Pharmacognostic, Physicochemical Standardization and Phytochemical Analysis of *Quercus infecoria* galls

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Abstract

Introduction: Medicinal plants and their bioactive molecules are always in demand and are a central point of research. To date, herbs have remained useful not only as remedy for different diseases that affect humans and animals, but also as good starting points for the discovery of bioactive molecules for drug development. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. The pharmacognostic parameters are necessary for confirmation of the identity and determination of quality and purity of crude drugs. The galls of the *Quercus infectoria* (Family *Fagaceae*), traditionally believed to have great medicinal value. Pharmacologically the galls are claimed to have various biological activities.

Objectives: In view of its diverse medicinal applications and in order to ensure the quality, authenticity and assay, and in view of lack of pharmacognostic study, the present investigation was undertaken with an objective to evaluate *Q. infectoria* galls on various pharmacognostic parameters, such as macroscopic, microscopic, physiochemical, determination of microbial contaminations, phytochemical screening studies, and analytical method development.

Results and Discussion: The micro and macro standards obtained here can be identifying parameters to substantiate and authenticate the drug. The total ash value, extractive values will be helpful in identification and authentication of the plant material along with the microscopic method, which is the cheapest method to establish the correct identification of the source material, and determination of microorganisms of great importance, because medicinal plant materials normally carry a great number of *bacteria* and *moulds*, often originating in soil. While a large range of *bacteria* and *fungi* form the naturally occurring *microflora* of herbs, aerobic spore-forming *bacteria* frequently predominate.   Also the extractive methods are useful to evaluate the chemical constituents of the crude drug. The preliminary phytochemical screening will be useful

in finding the chemical nature of the *Q. infectoria* galls. In this study, the preliminary phytochemical screening ascertained the presence of tannins, flavonoids, saponins, triterpenes, anthraquinones and cumarines.

Conclusion: The present study on pharmacognostical evaluation of *Q. infectoria* galls will provide useful information for its identification of this medicinally important plant. Additionally the physicochemical, phytochemical parameters and analytical method development were reported for the first time. These sets of standards can used in future to assess quality and purity of *Q. infectoria* galls and analytical method for determination of *Q. infectoria* galls extract –based formulations. The results of the present study serve as a valuable source of information and provide suitable standards for identification of this medicinally important plant drug material for future investigations and applications.

Keywords: *Quercus infectoria* galls, Biological activities, Medicinal plants, Phytochemical screening, Pharmacognostic evaluation, Physicochemical studies.

Introduction

Some plant-based drugs have been used for centuries and for some like cardiac glycosides, there is no alternative conventional medicine. Therefore, medicinal plants and their bioactive molecules are always in demand and are a central point of research. As a result, there is a recent [1] surge in the demand for herbal medicine. To date, herbs have remained useful not only as remedy for different diseases that affect humans and animals, but also as good starting points for the discovery of bioactive molecules for drug development. The scientific exploitation of herbs used ethnomedicinally for pain relief, wound healing and abolishing fevers has resulted in the identification of a wide range of compounds that have been developed as new therapies for cancer, hypertension, diabetes and as anti-infectives [2]. The study of plant drugs from the pharmacognostical stand point would include the study of the habitat, general characters of the plant from which the drug is derived, its place in the botanical system, the organ or the organs of the plant used, their gross, minute structures in the whole and in the powdered conditions and the chemistry of the constituents especially of those which may be used in therapeutics. Authentication and standardization are prerequisite steps while considering source materials for herbal formulation in any system of medicine [3]. For developing drug standardization, the quality of base material used for formulating the herbal products is a prerequisite. Since the materials used

in herbal drugs are traded mostly as roots, bark, twigs, flowers, leaves, and fruits and seeds, visible authentication of the material used is difficult and has led to a high level of adulteration. Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To ensure quality reproduction of herbal products, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification. The most common error is one common vernacular name is given to two or more entirely different species [4]. All these problems can be solved by pharmacognostic studies of medicinal plants. It is very important and in fact essential to lay down pharmacognostic specifications of medicinal plants which are used in various drugs. Pharmacognostic studies basically deals with standardization, authentication and study of natural drugs. Most of the research in pharmacognosy has been done in identifying controversial species of plants, authentication of commonly used traditional medicinal plants through morphological, phytochemical and physicochemical analysis. Pharmacognostic studies ensures plant identity, lays down standardization parameters which will help and prevents adulterations. Such studies will help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products.

