Asian Journal of Pharmaceutical Education and Research
Vol -11, Issue-3, July- September 2022
ISSN:2278 7496

RESEARCH ARTICLE Impact Factor: 7.014

STUDY OF PHYTO BIOACTIVE COMPOUNDS AND DIURETIC ACTIVITY OF FLOWER EXTRACT OF *CHRYSANTHEMUM MORIFOLIUM*

Nagendra Yadav*, Sailesh Kumar Ghatuary, Satkar Prasad, Amit Dubey

RKDF School of Pharmaceutical Sciences, Bhopal (M.P.)

*Corresponding Author's E mail: sitmdirector@rediffmail.com
Received 22 May 2022; Revised 28 May 2022; Accepted 15 June 2022, Available online 15 July 2022.

Cite this article as: Kumar S, Ghatuary SK, Prasad S, Dubey A. Study of Phyto Bioactive Compounds and Diuretic Activity of Flower Extract of Chrysanthemum Morifolium. Asian Journal of Pharmaceutical Education and Research. 2022; 11(3): 157-167.
https://dx.doi.org/10.38164/AJPER/11.3.2022.157-167

ABSTRACT

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is one of the most popular decorative plants and is only outsold by roses in terms of market value by other members of the Asteraceae family. The pharmacological effects of *Chrysanthemum morifolium* are hypothesised to be influenced by the flavonoids, alkaloids, and sesquiterpene lactones. According to a recent study, the flavonoids in the extract of *Chrysanthemum morifolium* shielded mice's brain, liver, and kidney from oxidative damage brought on by lead. Diuretic are very beneficial to treat illnesses including congestive heart failure, nephritis, pregnancy toxaemia, premenstrual tension, and hypertension linked with oedema. to investigate the diuretic effects of *Chrysanthemum morifolium* flower alcohol extract in albino rats. The current study suggests that *Chrysanthemum morifolium* hydroalcoholic extract has a significant potential as an excellent diuretic.

Keywords: *Tri Chrysanthemum morifolium*, Diuretic activity, Extract, Flower

INTRODUCTION

Renal handling of sodium and water nephron sites for diuretic actions.To understands the action of diuretics, it is first necessary to review how the kidney filters fluid and forms urine. The following discussion and accompanying illustration provide a simple overview of how the kidney handles water and electrolytes. For more detailed explanation, particularly related to ion and fluid movement across the renal tubular cells[1].

As blood flows through the kidney, it passes into glomerular capillaries located within the cortex (outer zone of the kidney). These glomerular capillaries are highly permeable to water and electrolytes. Glomerular capillary hydrostatic pressure drives (filters) water and electrolytes into Bowman's space and into the proximal convoluting tubule (PCT). About 20% of the plasma that enters the glomerular capillaries is filtered (termed filtration fraction). The PCT, which lies within the cortex , is the site of

sodium, water and bicarbonate transport from the filtrate (urine), across the tubule wall, and into the interstitium of the cortex. About 65-70% of the filtered sodium is removed from the urine found within the PCT (this is termed sodium reabsorption). This sodium is reabsorbed isosmotically, meaning that every molecule of sodium that is reabsorbed is accompanied by a molecule of water[2].

As the tubule dives into the medulla, or middle zone of the kidney, the tubule becomes narrower and forms a loop (Loop of Henle) that reenters the cortex as the thick ascending limb (TAL) that travels back to near the glomerulus. Because the interstitium of the medulla is very hyperosmotic and the Loop of Henle is permeable to water, water is reabsorbed from the Loop of Henle and into the medullary interstitium. This loss of water concentrates the urine within the Loop of Henle[3].

The TAL, which is impermeable to water, has a cotransport system that reabsorbs sodium, potassium and chloride at a ratio of 1:1:2. Approximately 25% of the sodium load of the original filtrate is reabsorbed at the TAL. From the TAL, the urine flows into the distal convoluting tubule (DCT), which is another site of sodium transport (~5% via a sodium-chloride cotransporter) into the cortical interstitium (the DCT is also impermeable to water). Finally, the tubule dives back into the medulla as the collecting duct and then into the renal pelvis where it joins with other collecting ducts to exit the kidney as the ureter. The distal segment of the DCT and the upper collecting duct has a transporter that reabsorbs sodium (about 1-2% of filtered load) in exchange for potassium and hydrogen ion, which are excreted into the urine. It is important to note two things about this transporter. First, its activity is dependent on the tubular concentration of sodium, so that when sodium is high, more sodium is reabsorbed and more potassium and hydrogen ion are excreted. Second, this transporter is regulated by aldosterone, which is a mineralocorticoid hormone secreted by the adrenal cortex[4].

