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EVALUATION OF MEDICINAL PROPERTIES OF *GREWIA NERVOSA* (LOUR.) PANIGRAHI

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ABSTRACT

Grewia nervosa (Lour) Panighrahi, belonging to the family Malvaceae s.l. is widely distributed along the Western Ghats of India. Although it has been commonly used in traditional medicine, the medicinal properties have not been scientifically evaluated. Phytochemical analysis established the presence of phenolic compounds, tannins, alkaloids and saponins in leaves. The aqueous and methanol extracts from leaves and bark of *G. nervosa* were investigated for medicinal properties using *in vitro* assays. The methanol extract of leaves demonstrated 97.5% inhibition of α-amylase activity. Additionally, the methanol extract of leaves also demonstrated antioxidant activity (5.41± 0.23 mmol/g, dw) that was higher compared to aqueous extract (3.32±0.45 mmol/g, dw). Further the methanol extract of bark exhibited anti-lipoxygenase activity indicative of its potential to control inflammatory activity. These results suggest that *Grewia nervosa* would be a potential source for treatment of diabetes and its associated complications such as oxidative stress and inflammation

KEYWORDS: ***Grewia nervosa*, α-amylase inhibition, anti-lipoxygenase, antioxidant activity**

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INTRODUCTION

Grevia nervosa (Lour.) Panighrahi, also known by its synonyms, viz. *Microcos nervosa* (Lour.) S.Y.Hu, *G. microcos* L. and *M. paniculata* L., belonging to the family Malvaceae s.l. is widely distributed from mainland India to China and up to Indonesia through Andaman islands, Sri Lanka, Myanmar, Thailand, Cambodia, Vietnam, and Malaysia[1]. The plant grows as a shrub or a tree and is commonly used in the preparation of Chinese herbal tea. Although the plant is traditionally claimed to have diverse medicinal values, most of it has been scientifically unproven. Traditional beliefs claim that it helps the digestive system to work well[2]. Additionally, it has been used for alleviation of health problems such as cold, hepatitis, diarrhoea, heat stroke and dyspepsia[3]. However none of these alleged claims have been evaluated scientifically.

Alkaloid, n-methyl- 6 beta-(deca-1', 3', 5'-trienyl) -3 beta-methoxy-2 beta-methylpiperidine isolated from the bark of *M. paniculata* (= *G. nervosa*) was associated with insecticidal activity against *Aedes aegypti* second instar larvae[4]. Furthermore, two new piperidine alkaloids, microcosamines A and B isolated from the leaves demonstrated larvicidal activity against *Culex quinquefasciatus*[5]. Additionally, five compounds isolated from the stem of *G.nervosa* have demonstrated free radical-scavenging activity [6].

Modern lifestyle has been the primary cause for high incidence of type 2 diabetes worldwide. Frequent occurrence of hyperglycemia associated with type 2 diabetes promotes oxidative stress and subsequent inflammations leading to increase morbidity and mortality in tissues. This in turn increases the risk of chronic disease such as heart diseases[7]. Thus oxidative stress and inflammation are commonly associated complications with diabetes.

The α-amylase inhibition assay has been commonly used to detect anti-diabetic activity[8]. Additionally, ferric reducing antioxidant power (FRAP) assay has been used to explore the antioxidant potential of plant extracts[9]. Lipoxygenase, a key enzyme in the biosynthesis of leukotrienes are known to play an important role in the several inflammatory and allergic diseases[10]. Thus compounds demonstrating lipoxygenase inhibition would be a potential anti-inflammatory drug.

The present study reports the major phytochemicals present in leaves of *G.nervosa* and investigates the anti-diabetic, antioxidative and anti-inflammatory activities of the plant using *in vitro* assays.

