Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

EFFECT OF SAUDI ARABIAN *Capparis Cartilaginea* FRUIT EXTRACTS ON SERUM PARATHYROID HORMONE AND 1α,25-DIHYDROXYVITAMIN D_3 LEVELS IN RATS

Noor Daifallah Al-Goufi[1], Dr. Hala Salim Sonbol[2]*

[1]76 the Quadrants, Ballincolling, Cork, Ireland
[2]Department of Biochemistry, Faculty of Science, King AbdulAziz University, P.O. Box No. 122522, Jeddah, 21332, Saudi Arabia

Received – December 07, 2017; Revision – January 26, 2018; Accepted – February 13, 2018
Available Online – February 20, 2018

DOI: http://dx.doi.org/10.18006/2018.6(1).230.235

KEYWORDS

Capparis cartilaginea

Parathyroid hormone

1α,25-Dihydroxyvitamin D_3

Medicinal plants

Flavonoids

ABSTRACT

Capparis cartilaginea is a shrub plant which used in traditional medicine to cure various diseases. Phytochemical analysis of *Capparis cartilaginea* plants extracts revealed the presence of isothiocyanates and flavonoids. A dose dependent study was designed to assess effects of *Capparis cartilaginea* fruit extract on serum parathyroid hormones (PTH) and 1α,25-Dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$) levels in adult male and female Wistar rats, which could describe a regulation effect on bone metabolism. Healthy adult Wistar rats (20 female and 20 male) were treated with 1000, 2000 and 3000 mg/kg body weight (BW) of fruit extract via gavage for 6 weeks. The rats were housed under standard laboratory conditions (22±1ºC and 60% humidity) for 2 weeks prior to the experiment. Blood samples were collected to determine the level of PTH and 1α,25$(OH)_2D_3$ in serum. Results of study revealed that serum PTH levels were increased in male and female rats treated with 1000, 2000 and 3000 mg/kg body *Capparis cartilaginea* fruit extract. The analysis of the hormones suggested a dose-dependent response in male serum PTH levels and it was significantly higher than the female PTH levels. On the other hand, all the three doses of *Capparis cartilaginea* fruit extract decreased 1α,25$(OH)_2D_3$ levels in both genders. The results of study indicated that *Capparis cartilaginea* fruit extracts have a potential to change the PTH and 1α,25$(OH)_2D_3$ hormonal levels.

* Corresponding author
E-mail: hsunbol@kau.edu.sa (Hala S Sonbol)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Traditional medicine covers a wide variety of therapies and practices, which vary from country to country and region to region (World Health Organization, 2013). The flora of Saudi Arabia is considered as the richest biodiversity area in the Arabian Peninsula that comprise important genetic resources of crops, medicinal plants and xerophytic vegetation which make up the prominent features of plant life in the kingdom (Zahran, 1982; Al-Yahya, 1984). According to Al-Yahya (1984), the Arabian Peninsula is the birthplace of herbal drugs and folk medicine. In addition to its large number of endemic species, the components of the flora are the admixture of the elements of Asia, Africa and Mediterranean region. Saudi Arabia is gifted with a wide range of flora, consisting of a large number of medicinal herbs, shrubs and trees. Saudi Arabian flora is expected to have more than 1200 medicinal species out of 2250 species in the flora (Mossa et al., 1987). Three hundred species have medicinal use (Rahman et al., 2004). Studies stated that about 24% of plants are medicinal in 15 families of which 30.1% are rare or threatened (Rahman et al., 2004; Yusuf et al., 2014). The total recorded vascular genera for the flora of Saudi Arabia stands at 855 and the number of species at 2,290, rising by ~ 2 % species throughout the past decade (Basahi et al., 2015).

Medicinal plants represent important health and economic components of biodiversity. In each country, it is essential to conduct an inventory of medicinal components of the flora, for conservation and sustainable use (Seighali & Zaker, 2010). The uses of plants in Saudi Arabia for the cure of many illnesses are ancient and still available among the tribal and local people and traditional healers (Hakim) (Rahman et al., 2004).