Increased aldosterone stimulates the reabsorption of sodium, which also increases the loss of potassium and hydrogen ion to the urine. Finally, water is reabsorbed in the collected duct through special pores that are regulated by antidiuretic hormone, which is released by the posterior pituitary. ADH increases the permeability of the collecting duct to water, which leads to increased water reabsorption, a more concentrated urine and reduced urine outflow (antidiuresis). Nearly all of the sodium originally filtered is reabsorbed by the kidney, so that less than 1% of originally filtered sodium remains in the final urine.

The overuse in automedication of phytotherapeutic preparations is the main means to cure about 80% population who is unable to get access to manufactured drugs. High blood pressure represents an important risk factor to development of other cardiovascular diseases and constitutes one of the main causes of mortality in the world.

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) belongs to the Asteraceae family of leading ornamental species, second only to the rose in terms of its market value. The flavonoids, alkaloids, and sesquiterpene lactones are thought to contribute to the pharmacological activities of *Chrysanthemum morifolium*. A recent report indicated that the flavonoids in the extracts of *Chrysanthemum morifolium* protected the brain, liver, and kidney against lead-induced oxidative damage in mice. Moreover, the extracts provided significant protection against cerebral ischemia and reperfusion injury in rats through their antioxidant effect.

In congestive heart failure, nephritis, toxemia of pregnancy, premenstrual tension and hypertension associated with oedema diuretic compounds are much helpful to relieve these conditions. To study the diuretic activity of alcoholic extract of flower of *Chrysanthemum morifolium* in albino rats.

Extraction (By Maceration Method)

Collected plant drugs namely *Chrysanthemum morifolium* Flowers were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drugs were converted into moderately coarse powder in hand grinder. Powdered plant drugs were weighed (300 gm) and packed in (1 liter) air tight glass Bottle. The plant drug was defatted with petroleum ether for about 12 hrs. The defatted plant drugs were subjected to extraction by ethanol and Water (ethanol: water; 70:30) as solvent for about 24 hrs. The liquid extracts were collected in a tarred conical flask. The solvent removed from the extract by evaporation method using hot plate. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated[5].

Preliminary Phytochemical Screening

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the Hydroalcoholic extract of Flowers of *Chrysanthemum morifolium*, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids[6].

6.5 Quantitative Estimation of Phenols and Flavonoids

Estimation of total phenolic content

The total phenolic content of dry extracts was performed with Folin-Ciocaltaeu assay. 1 ml of sample (1 mg/ml) was mixed with 1 ml of Folin Ciocalteu's phenol reagent. After 5 minutes, 10 ml of 7% sodium carbonate solution was added to the mixture followed by the addition of 13ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 minutes at 23^0C, after which the absorbance was read at 760 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of Gallic acid equivalents (GAE)/g of dried sample[7].

Estimation of total flavonoids content

Preparation of standard solution 10mg quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pippeted out and made up to 10ml with Methanol to get 100mcg/ml Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 25, 50, 75, 100, 125 and 150 mcg/ml were prepared. To each of these 4ml water was added followed by 0.3ml of 5% sodium nitrite. After 5min 0.3ml of 10% Aluminium chloride solution and at the 6th minute 2ml of 1M Sodium hydroxide was added. The total volume was made up to 10ml with distilled water. A blank was prepared without addition of aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 510nm using UV-Visible spectrophotometer. A standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance[8].

Pharmacological activity

Animals:

Wistar rats (180-200 g) and Swiss albino mice (males; 20–25 g) were used in the present study. The animals were procured from College of Veterinary Science and Animal Husbandry Mhow, Indore (M.P), India. They were provided normal diet and tap water ad labium and were exposed to 12-h light and 12-h dark cycle. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals

(CPCSEA), Ministry of Environment and Forests, Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee.

