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COMPATIBILITY ASSESSMENT OF *QUERCUS INFECTORIA* GALLS (*FAGACEAE*) ETHANOLIC EXTRACT WITH CARBOPOL 940 AND XANTHAN GUM POLYMERS AS PRE-FORMULATION STUDY

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ABSTRACT

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. The our previous preliminary phytochemical screening study done was performed to establish the profile of galls extract for its chemical composition, the *Q. infectoria* galls extract showed the presence of tannins, flavonoids, saponins, anthraquinones, triterpenes and cumarines. These secondary metabolites exert antimicrobial activity through different mechanisms. Also in our previous study the ethanolic extract of the *Q. infectoria* galls was tested for their bioactivity against *C. albicans*. Extract of *Q infectoria* galls showed potential activity against the tested fungal isolate. Medicinal plant extracts are API's of great interest for the pharmaceutical industry and have been used as final and intermediate products, resulting in different pharmaceutical forms. Thus, the aim of this study is to evaluate the physical and chemical compatibilities of the ethanolic extract of *Q. infectoria* galls, with pharmaceutical excipients (carbopol 940 and xanthan gum) used in the formulation of pharmaceutical oral gel dosage form. In physical compatibility study each excipient was thoroughly blended with *Q. infectoria* galls extract to

increase drug-excipients molecular contacts and also to accelerate the reaction if possible. Chemical compatibility of the *Q. infectoria* galls extract with carbopol 940 and xanthan gum was determined by FT-IR spectral analysis. Physical compatibility study showed that after 30 days storage of drug extract with excipients in various ratio at room temperature, there is no physical changes observed in the mixture of extract and polymer combinations. FT-IR study showed that there was no major change in the position of peak obtained in the drug alone and in a mixture of extract with excipients, which shows that were no chemical instabilities in drug – excipient combinations, suggesting no evidence of interactions between the functional groups of these components. Based on our results, all mentioned excipients were found to be compatible with extract. This study shows the importance of using instrumental analytical techniques in the early stages of development of herbal medicines to ensure the effectiveness, safety and quality of the final product. The FTIR method, it is proven that FTIR as fast screening tools to check compatibility in early stages of a pre-formulation process. This study also demonstrates the importance of using instrumental techniques in the early stages of development of herbal medicine, selecting excipients that can optimize the activity of *Q. infectoria* galls extract. Thus, this holds great promise for future research for the formulation of potent antifungal drug for the present plant.

KEYWORDS: *Quercus infectoria galls*, Excipients, Analytical techniques, Medicinal plants, Compatibility study, Pre-formulation, FT-IR spectral analysis.

INTRODUCTION

Assessment of possible incompatibilities between the drug and different excipients is an important part of pre-formulation. The formulation of a drug substance frequently involves it being blended with different excipients to improve manufacturability, and to maximize the product's ability to administer the drug dose effectively. Excipients (additives) are used for the production and dispensing of pharmaceuticals. Preferably they should, have no therapeutic effect as well they should be inert towards active pharmaceutical ingredient. Compatibility study of active drug and excipient is vital stage in pre-formulation studies.

Studies of active drug/excipient compatibility represent an important phase in the pre-formulation stage of the development of all dosage forms. The potential physical and chemical interactions between drugs and excipients can affect the chemical nature, the stability and bioavailability of drugs and, consequently, their therapeutic efficacy and safety. If interactions occur between drug and excipient, in such case these interactions (or

degradation) products must be evaluated for safety, and suitable analytical procedures for its identification and quantitation needs to be developed reactions between active drug substances and excipients are of interest in the drug formulation process and should also be considered in the following storage of final preparations.[1-3] The active substance/excipients compatibility studies play the role of identifying in an as short as possible time interactions between potential formulation excipients and the active substance.[4-6] The most frequently methods for the physical-chemical investigations with the view of detecting the possible interactions between the excipients and the active drug substance are thermal analysis (DSC, DTA, DTG, ITC), spectroscopic methods (FT-IR, X-ray diffraction, NMR), chromatographic methods (LC, LC-MS/MS), the dissolution tests etc.[7-12] Fourier Transform Infrared, Raman and Near Infrared Spectroscopy are sensitive to the structure and the environment of organic compounds. These techniques are not only focused on solid state behavior of APIs and their formulations, but are also used as compatibility screening tool as the vibrational changes serve as probe of potential intermolecular interactions among the components. Thus, pharmaceutical interactions that result in desalting, hydrate formation, dehydration, polymorphic changes or transformation of crystalline to amorphous forms and vice versa during processing can easily be detected with the aid of these spectroscopic techniques. However, the presence of overlapping peaks in the spectra may hinder the analysis. Thus, FT-IR helped in the choice of suitable excipients for a stable formulation.

