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Full Length Research Paper

Xanthine oxidase inhibition of selected Philippine medicinal plants

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Extracts from selected species of Philippine plants traditionally used for their anti-inflammatory properties were examined for their ability to inhibit the action of xanthine oxidase. The degree of inhibition using *Adenanthena payonina*, *Antegonon leptopus*, *Blumea balsamifera*, *Calophyllum inophyllum*, *Cassia alata*, *Cassia fistula*, *Gliricidia sepium*, *Michelia alba*, *Mimosa pudica*, *Portulaca olercea*, *Pogostemon cablin*, *Solanum tornum*, *Tinosphora rumphii* and *Vitex negundo* extracts were determined by measuring the increase in absorbance at 295 nm which is associated with uric acid formation which is linked to gout. *B. balsamifera* has the highest percent inhibition at 79.67% followed by *M. pudica* with 62.36% inhibition. The xanthine oxidase inhibitory potential and IC_{50} values of the extracts are reported.

Key words: Gout, medicinal plants, plant extracts, xanthine oxidase.

INTRODUCTION

Herbal remedies obtained from traditional herbs and medicinal plants is commonly use in the Philippines. In rural areas, health and healing are usually in the alternative form of a hand-me-down herbal concoction. Even in the capital city of Manila, herbal vendors trade fresh plants and preparations for various conditions ranging from fever to abortifacients. There are thousands of herbal plants that folklore had attributed medicinal benefits to (Quisumbing, 1978). However, a considerable number of plants still needs to be scientifically validated, hence, much work is still needed to investigate the bioactivity and phytochemicals of these plants.

Xanthine oxidase plays a major role in the purine nucleotide metabolism in humans. Its major function is to catalyze the oxidation of hypoxanthine to xanthine and of xanthine to uric acid (Rundes and Wyngaarden, 1969). Overproduction of uric acid leads to hyperuricemia which is linked to gout (Harris et al., 1999). Gout is characterized by the deposition of uric acid in the joints leading to severe and episodic painful inflammation (Klippel, 2008). This metabolic disease is a common disease with a higher prevalence in men older than 30 years and in women older than 50 years (Kramer and Curham, 2002; Packer et al., 2006). Recent epidemiological studies revealed that the overall disease burden of gout worldwide is increasing (Kramer and Curham, 2002).

The employment of xanthine oxidase inhibitors which hinders the formation of uric acid in the body is the foremost therapeutic approach in the treatment of hyperuricemia (Emmerson, 1996). Gout is commonly treated using allopurinol or 1,5-dihyroxu-4H-pyrazolo[3,4-d]pyrimidin-4-one (Choi and Curham, 2005). However, medication using this drug is coupled with adverse side effects which includes fever and rash, progressively developing leukocytosis, eosinophilia, vasculitis, aseptic meningitis, nephritis and renal dysfunction, and hepatic dysfunction (Boyer et al., 1977; Duchene et al., 2000; Greenberg et al., 2001; Jarzobski et al., 1977; Khoo and Leow, 2000; Para et al., 1995; Wolkenstein and Revuz, 1995). On top of these, “allopurinol hypersensitivity syndrome”, a condition which leads to renal failure and impaired liver function is fatal (Arellano and Sakristan, 1993).

Several plant extracts from China (Kong et al., 2000), North America (Owen and Johns, 1999), Brazil (Filha et al., 2006) and Australia (Sweeney et al., 2001) have shown

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Table 1. Percent inhibition and IC_{50} of the 14 plant extracts.

Plant species	Inhibition at 100 (µg/mL)	IC_{50}
A. payonina	47.15	
A. leptopus	59.0	65.3
B. balsamifera	79.67	192.1
C. inophyllum	25.63	
C. alata	24.81	
C. fistula	61.9	
G. sepium	6.94	
M. alba	22.49	
M. pudica	62.36	32.8
P. olercea	39.0	
P. cablin	33.16	
S. tornum	38.45	
T. rumphii	39.99	
V. negundo	50.42	38.4
Allopurinol		6.1

inhibitory effects on xanthine oxidase. Lignans and iridoids isolated from the *Sterospermum personatum* were responsible for the xanthine oxidase inhibitory action of the plant (Sampath et al., 2005). A chalcone compound from *Caesalpinia sappan* had shown comparable IC_{50} values to that of allopurinol (Nguyen et al., 2005). Notably, aqueous extracts of *Lagerstromia speciosa*, yielded two known compounds with xanthine oxidase inhibitory action (Unno et al., 2004).