Acute toxicity study:

Five groups (n = 5) of male albino mice were used in the acute toxicity study of Flowers of *Chrysanthemum morifolium* Hydroalcoholic extract. Animals from all groups were fasted overnight and administered (p.o) with single dose (250, 500, and 2000 mg /kg) of the extract. A group of animals which received equal volume of PBS served as control. Changes in the behavior of animals were observed for 24 h after extract administration. For any signs of toxicity and mortality, animals were observed for 14 days[9].

Material and Methods

Assessment of diuretic activity

Adult Albino rats of either sex weighting 200 – 220 g were divided into five groups of six animals each. Prior to experimentation, the animals were screened for any visible signs of disease (i.e. sneezing, runny nose, discoloration of skin and eyes, and lazy in movements). Only the healthy animals were selected for the study.

The study was performed at a normal room temperature (25 ± 5^{o}C). Before experimentation, the bladder of the rats was emptied by gentle compression of the pelvic area and by the pull of their tails.

Group I (control group) was administered 10 ml/kg of normal saline. Group II (reference group) was administered 10 mg/kg of furosemide and the test groups (III and IV) were administered different doses of Hydroalcoholic extract of *Chrysanthemum morifolium*. (50 and 100 mg/kg), respectively. All doses were prepared in the same volume of normal saline to administer the same volume in each group. The IP route was used for the administration of Hydroalcoholic extract of *Chrysanthemum morifolium*. Normal saline and furosemide because of its benefits (i.e., ease of administration and freedom to administer large volume of fluids) over other routes[10].

Group I: (control group) receives 10 ml/kg of normal saline

Group II: (reference group) receives 10 mg/kg of furosemide

Group III: Receives 50 mg/kg of HACM

Group IV: Receives 100 mg/kg of HACM

One week prior to the study, the albino rats were individually placed in the metabolic cages for 6 hours to adapt them to the experimental conditions. During the study, the animals were placed in the metabolic cages (i.e. one animal per cage) to separate urine and faeces. The volume of urine collected in graduated vials was measured at the end of 6 h and expressed as ml/100 g of body weight per 6 h.

We kept the animals in an isolated area away from normal flow of students to avoid the stress and other psychological-related effects on diuresis.

Determination of electrolytes level

A calibrated flame photometer was used to estimate concentration of Na^+ and K^+ in the fresh urine samples. Before estimating the electrolyte levels, the samples were filtered to remove debris and shedding. The concentration of electrolytes in the urine was expressed in parts per million (ppm)[11].

Determination of pH of urine

A calibrated pH meter was used to estimate pH of the fresh urine samples.

Computation of diuretic index, Lipchitz value, saliuretic index and Na^+ /K^+ ratio

The following equations were used to compute these parameters.

Diuretic index = (UVt/UVc)

Lipchitz value = (UVt/UVr)

Saliuretic index = (CUEt/CUEc)

Na^+ /K^+ ratio = (UNa^+ /UK^+)

where UVt = mean urine volume of test group, UVc = mean urine volume of control group, UVr = mean urine volume of reference group, CUEt = concentration of electrolytes in urine of test group, CUEc = concentration of electrolytes in urine of control group, UNa+ = concentration of Na^+ in urine of a group, and UK^+ = concentration of K+ in urine of a group[12].

Statistical analysis Graph

Pad Prism software was used for statistical analysis. The data were expressed as mean ± standard error of mean (S.E.M.) at 95 % confidence interval (CI). Student t-test was applied to test difference among the groups. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Chrysanthemum morifolium is extensively studied for a variety of secondary metabolites, and it is reported to contain Phenols, flavonoids, steroids, terpenoids and saponins. The sugar moiety of the saponins is composed of glucose and galactose. Our preliminary phytochemical investigation revealed the presence of Phenols, flavonoids, saponins and tannins in the Hydroalcoholic extracts of *Chrysanthemum morifolium* (Flowers). Various studies have explained that the flavonoids, saponins, Phenols and organic acids are responsible for the diuretic activity of a plant extract.

Such findings advocate that the presence of these secondary metabolites might be responsible for the diuretic activity of *Chrysanthemum morifolium* (Flowers). Diuretics are usually used for the treatment of pathological conditions like nephritic syndrome, hepatic cirrhosis, congestive heart failure and hypertension. It is now evident that the renal dysfunction is a common comorbidity accompanying the uncontrolled hypertension. By controlling high blood pressure, progression of renal disease may be halted. Likewise, the antioxidants are scientifically proved to have renoprotective effects in numerous animal models and may be administered as an adjuvant therapy to hypertensive patients with compromised renal function.