According to WHO [5, 6], standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion. Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity. Of these, the phytochemical profile is of special significance since it has a direct bearing on the activity of the herbal drugs.

Quercus infectoria (Family *Fagaceae*) is a small tree or shrub about 2 M high, with many spreading branches. The bark is slightly grey in color. The galls are collected for medicinal use before the escape of the insect and well dried. The surface of mature dry gall may be smooth and shining, as though varnished and chestnut brown, but more usually it is rough and of a greyish brown in color. When the galls are gathered at the correct stage, i.e. before the insect emerges, the inner tissue is soft, of a deep greenish yellow color, with a very astringent taste and slightly sweet aftertaste [7]. The galls of *Qurercus infectoria* have also been pharmacologically documented to possess astringent, antibacterial, antifungal, larvicidal, antidiabetic, local anaesthetic, antiviral, and anti- inflammatory [8]. In addition, *Q. infectoria* galls extract have potential anti-ulcer activity [9].

In view of its diverse medicinal applications and in order to ensure the quality, authenticity and assay [10], and in   view of lack of pharmacognostic study, the present investigation was

undertaken with an objective to evaluate galls of *Q. infectoria* on various pharmacognostic parameters, such as macroscopic, microscopic, physiochemical, determination of microbial contaminations, phytochemical screening studies, and analytical method development.

Materials and Methods

Plant material collection, authentication and identification

The galls 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 *Q. infectoria* galls was washed cleaned, made free from lichens, mosses, dried (Garbling process), the plant material were subjected to size reduction to get coarse powder and then passed through sieve no. 43 to get uniform powder. Some of fresh and dried plant material was used for pharmacognostic characteristics, physicochemical standardization and powdered materials were utilized for determination of identity, purity and quality of *Q. infectoria* galls, preparation of extract and then for subsequent phytochemical screening studies and analytical method development. The dried powder of *Quercus infectoria* galls were standardized for pharmacognostical and physicochemical parameters as per standard methods WHO [5, 6].

All the chemicals used were of analytical grade. Chloroform (SD Fine India), Ferric Chloride (BDH England), Acetic anhydride (SD Fine England), Sulphuric acid (SD Fine India), Hydrochloric acid (Romile EU), Alumminum 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).

Pharmacognostic and Physicochemical Standardization [5, 6]

Pharmacognostic and physicochemical standardization include macro and microscopic evaluations, determination of organoleptic and physicochemical characteristics, determination of microbial contamination of *Q. infectoria* galls and phytochemical analysis, and also analytical method development.

Pharmacognostical Evaluation
Macroscopic, Organoleptic Properties and Microscopic study of plant material

Macromorphological characters of the *Q. infectoria* gall like shape, size, colour, texture were observed. Measurements were carried out using line ruler. In some cases, general appearance of the herb is similar to related species. Thus, detailed study of the morphological characters can be helpful in differentiating them. The organoleptic evaluation of *Q. infectoria* galls includes its visual appearance to the naked eye along with its characteristics likes odour, colour, taste, texture etc. Microscopic analysis was carried out on transverse sections of *Q. infectoria* gall.

Physicochemical Study

Various physicochemical parameters such as pH, loss on drying (LOD), ash values (total ash, acid insoluble ash, water soluble ash) extractive values (by cold extraction) of *Q. infectoria* galls were established by using the powdered crude drug as per the method mentioned in WHO guidelines.

Determination of Microbial Contamination of *Q. infectoria* galls

Tests for Specific Microorganisms

i. Test for *Escherichia coli* Contamination of *Q. infectoria* galls

A suitable quantity (0.5g) of the powdered *Q. infectoria* galls was placed in a sterile screw-capped container, 50ml of nutrient broth were added, and the mixture was incubated for 24 hours at 37oC (enrichment culture). Then 0.1ml of the enrichment culture was transferred to a tube containing 5ml of MacConkey broth and incubated at 36-38°C for 48 hours. The contents of the tube were examined for the presence of acid and gas which indicated the possible presence of *Escherichia coli*.

ii. Test for *Salmonella spp*. Contamination of *Q. infectoria* galls

A suitable quantity (0.5g) of the powdered *Q. infectoria* galls was placed in a sterile screw-capped container, 100ml of nutrient broth were added, shaken and allowed to stand for one hour, shaken again, then the cap was loosened, and the container was incubated for 24 hours at 37oC (enrichment culture). Then 0.1ml of the enrichment culture was transferred to each of two tubes containing either a) 10ml of Selinite broth, or b) 10ml of Tetrathionate broth and incubated at 36-38°C for 48 hours. Then from each of these cultures one plate was inoculated containing a layer of Deoxycholate citrate agar; the plates were incubated at 36-38°C for 24 hours, and the colonies, if any, were examined for the possible presence of *Salmonella spp*.