MATERIALS AND METHODS

(i) Collection and preparation of plant material

The bark of the main stem measuring from 0.2 to 0.3 cm in thickness and healthy mature leaves of *G. nervosa* were collected from the Goa University campus (15°27’35.05” N, 73°49’58.46” E, 52 m) in June, 2011. The weather on the plateau is generally hot and humid throughout the year. Fresh mature leaves of the plant and bark were extracted in methanol or water by the Soxhlet extraction method. Finally, the extracts were dried using rota-evaporator (Roteva, Equitron®) at 60°C and immediately used for investigating medicinal properties.

(ii) Qualitative and quantitative phytochemical studies

(a) Total phenols and tannins estimation

Qualitative detection of total phenols and tannins in leaf samples was performed as per standard protocols[11, 12]. Quantitative estimation of total phenols by Folin-Ciocalteu method was carried out as follow: 10 ml of 70% acetone was added to 200 mg fine ground powder of mature leaves of *G. nervosa*. The mixture was subjected to ultrasonic treatment (VC300, Sonic & Material.INC) for 20 min at room temperature and then centrifuged at 3000 g for 10 min at 4°C. The pellet was again extracted by 70% acetone treatment and again subjected to centrifugation as mentioned above. After centrifugation, the supernatant was pooled and used immediately for further study.

Although, tannins were also estimated by the Folin-Ciocalteu method, the tannin concentration was obtained by subtracting the values for total phenolics obtained, before and after addition of polyvinylpyrollidone (Sigma grade) to plant extract[13]. The total phenols and tannins in the plant were determined from the tannic acid standard calibration curve and expressed as tannic acid equivalent (TAE).

(b) Saponin

The presence of saponin was detected by frothing test[14]. For quantitative estimation, 20 g of dried leaf sample of *G. nervosa* was resuspended in 200 ml of 20% ethanol. The suspension was heated at 90°C for 4 h. The suspension was transferred to a separating funnel followed by the addition of 20 ml of diethyl ether. After vigorous mixing, the aqueous layer was recovered whereas ether layer was discarded. This extraction process was repeated by adding 60 ml of n-butanol to the aqueous layer. The suspension was extracted twice with 5% NaCl solution. The remaining solution was heated on a water bath and air dried in hot oven. The yield of saponin was denoted as %.

(d) Alkaloids

Alkaloids were qualitatively estimated by Dragendorff's test[15]. For quantitative estimation, 200 ml of 20% acetic acid in ethanol was added to 5 g of dried leaf powder and incubated for 4 h at 30°C. The suspension was filtered through Whatman filter paper no. 1 and the filtrate was incubated in boiling water bath to decrease the content to one fourth of its initial volume. Later, concentrated ammonium hydroxide was added drop wise and incubated to obtain the precipitate. The precipitate was collected on Whatman filter paper no.1 and weighed[16]. The yield of alkaloids was expressed as %.

(iii) In vitro anti-diabetic assay

Amylase inhibition activity was determined by 3, 5-dinitrosalicylic acid colorimetric method[17]. Methanol and aqueous extracts of leaves and bark were re-suspended in methanol and water respectively and used as test samples. 0.2% (w/v) maltose was used as standard. 50 mg of Acarbose tablet (Glucobay®50, Bayer pharmaceuticals Pvt. Ltd.) was used as a standard α-amylase inhibitory drug. Results were expressed as % inhibition and was calculated as % inhibition = (Test control- test sample/ Test control) × 100.

(iv) In vitro anti-inflammatory assay

The anti-lipoxygenase assay was performed using linoleic acid (Himedia, India) as substrate, lipoxidase (Himedia, India) as an enzyme and 10 mM ascorbic acid as a standard[18]. 2% methanol or aqueous extract from bark of *G. nervosa* resuspended in 0.2 M borate buffer (pH 9.0) was used as a test sample to determine the anti-lipoxygenase activity.

(v) In vitro antioxidant activity

Total antioxidant activity in the plant extract was estimated using ferric reducing antioxidant power (FRAP) assay[19]. Methanol and aqueous extract of *G. nervosa* leaves (0.1%) suspended in 300 mM acetate buffer (pH 3.6) was used as a test sample. Ascorbic acid (100μM-1mM) was taken as the standard. Results were expressed as μg ascorbic acid equivalent (μg AAE/ml).

