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Protective Potentials of *Bryophyllum pinnatum* In Wistar Albino Rats Against Gentamicin-Induced Biochemical Injury

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ABSTRACT

The protective potentials of ethanol leaf extract of *Bryophyllum pinnatum* against gentamicin induced biochemical injury in Wistar albino rats were evaluated using serum biochemical parameters. Twenty-five male albino rats were divided randomly into five groups A to E. Groups C and D received 150 and 300 mg/kg *B. pinnatum,* respectively. Groups A and B were fed with chow (normal and positive controls) while Group E received 100 mg/kg of Vitamin C. Biochemical injury was induced in rats in groups B-E with gentamicin (i.p) at the dose of 80 mg/kg body weight on the 16th day of study. The rats were then fasted for 48 h and sacrificed by cervical dislocation. Serum was collected for biochemical analysis using standard methods and analytical biochemical kits. There was a significant increase in the serum levels of AST, ALT and ALP of the rats administered with gentamicin (Group B - positive control) compared to the Group A- normal control. However, treatment of rats with 150 and 300 mg/kg body weight of ethanol leaf extract of *B. pinnatum* significantly decrease these biochemical parameters compared to Group B ($p < 0.05$). Also, there was a significant increase in the serum levels of conjugated bilirubin and total bilirubin in Group B compared to the normal control. Rat pretreated with 150 and 300 mg/kg body weight of extract showed significant decrease in the conjugated and total bilirubin compared to the non-treated rats in Group B ($p <0.05$). Serum level of urea and creatinine significantly increased in Group B compared to normal control. Rats in groups C and D pretreated with 150 and 300 mg/kg body weight of extract showed significant decrease in the levels of urea and creatinine compared to Group B ($p <0.05$). There was significant decrease in the serum levels of albumin and total protein of the rats administered with gentamicin compared to the normal control. Rats pretreated with 150 and 300 mg/kg body weight of extract showed significant increase compared rats in non-treated Group B ($p <0.05$). The results obtained from this study shows that the ethanolic leaf extract of *B. pinnatum* has protective functions against gentamicin-induced hepatic and nephrotic damage in Wistar albino rats

Key words: *Bryophyllum pinnatum,* Gentamicin-induced injury, Conjugated bilirubin, Total protein and Creatinine.

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INTRODUCTION

Bryophyllum pinnatum (*Lam*) is a glabrous, ornamental herb that is cultivated in houses and gardens. It is about 1 to 1.5 m in height with four obtuse angled stems (kritikar and Basu, 1975). It belongs to the family Craussulacae, genus *Bryophyllum* and Species *pinnatum*. It grows widely and is used as folk medicine in tropical Africa, India, China, Australia and tropical America, Madagascar, Asia and Hawaii (Lans, 2006; Yadav and Dixit, 2003). The flowers are 5 cm long, reddish purple. It is commonly known as air plant, resurrection plant, green love and miracle leave. Preliminary phytochemical investigation of different parts of plant extracts of *B. pinnatum* has reported to contain alkaloids, phenols, flavonoids, saponins, tannins, carotenoids, glycosides, triterpenoids and phenanthrenes

(Kanika, 2011; Nwali et al., 2012), sitosterol, anthocyanins (Nielsen et al., 2005), malic acid, quinines, tocopherol (Pal et al., 1999), lectins (Adinike and Eretan, 2004) and coumarins (Liu et al., 1989). The herbal remedies of *B. pinnatum* have been seen in various cultures and still serve as the main means of therapeutic medical treatment. Traditional healers in northern parts of Nigeria claimed that the leaves and roots serve effectively as asthmatic remedy. It is also used for all set of respiratory conditions, hypertension, kidney stone, gastric ulcer, skin disorder and dysmenorrhea (Gill, 2003; Lans, 2006). It was also claimed to be useful for the treatment of other cardiovascular diseases (Sofowora, 1993).