Phytochemical Analysis

i. Preparation of Extract of *Q. infectoria* galls

Extraction was carried out according to method descried by [11]: 500 g of the *Q. infectoria* galls 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.

ii. Determination of Percentage Yields of Crude Extracts

Five hundred grams of powdered *Q. infectoria* galls were used to obtain crude extract. The percentage yield for the plant sample as the amount of crude extract recovered in mass compared with the initial mount of powdered plant materials used. It is presented in percentage (%) and the yield percentages were calculated as followed:

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

iii. Preliminary Qualitative Phytochemical Screening Study

Phytochemical screening of *Q. infectoria* galls ethanolic extract for the active constituents was carried out using the methods described by [12,13,14,15], with many few modifications.

Analytical Method Development [16, 17]

i. Determination of λmax for ethanolic extract of *Quercus infectoria* galls
Estimation was carried out by SHIMADZU-1700 UV spectrophotometer, weight accurately 1 gm from the *Q. infectoria* galls extract and dissolve in volumetric flask 100 ml , shake for 10 min by mechanical mean ,complete to volume by ethanol, read the absorbance after determine the maximum wave length by scan the same solution in the range 200-400 nm

ii. Preparation of Standard Curve
To construct the calibration curve, *Q. infectoria* galls extract was weighed and a solution of concentration 100 mg/ml was prepared. and then various dilutions were made, Spectra was run on UV spectrophotometer, and absorbances were noted. A standard calibration curve was developed and can be used to calculate the concentration of the dug during the study of different *Q. infectoria* galls extract-based formulations.

Results and Discussion

Pharmacognostical Evaluation

The pharmacognostical study is a major and reliable criterion for identification of plant drugs. The pharmacognostic parameters are necessary for confirmation of the identity and determination of quality and purity of crude drugs [18]. Evaluation of drug means confirmation of its identity and determination of its quality and purity and detection of nature of adulteration. The evaluation of a crude drug is necessary because of these main reasons i) biochemical variation in the drugs ii) deterioration due to treatment and storage, and iii) substitution and adulteration , a result of carelessness, ignorance or fraud. In the traditional text, plant species of same genus or other plant with similar therapeutic activity were known by same name , further plants morphologically looks alike. Thus, there has been an emphasis on standardization of medicinal plants, and evaluation of plant drugs by pharmacognostic studies is still more reliable, accurate, and inexpensive means.
Over the years the nature and degree of evaluation of crude drugs has undergone a systematic changes. Initially, the crude drugs were identified by comparison only with the standard description available. Due to advancement in the chemical knowledge of crude drugs, at present, evaluation also includes method of estimating active constituents present in the crude drug, in addition to its morphological, microscopic analysis and toxicological profile. With the advent of separation techniques and instrumental analysis, it is possible to perform physical evaluation of a crude drug, which could be both of qualitative and quantitative in nature. The plant may be considered a biosynthetic laboratory not only for the chemical compound such as carbohydrate, proteins and lipids that are utilized as food by man but also for a multitude of compounds like glycosides, alkaloids, volatile oils, tannins etc. that exerts a physiologic effect. The compounds that are responsible for therapeutic effect are usually secondary metabolite. The plant material may be subjected to preliminary phytochemical screening for the detection of various plant constituents.