(vi)Statistical measurements

All assays were done in triplicates and standard deviation was calculated.

RESULTS AND DISCUSSION

1. Qualitative & Quantitative screening for phytochemicals from G.nervosa

Qualitative analysis for detection of phytochemicals demonstrated the presence of phenols, tannins, saponins and alkaloids in the mature leaves of *G. nervosa*. Results of quantitative estimation of phytochemicals are as shown in Table 1.

Table 1
Phytochemical constituents in the leaf extract of Grewia nervosa

Constituents	Concentration (dry weight) * mg.g^{-1} / # %
Total phenols	7.11±1.76*
Tannins	5.15±0.88*
Saponins	0.44±0.04#
Alkaloids	3.84±0.01#

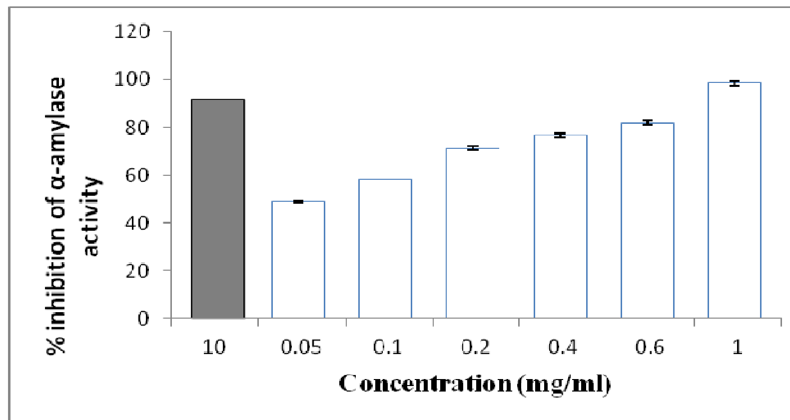
As observed from Table 1, the concentration of phenols and tannins were 7.11±1.76 mg/g and 5.15±0.88 mg/g respectively. The above values are within the range of 2.2 to 13.3 mg/g reported for phenols and tannins in eleven different plants belonging to Malvaceae family[20]. The phenolic compounds have been commonly linked with medicinal properties such as anti-apoptosis[21], anti-aging[22], anti-carcinogenic[23], anti-inflammatory[24], anti-atherosclerosis[25] and cardiovascular protective activity[26]. Additionally, a direct association between polyphenols and antioxidant activity has been described in jackfruit (*Artocarpus heterophyllus* Lam.), the most ancient fruit in Western Ghats of India[27]. Although there are no reports of saponins from medicinal plants belonging to Malvaceae family, the saponin content in medicinal plants belonging to non-Malvaceae families were in the range of 1.12±0.22% to 3.92±0.11%[28]. In contrast, the saponin content in *G. nervosa* leaf extract is comparatively lower (0.44±0.04% dw). The saponins have been concurrent with medicinal properties such as agglutination, formation of foam in aqueous solutions, haemolytic activity and cholesterol binding properties[29, 30]. The concentration of alkaloids in the leaf extract of *G. nervosa* was 3.84±0.01% per gram of dried leaves and is comparatively higher than other medicinal plants, which reported an average of 1.12 %[31]. Alkaloids have been associated with analgesic, antispasmodic and bactericidal activities[32].

2. Inhibition of α-Amylase activity

Varying concentrations of methanol and aqueous extract of leaves and bark (0.05–1 mg/ml) were evaluated for α-amylase inhibition activity. At a concentration of 1mg/ml, 97.5% inhibition of α-amylase activity was observed with methanol extract of leaves (Fig.1). The aqueous/methanol extract of bark and aqueous extract of leaves did not demonstrate α-amylase inhibition activity. Acarbose, a standard anti diabetic drug was used as positive control and demonstrated 91.3% inhibition of α-amylase activity (Fig. 1).

