



PRELIMINARY PHYTOCHEMICAL SCREENING OF QUERCUS INFECTORIA GALLS

Fateh AL Rahman F. Magbool*¹, Elamin Ibrahim Elnima², Shayoub M. E.³ and Salah
Eldin Omar Hussein⁴

¹PhD Student, Department of Pharmaceutics, Faculty of Pharmacy, University of Khartoum –
Sudan.

²Professor of Microbiology, Faculty of Pharmacy, University of Khartoum – Sudan.

³Associated Professor of Pharmaceutics, Alyarmouk College of pharmacy – Sudan.

⁴Assistance professor - AL-Ghad International College for Applied Medical Sciences –
Medical laboratory science Department (Almadina Elmanwra - KSA)

Article Received on
30 October 2017,

Revised on 20 Nov. 2017,
Accepted on 11 Dec. 2017

DOI: 10.20959/wjpps20181-10775

*Corresponding Author

Fateh AL Rahman F.

Magbool

PhD Student, Department of
Pharmaceutics, Faculty of
Pharmacy, University of
Khartoum – Sudan.

ABSTRACT

Introduction: Medicinal plants are a rich source of producing wide number of chemical constituents, the beneficial medicinal effects of the medicinal plant materials typically result from the combinations of secondary products present in the plant making the medicinal actions of plants unique to particular plant species or groups. Recently, many plant extracts have shown to have therapeutic values indifferent disease conditions. *Quercus infectoria* is a small tree or a shrub belonging to the *Fagaceae (Quercaceae)* family. The Gall of *Quercus infectoria* is described in detail in ethnobotanical and literature to possess various pharmacological actions such as analgesic, anti-inflammatory, antipyretic, antiseptic, antistomatitis, deodorant,

derivative, desiccant, expectorant, germicidal, hypnotic, hypoglycaemic, powerful astringent, sedative, local anesthetic, antiviral, antibacterial, larvicidal and antifungal activities. The main constituents found in the galls of *Q. infectoria* are tannin (50-70%) and small amount of free gallic acid and ellagic acid. **Methods:** Ethanolic extract prepared from *Quercus Infectoria* Galls as well as the bioactive compounds screened from these crude extracts. **Results and Discussion:** An evaluation on the phytochemical screening of Galls of *QI* extracts revealed the presence of medicinally active constituents, in these screening process tannins, flavonoids, saponins, triterpenes, anthraquinones and coumarins gave positive

results, while sterols and alkaloids gave negative results. The various phytochemical compounds detected are known to have beneficial importance in industrial and medicinal sciences. The galls of the *Quercus infectoria* (QI) tree are traditionally believed to have great medicinal value. The galls produced on the tree are strongly astringent and can be used in treatment of hemorrhages, chronic diarrhea, dysentery etc. Tannins mainly contribute to the anti-microbial activities of the drug. **Conclusion:** This study thus provides a useful database of the therapeutic bioactivity of *Quercus Infectoria* Galls, Thus effort must be making for isolation, standardization and clinical evaluation of such phytochemicals in order to obtain lead compounds for further new drug discovery.

KEYWORDS: *Quercus Infectoria* Galls, phytochemicals, ethno botanical, bioactivity, Phytotherapy.

INTRODUCTION

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend themselves against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. Medicinal plants are a rich source of producing wide number of chemical constituents in most efficient way and with precious selectivity. According to the World Health Organization more than 80% of the world's people depend on traditional medicine for their primary healthcare needs. The beneficial medicinal effects of these plant materials typically result from the combinations of secondary products present in the plant making the medicinal actions of plants unique to particular plant species or groups. Phytotherapy is referred to as the study of the use of plant extracts from natural origin as medicines or health-promoting agents. The main difference between phytotherapy medicines and the medicines containing the herbal elements lies in the methods of plants processing. The preparation of medicines containing herbal elements involves the extraction of the chemically clean active substances, while in the case of phytotherapy medicines all complex active substances of plant are incorporated in the crude natural form. The study of plants used in traditional medicine requires the effective integration of information on chemical composition of extracts, pharmacological activities of isolated compounds, as well as indigenous knowledge of traditional healers. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound

therapeutic benefits and more affordable treatment. The quality and quantity of the biologically active compounds from the plant extracts significantly depend on the species, the plant organ and harvest time.^[1,2] There are global problems of multiple resistance as well as emergence of new and resurrection of previously eradicated diseases.