Macroscopic, Microscopic and Organoleptic Characteristics

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus in recent years there has been an emphasis in standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostic studies is still more reliable, accurate and inexpensive means. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken [5, 6].

i. Macroscopic Characteristics

Galls are spherical or pear shaped, hard and brittle having 1.2 to 2.5cm in diameter They have a short basal stalk and numerous rounded projections on the upper part of the gall; they usually sink in water; surface is smooth rather shining, bluish green, olive green or white brown, a few galls show the escape route of the insect, in the form of a small rounded hole leading to cylindrical canal which passes to the centre of the gall. The taste of the drug is astringent, followed by sweetness. The average weight of ten galls picked at random should not be less than 2.5gms.

ii. Microscopic Characteristics

Transverse section of the gall shows an outer zone of small thin walled, irregularly shaped parenchymatous cells. Oval shaped sclerenchymatous cells are arranged as a ring in the center. Also small thick walled parenchymatous cells were present in the center zone. The outer zone of parenchyma has 3 types of cells arranged as layers. The uppermost cells are small, irregular and thin walled. Middle cells are large and oval in shape. Innermost cells are long parenchymatous cells, all having intercellular spaces. Vascular bundles consisting of xylem and phloem are irregularly distributed. Around the central cavity, sclerenchymatous cells are arranged as a ring. They vary in size and shape. Rectangular, ovoid, elongated and thick walled scleroses having pits and large lumen usually filled with dense brown material. Rosette crystals of calcium oxalate are present in the outer and middle region and prismatic crystals in the inner parenchymatous cells. Starch grains are either simple or compound with central hilum. Simple grains are present abundantly in the innermost zone of the parenchyma [19].

iii. Organoleptic Characteristics

Table 1: Various organoleptic properties of extract

Appearance	Colour	Odour	Texture	Taste
Powder	Light Brownish yellow	Not characteristic	Mixture of coarse and fine	Bitter astringent

Identification of the crude drug by organoleptic characters is one of the important aspects of pharmacognostical study. The term organoleptic evaluation refers to the sensory evaluation. Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. The Organoleptic studies show the important characteristics of the drugs, the typical tongue sensation and the odour may screen the preliminary phytochemical constituents. The characteristics which are evaluated with a help of sense organ such as color, odour, taste, size, shape, texture etc. It is Qualitative Evaluation. The majority of information on the identity, purity and quality of the material can be drawn from these observations; they are of primary importance before any further testing can be carried out. In this study the organoleptic features indicate Light Brownish yellow colour. The powder appeared mixture of coarse and fine in texture, Bitter astringent taste with not characteristic odour. The results of Organoleptic properties were summarized in (Table 1).

Physicochemical Study

Physicochemical parameters were determined as per guidelines of WHO, air dried coarse powdered sample of *Q. infectoria* galls was subjected for determination of physicochemical parameters such as pH, foreign organic matter, ethanol soluble extractives, water soluble extractives, total ash content, acid insoluble ash, water soluble ash, loss on drying and % moisture content were determined. According to standard method. All values were calculated and shown in (Table 2).

Table 2: Various observed values for the physico-chemical constants of *Q.infectoria* galls extract

Test	Observed value in %*
pH	5.9
Total Ash	5.03
Water soluble Ash	2.21
Acid insoluble Ash	0.12
Moister content	8.2
Foreign matter	0.1

*Mean values of three determinations

Physical tests are performed to establish and determine quality and purity of a crude drug. Physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The term pH refers to the relative amounts of hydrogen in a given chemical environment. It is a measure of the acidity or alkalinity of a given formulation or product. The pH is important in aqueous drug product formulation, especially since it involves drug solubility, activity absorption, stability, sorption and patient comfort. The rate of hydrolysis of product may vary depending on the pH of the solution. Determination of the pH will give information as to which excipients could be added to a drug to ensure that the product is stable and can be tolerated physiologically. The pH value of a product is becoming increasingly recognized for its important

contribution to product quality. This is because it plays a key role in prevention of microbial spoilage. The pH of *Q.infectoria* galls extract was found to be 5.9 for the ethanol extract. Extract whose pH 5.9 is slightly acidic because the extract majorly contains polyphenols which are acidic in nature.

Medicinal plants are drug from natural sources, has undergone through the cultivation, harvesting. There is obvious chance that plant materials get contaminated with part of same or other plant which are not therapeutically active or with insects, *moulds*, animal excreta and other contaminants like soil, stone, dust, metal parts etc. According to WHO it should be within with prescribed limits [20]. In present study the content of foreign matter was found very negligible (Table 2).

The total ash usually consists of carbonates, phosphates, silicates and silica, which include both physiologic ash and non-physiologic ash. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the crude drug for marketing. Acid insoluble ash indicates contamination with silica, for example, earth and sand. Comparison of this with the total ash value of the same sample will differentiate between contaminating materials and variations of the natural ash of the drug. Water soluble ash is that part of the total ash content, which is soluble in water. It is a good indicator of the water soluble salts in the drug. The percentage of total ash, acid insoluble ash and water soluble ash of *Q.infectoria* galls extract were shown in (Table 2). The ash values of a drug give an idea of the purity of drug from earthy matter or the inorganic composition and other impurities present along with the drug.