Among natural health products, *Capparis cartilaginea* (family Capparidaceae), has been found in the Saudi Arabian flora (Rahman et al., 2004). It has been used as important medicinal plants against various human diseases such as rheumatism, gout, paralysis, treating enlarged spleen and tuberculosis (Said, 1969; Nadkarni 1976; Al-Shayeb, 2012). It has also been reported that the crude extract of whole plant of *Capparis cartilaginea* produces a dose-dependent decrease in blood pressure and slight bradycardic rhythm in anaesthetised rats (Gilani & Aftab, 1994).

The presence of various phytochemicals has been reported from the crude extract of *Capparis cartilaginea*, among these isothiocyanates is most commonly reported one (Hamed et al., 2007; Al-Shayeb, 2012).Therapeutic use of isothiocyanates is also well reported and it can be used to treat arthritis and reduce the inflammatory status of synovium without disrupting the cellular homeostasis (Balar & Nakum, 2010). Rutin and quercetin are flavonoids that have been found in *Capparis cartilaginea* extract (Ahmed et al.,1972; Sharaf et al., 1997).

Beneficial health effects of these flavonoids are well reported and are known to have anticarcinogenic (Webster et al., 1996), anti-inflammatory, analgesic (Pietta & Gardana, 2003) and anti-mutagenic properties (Brindzova et al., 2009), in addition to this, it has partial protective effect against the development of diabetes (Srinivasan et al., 2005). These chemicals were also used to treat skin inflammation, bruises, swellings, rheumatism, joint inflammation, knee problems, tendinitis, sprains, muscular contractions, paralysis, headaches, and earaches (Rivera et al., 2003).

The role of phyto-flavonoids in regulation of various hormones such as estrogens, androgens and thyroid is well reported (Narayana et al., 2001). This regulation in humans is performed by binding to 17 beta-hydroxysteroid dehydrogenases, which in turn controls estrogen and androgen levels. Furthermore, it binds to beta-hydroxysteroid dehydrogenase, as a step towards regulating progestin and androgen levels (Noro et al.,1983). Furthermore, Quercetin and rutin influence the transport, metabolism and function of thyroid hormones (Tripathi & Rastogi, 1981).The current study has been undertaken to evaluate the impacts of different doses of ethanolic fruit extract of *Capparis cartilaginea* on the levels of PTH and 1α,25(OH)2D3 in adult Wistar male and female rats. PTH and the active form of vitamin D; 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], (also called calcitriol) control the mineral fluxes through the intestine, bone, kidney and blood (Favus et al., 2006). PTH promotes the reabsorption of Ca^{2+} from the bone into the circulation. In the kidney, it stimulates Ca^{2+} reabsorption and inorganic phosphate excretion in the urine. PTH induces the hydroxylation of 25-hydroxyvitamin D at the 1-position, forming the active form of vitamin D (calcitriol). In intestine, absorption of dietary Ca^{2+} increases by increasing the level of Vitamin D. It also enables the renal reabsorption of filtered Ca^{2+}. In the bones, vitamin D releases Ca^{2+} into the circulation by increasing bone reabsorption. Consequently, bone reabsorption is inhibited and the action of calcitonin, which amplifies the renal Ca^{2+} excretion. The communications between PTH, vitamin D and calcitonin leads to the maintenance of normal concentration of Ca^{2+} in blood plasma (Molina, 2013). Present study has been carried out to access the effect of *Capparis cartilaginea* fruit extract on the levels of these two hormones.