In this paper, we evaluated the xanthine oxidase inhibitory action of *Adenanthena payonina*, *Antegonon leptopus*, *Blumea balsamifera*, *Calophyllum inophyllum*, *Cassia alata*, *Cassia fistula*, *Gliricidia sepium*, *Michelia alba*, *Mimosa pudica*, *Portulaca olercea*, *Pogostemon cablin*, *Solanum tornum*, *Tinosphora rumphii* and *Vitex negundo*. These plants are traditionally used to treat anti-inflammatory conditions like asthma, gout, rheumatism in the Philippines (Quisumbing, 1978).

MATERIALS AND METHODS

Plant material

A. payonina, *A. leptopus*, *B. balsamifera*, *C. inophyllum*, *C. alata*, *C. fistula*, *G. sepium*, *M. alba*, *M. pudica*, *P. olercea*, *P. cablin*, *S. tornum*, *T. rumphii* and *V. negundo* leaves were collected from the University of the Philippines, Diliman Campus between the period of November 2009 and February 2010 and submitted to Dr. Jose Vera Santos Herbarium, Institute of Biology, University of the Philippines, Diliman for authentication. Voucher specimens for each plant were also deposited.

Plant extraction

The leaves were washed with running water and allowed to drip dry. The air-dried samples were weighed then homogenized for overnight soaking in methanol using clean glass jars. The crude methanolic extracts were concentrated *in vacuo* using a rotary evaporator (Heidolph).

Phytochemical analysis

The phytochemical screening methods used were based on Harborne (1984) and Edeoga (2005). Qualitative test for terpenoids, saponins, tannins, flavonoids, steroids, phenolic compounds, alkaloids and cardiac glycosides were performed.

Xanthine oxidase inhibitory assay

The xanthine oxidase activity with xanthine as the substrate was measured spectrophotometrically using the procedure of Owens and Johns (1999) with the following modifications. The positive control, allopurinol solution, was prepared by dissolving 5.0 mg of allopurinol in 5.0 ml of 0.15 M phosphate buffer (pH 7.5). Xanthine oxidase from bovine milk was purchased from Sigma (×4500). The enzyme solution was prepared by diluting 30 µl of a 5.0 U/0.2 ml xanthine oxidase solution to a final volume of 3.0 ml. The substrate solution was prepared by addition of 5 drops of 1.0 M NaOH to 22.7 mg of xanthine to aid its dissolution with deionized water to a final volume of 250 ml. The plant extracts were dissolved in 1% dimethyl sulfoxide (DMSO) to a final concentration of 1 mg/ml. All solutions were prepared immediately before use.

Total volume of the assay mixture is 3.4 ml and consists of the plant extract under study (apportioned concentrations of 200, 100, and 75 µg/ml), 0.15 M phosphate buffer (pH 7.5) and 100 µl of 0.03 U/ml xanthine oxidase enzyme solution. After preincubation of the test solution at 25˚C for 10 min, the reaction was initiated by addition of 1 ml of 0.6 mM substrate solution of xanthine, mixed thoroughly, and monitored through absorption increments read every 30 s for 10 min at 295 nm indicating the formation of uric acid using a Shimadzu UV-1700 series spectrophotometer. Allopurinol was used at a final concentration of 30 µg/ml in the assay mixture.

The percent xanthine oxidase inhibitory activity of the assayed samples was determined through the slope of the plot of absorbance against time (seconds). IC50 values were obtained through linear regression analysis the plot of concentration (200, 100, 25 µg/mL) against percent inhibition.

RESULTS AND DISCUSSION

Fourteen Philippine plants used in folkloric medicine to alleviate gout were evaluated for their xanthine oxidase inhibitory action. Phytochemical profiles of all plants tested were also determined. Allopurinol was used as the positive control and its IC_{50} value was calculated as 6.1 µg/ml. The percent xanthine oxidase inhibitory activity of the assayed samples was determined through the slope of the plot of absorbance against time (seconds). IC_{50} values were obtained through the slope of the plot of concentrations used (200, 100, 25 µg/m) against percent inhibition determined at each concentration.

A. payonina, *A. leptopus*, *B. balsamifera*, *C. inophyllum*, *C. fistula*, *M. pudica*, *P. olercea*, *P. cablin*, *S. tornum*, *T. rumphii* and *V. negundo* methanolic extracts had greater than 25% inhibition at 100 µl as shown in Table 1.

It had been reported previously that extracts causing>50% enzyme inhibition at concentration of

Table 2. Phytochemical profile of the 14 plant extracts.