Quercus infectoria is a small tree or a shrub belonging to the *Fagaceae* (*Quercaceae*) family. Gall of *Quercus infectoria* (QI) is known by different vernacular names, locally known as Ifas. The plant is found in Turkey, Syria, Persia, Cyprus and Greece.^[3] The various *Quercus* species originated in Iran, Iraq and Turkey, but are now widespread and particularly common in Asia Minor, Europe and North Africa.^[4] Galls are irregular plant growth, which is stimulated by the reaction between plant hormones and powerful growth regulating chemicals produced by insects or mites.^[5] The QI galls are produced by the insect, *Cynipsquercifolii*, for depositing its eggs.^[6]

The Gall of *Quercus infectoria* is described in detail in ethnobotanical and literature to possess various pharmacological actions such as analgesic, antidote, anti-inflammatory, antipyretic, antiseptic, antistomatitis, deodorant, derivative, desiccant, expectorant, germicidal, hypnotic, hypoglycaemic, powerful astringent, sedative, styptic, tonic, tonic to teeth and gum, and wound healing.^[7,8,9,10,11,12] The galls of *Q. infectoria* have also been pharmacologically documented to possess antitremorine, local anesthetic^[13], antiviral^[14], antibacterial^[15], larvicidal^[16] and antifungal^[17] activities. The main constituents found in the galls of *Q. infectoria* are tannin (50-70%) and small amount of free gallic acid and ellagic acid.^[18,19] The galls contain 50-70% of the tannin known as gallotannic acid. This is a complex mixture of phenolic acid glycosides varying greatly in composition. The galls also contain gum, sugar and essential oil.^[20] Preliminary screening for the galls had been focused considering their widespread use.

MATERIAL AND METHODS

Plant material collection and identification

The Gall of *Quercus infectoria* were collected. The plant was identified by a taxonomist at medicinal and aromatic plants institute, National Center for Research - Khartoum, Sudan. The tested plant part then ground into powder and was used for the subsequent experimentation.

Reagents

Chloroform (SD Fine India), Ferric Chloride (BDH England), Acetic anhydride (SD Fine England), Sulphuric acid (SD Fine India), Hydrochloric acid (Romile EU), Aluminium Chloride (BDH England), Potassium Hydroxide (Sharlau Spain), Hydrogen Peroxide (Sharlau Spain), Ammonium Hydroxide (SD Fine India), Benzene (Sharlau Spain), Sodium Chloride (Sharlau Spain), Gelatin salt (Sharlau Spain), Potassium chloride (BDH England), Mercuric iodide (BHD England), Ethanol (National Distillation Company).

Preparation of extract

Extraction was carried out according to method described by^[21]: 500 g of the plant sample was extracted by soaking in 2500 ml 80 % ethanol for about seventy two hours with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus, In order to obtain a completely dry extract, the resultant extract were transferred to glass dishes. The yield percentages were calculated as followed:

$$\text{Weight of extract / weight of sample} * 100$$

Phytochemical screening

Phytochemical screening for the active constituents was carried out using the methods described by^[22,23,24,25], with many few modifications.

1. Phytochemical screening

1.1 Identification of tannins

0.5 g of the extract was washed three times with petroleum ether, dissolved in 10 ml hot saline solution and divided in two tests tubes. To one tube 2-3 drops of ferric chloride added and to the other one 2 – 3 drops of gelatin salts reagent added. The occurrence of a blackish blue color in the first test tube and turbidity in the second one denotes the presence of tannins.

1.2 Test of sterols and triterpenes

0.5 g of the extract was washed three times with petroleum ether and dissolved in 10 of chloroform. To 5 ml of the solution, 0.5 ml acetic anhydride was added and then 3 drop of conc. Sulphuric acid at the bottom of the test tube. At the contact zone of the two liquids a The gradual appearance of green, blue pink to purple color was taken as an evidence of the presence of sterols (green to blue) and or triterpenes (pink to purple) in the sample.

1.3 Test for Alkaloids

0.5 g of the extract was heated with 5 ml of 2N HCl in water bath and stirred for about 10 minutes, cooled filtered and divided into two test tubes. To one test tube few drops of Mayer's reagent was added while to the other tube few drops of Valser's reagent was added. A slight turbidity or heavy precipitate in either of the two test tubes was taken as presumptive evidence for the presence of alkaloids.

