OPEN ACCESS

Phytopharmacological Communications

DOI: doi.org/10.55627/ppc.003.001.0298

Research Article

Pharmacological Investigation of Aqueous Methanolic Extract of *Ficus carica* against Thyroxine-induced Hyperthyroidism

Qaiser Jabeen*, Muhammad Umair, Mariya Anwaar, Maria Qadeer

Department of Pharmacology, Faculty of Pharmacy, the Islamia University of Bahawalpur, Pakistan
*Correspondence: qaiser.jabeen@iub.edu.pk

Abstract

Plants have been considered the best alternatives to chemical entities. Therefore, *Ficus carica* L. (Fig) was selected to investigate its protective role against experimentally-induced hyperthyroidism. A 70% aqueous methanolic extract of *F. carica* (Fc.Cr) was prepared, and a phytochemical analysis was performed. A thyroxine-induced hyperthyroidism model was used for the pharmacological evaluation of Fc.Cr. Rats were divided into different groups; the normal control group was given distilled water (5ml/kg; p.o.), and other groups were administered thyroxine (600µg/kg; p.o.) for 14 days. After induction of hyperthyroidism, the intoxicated group was administered distilled water (5ml/kg; p.o.), whereas treatment groups were administered Fc.Cr (30, 100, and 300 mg/kg; p.o.) and carbimazole, the standard drug (20mg/kg; p.o.) individually for the next 21 days. After induction and treatment, blood was drawn through the retro-orbital puncture, sera were separated, and the levels of thyroid hormones were determined using an enzyme-linked immunosorbent assay. Phytochemical analysis showed the presence of secondary metabolites, i.e., alkaloids, flavonoids, glycosides, phenols, saponins, terpenes etc. The results showed dose-dependent effects of Fc.Cr, as evident from the increase in serum thyroid stimulating hormone (TSH) and decrease in T3 and T4 levels, indicating its potential for managing hyperthyroidism. Acute toxicity assay of Fc.Cr was also performed and found to be safe up to 10 g/kg. Hence, the study's results rationalize the traditional use of *Ficus carica* to manage hyperthyroidism.

Keywords: Hyperthyroidism, T3, T4, TSH, *Ficus carica*, Thyroxine

1. Introduction

Cardiovascular disorders The thyroid gland is the largest gland in the body, weighing 15 to 20g in adults. It releases two main hormones, i.e., thyroxine (T4) and triiodothyronine (T3). These hormones control the body's metabolic processes, growth, and development and produce energy (Bhaigyabati, Ramya, and Usha 2012). Thyroid disorders arise due to disturbances in the production of thyroid hormones, i.e., hyperthyroidism and hypothyroidism. Hyperthyroidism, also known as thyrotoxicosis, is presented with excessive production of thyroid hormones. This can increase metabolic activities leading to sweating, elevated heart rate, tremors, nervousness, increased appetite, and weight loss (Truter 2011). Grave's disease, a common reason for hyperthyroidism, is an autoimmune disorder (Chiari et al. 2023). According to World Health Organization (WHO), plants are the cheapest source of medicine for about 75% of the world population, and herbal medicines are more effective and safe (Gilani and Rahman 2005).

Ficus carica, an edible and medicinal plant, belongs to Angiosperms and consists of the largest genera from the mulberry family, *Moraceae*, which contains more than 800 species of shrubs, hemi-epiphytes, trees, climbers, and creepers in subtropical and tropical areas all over the world (Frodin 2004). Fig fruit (locally known as Anjeer)

is pear-shaped, 2.5-4cm long, with different colors, yellowish-green to coppery or dark purple. Small scales partly enclose the fruit. The fruit's phytochemical analysis studies have reported alkaloids, saponins, phenolic contents, flavonoids, and other secondary metabolites, indicating its high antioxidant potential. Volatile components present in fig fruit have been studied with the help of gas chromatography-mass spectroscopy, i.e., β-amyrin, oleic acid, vitamin E, stigmasterol, campesterol, and isoamyl laurate. The phytochemical analysis and high antioxidant value of figs affirm their potential to be a rich source of nutrients and their use for therapeutic benefits (Soni et al. 2014). Fig fruit has been reported to be used in traditional medicine to treat different disease conditions like gastrointestinal, respiratory, and cardiovascular problems and has anti-inflammatory and antispasmodic activity (Duke 2002). The present study aimed to assess the potential of aqueous methanolic extract of *Ficus carica* L. (Fc.Cr) for managing hyperthyroidism in thyroxine-induced hyperthyroidic rats.