2 Materials and Methods

2.1 Animals

In the present study, 20 males and 20 females (3-month-old; ~300g) adult Wister rats were obtained from the Animal House Unit at King Fahad Medical Research Centre (KFMRC), Jeddah, Saudi Arabia. The rats were stratified into four groups of each gender based on the dose of *Capparis cartilaginea* fruit extract

(control, 1000, 2000 and 3000 mg/kg BW). The rats were accommodated under standard laboratory conditions (22±1ºC and 60% humidity) for 2 weeks prior to the experiment. They were under 12 h dark-light cycle (lights on at 0700 h), given a slandered pelleted diet (Grain Soils and Flour Mills Organization Jeddah, Saudi Arabia), with free access to water. Animals received care according to institutional guidelines for the care and the use of laboratory animals in KFMRC. The Research Ethics Committee, Unit of Biomedical Ethics, KAU, and Jeddah, Saudi Arabia have approved the experimental protocol.

2.2 Plant Material

The fresh fruits of *C. cartelaginea* were collected from Umluj Mountains in Tabuk province, Northwest Saudi Arabia. All the collected fruit were freeze-driedat -64°C under 5m Torr pressure and grounded by Waring blender (USA). The freeze-dried fruit (50g) was used for extraction purpose, and extraction was carried out by 70% ethanol for 6-8 hours at 70°C using Soxhlet apparatus (Sigma, USA). After extraction, the mixture was evaporated by a rotary evaporator (Hahnapor, USA) at 60°C, concentrated under reduced pressure (100 torr), and dried by the freeze dryer. The dried extract was stored at -20°C until it is used.

2.3 Acute Oral Toxicity Test

Acute oral toxicity test of *C. cartelaginea* fruit extract was performed (Organisation for Economic Co-operation and Development - OECD – 420, 2008), to select a proper dose for oral gavage. Groups of animals were dosed using the fixed doses of 5, 50, 300, 2000 and 5000 mg/kg body weight (BW).

2.4 Experimental Design

After 2 weeks of acclimatisation, the rats were divided into four equal groups (n=5). Group one (control) was administrated with 2 ml of distilled water via oral gavage once daily. Group two was administrated with 1000mg/kg BW of *C. cartelaginea* fruit extract. Group three was administrated with 2000mg/kg BW of *C. cartelaginea* fruit extract. Group four was administrated with 3000mg/kg BW of *C. cartelaginea* fruit extract. The extract was administrated daily to the rats using oral gavage from Sunday to Thursday for 6 weeks.

After the treatment period, blood was collected via the intraorbital sinus (Parasuraman et al., 2010) of the rats, using a capillary tube (75mm, Hirschmanlaborgerate, Germany) under ether anaesthesia. The blood was withdrawn into a plain tube for serum preparation. A collected blood sample was centrifuged at 3000 rpm for 15 min. The serum was then stored in adeep freezer at -80°C until further use.

2.5 Measurement of $1\alpha,25(OH)_2D_3$

The $1\alpha,25(OH)_2D_3$ was measured from the rats' serum by competitive inhibition enzyme immunoassay technique using the commercial $1\alpha,25(OH)_2D_3$ ELISA kit (CUSABIO Biotech CO. Ltd, China). The analysis was carried out according to the manufacturer's instructions (https://www.cusabio.com/ELISA-Kit/Rat-25-hydroxy-vitamin-D325-HVD3ELISA-Kit-62299.html).

2.6 Measurement of PTH

The levels of PTH was measured from the rats' serum, employing the quantitative sandwich enzyme immunoassay technique by using commercially available PTH ELISA kit (CUSABIO Biotech CO. Ltd, China). The analysis was carried out according to the manufacturer's instructions (https://www.cusabio.com/ELISA-Kit/Rat-Parathyroid-hormonePTH-ELISA-Kit-98934.html).

2.7 Statistical Analysis

The data were analysed using the Statistical Package for the Social Sciences program version 21 (SPSS 21). Weight difference and biochemical parameters were analysed using one-way ANOVA. Post hoc testing was performed for inter-group comparisons. Results were expressed as themean ± standard deviation (SD), and the level of significance was set at $P<0.05$.

3 Results

The doses used in the acute oral toxicity test performed on the rats did not show any visible sign of toxicity.