The leaf juice of the plant was used traditionally as antiviral, antipyretic, antimicrobial, anti-inflammatory, antitumor, hypocholesterolemic, antioxidant, diuretic, antiulcer, antidiabetic, antiseptic, cough suppressant, antihistamine and anti-allergic (Okwu and Josiah, 2006). The plant has also been used for the treatment of edema of legs (Okwu and Nnamdi, 2011). Leaf juice is used in the treatment of coughs, bronchial affections, blood dysentery, jaundice and gout (Ghani, 2003). In Southeastern Nigeria, the herb is used to facilitate the expulsion of the placenta after delivery and applied on the body of young children when they are sick (Agoha, 1974). Gentamicin is an aminoglycoside antibiotic widely used for the treatment of bacterial infections. Therapeutic doses of gentamicin and other aminoglycoside antibiotics can produce nephrotoxicity in humans and animals. Its use is known as one of the most common causes of acute renal failure and nephrotoxicity (Cuzzocrea et al., 2002). Vitamin C, selenium, vitamin E, taurine and the carotenoids (beta-carotene, lutein and lycopene) were reported to decrease the gentamicin-induced reduction in the glomerular filtration rate and the severity of the tubular damage (Anganeyulu and Chopra, 2004; Ekor et al., 2006). This study was aimed at evaluating the possible protective potentials of *B. pinnatum* leaf extract against gentamicin-induced biochemical injury in Wistar rats.

MATERIALS AND METHODS

Animals

Twenty-five Wistar Albino male rats weighing 104 to 132 g were obtained from the Animal House of the University of Nigeria, Nsukka (UNN), Enugu Campus. The animals were house in stainless cages and maintained under standard conditions for the period of the study. The animals were acclimatized for two weeks and maintained at the temperature of 25± 2ºC, 45 ± 5% relative humidity and 12 h light/dark cycle. The animals had access to water and food freely.

Chemicals

Absolute ethanol was product of BDH Chemical Company Ltd, Poole, England. Rat chow was purchased from Pfizer Nigeria Plc.

Collection of Plant Materials

The plant *B. pinnatum* was collected from Amassoma, Southern Ijaw Local Government Area, Bayelsa State, Nigeria and was identified by Dr. Ebi Baraka in the Department of Crop and Soil Science, Faculty of Agriculture, Niger Delta University, Bayelsa State, Nigeria. Voucher no: NDU 096.

Extract Preparation

The leaves of *B. pinnatum* were collected and air dried under shade for three weeks. The dried leaves were ground into powder using an electric blender. 600 g of the powdered leave were macerated in 1800 ml of ethanol at room temperature for 24 h. It was continuously mixed and then filtered using a filter paper (Whatman size No.1). The filtrate was dried in a water bath at 37ºC, and the 50 g viscous concentrate was kept in air tight bottle at 4°C until use.

Experimental Design

Twenty-five (25) adult male Wistar albino rats (eight weeks old) were randomly divided into five (5) groups of five (5) animals per group, labeled A to E and were treated as follows: Group A: served as the normal control (fed and water only). Group B: served as the positive control. Group C: received 150 mg/kg body weight of extract orally. Group D: received 300 mg/kg body weight of extract orally.
Group E: received 100 mg/kg body weight Vitamin C orally (Standard antioxidant). All treatments lasted for fifteen (15) days. Biochemical injury in animals in Group B to E were induced with gentamicin (i.p) at the dose of 80 mg/kg body weight on the 16th day of study, fasted for 48 h and then sacrificed through cervical dislocation.

Collection of Samples

Blood samples were collected in plain bottles through the cardiac puncture and were centrifuged at 2,800 rpm for 10 min. Serum obtained was used for biochemical analysis.

Biochemical Assay

Serum transaminase (ALT and AST) was determined by method of Reitman and Frankel (1957). ALP by the phenolphthalein monophosphate method (Babson, 1965). Total protein was determined by colorimetric method (Biuret method), as modified by Gornallet et al. (1994) method. Bilirubin was estimated by colorimetric method of Jendrassik and Grof (1938). Serum Urea was estimated by Natelson (1951) method, and serum creatinine by Jelliffe (1971).

Table 1. Effect of *B. pinnatum* on liver indices in gentamicin-induced wistar albino rats.