Within this context, plants like *Chrysanthemum morifolium* containing secondary metabolites with diuretic and antioxidant activities are expected to be the ideal candidates for the treatment of hypertension associated with renal disorders. The results of our study showed that *Chrysanthemum morifolium* has notable diuretic effects in the given animal model. The maximum diuretic effects were observed at the dose of 100 mg/kg. Patel *et al* stated that if the diuretic index value is > 1.50, it indicates a good diuretic activity. Whereas the diuretic index values ranging from 1.00–1.50 and 0.72–0.99 demonstrate moderate and mild diuretic activity, respectively. A diuretic index value of < 0.72 indicates no diuretic activity.

In the present study, the diuretic index values of the treated groups (III and IV) were 2.4 and 3.2 respectively. Thus, the extract demonstrated 3 times increase in urine volume. Lipschitz values showed that at maximal dose (100 mg/kg), the plant showed 48 % of diuretic activity as compared with furosemide. This finding advocated that there is a need for further fractionation and isolation of pure secondary metabolite responsible for the diuretic activity of this plant extract.

In primary hypertension, Na^+ is considered to be one of the important external factors. Increased Na^+ uptake has been known to produce adverse effects on arterial blood pressure. Our study showed that, compared with the saline treated group, the IP administration of Hydroalcoholic extract of *Chrysanthemum morifolium* produced significant natriuretic effects especially at the doses of 50 mg/kg

and 100 mg/kg. However, at the dose of 100 mg/kg, the amount of urinary Na^+ was significantly lower than that of the reference standard indicating lower probability of hyponatremia in of Hydroalcoholic extract of *Chrysanthemum morifolium* treated animals, which is indeed a common problem associated with thiazide diuretics.

Similarly, K^+ in the urine samples significantly increased with the increasing dose of of Hydroalcoholic extract of *Chrysanthemum morifolium* . However, it is noteworthy that excretion of K^+ in the treated groups was less than that of the reference group suggesting potassium-sparing properties of Hydroalcoholic extract of *Chrysanthemum morifolium* . Based on these findings, it is hypothesized that the diuretic action of of Hydroalcoholic extract of *Chrysanthemum morifolium* might be the consequence of inhibition of epithelial sodium channels or aldosterone action.

Table 1: Yield of crude extracts

Extracts	Colour	Consistency	Yield(% w/w)
***Chrysanthemum Morifolium* (Flowers)**			
Pet ether	Dark brown	Semisolid	16.25
ethanol: water; 70:30	Brown	Semisolid	18.19

Table 2: Summary of preliminary qualitative phytochemical tests for *Chrysanthemum morifolium* (Flowers) extracts

Phytoconstituents	*Chrysanthemum morifolium* (Flowers)
i)Primary Metabolites	
Carbohydrates	(+)Present
Amino acids	(-)Absent
Proteins	(-)Absent
Fats and oils	(-)Absent

ii)Secondary metabolites	
Steroids	(+)Present
Triterpenoids	(+)Present
Volatile oils	(-)Absent
Gums and mucilage	(-)Absent
Glycosides	(-)Absent
Saponins	(+)Present
Flavonoids	(-)Absent
Tannins & Phenolics	(+)Present
Alkaloids	(-)Absent

HE = Hydroalcoholic extract; '+' = Present; '-' = Absent

Table 3: Total Phenolic Content of Hydroalcoholic extract of *Chrysanthemum morifolium* (Flowers)

Sample	Total phenolic content GAE mcg/ml
Hydroalcoholic extract 100µg/ml	19.25± 0.112

n=3, values are given in SEM

Table 4: Total Flavonoid content of Hydroalcoholic extract of *Chrysanthemum morifolium* (Flowers)

S. No.	Extracts 100µg/ml	Flavonoid content Quercetin equivalent mcg/ml
1	Hydroalcoholic extract (100µg/ml)	18.9251 ± 0.220

n=3, values are given in SEM

Table 5: Effect of *Chrysanthemum morifolium* on urinary volume and electrolyte concentrations