2. Material and Methods

2.1. Chemicals/Equipment

All the chemicals used in experiments were of analytical grade, including Carbimazole (Ray Pharma, UK), Ketamine (Budapest, Hungary), Thyroxine (GSK, Pakistan), Xylazine (Prix Pharmaceutica, Pakistan), and Enzyme immune assay kits for thyroid stimulating hormone (TSH), T3 and T4 (Bio-check, USA). Apparatus used include centrifuge machine (Hettich-EBA 20, Germany), digital weighing balance (Shimadzu, Japan), enzyme-linked immunosorbent assay (ELISA) reader (Bio Tek, USA), deep freezer (Haier, Pakistan), incubator (Memmert, Germany), refrigerator (Dawlance, Pakistan), rotary evaporator (Heidolph Laborota, Germany) and Vortex mixer (Eeoulin Biosciences, Korea).

2.3. Collection of Plant Material

Dried fruit of *Ficus carica* (Anjeer) was purchased from the local market of Bahawalpur, followed by identification from the botanist Mr. Abdul Hameed, Department of Life Sciences, the Islamia University of Bahawalpur (IUB), Pakistan. After cleaning of extraneous substances, the dried plant sample was submitted to the Herbarium of Pharmacology research laboratory, Department of Pharmacology, Faculty of Pharmacy, IUB, and a voucher number for the specimen was issued for future reference, i.e., FC-FT-04-15-88.

2.4. Preparation of the Crude Extract

1kg of the dried fruit of *Ficus carica* was soaked in aqueous methanol (30:70) for three days with occasional shaking at room temperature, followed by filtration, first through muslin cloth and then through filter paper. This process of soaking and filtration was repeated twice. The residue was then discarded, and the filtrate was concentrated by evaporation under reduced pressure at 40-50ºC using a rotary evaporator. A thick semisolid paste (Fc.Cr) was obtained, percent yield was calculated, labeled properly, and stored in a freezer below 0°C for future use.

2.5. Phytochemical Analysis

Phytochemical screening of Fc.Cr was performed to detect the secondary metabolites, i.e., alkaloids, glycosides, flavonoids, tannins, saponins, anthraquinone glycosides, coumarins, fats, oils, and phenolic compounds according to the previously described methods with slight modifications (Gilani et al. 2008, Tiwari et al. 2011).

2.6. Experimental Animals

Male Wistar albino rats weighing 150-200 g and Swiss albino mice of either sex weighing 18-30g were selected and kept in the animal house of the Pharmacology research laboratory, Department of Pharmacology, Faculty of Pharmacy, IUB. Animals were kept in polycarbonate cages under standard temperature (23±2°C) and humidity (55±5%) along with 12 h light and dark cycle and were given a standard rodent pellet diet and tap water *ad libitum*. The study was conducted according to the institutional animal ethics

Table 1: Effects of Fc.Cr on Serum TSH, T3 andT4 Levels.

Groups	TSH (μIU/ml)			T3 (ng/ml)			T4 (μg/dl)		
	14th day	28th day	35th day	14th day	28th day	35th day	14th day	28th day	35th day
Control (DW 5ml/kg; p.o.)	0.039± 0.005	0.04±0 .001	0.04±0 .001	1.4±0. 006	1.402± 0.005	4.7±0. 055	4.96± 0.006	4.92± 0.005	4.92± 0.006
Intoxicated (DW 5ml/kg; p.o.)	0.014± 0.001	0.013± 0.001	0.01±0 .001	4.8±0. 04	4.72±0 .033	4.7±0. 055	8.91± 0.037	8.91± 0.03	8.96± 0.05
Carbimazole (20mg/kg; p.o.)	0.013± 0.005	0.03±0 .001***	0.04±0 .001***	4.72± 0.04	2.512± 0.06***	1.53± 0.045*	8.90± 0.04	5.62± 0.06***	4.79± 0.05***
Fc.Cr (30mg/kg; p.o.)	0.013± 0.005	0.02±0 .001**	0.02±0 .001***	4.76± 0.05	4.3±0. 032**	3.92± 0.025*	8.82± 0.05	7.70± 0.03**	7.05± 0.03***
Fc.Cr (100mg/kg; p.o.)	0.012± 0.005	0.02±0 .001***	0.03±0 .001***	4.78± 0.002	3.9±0. 02***	3.27± 0.031*	8.73± 0.002	6.59± 0.03***	5.80± 0.03***
Fc.Cr (300mg/kg; p.o.)	0.012± 0.006	0.03±0 .001***	0.03±0 .001***	4.78± 0.008	2.64±0 .021***	1.85± 0.036*	8.73± 0.008	5.58± 0.02***	5.01± 0.04***

Mean±SEM; n=4, significant (*) if p<0.05, more significant (**) if p<0.01, highly significant (***) if p<0.001. *: Comparison with intoxicated group

committee (PAEC) guidelines under reference no. 35-2-015/PREC.

