April 30, 2018

PharmacologyOnLine

Archives • 2018 • vol.1 • 117-127

ASSESSMENT OF THE DIURETIC AND URINARY ELECTROLYTE EFFECTS OF HYDROALCOHOLIC EXTRACT OF *Oreocallis grandiflora* (Lam.) R. Br. IN WISTAR ALBINO RATS

Vinueza Tapia, D. R.[1*]; Allauca Allauca, A. A.[2]; Pilco Bonilla G.A.[1]; Acosta León K.L.[1]; Abdo López S.P.[1]

[1]*Natural Products Laboratory, Polytechnic School of Chimborazo (ESPOCH), Panamericana Sur km 1 ½, Riobamba-Ecuador*

[2]*Laboratory Animal Center, Polytechnic School of Chimborazo (ESPOCH), Panamericana Sur km 1 ½, Riobamba-Ecuador*

drvinueza@espoch.edu.ec

Abstract

The aim of this research was to perform phytochemical screening and to evaluate the diuretic effect of the hydroalcoholic extract of flowers of *Oreocallis grandiflora* on Wistar albino rats (*Rattus norvegicus*).

Hydroalcoholic extract of the dry powered flowers of *O. grandiflora* was obtained by cold maceration method and was subjected to preliminary phytochemical screening. The diuretic activity of the plant extract at doses of 25, 100 and 200 mg/kg and control (furosemide 10 mg/kg) was carried out using metabolic cages, determining the urine volume excreted and the Na^+, K^+, Cl^- and Ca^{2+} urine concentrations. Furthermore, total phenolic content was estimated by using Folin-Ciocalteu reagent, total flavonoid content was evaluated through of $AlCl_3$ complexation method and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was used to determine the *in vitro* antioxidant activity of plant extract.

Phytochemical analysis of extract revealed the presence of major classes of phytochemicals such as flavonoids, tannins, triterpenes, steroids, etc.; but no alkaloids were detected. The hydroalcoholic extract showed a high phenolics and flavonoids content, also exhibited an appreciable antioxidant activity. The diuretic activity of the plant extract was significant in comparison with the standard diuretic drug, furosemide; due it does not show a statistical difference ($p<0.05$) when comparing the lower dose of the hydroalcoholic extract (25 mg/kg) v.s. furosemide (10 mg/kg). The diuretic effect of the plant extract was inversely proportional to the dose.

Based on the results of this preliminary study, it can be concluded that *O. grandiflora* is a very interesting source of natural diuretic compounds which can be used to prevent many chronic disorders. Further detailed phytochemical studies are needed to identified the chemical compounds responsible of the potent diuretic activity showed.

Keywords: *Oreocallis grandiflora, diuretic activity, furosemide, Rattus norvegicus*

Introduction

According to the World Health Organization (WHO), the wellbeing of 80 % of the population in developing countries depends mostly on the use of medicinal plants (1). Medicinal plants have been used extensively for traditional treatment of renal diseases and a significant number of vegetal species have been recognized and reported to show diuretic activity (2).

A recent analysis, associates low doses of a selected group of diuretics with a substantial improvement of primary hypertension and reduction of cardiovascular outcomes; as well as an appreciable decrease in side effects associated with the use of diuretics (3), hence the importance of studying new sources of diuretics.

O. grandiflora is a vegetal species belonging to the Proteaceae family and it is native of Ecuador. The common names with which is known at Ecuador are: cucharillo (at Loja and Zamora provinces), cucharilla (along to the Sierra region of Ecuador), gañal (at Bolívar province) and algil (at Chimborazo province). This plant is widely used for gastric ulcer and liver (complaints), injuries and kidneys in association with *Equisetum sp.* by the indigenous communities of Ecuador; in accordance at Useful Plants of Ecuador compendium made by Ríos et al. 2007 (4), also according to ethnobotanical information it has been established that it may be useful to treat: hernias, liver diseases, intestinal tract infections, cholesterol, nephritis, diabetes, gastric ulcer, inflammations, eye problems, influenza (5), strong sensation of cold, after hard work in the sun, bath against cold (6), vaginal bleeding, ovary/uterus inflammation (7) are remarked. Moreover, *O. grandiflora* is one of the 71 species that make up the traditional drink called "Horchata". A recent study on this preparation mention the following traditional uses for *O. grandiflora* such as anti-inflammatory, digestive, diuretic, hepatic, hypoglicemic agent and vulnerary (8).