The moisture content of the *Q.infectoria* galls ethanolic extract (Table 2) was 8.2%v/. the value was within the generally accepted limit of 10 %v/w. Moisture content is an important quality control parameter for herbal extracts as it gives an indication of the stability of extracts on storage because high moisture content of an extract could lead to microbial contamination and/or chemical instability on storage [21]. Lower moisture content is always desired in powders. Insufficient drying leads to spoilage by *molds* and *bacteria* and makes possible the enzymatic destruction of active principles.   Though the value obtained are within limit, they are reasonably high to warrant protection of the extract from environmental moisture by storing in tightly closed containers or in desiccators.

Determination of Microorganisms in *Q. infectoria* galls

Medicinal plant materials normally carry a great number of *bacteria* and *moulds*, often originating in soil. While a large range of *bacteria* and *fungi* form the naturally occurring *microflora* of herbs, aerobic spore-forming *bacteria* frequently predominate**.**

i. Determination of *Escherichia coli* in *Q. infectoria* galls

The examined plant material of *Q. infectoria* galls passed the test for the absence of *Escherichia coli* since no such colonies were detected, and the confirmatory biochemical reactions were negative [22, 23].

ii. Determination of *Salmonella species* in *Q. infectoria* galls

The examined plant material of *Q. infectoria* galls passed the test for the absence of *Salmonella species* as cultures of the type described [22, 23] did not appear in the primary test, and the confirmatory biochemical tests in the secondary test were negative.

Phytochemical Analysis

i. Extract of *Q. infectoria* galls

Extraction is the crucial first step in the analysis of *Q. infectoria* galls, because it is necessary to extract the desired chemical components from the plant material for further characterization and biological study. The powdered galls of *Q. infectoria* was extracted and the properties of extract was given in (Table 1).

ii. Determination of Extraction Yield

Extractive values will be helpful in identification and authentication of the plant material along with the microscopic method, which is the cheapest method to establish the correct identification of the source material; also the extractive methods are useful to evaluate the chemical constituents of the crude drug. Extractive values are representative of the presence of the polar or nonpolar extractable compounds in a plant material. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Extractive value useful for the evaluation of a crude drug and at the same time give idea about the nature of the chemical constituents present, which is helpful for the estimation of specific constituents, soluble in that particular solvent used for extraction. For this purpose we have to determine alcohol-soluble and water soluble extractives. Water soluble extractive value gives idea about presence of tannins, sugars, plant acids, mucilage and other water soluble phytochemicals. It also indicates about drug quality, adulteration and or incorrect processing. The alcohol soluble extractives are also indicatives of the same purpose. The result indicated that *Q. infectoria* galls have high ethanolic extractive value 15.26 % in comparison to the water 9.24% extractive values (Table 3). By following the extraction method, the yield of alcohol soluble extractive is greater than the water soluble extractives. It indicates the possibility of considerable amount of polar and non-polar compounds and presence of large quantity of alcoholic soluble constituents.

Table 3: *Quercus infectoria* galls ethanol and water Extractive values.

Solvent	Weight of sample	Weight of extract	Extraction yield %
Ethanol	500 gm	76.3 g	15.26 %
Water	500 gm	46.2g	9.24 %

The higher extraction yield of ethanol as compared to that of aqueous solvent can be attributed to that ethanol was more efficient in cell walls and seeds degradation which have un polar character and cause phytochemicals especially polyphenols to be released from cells. More useful

explanation for the decrease in activity of aqueous extract can be ascribed to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts, whereas in methanol and ethanol they are inactive. Moreover, water is a better medium for the occurrence of the micro-organisms as compared to ethanol [24]. Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material [25]. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. Methanol is more polar than ethanol but due to its cytotoxic nature, it is unsuitable for extraction in certain kind of studies as it may lead to incorrect results. The higher ethanolic extractive suggests that the ethanolic extract can be used for further phytochemical investigation.

iii. Preliminary Qualitative Phytochemical Screening Study

The quality and quantity of the biologically active compounds from the plant extracts significantly depend on the species, the plant organ and harvest time [26, 27].