2.7. Animal Model of Hyperthyroidism

The animal model of hyperthyroidism described previously was used with slight modifications Bhaigyabati, Ramya, and Usha (2012). Rats were divided into six groups, each comprising six animals. The normal control group was given distilled water (5 ml/kg/day), while all other groups were orally given thyroxine (600 μg/kg/day) for 14 days. After 14 days of intoxication, treatments were given to all the groups for the next 21 days; i.e., the normal control group and the intoxicated group were given distilled water (5 ml/kg/day; p.o). The standard control group was given a standard drug, Carbimazole (20mg/kg; p.o), and the treatment groups were given Fc.Cr at the doses of 30, 100, and 300 mg/kg; p.o. for the next 21 days.

2.8. Determination of Serum TSH, T3, and T4 Levels

All the animals were anesthetized with 0.2 ml/100g Ketamine/Xylazine combination (10:1). At the end of the study, blood was collected by cardiac puncture, whereas at days 0, 14 and 28, blood was collected by retro-orbital puncture. Blood was allowed to clot for 15 minutes. Serum was separated by centrifuging the blood at 5000 rpm for 15 minutes. Serum thyroid hormone levels, i.e., TSH, T3, and T4, were determined by using ELISA.

2.9. Acute Toxicity Assay

Acute toxicity assay was performed according to the Organisation for Economic Cooperation and Development guidelines to determine the safety of Fc.Cr. Mice of either sex were divided into five groups and were kept on overnight fasting mode. The normal control group was given normal saline (10 ml/kg; p.o.), while other groups were given Fc.Cr at the doses of 0.5, 1, 5, and 10 g/kg; p.o. Animals were monitored for any physiological or behavioral changes like changes in body weight, grooming, alertness, convulsions, hyperactivity, lacrimation, salivation, sweating, urination, touch response, pain response, writhing reflex, corneal reflex, gripping reflex, and righting reflex at 0, 0.5, 1, 2, 4, 6, 12, 24 and 48 hours up to 14 days (Jabeen et al. 2021).

2.10. Statistical Analysis

All the results were expressed as mean ± SEM. Data were analyzed using one-way ANOVA followed by the student t-test. P <0.05 was considered statistically significant.