The present study was conducted with the objectives (i) to identify the major classes of phytochemicals present in the flowers of *O. grandiflora* grown in Ecuador, (ii) to determine the total phenolic and flavonoid content (TPC) and radical scavenging activity in flowers of *O. grandiflora* and (iii) to evaluate the diuretic activity of *O. grandiflora* flowers by *in vivo* model using *Rattus norvegicus* to validate its use in traditional medicine.

Materials and methods

Drugs and chemicals.

Furosemide and 2,2-Diphenyl-1-picrylhydrazyl were purchased from Sigma-Aldrich, S.L. (USA), deionized water was used in all experimental procedures. All other reagents were of analytical or high performance liquid chromatography grade as appropriate.

Collection of plant material

Oreocallis grandiflora (Lam.) R. Br., Proteaceae, was collected at Ecuador, Loja province, Olmedo town, sector S 03° 56' 0.172" W 079° 38' 25.814", at 1314 meters above sea level. The plant material was taxonomically identified by the botanist Jorge Caranqui at Polytechnic School of Chimborazo and a specimen was deposited at Herbarium, number of deposit ESPOCH 4323. *O. grandiflora* flowers were collected, dried at 50 °C in a forced convection oven for 8 h, and it were ground in a knife mill until particle size of 2-3 mm.

Extraction of the plant material

The dried powdered flowers (100 g each) were extracted by maceration with 1000 mL 70% v/v ethanol for 72 h at room temperature with occasional shaking. Then, the extracts were filtered and the process was repeated on the marc until material were exhausted. The collected filtrates were polled and evaporated under reduced pressure (50°C, -0.5 bar) to yield the dry extract (1.82%). The obtained solids were stored at 4°C and vacuum until use.

Preliminary phytochemical screening

Phytochemical screening of the freshly prepared crude extracts of flowers was carried out to investigate the presence of secondary metabolites such as flavonoids, alkaloids, terpenoids, saponins, and tannins using standard procedure (9) (10).

Total flavonoid determination

The total flavonoids were measured by a colorimetric assay modified by Boukhris et al. (2013). 1 mL aliquot of the diluted sample or standard solution of quercetin (20, 40, 60, 80 and 100 mg/L) was added to a 10 mL volumetric flask containing 4 mL of H_2O. At zero time, 0.3 mL of $NaNO_2$ (5%, w/w) was added to the flask. After 5 min, 0.3 mL $AlCl_3$ (10% w/w) was added.

At 6 min, 2 mL of NaOH (1 M) was added to the mixture. Immediately, the reaction flask was diluted to volume with the addition of 2.4 mL of of H_2O and thoroughly mixed. The absorbance of the mixture, characterized by a pink colour, was determined at 510 nm compared to a water control (11). Total flavonoids were expressed as mg quercetin equivalents (QE)/100 g of hydroalcoholic extract.

Total phenolics determination

The Folin-Ciocalteau method for determination of total phenolics was used in accordance with the description of Waterman and Mole (12). To 200 mL of deionized water were added 50 g of Na_2WO_4, 6.13 g of $H_3PMo_{12}O_{40}$, 25 mL of concentrated HCl, and 12.5 mL of 85% o-H_3PO_4, and the solution was refluxed for 10 h. A few drops of Br_2(liq) was added, and the final volume was adjusted to 250 mL. Sample (200 µL) was vortexed with about 10 mL of destilled water and 1 mL of Folin-Ciocalteu reagent. After 1 min and before 8 min, 3.75 mL of a 20 g/100 mL Na_2CO_3 solution was added, and time was recorded as time zero. The volume was made up to 20 mL with distilled water, and the solution was vortexed three to four times during the next 2 h. After exactly 2 h, the absorbance was recorded at 760 nm (13). Total phenols were expressed as mg gallic acid equivalents (GAE)/100 g of hydroalcoholic extract, using a calibration curve of a freshly prepared gallic acid solution.