3.1 Biochemical Parameters

The effects of *Capparis cartilaginea* fruit extract on the hormones levels in the serum of the male and female rats are summarised in table 1. Briefly, there was a dose dependent increase in serum PTH levels in male groups treated with *Capparis cartilaginea* fruit extract when compared to control group. *Capparis cartilaginea* fruit extract at a dose of 1000, 2000 and 3000 mg/kg raised the PTH levels by 14.5±5.4 pg/ml, 19±7 and 24.5±7.8 pg/ml respectively. However, the differences between various treatment are not statistically significant (p>0.05).

The serum PTH levels increased in female groups treated with *Capparis cartilaginea* fruit extract as compared to the control group. A dose of 1000, 2000 and 3000 mg/kg of *Capparis cartilaginea* fruit extract raised the PTH levels to 12±4, 10±5 and 17.7±9.5 pg/ml respectively. This is also not showing statistically significant differences (p>0.05).

Table 1 Effect of oral administration of *Capparis cartilaginea* fruit extract on serum PTH and 1α,25$(OH)_2D_3$ in Wistar rats

Groups	Female				Male			
	PTH (pg/ml)	*P-value*	1α,25$(OH)_2D_3$ (pg/ml)	*P-value*	PTH (pg/ml)	*P-value*	1α,25$(OH)_2D_3$ (pg/ml)	*P-value*
Group one (Control)	8±2.4	Ref	34.4±3.6	Ref	11.6±2.37	Ref	23.6±4.5	Ref
Group two (1000 mg/kg)	12.0±4.1	0.67	33.5±3.60	0.98	14.5±5.4	0.99	23.4±1.8	0.99
Group three (2000 mg/kg)	10.30±5.1	0.92	28.3±4.3	0.1	19.0±7.0	0.23	22.3±1.9	0.89
Group four (3000 mg/kg)	17.70±9.5	0.09	27.9±2.80	0.07	24.5±7.8	0.63	18.7±2.1	0.06

Data are expressed as mean±SD, SD: standard deviation, PTH: parathyroid hormone, 1α,25$(OH)_2D_3$: 1 α,25-Dihydroxyvitamin D_3

A dose of 1000 mg/kg *Capparis cartilaginea* fruit extract did not change the mean serum 1α,25$(OH)_2D_3$ levels (23.4 vs 23.6 pg/ml respectively) of group two male rats when compared to control, as shown in table 1. However, as the dose of *Capparis cartilaginea* fruit extract increase in male group three and four (2000 and 3000 respectively), the mean serum 1α,25$(OH)_2D_3$ levels decrease compared to control group (22.3, 18.7 vs 23.6 pg/ml respectively).

The reduction in the mean was more apparent in the female groups treated with *Capparis cartilaginea* fruit extract as compared to control group. A dose of 1000, 2000 and 3000 mg/kg *Capparis cartilaginea* fruit extract reduced the mean serum 1α,25$(OH)_2D_3$ levels to 33.5±3.6, 28.3±4.3 and 27.9±2.8 pg/ml respectively. The serum1α,25$(OH)_2D_3$ levels in both male and female groups did not reach statistical significances ($p>0.05$).

4 Discussion

Saudi Arabian *Capparis cartilaginea* has been well known for its medicinal properties such as in rheumatism and diabetes *(*AbdulLatif et al., 2014). Flavonoids are major constituents of *Capparis cartilaginea* and are known to participate in the regulation of a variety of hormones (Narayana et al., 2001). PTH and 1α, 25$(OH)_2D_3$ are two important communicators in bone regulation (van Driel et al., 2017; Walsh, 2018). This study used animal model and evaluated the effect of *Capparis cartilaginea* fruit extract on the serum levels of PTH and 1α, 25$(OH)_2D_3$ in female and male rats. By analysing the levels of these two hormones, this study aimed to find out beneficial effects of *Capparis cartilaginea* fruit extract on bone regulation.