1.4 Tests for Flavonoids

0.5 g of the extract was washed three times with petroleum ether and dissolved in 30 ml of 80% ethanol.

The filtrate was used for following tests

A/ to 3 ml of the filtrate in a test tube 1ml of 1% aluminum chloride solution in methanol was added. Formation of a yellow color indicated the presence of Flavonoids. Flavones or chalcone.

C/ to 2 ml of the filtrate 0.5ml of magnesium turnings were added. Producing of defiant color to pink or red was taken as presumptive evidence that flavonenes were present in the plant sample.

1.5 Test for Saponins

0.5 g of the extract was placed in a clean test tube. 10 ml of distilled water was added, the tube stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of foam, which persisted for least an hour, was taken as evidence for presence of saponins.

1.6 Test for Coumarins

0.5 g of the extract was dissolved in 10 ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of 0.5N KOH put on it. Then the filter paper was inspected under UV light, the presence of coumarins was indicated if the spot have found to be adsorbed the UV light.

1.7 Test for Anthraquinone glycoside

0.5 g of the extract was boiled with 10 ml of 0.5N KOH containing 1ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene. 5ml of the benzene solution was shaken with 3ml of 10% ammonium hydroxide solution and the two

layers were allowed to separate. The presence of anthraquinones was indicated if the alkaline layer was found to have assumed pink or red color.

RESULTS AND DISCUSSION

Table 1: Yield percentages of ethanolic extracts.

Sample	Weight of sample	Weight of extract	Yield %
<i>Quercus infectoria</i> galls	500 g	76.3 g	15.26 %



Fig (1): Rotary Evaporator apparatus.

Table 2: Phytochemical screening of *Quercus infectoria* galls.

Test	Results	Observation
Saponins	++	Foam
Cumarins	+	UV absorption
Alkaloids	-	No observation
Anthraquinones	++	Pink colour
Tannins	+++	blue colour
Flavonoids	+++	Yellow color
Sterols	-	No observation
Triterpenes	++	Purple colour

An evaluation on the phytochemical screening of Galls of QI extracts revealed the presence of medicinally active constituents. The phytochemical active compounds of *Quercus*

Infectoria Galls were screened and the results are presented in table 2. In analysis of tannin compounds the occurrence of a blackish blue color in the first test tube and turbidity in the second one denotes the presence of tannins. Similarly based on the presence or absence of colour change indicate positive and negative results are indicate. In these screening process tannins, flavonoids, saponins, triterpenes, anthraquinones and coumarins gave positive results, sterols and alkaloids gave negative results. The various phytochemical compounds detected are known to have beneficial importance in industrial and medicinal sciences. These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins are reported to possess physiological astringent and haemostatic properties, which hasten wound healing and ameliorate inflamed mucus membrane and also inhibit the growth of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them; they form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. They have important roles such as stable and potent anti-oxidants.^[26,27,28] They act as binders and for treatment of diarrhea and dysentery.^[29] Tannins also reported to exhibit antiviral, antibacterial, anti-tumor activities. It was also reported that certain tannins are able to inhibit HIV replication selectivity and is also used as diuretic.^[30] Plant tannin has been recognized for their pharmacological properties and is known to make trees and shrubs a difficult meal for many caterpillars.^[31] Plant phenolic compounds especially flavonoids are currently of growing interest owing to their supposed properties in promoting health (anti-oxidants)^[32]; Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity. The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages.^[33] Saponins have expectorant action which is very useful in the management of upper respiratory tract inflammation; saponins present in plants are cardiogenic in nature and are reported to have anti-diabetic and anti-fungal properties.^[34,35] They are stored in plant cells as inactive precursors but are readily converted into biological active antibiotics by enzymes in response to pathogen attack. A large number of studies have been done in recent years on the antifungal and antibacterial activity of terpenoids of natural origin. The mechanism of action of triterpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic nature. Coumarins have been reported to stimulate macrophages which could have an indirect negative effect on infections. Plant steroids are known to be important for their cardiogenic, insecticidal and anti-microbial