Groups/ treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	ALBUMIN (g/dl)	Total Protein (g/dl)	CONJ. Bilirubin (µmol/l	Total Bilirubin (µmol/l)
A (normal control)	20.20 ± 2.94[a]	18.00 ±1.58[a]	40.19 ± 2.10[a]	36.89 ± 1.00[c]	63.25± 1.39[c]	1.45±0.27[a]	5.86±0.54[a]
B (positive control)	27.60 ± 6.06[c]	30.20 ± 4.76[c]	49.50 ± 2.95[c]	24.19 ± 1.55[a]	46.82± 2.78[a]	1.94±0.05[b]	9.12±1.30[b]
C (150 mg/kg body weight of extract)	23.60 ± 2.19[b]	23.00 ± 4.30[b]	43.20± 3.07[b]	30.46 ± 6.23[b]	58.41± 4.67[b]	1.53±0.12[a]	6.29±0.66[a]
D (300 mg/kg body weight of extract)	23.20 ± 2.38[b]	22.80 ± 4.15[b]	43.58 ± 4.64[b]	33.15± 6.50[b]	56.80± 1.77[b]	1.51±0.15[a]	6.15±0.63[a]
E (100 mg/kg body weight of vitamin C)	24.20 ± 2.38[b]	22.40 ± 4.04[b]	41.64 ± 2.09[a]	34.24± 5.43[b]	57.94± 3.75[b]	1.53±0.13[a]	6.47±1.15[a]

Data are mean ± SD (n = 5). Means values in the same column with different superscript letter(s) are significantly different; $p < 0.05$

Table 2. Effect of *B. pinnatum* on kidney indices in gentamicin-induced wistar albino rats.

Groups/treatment	UREA (mmol/l)	CREATININE (mmol/l)
A (normal control)	5.30 ± 0.49[a]	68.81± 4.35[a]
B (positive control)	9.10± 0.38[b]	91.47± 7.67[c]
C (150mg/kg body weight of extract)	6.26±0.90[a]	75.36±6.38[b]
D (300mg/kg body weight of extract)	6.05± 1.06[a]	74.32± 5.01[b]
E (100mg/kg body weight of vitamin C)	6.32±0.37[a]	71.16±4.52[a]

Data are mean ± SD (n = 5). Means values in the same column with different superscript letter(s) are significantly different; $p < 0.05$.

Statistical Analysis

The results were expressed as mean ± SD. Data was analyzed by one-way analysis of variance (ANOVA). Sequential differences among means were calculated at the level of $P < 0.05$, using Turkey contrast analysis as needed.

RESULTS

The results of the effect of ethanol leaf extract of *B. pinnatum* on liver indices in wistar rats are presented in Table 1. There was significant increase in serum levels of AST, ALT, ALP of the rats administered with gentamicin) (27.60 ± 6.06, 30.20 ± 4.76, 49.50 ± 2.95), respectively when compared to the normal control group (20.20 ± 2.94, 18.00 ± 1.58, 40.19 ±2.10), ($p < 0.05$). However, pretreatment of rats with 150 and 300 mg/kg body weight of ethanol leaf extract of *B. pinnatum* significantly decrease AST, ALT, ALP (23.60±2.19, 23.00 ±4.30, 43.20 ± 3.07) and (23.20 ±2.38, 22.80 ± 4.15, 43.58 ± 4.64), respectively, compared to non-treated positive control group ($p < 0.05$). Also, there was a significant increase in the serum levels of conjugated bilirubin and total bilirubin in non-treated rat positive control group (1.94 ±0.05 and 9.12 ± 1.30), respectively, compared to the normal control (1.45 ±0.27 and 5.86 ± 0.54). Rats in the groups treated with 150 and 300 mg/kg body weight of extract showed significant decrease in the conjugated and total bilirubin (1.53 ±0.12 and 6.29 ±0.66) and (1.51±0.15 and 6.15 ±0.63), respectively, compared to the non-treated positive control group ($p < 0.05$). Serum level of urea and creatinine significantly increased in gentamicin induced rats group (9.10 ± 0.33 and 91.47 ± 7.67), respectively, compared to normal control (5.30 ±0.49 and 68.81 ± 4.35), ($p < 0.05$) (Table 2). Rat in groups C and D treated with 150 and 300 mg/kg body of extract showed significant decrease in the levels of urea and creatinine (6.26 ± 0.90 and 75.36±6.38) and (6.05 ± 1.06 and 74.32 ±5.01), respectively, compared to the positive control group ($p < 0.05$). There was a significant decrease in the serum levels of albumin and total protein of the rats administered with gentamicin (24.19 ± 1.55 and 36.89 ±1.00), respectively, compared to the normal control (46.82 ± 2.78 and 63.25 ± 1.39). Rats treated with 150 and 300 mg/kg body weight of extract showed significant increase (30.46±6.23 and 58.4±4.67) and (33.15±6.50 and 56.80±1.77), respectively, compared to rats in non-treated group ($p < 0.05$).