The result of the study revealed a dose dependent reduction in 1α,25$(OH)_2D_3$ concentration and increase in serum PTH in both male and female rats.The inverse relationship between 25$(OH)_2D_3$ and PTH is interesting, because PTH is a strong bone-resorbing mediator, and a small increase in serum PTH is associated with an elevation in bone turnover and enhanced bone loss. Accordingly, this can be stopped by the supplementation of vitamin D (Dawson-Hughes et al., 1997; Chapuy et al., 1997).

The reduction in 1α, 25$(OH)_2D_3$ levels in the present study in female and male rats were minor compared to the control, yet still dose dependent. A study conducted by Nagano et al., (2001) on male Sprague-Dawley rats, stated a higher control concentration of 1,25$(OH)_2$D3 (54.7±3.28 pg/ml) than the present study (23.6±4.5pg/ml).

Further, Schultz et al. (1994) determined the bioactive rats' parathyroid hormone concentrations *in vivo* and *in vitro*, by a 2-site homologous immunoradiometric assay. They indicated that the level of circulating PTH in the female adult rats, measured with homologous two-site immunoradiometric assay method, was 10-15pg/ml. Also, Elkomy & Elsaid, (2015) showed that the control PTH value was 16.86pg/ml in adult female albino rats. The two previous studies presented a higher control PTH value than the present study (female) (7.97pg/ml). However, control male PTH (11.57pg/ml) was within the same range.

As per the findings of the present study, 1000 and 2000 mg/kg of *Capparis cartilaginea* fruit extract showed no direct effect on serum PTH value in female rats. However, a higher dose *Capparis cartilaginea* (3000mg/kg) present insignificant elevation in serum PTH. In male rats, 1000, 2000 and 3000mg/kg of *Capparis cartilaginea* fruit extract showed a dose dependent increase. However, the rise was insignificant.

Capparis cartilaginea fruit extract contains flavonoids such as rutin, quercetin and isothiocyanates (Hamed et al., 2007). Flavonoids are natural compounds that are produced by plants, they are used therapeutically and can be consumed as food. De Souza Dos Santos et al. (2011) have shown that flavonoids can reduce the thyroid hormones levels, and consequently increase TSH and causegoiter. They proposed that flavonoids have been shown to have a role in thyroid hormones synthesis and availability in *vivo* and *in vitro* models (de Souza dos Santos at al., 2011). Plants flavonoids perform many biological activities such as regulation of gene expression by interacting with protein transcription factors (Kuo, 2002). Other studies have shown that

the apigenin, quercetin and fisetin (flavonoids) might have an inhibitory effect on the expression of vitamin D receptor in human keratinocytes (Segaert et al., 2000).The effect that was detected at the protein and mRNA levels led to complete destruction of the vitamin D sensitivity in these cells (Kuo, 2002).

It can be concluded that flavonoids in *Capparis cartilaginea* fruit extract may influence the hormonal levels in male and female Wister rats. However, further conformational studies are needed to better define these effects. It is possible that the influence on the serum PTH and $1\alpha,25(OH)_2D_3$ levels may depend on the length of the exposure, the plant dose, the way of treatment administration (diet, injection, *in vitro* and *in vivo* treatment) and the number of rats. Also, more studies by using different parts of *Capparis cartilaginea* should be designed to compare the results achieved from different parts of the plants.

Acknowledgement

The authors would like to sincerely thank Dr. Suad A Alabsi for her kind assistance and contribution to this research. She suggested looking into this plant as it has great therapeutic properties. The study was funded by King Abdulaziz City for Science and Technology (KACST) for the generous financial support they provided for this research project under grant number (AO -36-175).

Conflict of interest

The authors have no conflicts of interest relevant to this article to disclose.

Disclosure Summary

The authors have nothing to disclose. This manuscript describes original work and is not under consideration by any other journal.

