Journal of Medicinal Plants Research Vol. 4(21), pp. 2204-2210, 4 November, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR10.321
ISSN 1996-0875 ©2010 Academic Journals

\

Full Length Research Paper

Antiproliferative activity of extracts of some Bolivian medicinal plants

Gloria C. Rodrigo[1,2,3], Giovanna R. Almanza[4], Bjorn Akesson[2]* and Rui-Dong Duan[3]

[1]Molecular Biology and Biotechnology Institute, University Major of San Andrés, La Paz, Bolivia.
[2]Biomedical Nutrition, Pure and Applied Biochemistry, Lund University, Lund, Sweden.
[3]Department of Clinical Sciences, Gastroenterology Laboratory, Lund University, Lund, Sweden.
[4]Chemical Research Institute, University Major of San Andres, La Paz, Bolivia.

Accepted 2 August, 2010

Twenty-six plant species from the native flora used in Bolivian folk medicine were selected for studies of bioactivity. They belonged to the following families namely: Asteraceae (11), Brassicaceae (1), Cactaceae (1), Caesalpinaceae (2), Chenopodiaceae (1), Frankeniaceae (1), Geraniaceae (1), Laureaceae (1), Oxalidaceae (1), Piperaceae (1), Plantaginaceae (1), Rosaceae (1), Solanaceae (1) and Verbenaceae (2). Their effects on the proliferation of colon cancer cells (Caco-2) were studied using the WST-1 reagent. The results indicated that four out of 26 ethanolic extracts had a significant antiproliferative activity in this assay from (*Schkuhria pinnata, Piper longestylosum, Parastrephia lepidophylla* and *Erodium cicutarium*). The bioactivity of the extracts was correlated with the phytochemical characterization. Further studies of the mechanism of action of the bioactive extracts are needed.

Key words: Bolivian plants, cell proliferation, colon cancer.

INTRODUCTION

Many biologically active herbal ingredients are employed in the pharmacological industry (Gurib-Fakim, 2006) e.g. those used in clinical trials for anticancer activity (Phillipson, 2007). In addition, there is also a continued search for new natural anticancer, chemopreventive and antimutagenic agents including those occurring in foods and medicinal plants (Zessner et al., 2008). In Bolivia, the flora has been estimated to comprise approximate 20,000 plant species (Larrea-Alcazar and Lopez, 2005) and among them approximate 3,000 plant species have a recognized use as medicinal plants but only little of this potential medicinal plant wealth has been scientifically studied. Studies of their chemical composition have been done e.g. in *Erythroxylum coca* var. *coca* (Sauvain et al., 1997), in *Minthostachys andina* and *Hedomea mandonianum* (Fournet et al., 1996), and in *Doliocarpus dentatus* (Sauvain et al., 1996).

*Corresponding author. E-mail: bjorn.akesson@tbiokem.lth.se. Tel: +46 46 2224523. Fax: +46 46 2224611.

Some studies of the bioactivity of Bolivian plants have also been performed, for example regarding antimalarial activity (Deharo et al., 2001), anti-chagasic activity (Fournet et al., 1996; Del Olmo et al., 2001), anti-inflammatory activity (Deharo et al., 2004), gastric cytoprotection (Gonzales et al., 2000), anti-viral activity (Gupta, 1995; Abad et al., 1999), and inhibition of tyrosinase activity (Kubo et al., 1995). Moreover, the radical scavenging and antioxidant activity of some plants (Parejo et al., 2003) have been studied, but to our knowledge none of their antiproliferative activity. Our research group is mainly interested in the properties of Bolivian plants growing in the highlands (over 3.000 meters above sea level) but also others that primarily are used to treat various diseases.

The most dominant plant biodiversity present in the Bolivian highlands includes bushes, cushions and the most prominent family of the herbaceous species, the Asteraceaewith its most dominant genera, the *Parastrephia* and *Baccharis* genus (Cabrera et al., 1973; Rocha, 2002). Several studies have also demonstrated that it is important considering the aspects of

ethnobotany and ethnomedicine during the selection of plants for chemical and biological studies.

Thus, in this paper we investigated the antiproliferative activity of extracts from 26 plants which are common in the Bolivian highlands and/or are frequently used in traditional Bolivian medicine.