properties. They are also used in nutrition, herbal medicines, cosmetics and they are routinely used in medicine because of their profound biological activities.^[36] Anthraquinones are structurally built from an anthracene ring (tricyclic aromatic) with a keto group each on carbon atom nine and ten. In plants, anthraquinones are found in a wide range of species. The effects of anthraquinones and anthrones are very diverse. Anthraquinones and anthrones are very reactive and have a broad pharmacological activities including, they are potent anticancer, antidiabetic, antimicrobial, antiinflammatory, and cathartic properties as well as its cardio-, hepato-, and neuroprotective qualities.^[37] Anthraquinones and xanthonones contain an aromatic core that serves as a scaffold for the attachment of diverse functional groups, resulting in a wide variety of molecules with distinct biological and biochemical characteristics.

CONCLUSION

This study has revealed the presence of many secondary metabolites in the Galls of *Quercus Infectoria* it has further confirmed that the plant extracts could be used for various conditions in folkloric medicine. The galls of the *Quercus infectoria* (QI) tree are traditionally believed to have great medicinal value, and possess various biological activities such as astringent effect, antidiabetic, antitremorine, local anaesthetic, antipyretic, anti-inflammatory, antifungal, antibacterial, antiviral and many more. These pharmacological activities of gall extracts were reported to be due to its excellent antioxidant activity with phytochemicals constituents of phenolic and flavanoid compounds. The galls produced on the tree are strongly astringent and can be used in treatment of hemorrhages, chronic diarrhea, dysentery etc. Tannins mainly contribute to the anti-microbial activities of the plant. Further studies are needed to explore its other medicinal and pharmacological activities, efforts must be making towards the discovery of pharmaceutical products or more appropriately biomedicines from biologically active plant crude extracts.

REFERENCES

1. Abubakar EL-MM. Efficacy of crude extracts of garlic (*Allium sativum* Linn.) against nosocomial *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. *J. Med. Plants Res.*, 2009; 3: 179-185.
2. Pârvu M, Pârvu AE, Roșca-Casian O, Vlase L, Groza G. Antifungal activity of *Allium obliquum*. *J. Med. Plants Res.*, 2010; 4: 138- 141.
3. Evans, W.C. Trease and Evans Pharmacognosy, Harcourt Publishers Limited, 15th

- edition, Edinburgh, 2002; 21: 224, 474.
4. Thomas, F. PDR for Herbal Medicine, Medical Economic Comp, 2000; 550-551.
 5. Townsend L, Extension Entomologist and Eliason E. (1998) Common Oak Galls. Department of Entomology, University of Kentucky, USA. <http://www.uky.edu/Ag/Entomology/entfacts/trees/ef408.htm>. Accessed on 15 June 2006.
 6. William Cook M D. (1869). The Physiomedical Dispensatory-*Quercus infectoria*. http://www.henriettesherbal.com/eclectic/cook/Quercus_Infectoria.htm. Accessed on 16 August, 2006.
 7. Khare, C.P. Encyclopaedia of Indian Medicinal Plants, Springer Berlin, Heideberg, New York, 2004; 395-396.
 8. Wallis TE. Textbook of Pharmacognosy 5th ed. New Delhi: CBS publisher and distributors, 2015; 101-103.
 9. Nadkarni, A.K., Indian Materia Medica, Bombay Popular Prakashan, Mumbai, 1982; I: 1041-1044.
 10. Khan, M.A. Moheet-e-Azam, published by Nizami Press, Kanpur, 1313H; II: 38.
 11. Kantoori, G.H. Tarjuma Qanoon bu Ali Sina, Book Printers, Lahore, 1992; II: 175.
 12. Agarwal, V.S. Economic Plants of India, Bishen Singh Mahindra Pal Singh, Dehradun, 1989; 321.
 13. Hussein G, Miyashiro H, Nakamura N, Hattori M, Kakiuchi N and Shimotohno K. Inhibitory effects of Sudanese medicinal plant extracts on hepatitis C virus protease. *Phytother Res.*, 2000; 14: 510-6.
 14. Fatima S, Farooqi AHA, Kumar R, Kumar TRS and Khanuja SPS. Antibacterial activity possessed by medicinal plants used in tooth powders. *J Med Aromatic Plant Sci.*, 2001; 22: 187-9.
 15. Digraki M, Alma MH, Ilcim A and Sen S. Antibacterial and antifungal effects of various commercial plant extracts. *Pharm Biol.*, 1999; 37: 216-20.
 16. Kaur G, Hamid H, Ali A, Alam MS and Athar M. Antiinflammatory evaluation of alcoholic extract of galls of *Quercus infectoria*. *J Ethnopharmacol*, 2004; 90: 285- 92.
 17. Redwane A, Lazrek HB, Bouallam S, Markouk M, Amarouch H and Jana M. Larvicidal activity of extracts from *Quercus lusitania* var. *infectoria* galls (Oliv.). *J Ethnopharmacol*, 2002; 79: 261-3.
 18. M.Ikram, F.Nowshad, "Constituents of *Quercus infectoria*", *Planta Med*, 1977; 31: 286-7.
 19. E.C. Bate-Smith and T. Swain, "Flavonoid compounds, In comparative Biochemistry, H.S. Mason and M. A. Florkin eds, Academic Press, New York, 1962; 755-809.