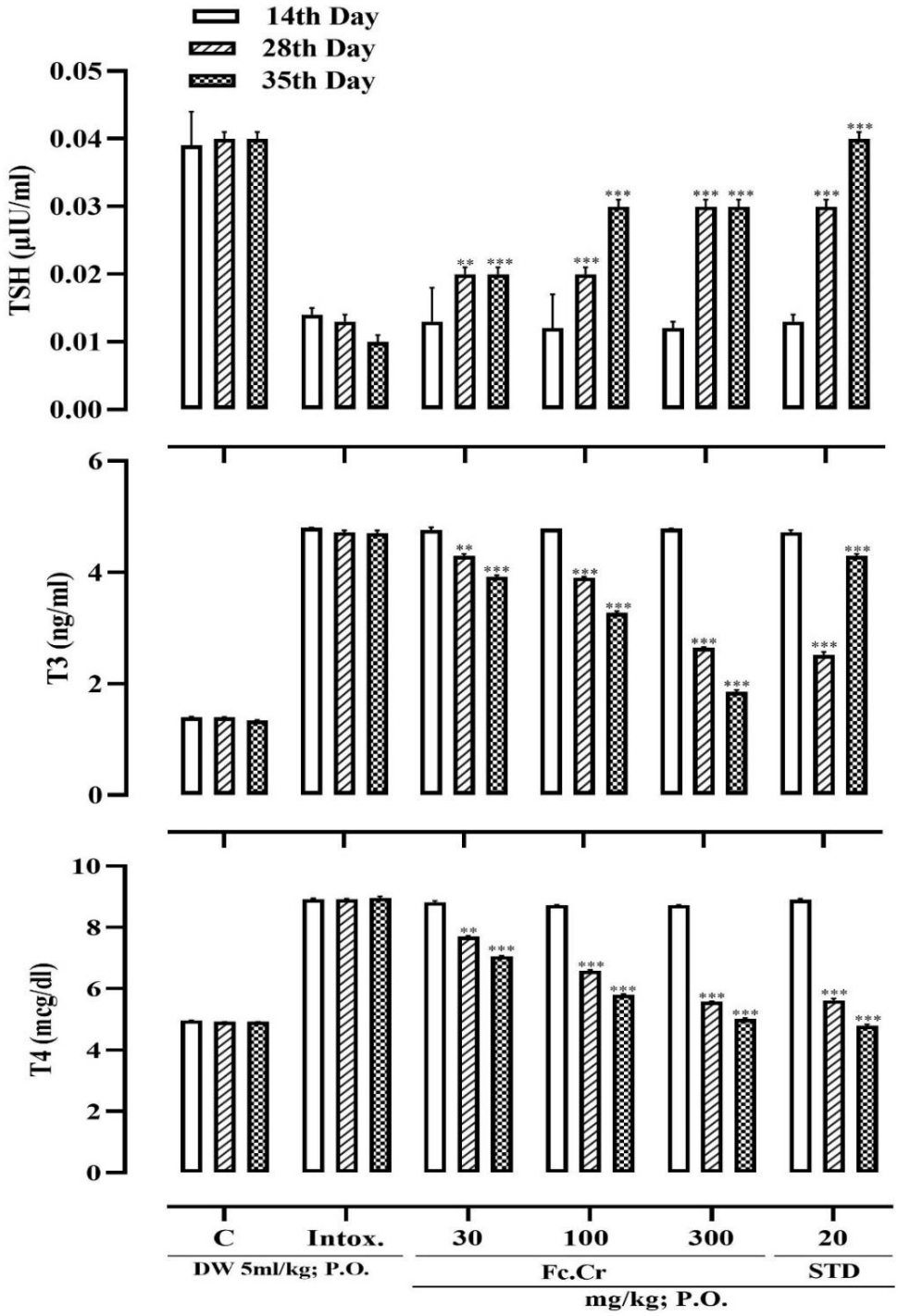

Figure 1: The graph showing serum TSH, T_3, and T_4 levels after 14 days of intoxication and after treatment with Fc.Cr and Carbimazole for the next 21 days. Mean±SEM; n=4, significance (*) if p<0.05, (**) if p<0.01, (***) if p<0.001, * Comparison with intoxicated group.

3. Results

The percent yield of Fc.Cr was calculated as 60%.

3.1. Phytochemical Analysis

The results of the phytochemical analysis showed the presence of secondary metabolites,

i.e., alkaloids, carbohydrates, coumarins, flavonoids, glycosides, phenols, quinones, saponins, and terpenes.

3.2. Effects of Fc.Cr on Serum TSH, T3 and T4 Levels

Serum levels of TSH, T3, and T4 were analyzed after intoxication and the treatment. The control group showed normal serum TSH, T3, and T4 levels throughout the study. The animals showed a decrease in TSH and increased T3 and T4 levels after intoxication on the 14th, 28^{th,} and 35th days of the study, which showed that the animals were under the influence of intoxication till the end of the study.

On the 14th day of intoxication, all animals showed a significant decrease in TSH and an increase in T3 and T4 levels. After intoxication, the treatment was started for the next 21 days. The standard group (Carbimazole 20mg/kg) showed an increase in TSH levels. On the other hand, a decrease in T3 and T4 levels on the 28th day was highly significant compared to the intoxicated group. Fc.Cr, at all the tested doses (30, 100, and 300 mg/kg/day p.o), showed increased levels of TSH and decreased T3 and T4 levels. The results were found to be dose-dependent. The results of Fc. Cr-treated groups on the 35th day of the study were highly significant ($p<0.001$) as compared to the intoxicated group.

3.3. Acute Toxicity Assay

Fc.Cr was found to be safe up to the dose of 10g/kg body weight. None of the animals showed signs of toxicity during the initial 24 hours, no lethal effect was recorded after the next 48 hours, and no such event was observed for the next 14 days.

