavenging and anti-lipid peroxidation activity of *G. montana* extracts was estimated spectrophotometricaly. Among the extracts, methanol extract shows good activity followed by water extract. The methanol extract of *G. montana* showed 82.03% of DPPH scavenging activity and 80.34% and 78.44% of reducing power and anti-lipid peroxidation activity respectively at 100µg concentration(Fig.1).

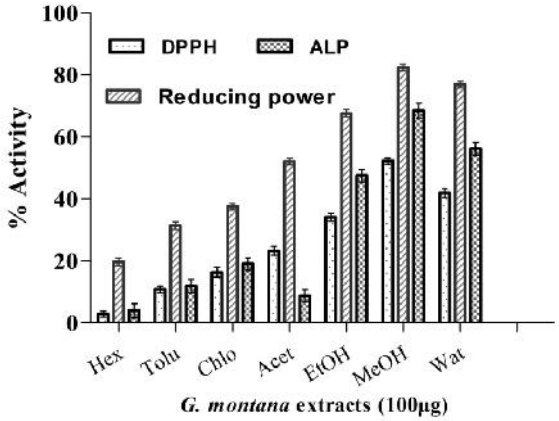

Fig.1 Estimation of antioxidant, reducing power and anti-lipid peroxidation activity of methanol extracts of *G. montana* at 100µg. Data represents mean±SD (n=3)

Further phytochemical separation of the extracts was carried out by Thin Layer Chromatography (TLC). The hexane: ethyl acetate (8:2) was exclusively used to detect flavonoids and the same was used to separation of the active constituents of *G. montana*. The TLC of methanol extract of *G. montana* shows different spots under UV light. Rf values of extracts separated in TLC spots was found to be 0.412, 0.602, 0.791 and 0.902.This data suggest that, the methanol extract of *G. montana* contains flavonoids (Fig.4).

The most NSAIDs target the either COX-1/2 or LOX enzymes or both the enzymes. The pro-inflammatory enzymes for soybean LOX (15-LOX) inhibition was measured in terms of amounts of 13-HPODE formed. Methanol extract of *G. montana* inhibited 15-LOX enzyme in concentration dependent manner & IC_{50} value of *G. montana* extract was 64.38 ±0.05µg/µl (Fig. 2). The complete LOX inhibition was observed at a concentration of 250µg/µl.

Table 1 Photochemical analysis of *G. montana* extracts

G. montana extracts	Phytochemicals						
	Tannins	Alkaloids	Glycosides	Saponin	Phenolics	Terpenoids	Flavonoids
Hexane	+	-	-	+	-	-	-
Benzene	-	-	-	-	+	-	-
Chloroform	-	-	+	-	+	+	-
Acetone	-	-	+	+	+	-	-
Ethanol	+	-	+	+	+	+	-
Methanol	+	+	+	+	+	+	+
Water	+	+	+	-	+	-	+

Table 1: The different solvents extracts of *G. montana* were dried using flash evaporator. The 100µg of extracts were re-dissolved in the respective solvents and used for analysis. (+) present; (-) absent

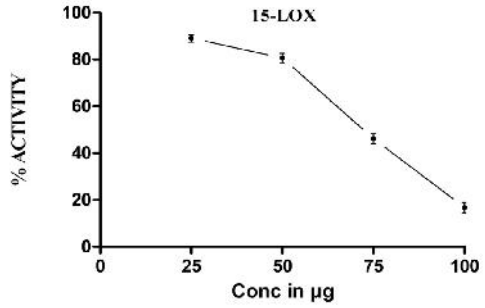

Fig. 2 Inhibition of 15-LOX by methanol extract of *G. montana*. 1 ml of reaction mixture contained, linoleic acid (100µg) and 15-LOX (5µg) in 200 mM borate buffer pH 9.0. Absorbance measured at 234 nm by µgole of 13-HPODE formed/min/mg protein

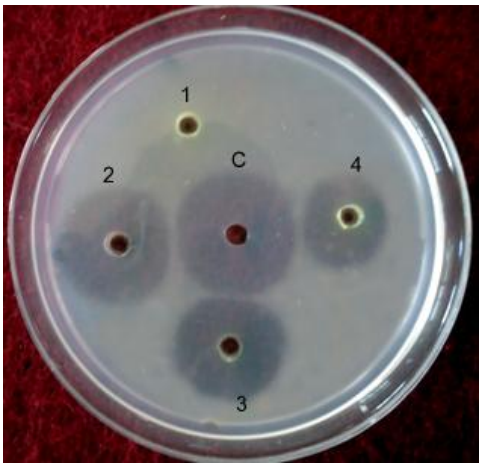

Fig.3 Inhibition of HPF-sPLA$_2$ from methanol extract of *G.montana* by egg yolk agar plate method. The wells contains 1) control 2) 25 µL of HPF-PLA$_2$ + 25 µg extract 3) 25 µL of HPF-PLA$_2$+ 50 µg extract 4) 25 µL of HPF-PLA$_2$+ 75µg extract and c) contains 25 µL of HPF-PLA$_2$ alone