The α-amylase inhibition activity observed in the leaf extract of *G. nervosa* can be attributed to the presence of phytochemicals observed in this plant. Previously, at least one of the phytochemicals such as alkaloids, glycosides, galactomannan gum, polysaccharides, hypoglycans, peptidoglycans, guanidine, steroids, glycopeptides or terpenoids from medicinal plants have been reportedly linked to anti-diabetic activity[33].

Figure1
α-amylase inhibition activity demonstrated by methanol leaf extract of G. nervosa (■ Acarbose; □ methanol leaf extract)

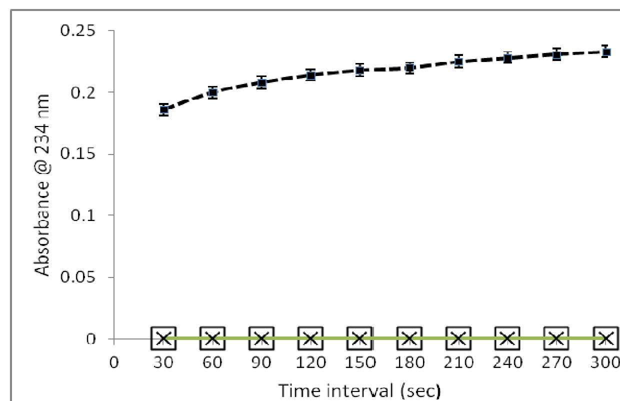


As observed from Fig. 1, a dose dependent inhibition of α-amylase activity was observed in the methanol leaf extract of *G. nervosa*. Likewise, 97.5% inhibition of α-amylase activity was also reported from *Cinnamomum tamala* (Lauraceae)[34]. Additionally, *Hibiscus sabdariffa* belonging to the Malvaceae family have also been reported to inhibit α-amylase activity by 100% at 10 ml/g, fresh wt[35]. Recently, 46 medicinal plants depicting very high α-amylase inhibition activity were reviewed [36]. The α-amylase inhibition activity in *Psidium guajava var. Pomiferum*, *Syzygium cumini* Skeels and *Cajanus cajan* were 98% at 200mg/ml, 98% at 200 mg/ml and 100% at 2 mg protein respectively. However, the concentrations used to demonstrate α-amylase inhibition in above plants were higher in comparison to the concentration of leaf extract of *G. nervosa* used in the present study. Thus methanol leaf extract of *G. nervosa* demonstrates higher α-amylase inhibition activity at lower concentration.

3. In vitro anti-lipoxygenase activity

Inhibition of 15-lipoxygenase enzyme by methanol as well as aqueous extract of bark and leaves of *G. nervosa* was analysed to evaluate anti-inflammatory activity. As observed from Fig.2, the methanol extract of the bark demonstrated anti-lipoxygenase activity. The aqueous extract of bark as well as the aqueous/methanol extract of leaves did not demonstrate any anti-lipoxygenase activity.

Figure 2
Anti-lipoxygenase activities demonstrated by the methanol extract of bark of G. nervosa. (—■— Control; —×— Methanol extract from bark; —□— Standard inhibitor (AA)