Radical scavenging capacity

Solutions of the *O. grandiflora* hydroalcoholic extract (flowers) in ethanol 70% v/v were prepared at concentrations in the range of 10-1000 µg/mL. 100 µL of each concentration was mixed with 3.9 mL of 60 µM DPPH in methanol, at room temperature. The samples were kept in the dark for 30 min, and only after that was the absorbance measured at 515 nm in UV-vis spectrophotometer (14). The blank solution was composed by methanol. The negative control solution was prepared by mixing 3.9 mL of 60 µM DPPH solution with 100 µL of ethanol 70% v/v. Similar solutions in the same medium (ethanol 70% v/v) of gallic acid in range of (10-100 µg/mL) and quercetin in range of (1-250 µg/mL) were prepared and tested for scavenging activity. The experiments were repeated 3 times to confirm the reproducibility of the data. The antioxidant activity was expressed as the percentage of DPPH radical inhibition. The IC50 was calculated by means of logarithmic regression of the curves obtained by plotting the results of percentage the DPPH inhibition (15). On these plots, the abscissa represents the concentration of *O. grandiflora* hydroalcoholic extract (flowers) and the ordinate represents the antioxidant activity.

Diuretic activity

Adult Wistar female albino rats weighing 180–230 g of equivalent age groups were used. The animals were kept under standard laboratory conditions, with standard diet and water *ad libitum*, under a 12 h light/12 h dark cycle.

The method described by Lipschitz with few modifications was employed for the assessment of the diuretic activity of hydroalcoholic extract of *O. grandiflora* (16). The experimental animals were fasted for 12 h prior to experiment allowing only water during the fasting period. Forty five minutes before to the treatment aministration to each animal were administrated 5 mL/ 100 g weight of isotonic saline solution (NaCl 0.9% p/v) to impose a uniform hydric load (17).

Twenty five healthy albino Wistar rats were selected and divided into five groups consisting of five rats in each group. Group I: Normal control group; received sodium carboxymethylcellulose (CMC, 0.5% w/v) orally at the dose of 10 mL/kg. Group II: Standard group: received furosemide orally at the dose of 10 mg/kg. Group III to Group V served as test groups and received *O. grandiflora* hydroalcoholic extract orally at the dose of 25, 100 and 200 mg/kg, respectively. After 1 h of respective treatments, the animals were kept in metabolic cages individually for the collection of urine. The urine was collected for 6 h after the dose was administered. The urine samples were filtered and finally stored at -20 °C for electrolyte analyses.

The diuretic action and diuretic activity were derived from the ratio of urine volume in the test group and that in the control and standard groups, respectively. It was decided prior to the start of the experiment that diuretic activity will be considered "nil", "little", "moderate", and "good", if the values were <0.72, 0.72–1.00, 1.00–1.5, and >1.5, respectively (18).

The sum of Na^+ and Cl^- urinary excretion was calculated as a parameter of saliuretic activity.

The ratio Na^+/K^+ was calculated for natriuretic activity. The ratio Cl/(Na+K) was calculated to estimate carbonic anhydrase inhibition (19).

Data Analysis

All values were expressed as mean values±SD (standard desviation) and data were analyzed by applying an analysis of variance (ANOVA) followed by Dunnett test to compare treatments v.s. standard. The results were considered statistically significant if $P<0.05$.

Results

Phytochemical screening

According to the results of preliminary phytochemical screening the extract showed the presence of flavonoids, phenolic compounds, terpenoids, steroids, carbohydrates, glycosides and saponins. Alkaloids, proteins and aminoacids were absent in the extract (see Table 1).