Preliminary phytochemical screening was performed to establish the profile of *Q. infectoria* galls ethanolic extract for its chemical composition. An evaluation on the phytochemical screening of galls of *Q. infectoria* extract revealed the presence of medicinally active constituents. The phytochemical active compounds of *Quercus infectoria* galls were screened and the results are presented in (Table 4). 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 cumarines 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. 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 [28, 29, 30]. They act as binders and for treatment of diarrhea and dysentery [31]. 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 [32]. Plant tannin has been recognized for their pharmacological properties and is known to make trees and shrubs a difficult meal for many caterpillars [33]. Plant phenolic compounds especially flavonoids are currently of growing interest owing to their supposed properties in promoting health (anti-oxidants) [34]; 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 [35]. Saponins have expectorant action which is very useful in the management of upper respiratory tract inflammation; saponins present in

plants are cardiotonic in nature and are reported to have anti-diabetic and anti-fungal properties [36, 37]. 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 cardiotonic, 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 [38]. 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 [39]. Anthraquinones and xanthones 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.

Table 4: Preliminary phytochemical screening of *Quercus infectoria* galls ethanolic extract

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

+++= appreciable amount, ++ = average amount, + = trace amount, - = absent

Analytical Method Development Using Spectrophotometric Technique

i. Determination of λmax for ethanolic extract of *Quercus infectoria* galls
The UV spectrum of solution of *Q. infectoria* galls extract (100mg/ml) was scanned between 200-400 nm regions on UV spectrophotometer. λmax of *Q. infectoria* galls ethanolic extract was found to be 296 nm (Figure 1).
ii. Calibration curve of ethanolic extract of *Quercus infectoria* galls
Scanning studies was carried out in UV region, the method for the estimation for the *Q. infectoria* galls extract showed maximum absorption at wavelength 296 nm (λmax) in alcohol. Standard

curve obeyed Beer's law at given concentration range of 0.02 – 0.12 mg/mL (Table 5) and when subjected to regression analysis, the value of regression coefficient was found to be 0.9988 were as shown in (Figure 2, Table 6), which showed linear relationship between concentration and absorbance, equation of a straight line as follows:

$$y = ax + b$$
$$y = 6.265x + 0.0423$$

$$R2 = 0.9988$$

Where (y) stands for absorbance and (x) for *Q. infectoria* galls crude extract concentration in the solution (Figure 2). Value of R2, determination coefficient, indicates the precision of the analytical method.

This developed analytical method is considered reproducible when dealing with analysis of different developed *Q. infectoria* galls crude extract formulations. The maximum absorption showed at wavelength 296 nm.

Table 5: Calibration curve concentration range of 0.02 – 0.12 mg/mL and corresponding absorbance

| Concentration mg/ml	Absorbance *
0.006	0.07
0.0125	0.123
0.025	0.213
0.05	0.3501
0.075	0.51
0.1	0.67

* Mean of three determinations.

Table 6: Data for Calibration curve of ethanolic extract of *Q.infectoria* galls extract

Sr.No.	Parameters	In ethanol
1	Limit of Detection	0.01
2	Limit of Quantitation	0.02
3	Regression Equation	y = 6.265x + 0.0423
4	Correlation Coefficient	0.9988
5	Slope	6.265
6	Beer's law limit (mg/ml)	0.02- 0.12
7	Absorbance maximum (λ max) in nm	296
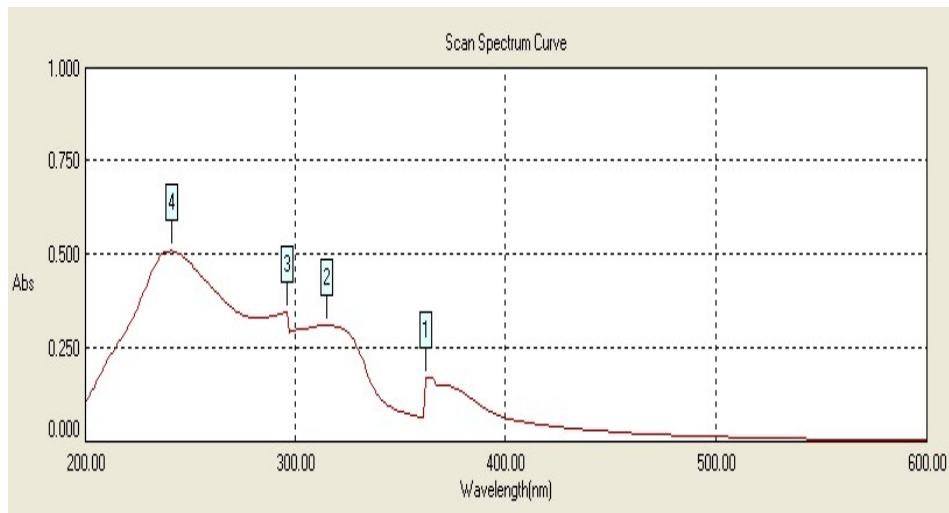
Figure 1:Determination of λmax for ethanolic extract of *Quercus infectoria* galls, UV spectrum of solution of QI gall extract (100mg/ml) was scanned between 200-400 nm regions on UV spectrophotometer.