MATERIALS AND METHODS

Plant materials

Plant species were collected in different Andean regions of Bolivia, mainly in the Lake Poopo basin and in the surroundings of La Paz city. All species were identified by the staff of the Herbario Nacional of Bolivia, La Paz, where specimens of each plant were deposited.

Cells and chemicals

Caco-2 human colon cancer cells were obtained from the European branch of American Tissue Culture Collection. Dulbecco's Modified Eagle's Medium (DMEM) and fetal calf serum (FCS) were purchased from Gibco (Stockholm), and the 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate reagent (WST-1) was purchased from Roche (Stockholm).

Preparation of plant extracts

The dried and ground plant material (100 g per sample) was first defatted by extraction with petroleum ether (40 - 60°C), 2 ml/g at room temperature with shaking for 72 h. The remaining plant material was extracted by maceration with ethanol (96% v/v, 2 ml/g) at room temperature for 72 h. The obtained ethanolic extracts were evaporated under vacuum and the residues submitted both to phytochemical characterization and experiments on their effects on cell proliferation. In other experiments some of the plant materials were extracted with either of dichloromethane, methanol, diethyl ether or water.

Phytochemical characterization

Sterols and triterpenes were identified by the Liebermann-Burchard reaction (Kenny, 1952). Tannins were qualitatively determined by examining the redox reaction between the ethanolic extracts (diluted 10 times) and a solution of ferric chloride (300 µM). Flavonoids were identified by Shinoda's reaction (Kumar et al., 2005), alkaloids by the Dragendorf reagent and saponins by shaking (2 min) the ethanolic extract (diluted 10 times) and observing the formation of stable foam (Flores et al., 2004).

Cell culture

Caco-2 cells were cultured in DMEM with L-glutamine, containing 100 IU/ml penicillin, 10 µg/ml streptomycin and 10% (v/v) heat-inactivated FCS. Cells were maintained in 25 cm^2 culture flasks at 37°C in a humidified incubator containing 95% air and 5% CO_2. For assays of cell proliferation the cells were detached with 0.05% trypsin / 0.02% EDTA, resuspended to a concentration of 1 x 10^5/ml and seeded into 96-well plates (Falcon) for 24 h. After 24 h, the plant extracts dissolved in dimethylsulfoxide (DMSO) (1% final concentration) were mixed with 200 µl medium and added to each well, followed by incubation for 24 h. Controls were treated with the same amount of DMSO. Ursolic acid (40 µM) was used as a positive control (Andersson et al., 2003).

Cell proliferation assay

The cell proliferation rate was assayed by use of WST-1 which is a tetrazolium salt, metabolized to a red formazan. The formation of formazan is proportional to the mitochondrial dehydrogenase activity, which in turn correlates with the number of the viable cells. After the incubation of the cells with plant extracts, 20 µl of WST-1 reagent was added to each well. The plate was incubated for 1 h at 37°C and the absorbance was read at 405 nm using 655 nm as background. The cell proliferation rate was expressed as a percentage of the negative control.

Statistical analysis

The results are presented as mean (SD) obtained from 3 - 6 determinations in at least two separate experiments. The statistical significance was determined by the Student t test and $p < 0.05$ was considered to be statistically significant.

RESULTS

The phytochemical characteristics of ethanol extracts are shown in Table 1. Sterols and triterpens were observed in 19 extracts, tannins in 8 extracts, flavonoids in 17 extracts, alkaloids in 1 extract and saponins in 9 extracts. The results of the effects of extracts on cell proliferation are presented in Table 2. Extracts from four out of 26 plant species inhibited the proliferation of Caco-2 cells (Figure 1). The most active one was an ethanolic extract from *Schkuhria pinnata* showing 53% inhibition of growth at 100 µg/ml, followed by *Piper longestylosum* with 43% inhibition at 200 µg/ml, *Parastrephia lepidophylla* with 19% inhibition at 200 µg/ml and *Erodium cicutarium* with 10% inhibition at 100 µg/ml. No significant effects could be observed for the rest of the ethanol extracts (Table 2). In each experiment ursolic acid (40 µM) was included as a positive control. The inhibition of cell proliferation by ursolic acid was 34.4% (14.7, mean (SD). For some plants also extractions with other solvents were made (Table 3), but no significant antiproliferative activity was observed in these extracts.