Total flavonoids and total phenolics determination

The total flavonoids content (TFC) and total phenolics (TPC) content of the extract expressed in terms of quercetin equivalents and gallic acid equivalents, respectively; as well yield (% w/w) are presented in Table 2. The TFC were calculated using the following linear regression equation based on the calibration curve of quercetin $A=0.0512C+0.0286$; $R^2=0.9991$. Where A is absorbance and C is amount of quercetin in µg/mL. The TPC were calculated using the following linear regression equation based on the calibration curve of gallic acid $A=0.0012C-0.0256$; $R^2=0.9990$. Where A is absorbance and C is amount of gallic acid in µg/mL. The extract was found to contain higher amounts of flavonoid and phenolic compounds.

Radical scavenging capacity

The scavenging capacity of different concentration of extract on the DPPH free radical was compared with standard antioxidant, gallic acid. The results are expressed as % inhibition and are shown in the Table 3. The extract showed a dose dependent scavenging activity and it exhibited 15.38% inhibition of free radicals at 200 µg/mL whereas at the same concentration gallic acid showed 92.08% inhibition. The scavenging ability of the extract was found to be non-significant ($P>0.05$) in comparison to gallic acid.

Diuretic activity

The diuretic effect of different doses of extract in terms of urine volume excretion, diuretic action and diuretic activity are summarized in the Table 4. The doses of extract showed a significant diuretic effect, inversely proportional to the dose. The diuretic activity and diuretic action of the extract at the dose level of 25 mg/kg were comparable to the furosemide (10 mg/kg), a reference diuretic drug. No significant differences in the urine excretion was observed in case of the groups treated with the extract doses of 25 and 100 mg/kg when were compared to the rats treated with furosemide.

The diuretic responses with its electrolyte excretion potency of the *O. grandiflora* extract were highly moderate in comparison to normal control rats. The *O. grandiflora* extract at all of tested doses showed a significant increase in Na^+, K^+ and Cl^- excretion but not in the Ca^{2+} excretion. The results of urinary electrolyte excretion after treatment with *O. grandiflora* extract at the dose of 25 mg/kg determine that both for Na^+ and Cl^- were comparable to the furosemide group; however, the K^+ and Ca^{2+} excretion was higher and lower, respectively, to all of extract doses compared to the group treated with furosemide (see Table 5).

The saliuretic activity, natriuretic activity and carbonic anhydrase inhibition are showed in the Table 6. The furosemide (10 mg/ kg) and extract of *O. grandiflora* at all doses showed potent saliuretic activity as compared to normal control. The extract of *O. grandiflora* didn't show carbonic anhydrase inhibition in this study.

Discussion

In the present study, the diuretic effect of three doses of hydroalcoholic extract of *O. grandiflora* was evaluated on Wistar albino rats. The results indicate that *O. grandiflora* at all dose levels (25, 100 and 200 mg/kg) significantly increased urine output in a manner inversely proportional to the dose over a period of 6 h, demonstrating a double effect on renal function, including the increase and inhibition of diuretic activity, differentiating it from diuretics commonly used in natural medicine (20). The co-extracted substances in the crude extract could interfere with the absorption, distribution or binding to the receptor of the