To confirm the anti-inflammatory activity, the sPLA$_2$ enzyme from Human Pleural Fluid (HPF-PLA$_2$) was subjected for inhibition by methanol extract of *G. montana*. The extract was significantly inhibited sPLA$_2$ enzyme at different concentration in egg yolk agar plate method. Percentage of HPF-PLA$_2$ inhibition was 22.36, 48.6 and 63.24% for 25, 50 and 75µg of methanol extract respectively (Fig. 3).the inhibition of sPLA$_2$ was concentration dependent manner. Since, certain bioactive molecule/s might have bind to the enzyme irreversibly, the enzyme inhibition was irrespective of the nature of substrate provided for its activity.

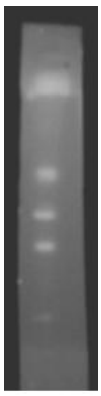

Fig.4 Thin layer chromatography of methanol extract of *G. Montana*

CONCLUSSION

In an overview, the bioactivity data obtained from the current investigation, the extract of *G. montana* exhibited very good *in vitro* sPLA$_2$ inhibition, anti- oxidant reducing power and anti-lipid peroxidation activity. In addition, the *G. montana* have good phytoconstituents and bioactive substances like phenolics, flavonoids, tannins etc. The methanol extract of *G. montana* inhibited the pro-inflammatory enzyme 15-LOX in concentration dependent manner. Based on TLC result, the flavonoids contents of *G. montana* extract might be responsible for sPLA$_2$ inhibition. But, the extract used in the assay is crude there is always a possibility of cumulative effect which may be the other reason for sPLA$_2$ inhibition. As a therapeutic source of details, standardized study is warranted in order to exhibit *G. montana* as an effective medicinal plant in near future.

References

1. Vadas P and Pruzanski W. 1986. Role of secretory phospholipases A_2 in the pathobiology of disease. Lab Invest. 55(4):391–404
2. Gonzalez-Buritica H, Khamashta M A and Hughes G R V. 1989. Synovial fluid phospholipase A2 and inflammation. Ann Rheum Dis. 48: 267-9
3. Hanahan, D. J. 1986. Platelet Activating Factor: A Biologically Active Phosphoglyceride. Annu. Rev. Biochem. 55: 483-520.
4. Wei, Y.; Quinn, D. M.; Sigler, P. B and Gelb, M. H. 1990. Kinetic and inhibition studies of phospholipase A2 with short-chain substrates and inhibitors.Biochemistry. 29: 6082
5. Viswanath BS, Fawzy AA and Franson RC. 1988. Edema inducing the activity of phospholipase A_2 purified from human synovial fluid and inhibition by aristolochic acid. Inflammation 12:549-61
6. Bomalaski, J. S., Lawton, P. and Browning, J. L. 1991. Human extracellular recombinant phospholipase A2 induces an inflammatory response in rabbit joints . J. Immunol. 146: 3904-10.
7. Davidson F.F, Dennis E.A, Powell M and Glenney J.R.1987. Inhibition of phospholipase A2 by "lipocortins" and calpactins. An effect of binding to substrate phospholipids. J Biol Chem. 262:1698-705
8. Glaser, K. B., Vedvick, T. S. and Jacobs, R. S. Kernan M.R and Faulkner D. J. 1988.Manoalide: structure-activity studies and definition of the pharmacophore for phospholipase A2 inactivation. Biochem. Pharmacol. 36(5):782-8.
9. Dharmappa K.K, Mohamed R, Shivaprasad H.V and Vishwanath B.S. 2010. Genistein, a potent inhibitor of secretory phospholipase A_2: a new insight in down-regulation of inflammation. Inflammopharmacology.18:25-31.
10. Viswanath B.S, Fawzy A.A and Franson R.C. 1988.Edema inducing the activity of phospholipase A2 purified from human synovial fluid and inhibition by aristolochic acid. Inflammation. 12:549-61.
11. Vishwanath, B. S., Kini, R. M. and Gowda, T. V. 1987. Toxicon 25, 501±515