Concentrations of *Q.I* galls *extract*, in milligrams
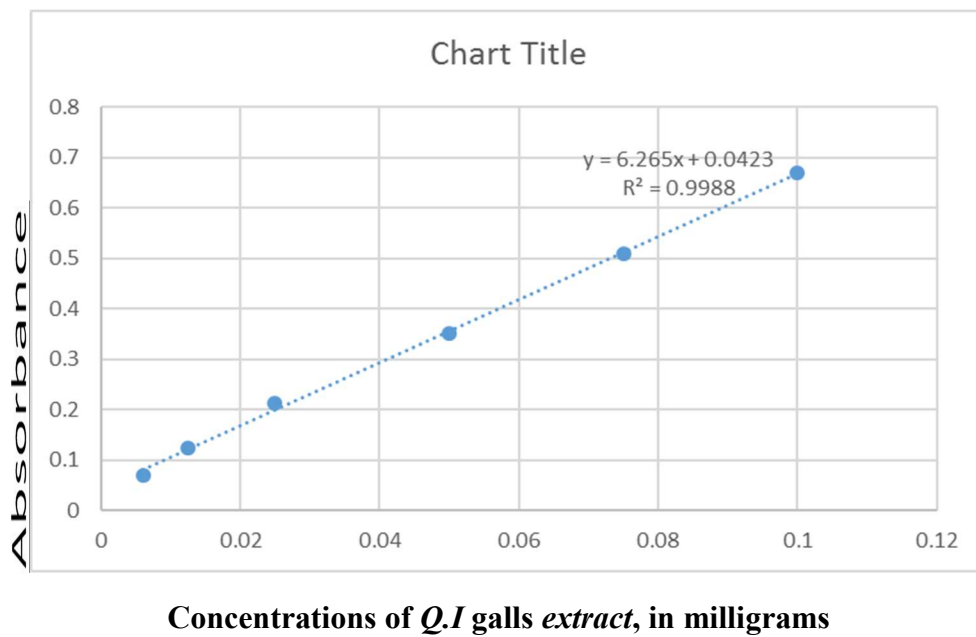
Figure 2: UV-Vis Standard Calibration curve of ethanolic extract of *Q.infectoria* galls

Conclusion

According to WHO, standardization and quality control of herbals is the process involved in the physicochemical evaluations of crude drug covering aspects, the galls of the *Quercus infectoria* (QIG), traditionally believed to have great medicinal value. Pharmacologically the galls are claimed to have 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 flavonoid compounds. Pharmacognostic studies ensures plant identity, lays down standardization parameters which will help and prevents adulterations. Such studies will help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products. The preliminary phytochemical screening will be useful in finding the chemical nature of the drug. In this study, the preliminary phytochemical screening ascertained the presence of tannins, flavonoids, saponins, triterpenes, anthraquinones and cumarines, the various phytochemical compounds detected are known to have beneficial importance in industrial and medicinal sciences. Consequently, the therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. As this drug material is used for various diseases, the established standards of the drug (*Q. infectoria* galls) by this study can be used as an useful information for identifying parameters to substantiate and authenticate the drug of this medicinally important plant. Thus exploring the usefulness of pharmacognostic evaluation to substantiate and authenticate drug. Additionally the physicochemical, phytochemical parameters and analytical method development were reported for the first time. These sets of standards can used in future to assess quality and purity of these plant and for determination of *Q. infectoria* galls extract –based formulations. The results of the present study serve as a valuable source of information and provide suitable standards for identification of this medicinally important plant drug material for future investigations and applications.

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