active components (17). Therefore, good diuretic activity was observed among rats treated with furosemide (21) and *O. grandiflora* extract at all doses, except 200 mg/kg dose, wherein moderate activity was observed. The diuretic activities of the extract were found to be highly potent when compared to the normal control group. However, significant differences in urinary excretion followed by diuretic action and diuretic activity were observed. It showed that the extract action was time and dose dependent. This can be explained by kinetic differences of the active principle presence in the extract (22). The instantaneous effect of furosemide due to its high bioavailability (23), as opposed to the administered doses of the extract, is probably related to the gastrointestinal absorption characteristics of the components of the extract or to containing active metabolites that have a long life and may even be long-acting in the action site (24). *O. grandiflora* extract increased the urinary excretion of Na^+, K^+ and Cl^- ions significantly. Therefore, *O. grandiflora* has been shown to possess significant saliuretic effect but not natriuretic effect. This may be due to the fact that most diuretics except potassium-sparing products cause an increase in the excretion of sodium and potassium ions, because to the inhibition of sodium reabsorption in the proximal segment of the nephron by increasing the secretion of K^+ in the tubular lumen at the distal segment in a flow-dependent manner. All doses of the hydroalcoholic extract of *O. grandiflora* resulted in a significant increase in urine volume, Na^+, K^+, and Cl^- ion excretion as compared to the normal control group. This is a characteristic of high ceiling diuretic. Furosemide acts by inhibiting electrolyte re-absorption in the thick ascending loop of Henle (25). The high concentration of electrolytes in urine is related to loop-type diuretics such as furosemide that act by inhibiting the Na^+, K^+ and Cl^- cotransporter located in the thick portion of the loop of Henle, thus inhibiting the reabsorption of electrolytes (26). The natural compounds present in *O. grandiflora* has not been studied, but a high flavonoid and phenolics content which could be responsible for the various properties, due to the high content of flavonoids in the extract, this could be related to diuretic activity because according to the literature these compounds act at the level of the nephron increasing urinary excretion. Furthermore, the diuretic effect of the *O. grandiflora* flowers could be attributed to the presence of phytochemical groups such as flavonoids, terpenoids and saponins since they provide favorable effects on the physiological processes of the kidney, probably increasing the potassium-saving capacity, the union with the A1 adenosine receptor or by inhibiting the tubular reabsorption of water and anions (27). Therefore, there is a need of further research to find out the active principles responsible for the diuretic activities. In the light of the above mentioned study, the hydroalcoholic extract of *O. grandiflora* constitute an effective diuretic due to it result in increased sodium, potassium and chloride ions in urine; which correlate well with the traditional use of the plant as a diuretic. However, lower concentrations of calcium and sodium were observed in the case of the extract, although higher potassium excreted than the positive control, and the time of action is longer when comparing the extract with the reference drug (furosemide). Therefore, it could be deduced that the extract of flowers of *O. grandiflora* could belong to the group of thiazide-type diuretics and within this group to the derivatives of sulphonamides, which do not increase their diuretic effect when increasing the dose, although additional studies are required to clearly determine its mechanism of action (28). The observations showed, *O. grandiflora* had a diuretic spectrum similar to that of tiazidic diuretic type. Finally, the extract of flowers of *O. grandiflora* achieves the elimination of a lower concentration of calcium ions against the standard control (furosemide) even lower than the negative control (carboxymethyl cellulose). By reducing the concentration of calcium ion could have the characteristic of preventing the formation and aggregation of calcium oxalate stones inhibiting the subsequent growth of kidney stones, it is also a scientific basis to classify it as a thiazide-type diuretic since these are characterized by eliminating less amount of calcium in urine, although measurement of other ions such as phosphate, uric acid, urea and creatinine is required to be granted these characteristics (29).

This study confirms the significant diuretic activity of the hydroalcoholic extract of *O.*

grandiflora (flowers) during the measurement period of the study (6 h). However, further studies are recommended for explaining the mechanism of diuretic activity and acute as well as chronic toxicity.

Acknowledgments

The authors thank the Polytechnic School of Chimborazo for providing the facilities to develop this research and recognize the support staff of the Laboratory Animal Center for their collaboration. A special thanks to Dennys Barragán for his contribution in the correct translation of the manuscript into English.