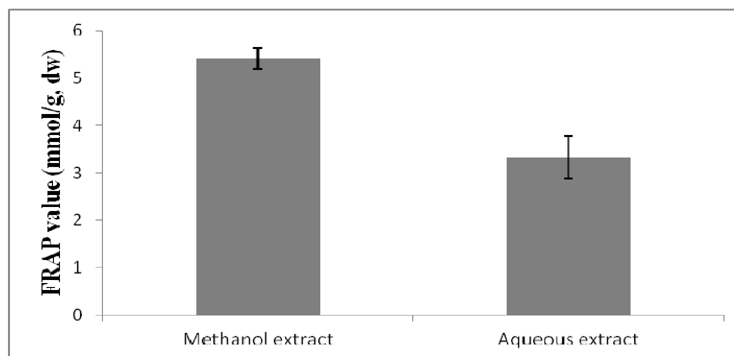


Lipoxygenase play an important role in the pathophysiology of several inflammatory diseases[37]. Plant derived chemical constituents such as flavonoids, coumarins, quinones, pentacyclic triterpenes, sesquiterpenes, alkaloids and polyacetylates have been reported to inhibit 15-lipoxygenase[38]. The anti-inflammatory activity in other medicinal plants belonging to Malvaceae family such as *Sida acuta Burm*.f, *Sida alba* L., *Sida cordifolia* L., *Sida rhombifolia* L., *Sida urens* L. and *Cienfuegosia digitata Cav*. have also been reported[39].

4. In vitro antioxidant activity

As observed in figure 3, the *in vitro* antioxidant assay of the methanol extract from leaf demonstrated antioxidant activity. The methanol extract of leaf demonstrated higher antioxidant activity [5.41± 0.23 mmol/g dry weight (dw)] compare to aqueous extract (3.32±0.45 mmol/g dw). Similarly the methanolic leaf extract of *Andrographis paniculata* and *Limonia crenulata* (Roxb.) have depicted a higher antioxidant activity than the aqueous leaf extract [40,41]. The antioxidant activities from fruit extract of *G. nervosa* have already been reported. However, the FRAP value (28.5 ± 0.43 µmol/g, dw) observed in the fruit extract is much lower than FRAP value of leaf extracts reported in the present study (Fig. 3) [42]. Similarly, the FRAP values reported in other plants belonging to Malvaceae family viz; *Sida acuta* Burm.f (3.70±0.15 mmol/g,dw), *Sida alba* L. (4.06±0.05 mmol/g,dw), *Sida cordifolia* L. (3.47±0.04 mmol/g,dw), *Sida rhombifolia* L.(3.69±0.38 mmol/g,dw), *Sida urens* L. (3.18±0.03 mmol/g,dw) and *Cienfuegosia digitata* Cav. (4.33±0.21 mmol/g,dw) [43] are lower than the FRAP values reported in the present study. Likewise *Acridocarpus orientalis*, a medicinal plant belong to the Malpighiaceae family also reported lower FRAP value (1.10 ± 0.01 mmol/g,dw)[43].

Figure 3
Antioxidant activity depicted by methanol & aqueous leaf extract of G. Nervosa



CONCLUSION

In the present study, the medicinal properties of *G. nervosa*, a plant predominantly found in the Western Ghats, was evaluated using various *in vitro* assays. Phytochemical screening of this plant revealed presence of alkaloids, polyphenols, tannins and moderate levels of saponin, The plant demonstrated potent antioxidant, anti-inflammatory and anti-diabetic properties. The plant under study is a possible source of novel compound with potential of treating diabetes as well as alleviating oxidative stress and inflammation that are commonly associated complications of diabetes. Further studies will focus on isolating, identifying, characterizing and elucidating the structure of the bioactive compounds.