Plant species	Alkaloids	Cardiac glycosides	Flavonoids	Phenolic compounds	Saponins	Steroids	Tannins	Terpenoids
A. payonina	-	+	-	-	-	-	-	-
A. leptopus	+	+	-	-	-	+	+	+
B. balsamifera	+	-	+	+	-	-	+	+
C. inophyllum	+	+	+	+	-	-	+	-
C. alata	+	-	+	+	-	-	+	-
C. fistula	+	+	-	-	-	-	+	+
G. sepium	-	+	-	-	-	-	+	-
M. alba	-	-	+	-	-	-	+	-
M. pudica	+	+	+	+	-	-	+	+
P. olercea	-	+	+	+	-	-	+	-
P. cablin	-	+	+	+	-	-	+	-
S. tornum	+	-	+	-	-	-	+	+
T. rumphii	+	+	-	-	-	-	+	+
V. negundo	+	-	+	-	-	+	+	+

50 µg/ml warranted further investigation (Sweeney, 2001). The IC_{50} value of plants exhibiting higher than 50% inhibition was determined. *B. balsamifera* has the highest percent inhibition at 79.67%. It is one of the herbal medicinal plants approved by the Philippine Department of Health and used as a diuretic. It used traditionally for colds, headache, stomach pains and rheumatism (Quisumbing, 1978). Flavonoids and sesquiterpenoids have been identified from its extracts (Nessa et al., 2004; Osaki et al., 2005). Extracts or constituents of *B. Balsamifera* have been found to possess several biological activities (Nessa et al., 2004; Noor Rain et al., 2007; Norikura et al., 2008; Osaki et al., 2005;

M. pudica exhibited 62.36% inhibition and showed the lowest IC_{50} of 32.8 µg/ml. In the Philippines, decoction of the entire plant is used as anti-asthma and the root as diuretic (Quisumbing, 1978). Its root extracts exhibited various biological activites which includes wound healing (Kokane et al., 2009), anti-fertility (Ganguly et al., 2007), anti-venom (Girish et al., 2004). Leaf extracts of *M. Pudica* also demonstrate anti-convulsant (Ngo et al., 2004), hyperglycemic (Amalraj and Ignacimuthu, 2002) and anti-depressant activities (Molina et al., 1999).

V. negundo likewise exhibited an IC_{50} of 38.4 µg/ml. It is a popular herbal plant in the Philippines and it also one of the ten herbal medicinal plants approved by the Philippine Department of Health. It is mainly use for the relief of cough and asthma. It is commercially marketed as Ascof Lagundi syrup and tablets for coughs. Its root is traditionally used for dyspepsia, colic, rheumatism and leprosy, the leaves as antiseptic for wounds and a remedy for headache (Quisumbing, 1978). Lignans, diterpenes, terpenes, iridoid glucosides have been identified from its extracts (Arif et al., 2008; Chawla et al., 1992; Khokra et al., 2008; Sehgal et al., 1983; Zheng et al., 2010). It was also found to possess anti-venom (Alam and Gomes, 2003), anti-inflammatory (Zheng et al., 2010), anti-bacterial (Khokra et al., 2008), hepatoprotective (Tandon et al., 2008) and nitric oxide scavenging activity (Zheng et al., 2009). It is possible that the inhibitory activities and IC_{50} values would improve once the compounds responsible for the activity are identified.

Phytochemical analysis of the different plant extracts as shown in Table 2 revealed that the extracts tested contained flavonoids, terpenoids, cardiac glycosides and tannins. Previous studies have shown that flavonoids interact with xanthine oxidase by competitively inhibiting its action (Jiao et al., 2006). However, tannins act through non-selective binding of the enzyme (Owens and Johns, 1999). It is possible that compounds belonging to these classes are responsible for the observed bioactivity. Literature search revealed no cardiac glycosides nor terpenoids have been previously identified with xanthine inhibitory action.

To the best of our knowledge, this is the first report of the evaluation of the xanthine oxidase inhibitory activity of the tested medicinal plant extracts. This work has scientifically validated the use of the plant extracts in folkloric medicine.

Conclusion

This study had established the xanthine inhibitory action of several plant extracts used in Philippine folk medicine. *A. leptopus, B. balsamifera, M. pudica* and *V. negundo* have shown higher than 50% inhibitory activity and further studies are underway to identify the compounds responsible for the observed bioactivity. The isolated and

purified compounds could then be used as a marker for standardization of herbal products or prototypes to develop more efficacious drugs with fewer side effects.