4. Discussion

Ficus carica possesses diverse phytoconstituents and is well-known for its multiple nutritional and therapeutic benefits. Bioactive substances present in the aqueous methanolic extract (Fc.Cr) can be used for therapeutic purposes. In the past few decades, the pharmacological value of medicinal plants has increased because of their secondary metabolites (Akinmoladun et al. 2007), and the antioxidant constituents of plants have been reported to prevent the damaging action of free radicals, which are involved in the pathological damages to various organs of the body (Chaudhary et al. 2023). Phytochemical screening of Fc.Cr revealed the presence of a variety of biochemical molecules, including phenolic compounds, flavonoids, glycosides, alkaloids and carbohydrates etc. Flavonoids have been reported to possess protective effects against oxidative stress-mediated organ damage (Rudrapal et al. 2022). The possible mechanisms of phenols as antioxidants mainly include free radical scavenging activity and their ability to affect the signaling pathways and gene expression (Soobrattee et al. 2005). Oxidative stress can be defined as the disturbance of equilibrium between radicals and antioxidants (Mancini et al. 2013). Cellular injury due to the production of free radicals and reactive oxygen species (ROS) may result in many diseases, such as atherosclerosis, cancer, diabetes, Graves' disease, inflammation, liver cirrhosis, and nephrotoxicity etc. (Sylvie et al. 2014).

In the thyroid gland, oxidation of iodide to iodine occurs in the presence of H_2O_2, which is further catalyzed by thyroid peroxidase (TPO), leading to the iodination of iodothyronine and the production of T_3 and T_4. In the case of hyperthyroidism, there is increased iodide peroxidation and production of thyroid hormones (Jamiu et al. 2022). The results of this study showed that Fc.Cr has the ability to decrease the levels of

T_3 and T_4, which may be due to its antioxidant potential, as oxygen consumption is directly affected by thyroid hormones that result in the enhanced production of superoxide radicals (Mono et al. 1997). The effects of Fc.Cr against hyperthyroidism may be due to the presence of flavonoids and phenols. The other possible mechanism involves blocking the TPO-mediated iodination of tyrosine residues in thyroglobulin, a key step in the synthesis of T_3 and T_4. The increased concentration of TSH may be due to the constituents involved directly or indirectly in the feedback mechanism or due to the stimulation of hypothalamic cells to secrete thyrotropin-releasing hormone (TRH). TRH stimulates the synthesis and release of thyroid stimulating hormone and the combination of antioxidants with anti-thyroidal drugs may increase the antioxidant defense system and also reduce the time of exposure of the thyroid gland to oxidative stress. Fc.Cr may also interfere with the peripheral conversion of T4 to T3, the active thyroid hormone (Cooper 2005, Katzung, Zehnder, and Trevor 2009). Hence, the results of the study show that Fc.Cr possesses therapeutic significance in the management of hyperthyroidism.

5. Conclusions

This study concludes that the aqueous methanolic extract of *Ficus carica* has antithyroid potential, evident by the increase in TSH levels and decrease in T3 and T4 levels in the albino rats. Phytoconstituents may be considered responsible for its antithyroid effects. Thus, the results lend scientific credence to the folkloric use of *Ficus carica* in traditional medicine. However, further studies are encouraged to elucidate the exact mechanism(s) of action.

Conflict of Interest

The authors declare that they have no competing interests.

Funding

No specific funding was received for this project.

Study Approval

This study was approved by the Department of Pharmacology, Faculty of Pharmacy, IUB.

Consent Forms

NA.

Authors Contribution

QJ conceptualized the study and wrote the final manuscript, MU, MA, MQ performed the experimental work, helped in the statistical analysis and writing the first draft.

Acknowledgments

The authors acknowledge the support of the Department of Pharmacology, Faculty of Pharmacy, IUB, for facilitating this study.

Reference

Akinmoladun, Afolabi C, EO Ibukun, Emmanuel Afor, BL Akinrinlola, TR Onibon, AO Akinboboye, EM Obuotor, and EO Farombi. 2007. "Chemical constituents and antioxidant activity of Alstonia boonei." *African Journal of Biotechnology* no. 6 (10).

Bhaigyabati, T, J Ramya, and K Usha. 2012. "Effect of methanolic extract of sweet corn silk on experimentally induced hyperthyroidism in Swiss albino rats." *International Research Journal of Pharmacy* no. 3 (3):241-245.

Chaudhary, Priya, Pracheta Janmeda, Anca Oana Docea, Balakyz Yeskaliyeva, Ahmad Faizal Abdull Razis, Babagana

Modu, Daniela Calina, and Javad Sharifi-Rad. 2023. "Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases." *Frontiers in Chemistry* no. 11.