DISCUSSION

Many researchers have directed their efforts towards the

provision of empirical proof to back up the use of many tropical plants for trado-medical practices (Ojo et al., 2005; Baladran et al., 1985; Madusolomuo and Okoye, 1995). This study was undertaken to explore the hepatoprotective and nephroprotective effect of *B. pinnatum* leaf extract in the hepatic and nephrotic damage caused by the administration of gentamicin. Administration of gentamicin to normal rats increased serum levels of AST, ALT, ALP, conjugated and total bilirubin, urea and creatinine while, and decreased serum levels of albumin and total protein. Elevation of certain liver enzymes in the blood serum gives invaluable diagnostic information for several disease conditions (David and Michael, 2008). AST and ALT for instances, are notably elevated in hepatocellular damage, and increase in ALP might be due to intrahepatic obstruction (Madukosiri, 2013). Elevation of these biochemical parameters may be due to the exposure of the rats to gentamicin which is considered toxic above therapeutic doses. The liver is one of the most important organs in the body and is responsible for the metabolism and detoxification of all toxins that enter the body. The liver is prone to xenobiotic- induced injury because of its central role in xenobiotic metabolism, its portal location within circulation, and anatomic and physiological structure (Godwin et al., 2010). However, pretreatment of rats with ethanol leaf extract of *B. pinnatum* significantly reduced these biochemical parameters. Present findings is in agreement with the earlier report that oral administration of aqueous and ethanolic leaves extract of *B. pinnatum* shows hepatoprotectivity in rats induced N-diethylnitosamine hepatic injury at doses of 250 and 500 mg/kg (Afzal et al., 2013). Also, ethanolic leaf extract of *Bryophyllum calycinum* was reported to confer hepatoprotectivity to rats in CCl_4-induced hepatic injury (Devbhuti et al., 2008).

In this study, there was a significant increase in the levels of conjugated and total bilirubin in untreated rat groups. Such elevation may be indicative of liver injury (Edward et al., 1995; Patrick-Iwuanyanwu et al., 2007). Bilirubin is formed from the breakdown of haemoglobin in the liver, spleen and bone marrow, and its measurement is an important index in determining the excretory function of the liver and assessment of haemolytic anaemia (Kpomah et al., 2012). Rats in groups treated with ethanol extract of *B. pinnatum* showed reduction in the levels of these parameters. Preliminary phytochemical investigation of different parts of plant extracts of *B. pinnatum* was reported to contain alkaloids, phenols, flavonoids, saponins, tannins, carotenoids, glycosides (Kanika, 2011; Nwali et al., 2012), sitosterol, anthocyanins, (Nielsen et al., 2005), malic acid, quinines, tocopherol (Pal et al., 1999), lectins (Adinike and Eretan, 2004), coumarins (Liu et al., 1989) and triterpenoids, phenanthrenes (Siddigui et al., 1989). Therefore, the herb may have exhibited hepatoprotective activity due to its antioxidant properties attributable to these powerful antioxidants. Babalola et al. (2001), reported that triterpenoids fraction of *V. amygdalina* leaf extract ameliorates carbon tetrachloride-induced hepatotoxicity in rats. The decreased levels of serum albumin and total proteins in non-treated rat group, is an indication of hepatotoxicity (Abatan et al., 1996). Albumin is essential for tissue growth and aids in preventing the leakage of fluids from blood vessels. It plays an important role in transporting both endogenous and exogenous substances, serving as protein reserves, as well as maintaining osmotic pressure (Kpomah et al., 2012). However, there was significant increase in the levels of albumin and total protein in extract treated rat groups.