DISCUSSION

Cytotoxicity screening models provide important initial data to help to select plant extracts with potential antineoplasia properties (Balunas et al., 2006). For the plants used in this study no data about their antiproliferative activity have been found in the literature. We found that the ethanolic extracts of *S. pinnata, P. longestylosum, P. lepidophylla* and *E. cicutarium* showed inhibitory activity against cell proliferation. Most of the plants studied contained sterols/triterpens and flavonoids, and some of them also tannins and saponins. Phenolic compounds have previously been found to have

Table 1. Phytochemical characterization of ethanol extracts from Bolivian medicinal plants. For the plants indicated in bold type antiproliferative activity was observed in Table 2.

Family (numbered) and species (in italics)	PP	Sterols Triterpens	Tannins	Flavonoids	Alkaloids	Saponins
Asteraceae						
Baccharis latifolia	L	++	-	+++	-	+
Baccharis papillosa	L	++	-	++	-	+
Mutisia acuminata	F	+	-	+/-	-	-
Mutisia orbigniana	L	+/-	-	+/-	-	-
P. lepidophylla	L	+	+	++	-	-
Pluchea fastigiata	L	++	-	+	-	-
S. pinnata (Lam.) Labr. Var. Abrot-anoides (roth) Cabrera	L	+	-	+	-	-
Xanthium spinosum L. Sin. Acanthoxanthium spinosum (L). Fourreau	L	++	-	+	-	-
Brassicaceae						
Lepidium meyenii	R	+	++	+	-	+
Caesalpiniaceae						
Caesalpinea spinosa (Mol.) Kuntze	L	++	++	+++	-	+
Caesalpinea pluviosa	B	++	++	+++	-	+
Frankeniaceae						
Frankenia trianda	W	++	++	+++	-	+
Geraniaceae						
E. cicutarium L'Herit	L	+/-	++	++	-	-
Lauraceae						
Persea americana	Fr	-	-	-	-	-
Oxalidaceae						
Hypseocharis pimpinellifolia	R	+	++	+	-	-
Piperaceae						
P.longestylosum	L	+	-	++	-	-
Rosaceae						
Tetraglochin cristatum	AP	++	+++	++	-	+
Solanaceae						
Cestrum parqui	L	++	-	-	+	+
Verbenaceae						
Junellia seriphioides	L	+++	-	-	-	+/-
Lampaya castellani	L	+++	-	+	-	-

PP: (Plant part), L: leaves, R: root, B: bark, F: flowers, S: stem, W: whole plant, AP: Aerial parts, Fr: Fruits. +++ High, ++ medium, + slight and +/- weak positive test, - negative test.

biological activities such as antibacterial, antiviral, immune-stimulating and estrogenic effects, and in addition antiproliferative and cytotoxic properties in several tumor cells (Manthey and Guthrie, 2002; Gomes et al., 2003). Also in this study of plant extracts, the antiproliferative effects were found to be mediated by

Table 2. Antiproliferative activity of ethanol extracts of plants in Caco-2 cells.

Family (numbered) and species (in italics)	Popular name	Plant part	Conc. (µg/ml)	inhibition[a] (%)
Asteraceae				
Baccharis genistelloides	Carqueja	W	1-200	NS
B. latifolia	Chi'llka	L	1-200	NS
Baccharis papillosa syn *B. obtusifolia*	Jathun- thola	L	1-100	NS
Baccharis pentlandii		L	5-20	NS
Baccharis santelices	Thola lejia	L	1-200	NS
Mutisia acuminata	Chinchir-cuma	F	0.01-200	NS
Mutisia orbigniana	Kutu kutu	L	1-200	NS
P. lepidophylla	Thola	S	1-200	19 ($p < 0.01$)
Pluchea fastigiata	Uri uri	L F	1-100 1-200	NS
S. pinnata (Lam.) Labr. Var. *abrotanoides* (roth) Cabrera	Jayakpic-hana	L	1-100	53 ($p < 0.001$)
Xanthium spinosum L.	Amorseco	L	1-100	NS
Brassicaceae				
Lepidium meyenii	Maca	R	1-200	NS
Caesalpiniaceae				
Caesalpinea spinosa (Mol.) Kuntze	Tara,taya	L	1-200	NS
Caesalpinea pluviosa	Momoqui	B	1-100	NS
Frankeniaceae				
Frankenia trianda	K'ota	W	0.01-200	NS
Geraniaceae				
E. cicutarium L'Herit	Aguja aguja	L	1-100	10% (NS)
Lauraceae				
Persea americana	Avocado, Palta	Fr	11.5-46	NS
Oxalidaceae				
Hypseocharis pimpinellifolia	Soltaqui, machoaltea	R L	1-200 1-100	NS
Piperaceae				
P. longestylosum	Thuda	L	2-200	43% ($p<0.01$)
Rosaceae				
Tetraglochin cristatum	Canguia	L	1-200	NS
Solanaceae				
Cestrum parqui	Andres huaylla	L	1.7-6.6	NS
Verbenaceae				
Junellia seriphioides	Anka-raya	L	1-200	NS
Lampaya castellani	Lampaya	L	0.01-50	NS