Chiari, Damiano, Barbara Pirali, Vittoria Perano, Roberto Leone, Alberto Mantovani, and Barbara Bottazzi. 2023. "The crossroad between autoimmune disorder, tissue remodeling and cancer of the thyroid: The long pentraxin 3 (PTX3)." *Frontiers in Endocrinology* no. 14:733.

Cooper, David S. 2005. "Antithyroid drugs." *New England Journal of Medicine* no. 352 (9):905-917.

Duke, James A. 2002. *Handbook of medicinal herbs*: CRC press.

Frodin, David G. 2004. "History and concepts of big plant genera." *Taxon* no. 53 (3):753-776.

Gilani, Anwarul Hassan, Qaiser Jabeen, Arifullah Khan, and Abdul Jabbar Shah. 2008. "Gut modulatory, blood pressure lowering, diuretic and sedative activities of cardamom." *Journal of Ethnopharmacology* no. 115 (3):463-472.

Gilani, Anwarul Hassan, and ur Atta Rahman. 2005. "Trends in ethnopharmacology." *Journal of Ethnopharmacology* no. 100 (1-2):43-49.

Jabeen, Qaiser, Muhammad Sohaib Khan, Abdul Wahid Qureshi, and Hafiz Muhammad Farhan Rasheed. 2021. "Effect of *Abutilon indicum* in thyroxine-induced hyperthyroidism in rat." *Bangladesh Journal of Pharmacology* no. 16 (3):103-113.

Jamiu, Abeeb Babatunde, Akinwale John Faniyi, Adedotun Afeez Aderanti, Adeola Kabirat Adedeji, Musediq Busari, and Ajoke Ruqayyah Olawale. 2022. "The Interplay of Oxidative Stress On Thyroid Hormone Disorders." *Global Scientific Journal* no. 10 (8).

Katzung, SM, J Zehnder, and A Trevor. 2009. *Basic and clinical pharmacology*: McGraw-Hill Medical, New York.

Mancini, Antonio, Sebastiano Raimondo, Chantal Di Segni, Mariasara Persano, Giovanni Gadotti, Andrea Silvestrini, Roberto Festa, Luca Tiano, Alfredo Pontecorvi, and Elisabetta Meucci. 2013. "Thyroid hormones and antioxidant systems: focus on oxidative stress in cardiovascular and pulmonary diseases." *International Journal of Molecular Sciences* no. 14 (12):23893-23909.

Mono, T, R Shinohara, K Iwase, M Kotake, M Hamada, K Uchimura, N Hayakawa, R Hayashi, A Nakai, and Y Ishizuki. 1997. "Changes in free radical scavengers and lipid peroxide in thyroid glands of various thyroid disorders." *Hormone and Metabolic Research* no. 29 (07):351-354.

Rudrapal, Mithun, Shubham J Khairnar, Johra Khan, Abdulaziz Bin Dukhyil, Mohammad Azam Ansari, Mohammad N Alomary, Fahad M Alshabrmi, Santwana Palai, Prashanta Kumar Deb, and Rajlakshmi Devi. 2022. "Dietary polyphenols and their role in oxidative stress-induced human diseases: insights into protective effects, antioxidant potentials and mechanism (s) of action." *Frontiers in pharmacology* no. 13:283.

Soni, Neha, Sanchi Mehta, Gouri Satpathy, and Rajinder K Gupta. 2014. "Estimation of nutritional, phytochemical, antioxidant and antibacterial activity of dried fig (*Ficus carica*)." *Journal of*

Pharmacognosy and Phytochemistry no. 3 (2).

Soobrattee, Muhammed Asin, Vidushi S Neergheen, Amitabye Luximon-Ramma, Okezie I Aruoma, and Theesan Bahorun. 2005. "Phenolics as potential antioxidant therapeutic agents: mechanism and actions." *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* no. 579 (1):200-213.

Sylvie, Djacbou D, Pieme Constant Anatole, Biapa Prosper Cabral, and Penlap Beng Veronique. 2014. "Comparison of in vitro antioxidant properties of extracts from three plants used for medical purpose in Cameroon: *Acalypha racemosa, Garcinia lucida* and *Hymenocardia lyrata*." *Asian Pacific Journal of Tropical Biomedicine* no. 4:S625-S632.

Tiwari, Prashant, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur, and Harleen Kaur. 2011. "Phytochemical screening and extraction: A review." *Internationale Pharmaceutica Sciencia* no. 1 (1):98-106.

Truter, Ilse. 2011. "Clinical review: hyper-and hypothyroidism: evidence-based pharmacy practice." *SA Pharmaceutical Journal* no. 78 (6):10-14.