The present study corroborates suggestions made by Davies and Goldberg, (1987) that the herb *B. pinnatum* may have strong protein oxidation inhibitory potency and thus, may be a good source of medicines against diseases in which lipids and protein oxidation are involved, such as toxic hepatitis since protein degradation seems to occur by distinct mechanism (Njayou et al., 2008). The significant increase in serum level of urea and creatinine in gentamicin induced rats group is also an indication of nephrotoxicity. Therapeutic doses of gentamicin and other aminoglycoside antibiotics can produce nephrotoxicity in humans and animals and use of this class of antibiotics is known as one of the most common causes of acute renal failure possibly due to increased renal uptake of the antibiotic mainly by the proximal tubules. Nephrotoxicity is the most common side effects associated with the use of gentamicin (Cuzzocrea et al., 2002). Rats in groups treated with extract showed significant decrease in the levels of urea and creatinine. Kanika, (2011) and Nwali et al. (2014), reported some phytochemical constituents of *B. pinnatum* to include; vitamin E, selenium, vitamin C, taurine and the carotenoids (beta-carotene, lutein and lycopene) which have the potentials to decrease the gentamicin-induced reduction in the glomerular filtration rate and the severity of the tubular damage (Anganeyulu and Chopra, 2004; Ekor et al., 2006). The aqueous extract of *Kalanchoe pinnata* leaves was earlier reported to possess potent nephroprotective activity in gentamicin-induced nephrotoxicity in rats (Harlaka and Patil 2007; Majaz et al., 2011). The results obtained from this study show that the ethanolic leaf extract of *B. pinnatum* has protective function against gentamicin-induced hepatic and nephrotic damage in wistar albino rats.

REFERENCES

Abatan MO, Arowolo ROA, Olorunsogo O (1996). Pathological Effects of *Lantana camara* and *Dichapetalum madagascasiense* in Goats. Trop. Vet. Med.,14:127 -132.

Adinike K, Eretan OB (2004). Purification and partial characterization of lectin from the fresh leaves of *Kalanchoe Crenata* (And.) Haw. J. Biochem. Mol. Biol., 37:229-233

Afzal M, Ahmad K, Saleem S, Kazmi I, Anwar F (2013). Effect of *Bryophyllum pinnatum* on N-diethylnitrosamine induced Hepatic Injury in Rats. J. Pharmacol., 4(2): 82-88.

Agoha RC (1974). Medicinal Plants of Nigeria, Offset Drakkerij Faculfcitder Wiskunde in Naturwetenschappen,the Netherlands 33:41.