L: leaves, R: root, B: bark, F: flowers, S: stem, Fr: fruits, W: whole plant, NS: without significant antiproliferative activity, [a]: at highest concentration indicated.

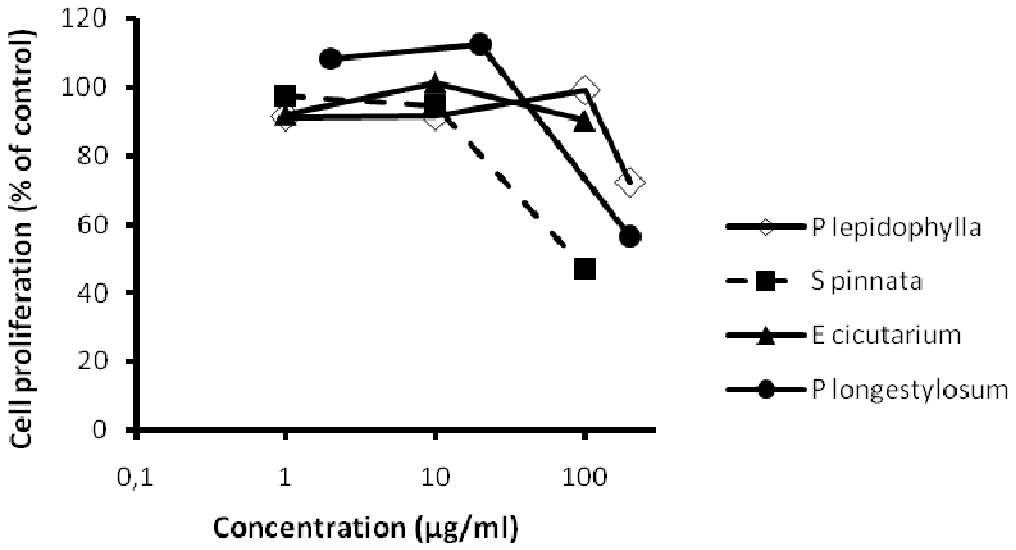

Figure 1. Antiproliferative activity of ethanol extracts from *P. lepidophylla, S. pinnata, P. longestylosum* and *E. cicutarium.* The results show the averages from two separate experiments.

Table 3. Extracts of medicinal plants by other solvents than ethanol which were studied for antiproliferative activity in Caco-2 cells.

Family (numbered) and species (in italics)	Popular plant name	Extract prepared	Plant part	Conc. (µg/ml)	inhibition (%)
Asteraceae					
B. latifolia	Chi'llka	PE	L	0.5-50	NS
		DE	L	1-100	NS
Baccharis papillosa syn *B. obtusifolia*	Jathun thola	EFPM	L	2-200	NS
Baccharis santelices syn *B. incarum, B. thola*	Thola lejia	EtE	L	1-100	NS
P. lepidophylla	Thola	EtE	L	1-100	NS
Cactaceae					
Opuntia soehrensii	Airampu	AE	S	0.05-5	NS
Chenopodiaceae					
Chenopodium ambrosoides	Paico	AE	L	0.66-67	NS
Lauraceae					
Persea americana	Palta	AE	Fr	1-100	NS
Plantaginaceae					
Plantago australis ssp. Hirtella, (Plantago mayor L.)	Llanten	AE	L	0.8-80	NS

AE: aqueous extract, DE: dichloromethane extract, EFPM: medium polarity ethanol extract; EtE: diethyl ether extract, PE: phenolic extract, L: leaves, S: stem, Fr: fruits, NS: without significant antiproliferative activity.

phenolic compounds in several cancer cell lines (Liu, 2004; De Kok et al., 2008).