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REFERENCES

1. Kirtikar KR and Basu BD, Indian medicinal plants, 2nd Edition, Dehradun, India, International Book Distributors and Booksellers, 383–386, (1987).
2. Nadkarni KM and Basu BD: Indian Materia Medica, 1st edition, Mumbai, India, Popular Prakashan Ltd, 94-97 (1996).
3. Ghani A, Medicinal Plants of Bangladesh, 1st edition, Dhaka, Bangladesh, The Asiatic Society of Bangladesh, 134-135, (1998).
4. Bandara KA, Kumar V, Jacobsson U and Molleyres LP, Insecticidal piperidine alkaloid from *Microcos paniculata* stems bark. Phytochemistry, 54; 29-32, (2000).
5. Feng SX, Lin LD, Xu HH and Wei XY, Two new piperidine alkaloids from the leaves of *Microcos paniculata*. J Asian Nat Prod Res, 10; 1155-1158, (2008).
6. Fan H, Yang GZ, Zheng T, Mei ZN, Liu XM, Chen Y and Chen S, Chemical constituents with free-radical-scavenging activities from the stem of *Microcos paniculata*. Molecules, 15; 5547-5560, (2010).
7. Scartezzini P and. Speroni E, Review on some plants of Indian traditional medicine with antioxidant activity. J Ethnopharmacol, 71(1-2): 23-43, (2000).
8. Subramanian R, Asmawi AZ, Sadikun A, *In vitro* α- glucosidse and α- amylase enzyme inhibitory effects of *Andrographis paniculata* extract Andrographolide. J Pol Biochem Soc, 55: 391-398, (2008).
9. Halliwell B. The antioxidant paradox. Lancet, 355: 1179-1180, (2000).
10. Sircar JC, Schwender CF and Johnson EA, Soybean lipoxygenase inhibition by nonsteroidal antiinflammatory drugs. Prostagalndins, 25(3): 393-396, (1983).
11. Martinez AG and Valencia, Manual Fitoquimia.1 Pharmacognosy and practices. Seattle: University of Antiquia. March fotiquimica, 59-65 (2003).
12. Trease GE and Evans WC, Pharmacognosy. 15th Ed. Saunders Publishers, London. 42- 44, 221-229, 246-249, 304-306, 331-332, 391-393, (2002).
13. Makkar HPS, Dawra RK, Singh B, Determination of both tannin and protein in a tannin-protein complex. J Agric Food Chem, 36; 523–525, (1988).
14. Obdoni BO and Ochuko PO, Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta states of Nigeria. Global J Pure Appl Sci, 8; 203-208, (2001).
15. Oguyemi AO, In: Sofowora A. Proceedings of a Conference on African Medicinal Plants. Ife-Ife: Univ Ife, 20-22, (1979).
16. Harborne JB, Phytochemicals Methods, Chapman and Hall Ltd., London pp. 49-188. (1973).
17. Miller GL, Use of di-nitro salicylic acid reagent for determination of reducing sugar. Analytical chemistry, 31: 426-428, (1959).
18. Shinde UA, Kulkarni KR, Phadke AS, Nair AM, Dikshit VJ and Saraf MN Mast cell stabilizing and lipoxygenase inhibitory activity of *Cedrus deodara.* (Roxb.) loud wood oil. Indian J Exp Biol, 37: 258-261, (1999).
19. Benzie FF and Strai JJ, Ferric reducing antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Method Enzymol. 299: 15-23, (1999).
20. Gacche RN, Kabaliye VN, Dhole Jadhav AD, antioxidant potential of selected vegetables commonly used in Asian subcontinent. IJNPR, 1(3): 306-313, (2010).
21. Sato M, Bagchi D, Tosaki A, Das DK, Grape seed proanthocyanidin reduces cardiomyocyte apoptosis by inhibiting ischemia–reperfusion-induced activation of JNK-1 and c-JUN. Free Radic Biol Med, 31: 729-737, (2001).
22. Alvarez P, Alvarado C, Puerto M, Schlumberger A, Jimenez L, De la Fuente M, Improvement of leukocyte functions in prematurely aging mice after five weeks of diet supplementation with polyphenol-rich cereals. Nutrition, 22: 913-921, (2006).