12. Dijkman, R., Cox, R., Van den Berg, L., Verheij, H. M. and De Haas, G. H. 1994. Competitive inhibition of lipolytic enzymes. X. Further delineation of the active site of pancreatic phospholipases A2 from pig, ox and horse by comparing the inhibitory power of a number of (R)-2-acylamino phospholipid analogues. Biochim. Biophys. Acta. 1212: 50-58
13. Yu, L., and Dennis, E. A. 1993.Effects of polar head groups on the interactions of phospholipase A_2 with phosphonate transition-state analogues, *Biochem.* 32: 10185-92
14. Kurumbail R.G, Stevens A.M and Gierse J.K. 1996. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature. 384(6610):644–48.
15. Marnett L.J. 2009; The COXIB experience: a look in the rearview mirror. Annu Rev Pharmacol Toxicol. 49:265–290.
16. Kitamura T, Itoh M, Noda T, Matsuura M and Wakabayashi K. 2004. Combined effects of cyclooxygenase-1 and cyclooxygenase-2 selective inhibitors on intestinal tumorigenesis in adenomatous polyposis coli gene knockout mice. Int J Cancer. 109(4):576–580.
17. Sano H, Noguchi T, Miyajima A, Hashimoto Y and Miyachi H. 2006. Anti-angiogenic activity of basic-type, selective cyclooxygenase (COX)-1 inhibitors. Bioorg Med Chem Lett. 16(11):3068–3072.
18. Chakraborti A.K, Garg S.K, Kumar R, Motiwala H.F and Jadhavar P.S. 2010 Progress in COX-2 inhibitors: a journey so far. Curr Med Chem.17 (15):1563–93.
19. Singh P and Mittal A. 2008 Current status of COX-2 inhibitors. Mini Rev Med Chem. 8(1):73–90.
20. Boolbol S.K., Dannenberg A.J, Chadburn A, Martucci C, Guo X.J, Ramonetti J.T, Abreu-Goris M, Newmark H, Lipkin M.L, DeCosse J.J and Bertgnolli M.M. 1996. Cyclooxygenase overexpression and tumor formation are blocked by sulindac in murine model of familial polyposis, Cancer Res. 56: 2556–60.
21. Goetano G.D, Donati M.B and Cerletti C. 2003. Prevention of thrombosis and vascular inflammation: benefits and limitations of selective or combined cox-1, cox-2 and 5- lox inhibitions. Trends in Pharm Sci. 24(5): 245-52.
22. Dinarello C.A. 2010. Anti-inflammatory Agents: Present and Future. Cell. 140(6): 935–950.
23. Giresha A. S, Narayanappa M, Vikram joshi, Vishwanath B. S and Dharmappa K. K. 2015. Human secretory phospholipase A_2 ($sPLA_2$) inhibition by aqueous extract of *Macrotyloma uniflorum* (seed) as an anti-inflammatory activity. Int J Pharm Pharm Sci. 7(1): 217-22.
24. Marshall, L. A., Bauer J, Sung M. L, and Chang J. Y. 1991. Evaluation of antirheumatic drugs for their effect in vitro on purified human synovial fluid phospholipase A2. J. Rheumatol. 18:59-65.
25. Schevitz, R. W, Bach N. J, Carlson D. G, Chirgadze N. Y, Clawson D. K, Dillard R. D, Draheim S. E, Hartley L. W, Jones N. D, Mihelich E. D, Olkowski J. L, Snyder D. W, Sommers C, and. Wery J.P. 1995. Structure-based design of the first potent and selective inhibitor of human non-pancreatic secretory phospholipase A2- Nat. Struct. Biol. 2:458-465.
26. Holmes M.V, Simon T, Exeter H.J, Folkersen L, Asselbergs F.W, Guardiola M.2013. Secretory phospholipase A2-IIA and cardiovascular disease a mendelian randomization study. J Am College Cardiol. 62:1966–76.
27. Giresha A.S, Anitha M.G and Dharmappa K.K.2015. Phytochemical composition, antioxidant and in-vitro anti-inflammatory activity of ethanol extract of *Ruta Graveolens* L. leaves. Int J Pharm Pharm Sci. 7(10): 272-76.
28. Kim H.P, Son K.H, Chang H.W and Kang S.S. 2004. Anti-inflammatory plant flavonoids and cellular action mechanisms. J Pharmacol Sci; 96:229-45.
29. Subrata.D. and Superna. D; G.montana, a potential hepatoprotective and anticancer drug –an over view. Asian J Pharm Clin Res.2012,3:20-24
30. Blios M.S.1958. Antioxidant determinations by the use of a stable free radical. Nature. 181:1199.
31. Buege J.A and Aust S.D. 1978. Microsomal lipid peroxidation. Method Enzymol.52:302-10.
32. Viswanath B.S, Fawzy A.A and Franson R.C.1988. Edema inducing activity of phospholipase A2 purified from human synovial fluid and inhibition by aristolochic acid. Inflammation.12:549-61.
33. Aharony D and Stein R.L.1986. Kinetic mechanism of guinea pig neutrophil 5-lipoxygenase. J Biochem. 261:11512-9.
34. Axelrod B, Cheesbrough T. M and Laakso S.1981. Lipoxygenase from soybeans. Method Enzymol. 71:441-51.
35. Boman H.G and Kaletta U. 1957. Chromatography of rattlesnake venom: a separation of three phosphodiesterases. Bioch Biophys Acta. 24:619-31.

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