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REFERENCES

Alam MI, Gomes A (2003). Snake venom neutralization by Indian medicinal plants (*Vitex negundo* and *Emblica officinalis*) root extracts. J. Ethnopharmacol., 86: 75-80.

Amalraj T, Ignacimuthu S (2002). Hyperglycemic effect of leaves of *Mimosa pudica* Linn. Fitoterapia, 73: 351-352.

Arellano F, Sakristan JA (1993). Allopurinol hypersensitivity syndrome: a review. Ann. Pharmacother., 27: 337-343.

Arif Lodhi M, Iqbal Choudhary M, Malik A, Ahmad S (2008). alpha-Chymotrypsin inhibition studies on the lignans from *Vitex negundo* Linn. J. Enzym. Inhib. Med. Chem., 23: 400-405.

Boyer TD, Sun N, Reynolds T (1977). Allopurinol-hypersensitivity vasculitis and liver damage. Western J. Med., 126: 143-147.

Chawla AS, Sharma AK, Handa SS, Dhar KL (1992). A lignan from *Vitex negundo* seeds. Phytochemistry, 31: 4378-4379.

Choi HK, Curhan G (2005). Gout: epidemiology and lifestyle choices. Curr. Opin. Rheumatol., 17: 341-345.

Duchene D, Smith C, Goldfarb R (2000). Allopurinol induced meningitis. J. Urol., 164: 2028.

Edeoga HO, Okwu DE (2005). Phytochemical Constituents of Some Nigerian Medicinal Plants. Afr. J. Biotechnol., 4: 685-688.

Emmerson BT (1996). The management of gout. New Eng. J. Med. 334: 445-451.

Filha ZS, Vitolo IF, Fietto LG, Lombardi JA, Saude-Guimaraes DA (2006). Xanthine oxidase inhibitory activity of Lychnophora species from Brazil ("Arnica").J. Ethnopharmacol., 107: 79–82.

Ganguly M, Devi N, Mahanta R, Borthakur MK (2007). Effect of *Mimosa pudica* root extract on vaginal estrous and serum hormones for screening of antifertility activity in albino mice. Contraception, 76: 482-485.

Girish KS, Mohanakumari HP, Nagaraju S, Vishwanath BS, Kemparaju K (2004). Hyaluronidase and protease activities from Indian snake venoms: neutralization by *Mimosa pudica* root extract. Fitoterapia, 75: 378-380.

Greenberg LE, Nguyen T, Miller SM (2001). Suspected allopurinol-induced aseptic meningitis. Pharmacotherapy, 21: 1007-1009.

Harborne JB (1984). Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, USA.

Harris MD, Siegel LB, Alloway JA (1999). Gout and hyperuricemia. Am. Fam. Physician, 59: 925-934.

Jarzobski J, Ferry J, Womboldt D, Fitch DM, Egan JD (1970). Vasculitis with allopurinol therapy. Am. Heart J., 79: 116-121.

Jiao RH, Ge HM, Shi Da H, Tan RX (2006). An apigenin-derived xanthine oxidase inhibitor from *Palhinhaea cernua*. J. Nat. Prod., 69: 1089-1091.

Khokra SL, Prakash O, Jain S, Aneja KR, Dhingra Y (2008). Essential Oil Composition and Antibacterial Studies of *Vitex negundo* Linn. Extracts. Indian J. Pharm. Sci., 70: 522-526.

Khoo BP, Leow YH (2000). A review of inpatients with adverse drug reactions to allopurinol. Singapore Med. J., 41: 156-160.

Klippel JH (2008). Primer on the Rheumatic Diseases 13th ed. Springer, New York, USA.

Kokane DD, More RY, Kale MB, Nehete MN, Mehendale PC, Gadgoli CH (2009). Evaluation of wound healing activity of root of *Mimosa pudica*. J. Ethnopharmacol., 15: 311-315.

Kong LD, Cai Y, Huang W, Cheng C, Tan R (2000). Inhibition of xanthine oxidase by some Chinese medicinal plants used to treat gout. J. Ethnopharmacol., 73: 199-207.

Kramer HM, Curhan G (2002). The association between gout and nephrolithiasis; the National Health and Nutrition Examination Survey III, 1988-1994. Am. J. Kidney Dis., 40: 37-42.

Mahanta M, Mukherjee AK (2001). Neutralisation of lethality, myotoxicity and toxic enzymes of Naja kaouthia venom by *Mimosa pudica* root extracts. J. Ethnopharmacol., 75: 55-60.