In some studies the triterpens were found to be responsible for the anticancer activity e.g. in Labrador tea extracts (Dufour et al., 2007). Moreover, triterpens isolated from apple peels showed high potential anticancer activities in HepG2 liver cancer cells, MCF-7 breast cancer cells and Caco-2 colon cancer cells (He and Lui, 2007) and triterpens isolated from *Nerium oleander* inhibited the cell growth of the lung carcinoma

cell line A549, VA-13 cells and HepG2 cells (Fu et al., 2005). The bioactive triterpenoid ursolic acid, used as a positive control in the present study, showed anticancer activity against colon carcinoma cells (Andersson et al., 2003).

In our study the ethanol extract from *S. pinnata* showed high antiproliferative activity inhibiting 53% of the Caco-2 cell growth. In other studies methanol and water extracts of the same plant also showed some cytotoxic activity against VeroE6 cells (Muthaura et al., 2007). In addition to the compounds mentioned above (Table 1), the genus *Schkuhria* contains germacranolides, elemanolides and labdanes (Ganzer and Jakupovic, 1990), heliangolides (Bohlman and Zdero, 1981), germacranolide, schkuhripinnatolide C and pectolarigenin (Pacciaroni et al., 1995), which may explain some biological activity of this plant.

Regarding plants from the genus Piper, an ethanol extract from *Piper rostratum* had cytoprotective effect *in vitro* against doxorubicin cardiotoxicity (Wattanapitayakul et al., 2005). Concerning other effects, *Piper chaba* showed strong activity against *Schistosome cercariae* (Atjanasuppat et al., 2009), and some compounds isolated from *Piper heterphyllum* and *Piper aduncum* showed high leishmanicidal activity and moderate anti-plasmodial and trypanocidal activity (Flores et al., 2009). Concerning *P. lepidophylla* it was found to contain flavones, scopoletin, umbelliferone and *p*-coumaro-yloxytremetone, triterpenoids and tannins (Flores et al., 2004) which is consistent with our phytochemical characterization. The genus Erodium has been reported to contain quercetin, kaempferol, myricetin and luteolin (Saleh et al., 1983) and our phytochemical characterization indicated the presence of sterols/triterpens, tannins and flavonoids. Extracts from it were shown to have antimicrobial activity but no activity in mammalian cells (Esmaeili et al., 2009).

It is possible that synergistic as well as antagonistic effects may occur among phenols and/or other phyto-chemical constituents of plant extracts by overlapping and complementary mechanisms (Liu, 2004; De Kok et al., 2008).

It is possible that anti-inflammatory agents such as plant extracts could act as cancer chemopreventive agents by reducing the initiation and promotion stages of tumour induction induced by DNA oxidation (Desmarchelier et al., 1997). The flavonoids alone or in combination with other compounds may cause suppression or elimination of tumor cells by inhibiting growth through induction of cell cycle arrest or apoptosis (De Kok et al., 2008).

In summary, the extracts of *S. pinnata* and *P. longestylosum* can be considered as the most promising potential sources of anticancer compounds studied in the present experiments. Further investigations are necessary for the detailed chemical characterization of the active principles and a more extensive biological evaluation.

ACKNOWLEDGEMENTS

This research was supported by Swedish International Development Agency (Sida) by funding a joint project on Plant Biodiversity between Major University of San Andres, La Paz, Bolivia and Lund University, Sweden. Some support was obtained from the EU NoE ECNIS (513943).

REFERENCES

Abad MJ, Bermejo P, Gonzales E, Iglesias I, Irurzun A, Carrasco L (1999). Antiviral activity of Bolivian plant extracts. Gen. Pharmacol., 32: 499-503.

Andersson D, Liu JJ, Nilsson Å, Duan R-D (2003). Ursolic acid inhibits proliferation and stimulates apoptosis in HT29 cells following activation of alkaline sphingomyelinase. Anticancer Res., 23: 3317-3322.