References

1. Tinitana F, Rios M, Romero-Benavides JC, de la Cruz Rot M, Pardo-de-Santayana M. Medicinal plants sold at traditional markets in southern Ecuador. J Ethnobiol Ethnomed [Internet]. 2016;12(29):1–18. Available from: http://ethnobiomed.biomedcentral.com/articles/10.1186/s13002-016-0100-4
2. Maghrani M, Zeggwagh NA, Haloui M, Eddouks M. Acute diuretic effect of aqueous extract of Retama raetam in normal rats. J Ethnopharmacol. 2005;99(1):31–5.
3. Mishra S. Diuretics in primary hypertension – Reloaded. Indian Heart J. 2016;68(5):720–3.
4. Rios M, Koziol MJ, Borgtoft H, Granda G. Plantas útiles del Ecuador aplicaciones, retos y perspectivas. 2007. 652 p.
5. Alejandro-Espinosa M, Jaramillo-Fierro X, Ojeda-Riascos S, Malagón-Aviles O, Ramírez-Robles J. Actividad antioxidante y antihiperglucemiante de la especie medicinal Oreocallis grandiflora (Lam.) R. Br., al sur del Ecuador. Bol Latinoam y del Caribe Plantas Med y Aromat. 2013;12(1):59–68.
6. Gonzales De La Cruz M, Baldeón Malpartida S, Beltrán Santiago H, Jullian V, Bourdy G. Hot and cold: Medicinal plant uses in Quechua speaking communities in the high Andes (Callejón de Huaylas, Ancash, Perú). J Ethnopharmacol [Internet]. 2014;155(2):1093–117. Available from: http://dx.doi.org/10.1016/j.jep.2014.06.042
7. Monigatti M, Bussmann RW, Weckerle CS. Medicinal plant use in two Andean communities located at different altitudes in the Bolívar Province, Peru. J Ethnopharmacol [Internet]. 2013;145(2):450–64. Available from: http://dx.doi.org/10.1016/j.jep.2012.10.066
8. Rios M, Tinitana F, Jarrín-v P, Donoso N, Romero-Benavides JC. "Horchata" drink in Southern Ecuador: Medicinal plants and people's wellbeing. J Ethnobiol Ethnomed [Internet]. 2017;13(18):1–20. Available from: http://ethnobiomed.biomedcentral.com/articles/10.1186/s13002-017-0145-z
9. Christy Jeyaseelan E, Justin Jashothan PT. In vitro control of Staphylococcus aureus (NCTC 6571) and Escherichia coli (ATCC 25922) by Ricinus communis L. Asian Pac J Trop Biomed [Internet]. 2012;2(9):717–21. Available from: http://dx.doi.org/10.1016/S2221-1691(12)60216-0
10. Chothani DL, Patel NM. Preliminary phytochemical screening, pharmacognostic and physicochemical evalution of leaf of Gmelina arborea. Asian Pac J Trop Biomed [Internet]. 2012;2(3 SUPPL.):1333–7. Available from: http://dx.doi.org/10.1016/S2221-1691(12)60411-0
11. Boukhris M, Simmonds MSJ, Sayadi S, Bouaziz M. Chemical composition and biological activities of polar extracts and essential oil of rose-scented geranium, pelargonium graveolens. Phyther Res. 2013;27(8):1206–13.
12. Waterman PG, Mole S. Analysis of Phenolic Plant Metabolites. Vol. 1. London, U.K: Blackwell Scientific Publications; 1994. 84 p.
13. Slimestad R, Vangdal E, Brede C. Analysis of phenolic compounds in six norwegian plum cultivars (Prunus