Molina M, Contreras CM, Tellez-Alcantara P (1999). *Mimosa pudica* may possess antidepressant actions in the rat. Phytomedicine, 6: 319-323.

Nessa F, Ismail Z, Mohamed N, Mas Haris MRH (2004). Free radical-scavenging activity of organic extracts and of pure flavonoids of *Blumea balsamifera* DC leaves. Food Chem., 88: 243-252.

Ngo Bum E, Dawack DL, Schmutz M, Rakotonirina A, Rakotonirina SV, Portet C, Jeker A, Olpe HR, Herrling P (2004). Anticonvulsant activity of *Mimosa pudica* decoction. Fitoterapia, 75: 309-314.

Noor Rain A, Khozirah S, Mohd Ridzuan MA, Ong BK, Rohaya C, Rosilawati M, Hamdino I, Badrul A, Zakiah I (2007). Antiplasmodial properties of some Malaysian medicinal plants. Trop. Biomed., 24: 29-35.

Norikura T, Kojima-Yuasa A, Shimizu M, Huang X, Xu S, Kametani S, Rho SN, Kennedy DO, Matsui-Yuasa I (2008). Anticancer activities and mechanisms of *Blumea balsamifera* extract in hepatocellular carcinoma cells. Am. J. Chinese Med., 36: 411-424.

Nguyen MT, Awale S, Tezuka Y, Le Tran Q, Kadota S (2005). Xanthine oxidase inhibitors from the heartwood of Vietnamese *Caesalphia sappan*. Chem. Pharm. Bull., 53: 984-988.

Osaki N, Koyano T, Kowithayakorn T, Hayashi M, Komiyama K, Ishibashi M (2005). Sesquiterpenoids and plasmin-inhibitory flavonoids from *Blumea balsamifera*. J. Nat. Prod., 68: 447-449.

Owen P, Johns T (1999). Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout. J. Ethnopharmacol., 64: 149-160.

Pacher P, Nivorozhkin A, Szabo C (2006). Therapeutic effects of xanthine oxidase inhibitors. Pharmacol. Rev., 58: 87-114.

Para E, Gota R, Gamen A, Moros M, Azuara M (1995). Granulomatous interstitial nephritis secondary to allopurinol treatment. Clin. Nephrol., 43: 350.

Quisumbing E (1978). Medicinal Plants of the Philippines. Katha Publishing Co., Inc., Quezon City, Philippines.

Rundes RW, Wyngaarden JB (1969). Drugs and uric acid. Ann. Rev. Pharmacol., 9: 345-362.

Sampath Kumar U, Tiwari AK, Venkat Reddy S, Aparna P, Jagadeeshwar Rao R, Zehra Ali A, Madhusudana Rao J (2005). Free radical-scavenging and xanthine oxidase inhibitory constituents from Stereospemum personatum. J. Nat. Prod., 68: 1615-1621.

Sehgal CK, Taneja SC, Dhar KL, Atal CK (1983). 6′-*p*-hydroxybenzoylmussaenosidic acid-an iridoid glucoside from *Vitex negundo*. Phytochemistry, 22: 1036-1038.

Sweeney AP, Wyllie SG, Halliker RA, Markham JL (2001). Xanthine oxidase inhibitory activity of selected Australian native plants. J. Ethnopharmacol., 75: 273-277.

Tandon VR, Khajuria V, Kapoor B, Kour D, Gupta S (2008). Hepatoprotective activity of *Vitex negundo* leaf extract against anti-tubercular drugs induced hepatotoxicity. Fitoterapia, 79: 533-538.

Unno T, Sugimoto A, Kakuda T (2004). Xanthine oxidase inhibitors from the leaves of *Lagestroemia speciosa*. J. Ethnopharmacol., 93: 391-395.

Wolkenstein P, Revuz J (1995). Drug-induced severe skin reactions: incidence, management and prevention. Drug Safety, 13: 56-68.

Zheng CJ, Huang BK, Han T, Zhang QY, Zhang H, Rahman K, Qin LP (2009). Nitric oxide scavenging lignans from *Vitex negundo* seeds. J. Nat. Prod., 72: 1627-1630.

Zheng CJ, Huang BK, Wang Y, Ye Q, Han T, Zhang QY, Zhang H, Qin LP (2010). Anti-inflammatory diterpenes from the seeds of *Vitex negundo*. Bioorgan. Med. Chem., 18: 175-181.