Atjanasuppat K, Wongkham W, Meepowpan P, Kittakoop P, Sobhon P, Bartlett A, Whitfield PJ (2009). *In vitro* screening for anthelmintic and antitumour activity of ethnomedicinal plants from Thailand. J. Ethnopharmacol., 123: 475–482.

Balunas MJ, Jones WP, Chin Y-W, Mi Q, Farnsworth NR, Soejarto DD, Cordell GA, Swanson SM, Pezzuto JM, Chai H-B, Kinghorn AD (2006). Relationships between inhibitory activity against a cancer cell line panel, profiles of plants collected, and compound classes isolated in an anticancer drug discovery project. Chem. Biodiversity, 3: 897-914.

Bohlman F, Zdero C (1981). A heliangolide from *Schkuhria pinnata*. Phytochemistry, 20: 2431-2432.

Cabrera AL, Willink A (1973). Biogeografía de América Latina, Monography N° 13. Biology Series, OEA, Washington D.C.

Deharo E, Bourdy G, Quenevo C, Muñoz V, Ruiz G, Sauvain M (2001). A search of natural bioactive compounds in Bolivia through a multidisiplinary approach. Part V. Evaluation of the antimalarial activity of plants used by the Tacana Indians. J. Ethnopharmacol., 77: 91-98.

Deharo E, Baelmans R, Gimenez A, Quenevo C, Bourdy G (2004). *In vitro* immunomodulatory activity of plants used by the Tacana ethnic group in Bolivia. Phytomedicine, 11: 516–522.

de Kok TM, van Breda SG, Manson M (2008). Mechanisms of combined action of different chemopreventive dietary compounds. Eur. J. Nutr., 47:51-59.

del Olmo E, García Armas M, López-Pérez JL, Ruiz G, Vargas F, Giménez A, Deharo E, San Feliciano A (2001). Anti-trypanosoma activity of some natural stilbenoids and synthetic related heterocyclic compounds. Bioorg. Med. Chem. Lett., 11: 2755–2757.

Desmarchelier C, Coussio J, Ciccia G (1997). Extracts of Bolivian plants, *Copaifera reticulate* and *Heisteria pallida* inhibit in vitro free radical-mediated DNA damage. Phytother. Res. 11:460–462.

Dufour D, Pichette A, Mshvildadze V, Bradette-Hébert ME, Lavoie S, Longtin A, Laprise C, Legault J (2007). Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Ledum groenlandicum* Retzius. J. Ethnopharmacol., 111: 22–28.

Esmaeili S, Naghibia F, Mosaddegh M, Sahranavard S, Ghafari S, Rain Abdullah N (2009). Screening of antiplasmodial properties among some traditionally used Iranian plants. J. Ethnopharmacol., 121: 400–404.

Flores Y, Villagomez J, Blanco N, Salcedo L, Sterner O, Almanza G (2004). Defense mechanisms of Bolivian highland plants. Rev. Bol. Quim., 21:36-41.

Flores N, Jiménez IA, Giménez A, Ruiz G, Gutiérrez D, Bourdy G, Bazzocchi IL (2009). Antiparasitic activity of prenylated benzoic acid derivatives from Piper species. Phytochemistry, 70: 621–627.

Fournet A, Rojas de Arias A, Charles B, Bruneton J (1996). Chemical constituents of essential oils of Muña, Bolivian plants traditionally used as pesticides, and their insecticidal properties against Chagas' disease vectors. J. Ethnopharmacol., 52: 145-149.