20. Anonymous. The Wealth of India – A Dictionary of Indian Raw Materials and Industrial Products, First Supplement Series, CSIR, New Delhi, Ph-Re, 2005; VIII: 351-352.
21. Sukhdev. S. H; Suman. P. S. K; Gennaro. L and Dev. D. R. Extraction technologies for medicinal and aromatic plants. United Nation Industrial Development Organization and the International Center for Science and High Technology, 2008; 116.
22. Martinez A, Valencia G: Marcha fitoquímica. (). In Manual de prácticas de Farmacognosia y Fitoquímica: 1999. 1st edition. Medellin: Universidad de Antioquia; Phytochemical screening methods, 2003; 59-65.
23. Sofowora, A. Medicinal Plants and Traditional Medicines in Africa. Chichester John, Willey & Sons New York, 1993; 256.
24. Harborne, J. B. Phytochemical methods. 2nd edition. Chapman and Hall, 1984.
25. Wall, M. E; Eddy, C. R; McClenna, M. L; & Klump, M. E. Detection and estimation of steroid and saponins in plant tissue. Analytical Chemistry, 1952; 24: 1337-1342.
26. Tyler VE, Brady LR, Roberts JE. Pharmacology. Lea and Ferbiger, Philadelphia, 1988; 85-90.
27. Awosika F. Local Medicinal plants and health of consumers. Clin. Pharm. Herbal Med., 1991; 9: 28-29.
28. Ogunleye DS, Ibitoye SF. Studies of antimicrobial activity and chemical constituents of *Ximenia Americana*. Trop. J Pharm Res., 2003; 2: 239-241.
29. Dharmananda S. Gallnuts and the uses of tannins in Chinese medicine. A paper Delivered at the Institute for Traditional Medicine, Portland, Oregon, 2003.
30. Heslem E. Plant Polyphenol: Vegetal Tannin Telisted- Chemistry and Pharmacology of Natural Products, 1st Edn., Cambridge University Press, Cambridge, Massachusetts, 1989; 169.
31. Aiyelaagbe O, Osamudiamen PM. Phytochemical Screening for Active Compounds in *Mangifera indica* Leaves from Ibadan, Oyo State, Plant Sciences Research, 2009; 1(2): 11-13.
32. Rauha JP, Remes S, Herinonen W, Hopia M, Kgjala T, Pitinlaja K et al. Antimicrobial effects of finished plant extract containing flavanoids and other phenolic compounds. Int. J Food Microbiol, 2000; 56: 3-12.
33. Mark Percival. Antioxidants. Clinical Nutrition Insights, 1998; 31: 01-04.
34. Trease GE, Evans MD. A text book of Pharmacognosy, 13th Edn. Baillier, Tindal and Caussel, London, 1989; 144 -148.
35. Kamel JM. An extract of the mesocarps of fruits of *Balanite aegyptiaca* exhibited a

- prominent anti-diabetic properties in Mice. *Chem. Pharmacol. Bull.*, 1991; 39: 1229-1233.
36. Denwick PM. *Natural Products A Biosynthetic Approach*. 2nd Edn., John Wiley and Sons, Ltd., England, 2002; 241-243.
37. Izhaki I Emodin - a secondary metabolite with multiple ecological functions in higher plants. *New Phytol*, 2002; 155(2): 205–217.