Anganeyulu M, Chopra K (2004). Effect of irbesartan on the antioxidant defense system and nitric oxide release diabetic rat kidney. Am. J. Nephrol., 24(5): 488-496.
Babalola OO, Anetor JI, Adeniyi FA (2001). Amelioration of carbon tetrachlorideinducedhepatotoxicity by terpenoid extract from leaves of *Vernonia amydgalina.* Afr. J. Med. Sci., 30:91-93.
Babson LA (1965). Estimation of Alkaline phosphatase activity. Clin. Chem., 11: 789.
Cuzzocrea S, Mazzon E, Dugo L, Serraino L, Paola RD, Britti D, Sarro Pierpaoli S, Caputi AP, Masini E, Savemini D (2002). A role of superoxide in gentamicin mediated nephropathy in rats. Eur. J. Pharmacol., 54(5): 469-473
David LN, Michael MC (2008). Metabolic fates of amino group. In W.H. Freeman and company (5th Eds). Principles of biochemistry. p. 678.
Davies KJA, Goldberg AL (1987). Oxygen radicals stimulate intracellular proteolysis and lipid peroxidation by independent mechanisms in erythrocytes. J. Biol. Chem.,262(17): 8220-8226.
Devbhuti D, Gupta JK, Devbhuti P, Bose A (2008). Phytochemical and acute toxicity study on *Bryophyllum calycinum* SALISB. Acta Poloniae Pharmaceutica-Drug Research, 65(4): 501-504.
Edward CRW, Bouchier IAD, Haslet C, Chilvers ER (1995). Davidson's Principles and practice of Medicine, 17th edn. Churchill Livingstone.pp.488-490.
Ekor M, Farombi EO, Emerole GO (2006). Modulation of gentamicin induced renal dysfunction and injury by the phenolic extract of soybean (Glycine max). Fundam. Clin. Pharmacol., 20(3): 263-271.
Ghani A (2003). Medicinal plants of Bangladesh. *The Asiatic Society of Bangladesh*, Dhaka 2: 382.
Gill LS (2003). Ethno-medical uses of plants in Nigeria. University of Benin press. p. 46
Godwin A, Lyiola S, Aghoghovwia B (2010). Histological and biochemical makers of liver of wistar rats on sub-chronic administration of green tea. North Amer. J. of Med.Sci., 2:376-380.
Harlalka GV, Patil CR (2007). Protective effect of *Kalanchoe pinnata pers.* (Crassulaceae) on Gentamicin induced nephrotoxicity in rats. Indian J. Pharmacol., 39(4): 201-205.
Jelliffe RW (1971). Estimation of creatinine clearance when urine cannot be collected. Lancet, 1:875.
Kanika P (2011). Pharmacognostic & Phytochemical Evaluation of *Bryophyllum pinnatum* Leaves. J. Adv. Sci. Res., 2(1): 42-49.
Kirtikar KR, Basu BD (1975). Indian medical plants periodical experts. Delhi. 2; 2nd edn,p. 999.
Kpomah ED, Arhoghro EM, Uwakwe AA (2012). Biochemical and histophathological changes following chronic administration of diherbal mixture of zanthoxylum leprieurii and piper guineense J. Nat. Sci. Res. 2(6):22-28
Lans CA (2006). Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. J. Ethnobiol. Ethnomed., 2: 45.
Liu KCS, Yang SL, Roberts MF, Phillipson JD (1989). Eupafolin rhamnosides from Kalanchoe gracilis. J. Natural Prod., 52:970-4.
Madukosiri CH (2013). The effect of hear-treated cassava diets and various levels of protein intake on some biochemical parameters in wistar rats. Int. J. Bio. Pharm. Allied Sci., 2(4):813-826.
Madusolomuo MA, Okoye ZS (1995). Anti-coagulant properties of bergenin from sacoglottis gabonsis stem bark extract J. Med Red., 23:443-444.
Majaz QA, Tatiya AU, Khurshid M, Nazim S (2011). The miracle plant (Kalanchoe pinnata): A photochemical and pharmacological review. Int. J. Res. Ayurveda Pharm. 2(5):1478-1482.
Natelson S, Scott ML, Beffa CA (1951). Rapid method for the estimation of urea in biologic fluids. Am. J. Clin. Pathol., 21: 275.
Njayou N, Fredric Paul F, Moundipa Angele N, Tchana Bonaventure, Ngadjui T Felicite M(2008). Tchouanguep Inhibition of microsomal lipid peroxidation and protein oxidation by extracts from plants used in BAMUN folk medicine (Cameroon) against hepatitis. Afr. J. Trad., Complem. Alternative Med., 5(3):278-289.
Nielsen AH, Olsen CE, Moller BL (2005). Flavonoids in flowers of 16 *Kalanchoe blossfeldiana* varieties. Phytochemistry, 66(24): 2829-35.
Nwali BU, Okaka ANC, Ibiam UA, Aja PM (2012). Phytochemical composition of *Bryophyllum pinnatum* Leaves. Int. J. Adv. Biol. Res., 2(4): 614-616.
Nwali BU, Okaka ANC, Offor CE, Aja PM, Nwachi UE (2014). Proximate and Mineral Compositions of *Bryophyllum pinnatum* Leaves. Am. Phytomed. Clin. Therapeut., 2(3): 286-289.
Okwu DE, Josiah C (2006). Evaluation of the chemical composition of two Nigerian medicinal plants, Afr. J. Biotechnol., 5(4): 357-361.
Okwu DE, Nnamdi FU (2011). Two novel flavonoids from *Bryophyllum pinnatum and* their antimicrobial Activity. Pharm. Chem. J., 3(2):1-10.
Pal S, Sen T, Chaudhari AK (1999) Neuropsychopharmacological profile of the methanolic fraction of *Bryophyllum Pinnatum* leaf extract. J. Pharm. Pharmacol., 51:313-318.
Patrick-Iwuanyanwu KC, Wegwu MO, Ayalogu EO (2007). Prevention of CCL4-induced liver damage by ginger, garlic and vitamin E. Pakistan J. Biol. Sci.,10(4):617-621.
Reitman S, Frankel S (1957). Colorimetic method for the determination of serum transaminases. Am. J. Clin. Pathol., 28: 56-63.
Siddiqui S, Faizi S, Siddiqui BS, Sultana N (1989) Triterpenoids and phenanthrenes from leaves of *Bryophyllum pinnatum*. Phytochem., 28: 2433-2438.
Sofowora A (1993). Medicinal plants and traditional medicine in Africa, New York. John Wiley and Sons. p.119.
Yadav NP, Dixit VK (2003) Hepatoprotective activity of leaves of *Kalanchoe pinnata* Pers, J. Ethnopharmacol., 86:197-202.