Fu L, Zhang S, Li N, Wang J, Zhao M, Sakai J, Hasegawa T, Mitsui T,

Kataoka T, Oka S, Kiuchi M, Hirose K, Ando M (2005). Three new triterpenes from *Nerium oleander* and biological activity of the isolated compounds. J. Nat. Prod., 68:198-206.
Ganzer U, Jakupovic J (1990). Schkuhripinnatolides, unusual sesquiterpene lactones from *Schkuhria pinnata*. Phytochemistry, 29: 535-539.
Gomes CA, Giraõ da Cruz T, Andrade JL, Milhazes N, Borges F, Marques MP (2003). Anticancer activity of phenolic acids of natural or synthetic origin: A structure-activity study. J. Med. Chem., 46: 5395-5401.
Gonzales E, Iglesias I, Carretero E, Villar A (2000). Gastric cytoprotection of Bolivian medicinal plants. J. Ethnopharmacol., 70: 329-333.
Gupta M (1995). 270 plantas medicinales Iberoamericanas. Publicación del programa Iberoamericano de Ciencia y Tecnología para el Desarrollo, Santafé de Bogota, D.C., Colombia.
Gurib-Fakim A (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. Mol. Aspects Med., 27: 1-93.
He X, Liu RH (2007). Triterpenoids isolated from apple peels have potent Antiproliferative activity and may be partially responsible for apple's anticancer activity. J. Agric. Food Chem. 55:4366-4370.
Kenny AP (1952). The determination of cholesterol by the Liebermann-Burchard reaction. Biochem. J., 52: 611-619.
Kubo I, Yokokawa Y, Kinst-Hori I (1995). Tyrosinase inhibitors from Bolivian medicinal plants. J. Nat. Prod., 58: 739-743.
Kumar RS, Sivakumar T, Sunderam RS, Gupta M, Mazumdar UK, Gomathi P, Rajeshwar Y, Saravanan S, Kumar MS, Murugesh K, Kumar KA (2005). Antioxidant and antimicrobial activities of *Bauhinia racemosa* L. stem bark. Braz. J. Med. Biol. Res., 38: 1015-1024.
Larrea-Alcázar D, López RP (2005). An estimation of the floristic richness of Bolivia's Andean dry valleys. Biodivers. Conserv., 14: 1923-1927.
Liu RH (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. J. Nutr., 134: 3479S-3485S.
Manthey JA, Guthrie N (2002). Antiproliferative activities of citrus flavonoids against six human cancer cell lines. J. Agric. Food Chem., 50: 5837-5843.
Muthaura CN, Rukunga GM, Chhabra SC, Omar SA, Guantai AN, Gathirwa JW, Tolo FM, Mwitari PG, Keter LK, Kirira PG, Kimani CW, Mungai GM, Njagi ENM (2007). Antimalarial activity of some plants traditionally used in Meru district of Kenya. Phytother. Res., 21: 60-867.
Pacciaroni ADV, Sosa VE, Ariza L, Oberti JC (1995). Sesquiterpene lactones from *Schkuhria pinnata*. Phytochemistry, 39: 127-131.
Parejo I, Viladomat F, Bastida J, Rosas-Romero A, Saavedra G, Murcia MA, Jiménez AM, Codina C (2003). Investigation of Bolivian plant extracts for their radical scavenging activity and antioxidant activity. Life Sci., 73: 1667–1681.
Phillipson JD (2007). Phytochemistry and pharmacognosy. Phytochemistry, 68: 2960-2972.
Rocha O (2002). Diagnóstico de recursos naturales y culturales de los lagos Poopo y Uru Uru, Oruro-Bolivia, pp. 11-36.
Saleh N, El-Karemy Z, Mansour R, Fayed AA (1983). A chemosystematic study of some Geraniaceae. Phytochemistry, 22: 2501-2505.
Sauvain M, Kunesch N, Poisson J, Gantier JC, Gayral P, Dedet JP (1996). Isolation of leishmanicidal triterpenes and lignans from the Amazonian liana *Doliocarpus dentatus* (dilleniaceae) Phytother. Res., 10: 1-4.
Sauvain M, Rerat C, Moretti C, Saravia E, Arrazola S, Gutierrez E, Lema A-M, Muñoz V (1997). A study of the chemical composition of *Erythroxylum coca* var. *coca* leaves collected in two ecological regions of Bolivia. J. Ethnopharmacol., 56: 179-191.
Wattanapitayakul SK, Chularojmontri L, Herunsalee A, Charuchongkolwongse S, Niumsakul S, Bauer JA (2005). Screening of antioxidants from medicinal plants for cardioprotective effect against doxorubicin toxicity. Basic Clin. Pharmacol. Toxicol., 96: 80–87.
Zessner H, Pan L, Will F, Klimo K, Knauft J, Niewöhner R, Hummer W, Owen R, Richling E, Frank N, Schreier P, Becker H, Gerhauser C (2008). Fractionation of polyphenol-enriched apple juice extracts to identify constituents with cancer chemopreventive potential. Mol. Nutr. Food Res., 52: S28-S44.