23. Stich HF, Rosin MP, Naturally Occurring Phenolics as Antimutagenic and Anticarcinogenic Agents. Adv Exp Med Biol, 177: 1-29, (1984).
24. Kim HY, Park EJ, Joe EH, Jou I, Curcumin suppresses Janus kinase-STAT inflammatory signaling through activation of src homology 2 domain-containing tyrosine phosphatase 2 in brain microglia. J Immunol, 171: 6072-6079, (2003).
25. Miura Y, Chiba T, Tomita I, Tea catechins prevent the development of atherosclerosis in apo-protein E-deficient mice. J Nutr, 131:27–32 (2001).
26. Becker, L.B. New concepts in reactive oxygen species and cardiovascular reperfusion physiology. Cardiovasc Res, 61: 461-470, (2004).
27. Gupta D, Mann S, Sood A, Gupta RK, "Phytochemical, nutritional and antioxidant activity evaluation of seeds of jackfruit (*Artocarpus heterophyllus* Lam.), "Int J Pharm Bio Sci, 2 (4); 336-345, (2011).
28. Edeoga HO, Okwu DE and Mbaebie BO, Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol, 4 (7); 685-688, (2005).
29. Sodipo OA, Akiniyi JA and Ogunbamosu JU, Studies on certain Characteristics of extracts of bark of *Pansinystalia macruceras* (K schemp) pierre Exbeille. Global J Pure Appl Sci, 6; 83-87, (2000).
30. Okwu DE and Okwu ME, Chemical composition of Spondias mombin linn plant parts. J Sustain Agric Environ, 6(2); 140-147, (2004).
31. Rasool R, Ganai BA, Akbar S, Kamili AN and Masood A, Phytochemical screening of *prunella vulgaris* l. –an important medicinal plant of Kashmir, Pak J Pharm Sci, 23; 399-402 (2010).
32. Stary F, The Natural Guide to Medicinal Herbs and Plants. Tiger Books International, London; 12-16. (1998).
33. Mentreddy SR. Medicinal plant species with potential antidiabetic properties. J Sci Food Agric, 87; 743-750, (2007).
34. Kumanan R, Manimaran S, Saleemulla K, Dhanabal SP and Nanjan MJ, Screening of bark of *Cinnamomum tamala* (Lauraceae) by using α-amylase inhibition assay for anti-diabetic activity. Int J Pharm Biomed Res, 1; 69-72, (2010).
35. Hansawasdi C, Kawabata J and Kasai T, α-Amylase Inhibitors from Roselle (*Hibiscus sabdariffa* Linn.) Tea. Biosc Biotechnol Biochem, 64: 1041-1043, (2000).
36. Sales PM, DESouza PM, Simeoni LA, Magalhaes PDO, Silveira D, α-Amylase Inhibitors: A Review of Raw Material and Isolated Compounds from Plant Source, J Pharm Pharmaceut Sci,15(1); 141 - 183, (2012).
37. Wedi B and Kapp A, Pathophysiological role of leukotrienes in dermatological diseases: potential therapeutic implications. Bio Drugs, 15(11): 729-43, (2001).
38. Werz O, Inhibition of 5-Lipoxygenase product synthesis by natural compounds of plant origin. Planta Med, 73; 1331-1357, (2007).
39. Kiessoun K, Souza A, Meda NTR, Coulibaly AY, Kiendrebeogo M, Meda AL, Lamidi M, Rasolodimby JM, Nacoulma OG, Polyphenol Contents, Antioxidant and Anti-Inflammatory Activities of Six Malvaceae Species Traditionally used to Treat Hepatitis B in Burkina Faso. Eur J Sci Res, 44; 570-580, (2010).
40. Sharma M and Joshi S, Comparison of anti-oxidant activity of *Andrographis paniculata* and *tinospora cordifolia* leaves. J Curr Chem Pharm Sc, 1; 1-8, (2011).
41. Merinal S and Viji SBG (2012). *In vitro* antioxidant activity and total phenolic content of leaf extracts of *Limonia crenulata* (Roxb.) J Nat Prod Plant Resour, 2; 209-214, (2012).
42. Fu L, Xu BT, Xu XR, Qin XS, Gan RY, Li HB, antioxidant capacities and total phenolic contents of 56 wild fruits from South China. Molecules, 15(12):8602-17, (2010).
43. Ksiksi T and Hamza AA, Antioxidant, lipoxygenase and histone de-acetylase inhibitory activities of acridocarbus orientalis from al ain and Oman. Molecules. 17, (2012).