Groups	Extract & dose (mg/kg)	Volume of urine (ml/6 h)	Urine Na+ (ppm)	Urine K+ (ppm)	pH
1	Normal saline 10(ml/kg)	1 2.0±0.6	479.3±1.6	25.3±0.2	7.02
2	Furosemide 10mg/kg	67.6±0.5*	569.3±1.6*	49.8± 0.5*	7.68
3	Extract 50mg/kg	2.4±0.2*	483.7±1.6	34.1±0.5*	6.92
4	Extract 100mg/kg	3.2±0.1*	514.0±2.2*	43.8±0.1*	7.02

Values given are as mean ± S.E.M of six observations. All the values are compared with the control group (normal saline treated); $p < 0.001$

Table 6: Effect of *Chrysanthemum morifolium* on electrolyte concentrations

Groups	Extract & dose (mg/kg)	Diuretic index	Lipschtiz value	Saliuretic index Na^+	Saliuretic index K^+	Na^+/K^+
1	Normal saline 10(ml/kg)	----	----	-----		18.38
2	Furosemide 10mg/kg	6.7	----	1.19	1.83	11.84
3	Extract 50mg/kg	2.4	0.36	1.00	1.24	15.02
4	Extract 100mg/kg	3.2	0.48	1.08	1.58	12.55

Values given are as mean ± S.E.M of six observations. All the values are compared with the control group (normal saline treated); $p < 0.001$

CONCLUSION

The present study indicates that of Hydroalcoholic extract of *Chrysanthemum morifolium* (Flowers) has a strong potential as an ideal diuretic. However, further studies are required to separate the antireproductive and diuretic phytochemical constituents to confirm its safe use in renal disorders.

REFERENCES

1. Ratnasooriya WD, Pieris KP, Samaratunga U, Jayakody JR. Diuretic activity of Spilanthes acmella flowers in rats. Journal of ethnopharmacology. 2004 Apr 1;91(2-3):317-20.
2. Brater, D.C., 2000. Pharmacology of diuretics. American Journal of the Medical Sciences 319, 38–50.
3. Lahlou, S., Tahraoui, A., Israili, Z., Lyoussi, B., 2006. Diuretic activity of the aqueous extracts of Carum carvi and Tanacetum vulgare in normal rats. Journal of Ethnopharmacology 110 (3), 458–463.

4. Materson, B.J., 1983. Insights into intrarenal sites and mechanisms of action of diuretic agents. Americal Heart Journal 106, 188–208.
5. Williams, B., Poulter, N.R., Brown, M.J., Davis, M., McInnes, G.T., Potter, J.F., Sever, P.S., Thom, S.M., 2004. British Hypertension Society guidelines for hypertension management 2004 (BHS-IV): summary. British Medical Journal 328, 634–640.
6. Schutz, K., Carle, R., Schieber, A., 2006. Taraxacum— a review on its phytochemical and pharmacological profile. Journal of Ethnopharmacology 107, 313– 323.
7. Hook, I., McGee, A., Henman, M., 1993. Evaluation of dandelion for diuretic activity and variation in potassium content. International Journal of Pharmacognosy 31, 29–34.
8. Racz-Kotilla, E., Racz, G., Solomon, A., 1974. The action of Taraxacum officinale extracts on the body weight and diuresis of laboratory animals. Planta Medica 26, 212–217.
9. Maghrani, M., Zeggwagh, N.A., Michel, J.B., Eddouks, M., 2005b. Antihypertensive effect of Lepidium sativum L. in spontaneously hypertensive rats. Journal of Ethnopharmacology 100, 193–197.
10. Li M, Zhao Y, Zhang S, Xu Y, Wang SY, Li BW, Ran JH, Li RT, Yang BX. A thienopyridine, CB-20, exerts diuretic activity by inhibiting urea transporters. Acta Pharmacologica Sinica. 2020 Jan;41(1):65-72.
11. El Ghouizi A, El Menyiy N, Falcão SI, Vilas-Boas M, Lyoussi B. Chemical composition, antioxidant activity, and diuretic effect of Moroccan fresh bee pollen in rats. Veterinary World. 2020 Jul;13(7):1251.
12. Welu GG, Yimer EM, Hailu HG, Bhoumik D, Lema MM. In Vivo Diuretic Activity of Hydromethanolic Extract and Solvent Fractions of the Root Bark of Clerodendrum myricoides Hochst.(L
13. amiaceae). Evidence-Based Complementary and Alternative Medicine. 2020 Dec 10;2020.