References

AbdulLatif, Amer HM, Hamad, ME, Alarifi SAR, Almajhdi FN (2014). Medicinal plants from Saudi Arabia and Indonesia: In vitro cytotoxicity evaluation on Vero and HEp-2 cells. Journal of Medicinal Plants Research 8: 1065-1073.

Ahmed Z, Rizk A, Hammouda F, Seif El-Nasr M (1972) Glucosinolates of Egyptian Capparis species. Phytochemistry11: 251–256.

Al-Shayeb A (2012) Chemical Composition of Essential Oil and Crude Extract Fractions and Their Antibacterial Activities of *Capparis spinosa* L. and *Capparis cartilaginea* Decne. Master thesis submitted to the University of Yarmouk, Irbid, Jordan.

Al-Yahya M (1984) Kuwait: Proceeding of the III International Conference on Islamic Medicine Pp. 349.

Balar C, Nakum A (2010) Herbal ingredients for the treatment of arthritis, Indian Patent Coden: INXXBQ.

Basahi JTR, Al-Ansari AE, Sivadasan M, El-Sheikh MA, Alfarhan AH, Al-Atar AA (2015) Additions to the Flora of Saudi Arabia: Two New Generic Records from the Southern Tihama of Saudi Arabia. National Academy Science Letters 38:513–516

Brindzova L, Mikulasova M, Takacsova M, Mosovska S, Pattova A (2009) Evaluation of the mutagenicity, antimutagenicity of extracts from the oat, buckwheat and wheat bran in the salmonella/microsome assay. Journal of Food Composition and Analysis 22: 87-90.

Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S, Meunier PJ (1997) Prevalence of vitamin D insufficiency in an adult normal population. Osteoporosis International 7:439-443.

Dawson-Hughes B, Harris SS, Dallal GE (1997) Plasma calcidiol, season, and serum parathyroid hormone concentrations in healthy elderly men and women. American Journal of Clinical Nutrition 65:67-71.

de Souza dos Santos MC, Gonçalves CFL, Vaisman M, Ferreira ACF, de Carvalho DP (2011) Impact of flavonoids on thyroid function. Food and Chemical Toxicology 49: 2495-2502

Elkomy M, Elsaid FG (2015) Anti-osteoporotic effect of medical herbs and calcium supplementation on ovariectomized rats Magda. The Journal of Basic & Applied Zoology 72: 81–88

Favus JM, Bushinsky DA, Lemann JRJ (2006) Regulation of Calcium, Magnesium, and Phosphate Metabolism. Chapter 13. Section of Endocrinology, University of Chicago, Chicago, Illinois; Nephrology Unit, University of Rochester School of Medicine, Rochester, New York; and 3 Nephrology Section, Tulane University School of Medicine, New Orleans, Louisiana. American Society for Bone and Mineral Research.

Gilani A, Aftab K (1994) Hypotensive and Spamolytic activities of Ethanolic extract of Capparis cartilaginea. Phytotherapy 8: 145- 148.

Hamed A, Abdel-Shafeek K, Abdel-Azim N, Ismail S, Hammouda F (2007) Chemical investigation of some Capparis species growing in Egypt and their antioxidant activity. Evidence-Based Complementary and Alternative Medicine 4: 25–28.

Kuo SM (2002) Flavonoids and gene expression in mammalian cells. Advances in Experimental Medicine and Biology 505:191–200.

Molina PE (2013) Chapter 5. Parathyroid Gland and Ca^{2+} and PO_4 Regulation. Endocrine Physiology, Fourth Edition, McGraw-Hill Companies, USA.

Mossa J, Al-Yahya M , Al-Meshal I (1987) Medicinal plants of Saudi Arabia. Riyadh: King Saud University Press.

Nadkarni KM (1976) Indian materiamedica with ayurvedic, unani-tibbi, siddha, allopathic, homeopathic, naturopathic and home remedies, appendices & indexes. Bombay, Popular Prakashan 810-816

Nagano N, Miyata S, Obana S, Ozai M, Kobayashi N, Fukushima N, Burke SK, Wada M (2001) Sevelamer hydrochloride (Renagel), a non-calcaemic phosphate binder, arrests parathyroid gland hyperplasia in rats with progressive chronic renal insufficiency. Nephrology Dialysis Transplantation 16:1870-1878.