domestica L.). J Agric Food Chem. 2009;57(23):11370–5.
14. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci Technol [Internet]. 1995;28(1):25–30. Available from: http://www.sciencedirect.com/science/article/pii/S0023643895800085
15. Silva R V., Costa SCC, Branco CRC, Branco A. In vitro photoprotective activity of the Spondias purpurea L. peel crude extract and its incorporation in a pharmaceutical formulation. Ind Crops Prod [Internet]. 2016;83:509–14. Available from: http://dx.doi.org/10.1016/j.indcrop.2015.12.077
16. Lipschitz WL, Hadidian Z, Kerpcsar A. Bioassay of diuretics. J Pharmacol Exp Ther. 1943;79(2):97–110.
17. Nedi T, Mekonnen N, Urga K. Diuretic effect of the crude extracts of Carissa edulis in rats. J Ethnopharmacol. 2004;95(1):57–61.
18. Hailu W, Engidawork E. Evaluation of the diuretic activity of the aqueous and 80% methanol extracts of Ajuga remota Benth (Lamiaceae) leaves in mice. BMC Complement Altern Med [Internet]. 2014;14(135):1–8. Available from: http://bmccomplementalternmed.biomedcentral.com/articles/10.1186/1472-6882-14-135
19. Somova LI, Shode FO, Ramnanan P, Nadar A. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from Olea europaea, subspecies africana leaves. J Ethnopharmacol. 2003;84:299–305.
20. Chen DQ, Feng YL, Tian T, Chen H, Yin L, Zhao YY, et al. Diuretic and anti-diuretic activities of fractions of Alismatis rhizoma. J Ethnopharmacol [Internet]. 2014;157:114–8. Available from: http://dx.doi.org/10.1016/j.jep.2014.09.022
21. Sadki C, Hacht B, Souliman A, Atmani F. Acute diuretic activity of aqueous Erica multiflora flowers and Cynodon dactylon rhizomes extracts in rats. J Ethnopharmacol [Internet]. 2010;128(2):352–6. Available from: http://dx.doi.org/10.1016/j.jep.2010.01.048
22. Nilvises N, Vamnatjinda V, Vanveerakul B, Pidech P. Diuretic Effect of Pluchea indica. Thai J Pharmacol. 1989;11:1–7.
23. Vargo DL, Kramer WG, Black PK, Smith WB, Serpas T, Brater DC. Bioavailability, pharmacokinetics, and pharmacodynamics of torsemide and furosemide in patients with congestive heart failure. Clin Pharmacol Ther. 1995;57(6):601–9.
24. Holford NHG, Sheiner LB. Understanding the Dose-Effect Relationship: 1981;453:429–53.
25. Shinkawa T, Yamasaki F, Notsu T, Nakakuki M, Nishijima K, Yoshitomi K, et al. Loop and distal actions of a novel diuretic, M17055. Eur J Pharmacol. 1993;238(2–3):317–25.
26. Novaes ADS, Da Silva Mota J, Barison A, Veber CL, Negrão FJ, Kassuya CAL, et al. Diuretic and antilithiasic activities of ethanolic extract from Piper amalago (Piperaceae). Phytomedicine [Internet]. 2014;21(4):523–8. Available from: http://dx.doi.org/10.1016/j.phymed.2013.10.014
27. Aziz MM, Saqib NU, Akhtar N, Asif HM, Jamshaid M, Sultana S, et al. Phytochemical screening and evaluation of the diuretic activity of aqueous methanol extract from aerial parts of mentha viridis linn (labiatae) in albino rats. Trop J Pharm Res. 2014;13(7):1121–5.
28. Morales-Olivas FJ. Diferencias y similitudes entre diuréticos. Hipertens y Riesgo Vasc. 2013;30(SUPPL.2):13–9.
29. Ghelani H, Chapala M, Jadav P. Diuretic and antiurolithiatic activities of an ethanolic extract of Acorus calamus L. rhizome in experimental animal

models. J Tradit Complement Med [Internet]. 2016;6(4):431–6. Available from: http://dx.doi.org/10.1016/j.jtcme.2015.12.004

Table 1: Results of phytochemical analysis of *Oreocallis grandiflora* flowers extract. +: present, -: absent.

Phytochemical test	Name of the test	*Oreocallis grandiflora* flowers
Tannins	$FeCl_3$ test, Lead acetate test	+
Steroids	Salkowski test	+
Flavonoids	Ammonia test, Alkaline reagent test	+
Saponins	Frothing test	+
Proteins and aminoacids	Ninhydrin test	-
Alkaloids	Dragendorff's, Hager's, Meyer's and Wagner's test	-
Carbohydrates	Molisch's test	+
Glycosides	Nitroprusside test	+
Cardiac glycosides	Keller Killiani test	-
Terpenoids	Salkowski test (modified)	+

Table 2: Yield (% w/w), Total Flavonoids Content and Total Phenolics Content of *O. grandiflora* flower extract expressed in terms of Quercetin equivalents and Gallic acid equivalents, respectively. Values are expressed as mean±SD, n=3.