Narayana KR, Reddy MS, Chaluvadi MR, Krishna DR (2001) Bioflavonoids Classification, Pharmacological, Biochemical Effects and Therapeutic Potential. Indian Journal of Pharmacology 33: 2-16.

Noro T, Oda Y, Miyasa UA, Fukushim S (1983) Inhibition of adinosine deaminase activity of aortic endothelial cells by selected flavonoids. Chemical and Pharmaceutical Bulletin 31:3984-91.

Parasuraman S, Raveendran R, Kesavan R (2010) Blood sample collection in small laboratory animals. Journal of Pharmacology and Pharmacotherapeutics 1: 87.

Pietta P, Gardana C (2003) Flavonoids in Herbs. In: Flavonoids in Health and Disease, New York, USA: Marcel Dekker Inc.

Rahman M, Mossa J, Al-Said M, Al-Yahya M (2004) Medicinal plant diversity in the flora of Saudi Arabia 1: A report on seven plant families. Fitoterapia 75: 149–161.

Rivera D, Inocencio C, Obón C, Alcaraz F (2003) Review of food and medicinal uses of Capparis L. subgenus Capparis (Capparidaceae). Economic Botany 57: 515–34.

Said HM (1969) Hamdard Pharmacopoea of Eastern Medicine, Karachi: Hamdard Foundation Press.

Schultz VL, Garner SC, Lavigne JR, Toverud SU (1994) Determination of bioactive rat parathyroid hormone (PTH) concentrations in vivo and in vitro by a 2-site homologous immunoradiometric assay. Bone and Mineral 27: 2, 121-132.

Segaert S, Courtois S, Garmyn M, Degree H, Bouillon R (2000) The flavonoid apigenin suppresses vitamin D receptor expression and vitamin D responsiveness in normal human keratinocytes. Biochemical Biophysical Research Communication 268:237-241.

Seighali N, Zaker S (2010) Contribution to the vascular and flora as well as habitat diversity of the Langarud and its environs (Guilan: Iran). Advances in Bioresearch 1: 110-122.

Sharaf M, El-Ansari M, Saleh N (1997) Flavonoids of four Cleome and three *Capparis* species. Biochemical Systematics and Ecology 25: 161-166.

Srinivasan K, Kaul CL, Ramarao P (2005) Partial protective effect of rutin on multiple low dose streptozotocin-induced diabetes in mice. Indian Journal of Pharmacology 37: 327.

Tripathi VD, Rastogi RP (1981) In vitro anti-HIV activity of flavonoids isolated from *Garcinia multifolia*. Journal of Scientific and Industrial Research 40:116-21.

van Driel M, Johannes PTM, van Leeuwen (2017) Vitamin D endocrinology of bone mineralization. Molecular and Cellular Endocrinology 453: 46-5.

Walsh JS (2018) Normal bone physiology, remodelling and its hormonal regulation. Surgery (Oxford) 36: 1-6.

Webster R, Gawde M, Bhattacharya R (1996) Protective effect of rutin, a flavonol glycoside, on the carcinogen-induced DNA damage and repair enzymes in rats. Cancer Letters 109: 185-191.

World Health Organization (2013) traditional medicine strategy. WHO, Geneva, Switzerland 2014-2023.

Yusuf M, Al-Oqail MM, Al-Sheddi ES, Al-Rehaily AJ, Rahman MA (2014). Diversity of medicinal plants in the flora of Saudi Arabia 3: an inventory of 15 plant families and their conservation management. International journal of environment 3: 2091-2854.

Zahran M (1982) Vegetation Types of Saudi Arabia, Jeddah, Saudi Arabia: King Abdel Aziz University Press.