Extract	Yield (% w/w)	Total flavonoid content mg of QE/g of extract	Total phenolics content mg of GAE/g of extract
Flowers	1.82%	158.51±1.53	150.0±0.30

Table 3: Percentage inhibition of DPPH free radical by *Oreocallis grandiflora* flower extract and gallic acid at 515 nm. Values are mean±SD, n=3.

Concentration (µg/mL)	Inhibition of DPPH (%)	
	***Oreocallis grandiflora* flower extract**	**Gallic acid**
10	3.53±0.14	19.71±0.11
20	3.72±0.23	27.96±0.15
40	4.94±0.55	36.94±0.28
60	7.13±0.71	70.91±0.54
80	8.32±0.64	77.96±0.77
100	9.62±0.82	89.59±0.61
200	15.38±0.85	90.48±0.73
500	34.99±0.33	91.29±0.15
1000	67. 14±0.59	92.17±0.76
IC_{50}	955.23±0.25	33.29±0.12

Table 4: Urine volume, diuretic action and diuretic activity of *Oreocallis grandiflora* flower extract at 25, 100 and 200 mg/kg, after 6 hours of the treatment administration. Urine volume values are mean±SD, n=5. * No significant difference at P<0.05 with respect to rats treated with furosemide.

Treatment	Urine volume (mL)	Diuretic action	Diuretic activity
CMC 0.5% (10 mL/kg)	2.12±0.303	1	-
Furosemide (10 mg/kg)	4.86±0.888	2.30	1
O. grandiflora flower extract (25 mg/kg)	4.62±0.768*	2.17	0.94
O. grandiflora flower extract (100 mg/kg)	4.14±0,483*	1.95	0.85
O. grandiflora flower extract (200 mg/kg)	3.62±0,449	1.72	0.75

Table 5: Effect of extract of *O. grandiflora* on urinary electrolyte excretion of Wistar albino rats at 6 h of urine sample collection. Values are mean±SD, n=5. * No significant difference at P<0.05 with respect to rats treated with furosemide.

Treatment	Urinary Na^+ (mmol/L)	Urinary K^+ (mmol/L)	Urinary Cl^- (mmol/L)	Urinary Ca^{2+} (mmol/L)
CMC 0.5% (10 mL/kg)	130.60±9.99	50.78±2.77	205.00±14.80	7.17±0.16
Furosemide (10 mg/kg)	183.60±12.34	128.76±18.22	262.67±9.50	9.30±0.19
O. grandiflora flower extract (25 mg/kg)	170.00±10.38*	177.66±9.55	249.00±17.34*	5.36±0.17
O. grandiflora flower extract (100 mg/kg)	153.50±11.32	165.52±8.19	240.33±14.57*	5.32±0.16
O. grandiflora flower extract (200 mg/kg)	148.94±8.00	159.06±6.28	230.67±14.29*	5.31±0.15

Table 6: Effect of extract of *O. grandiflora* on natriuretic effect, saliuretic effect and carbonic anhydrase inhibition (CAI) of Wistar albino rats at 6 h of urine sample collection. Values are mean±SD, n=5. * No significant difference at P<0.05 with respect to rats treated with furosemide.

Treatment	Saliuretic effect ($Na^+ + Cl^-$)	Natriuretic effect (Na^+/K^+)	CAI ($Cl/[Na^+ + K^+]$)
CMC 0.5% (10 mL/kg)	337.67±16.50	2.58±0.26	0.61±0.03*
Furosemide (10 mg/kg)	449.33±6.43	1.45±0.23	0.59±0.04*
O. grandiflora flower extract (25 mg/kg)	425.40±12.04*	0.96±0.07	0.59±0.04*
O. grandiflora flower extract (100 mg/kg)	402.97±16.09	0.93±0.09	0.61±0.04*
O. grandiflora flower extract (200 mg/kg)	385.03±11.44	0.94±0.03	0.61±0.04*