Quercus infectoria is a small tree or a shrub belonging to the *Fagaceae* (*Quercaceae*) family. *Quercus infectoria* gall (QIG) is known by different vernacular names, locally known as Ifas. The plant is found in Turkey, Syria, Persia, Cyprus and Greece.[13] The various *Quercus species* originated in Iran, Iraq and Turkey, but are now widespread and particularly common in Asia Minor, Europe and North Africa.[14] Galls are irregular plant growth, which is stimulated by the reaction between plant hormones and powerful growth regulating chemicals produced by insects or mites.[15] The QI galls are produced by the insect, *Cynipsquercufolii*, for depositing its eggs.[16] The *Quercus infectoria* galls is described in detail in ethnobotanical and literature to possess various pharmacological actions such as analgesic, antidote, anti-inflammatory, antipyretic, antiseptic, anti-stomatitis, deodorant, derivative, desiccant, expectorant, germicidal, hypnotic, hypoglycaemic, powerful astringent, sedative, styptic, tonic, tonic to teeth and gum, and wound healing.[17-22] The galls of *Q. infectoria* have also been pharmacologically and in folk medicine documented to possess antitremorine, local anesthetic[23], antiviral[24], antibacterial[25], larvicidal[26] and antifungal[27] 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.[28,29] 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 our previous preliminary phytochemical screening study done was performed to establish the profile of galls extract for its chemical composition. The *Q. infectoria* galls extract showed the presence of tannins, flavonoids, saponins, anthraquinones, triterpenes and cumarines. These secondary metabolites exert antimicrobial activity through different mechanisms. Also in our previous study the ethanolic extract of the plant was tested for their bioactivity against *C. albicans*. Extract of *Q. infectoria* galls showed potential activity against the tested fungal isolate. It is intriguing to note that crude extracts of *Q. infectoria* galls hold an *anti-Candida* potential, and contain more active compounds allowing recommended therapeutic alternatives to antifungal chemical drugs. Thus effort must be making for isolation, standardization and clinical evaluation of such phytochemicals in order to obtain lead compound/s for further new drug discovery and different formulations can be made in form of gels, ointments, mouth washes and powder to be effectively used for the treatment of different form of oral *candidiasis* in particular and other fungal infections. The incorporation of these dry extracts in formulations is recommended because they are easily obtained, standardized and embeddable in dosage forms. Pre-formulation studies were conducted in order to allow the detection of chemical changes in the formulation of a compound, even at very low levels.[30] In this stage of pharmacotechnical development, it is necessary to evaluate the compatibility between drugs and excipients. This is because interactions can affect the nature of the chemical composition and hence its safety and efficacy.[31]

Thus, the aim of this study is to evaluate the physical and chemical compatibilities of the ethanolic extract of *Q. infectoria* galls, with potential pharmaceutical excipients (carbopol 940 and xanthan gum) used in the formulation of pharmaceutical gel dosage form. Chemical compatibility of the extract with carbopol 940 and xanthan gum was determined by FT-IR spectral analysis, this study was carried out to detect any changes on chemical constitution of the drug after combining it with the excipients.

MATERIALS AND METHODS

Plant Material Collection and Identification

The *Quercus infectoria* galls 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.

All the chemicals used were of analytical grade.

Ethanol was obtained from National Distillation Company. Carbopol 940 and xanthan gum were obtained from CDH – India.

Preparation of Extract

Extraction *Q. infectoria* galls was carried out according to method descried by [32]: 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:

Weight of extract / weight of sample * 100

Drug-Polymers Compatibility Assessment

Excipients are integral components of almost all pharmaceutical dosage forms. The successful formulation of a stable and effective semisolid dosage form depends on the careful selection of the excipients, which are added to facilitate administration, to promote the consistent release and bioavailability of the drug and protect it from degradation. Excipients from different suppliers can propagate or participate in chemical and physical interactions with drug compounds differently due to their different manufacturing conditions. The need to have a physical and chemical compatibility study for the *Q. infectoria* galls extract and excipients was necessary.

a. Physical Compatibility Study

Each excipient (polymer) used in the formulations was blended with the *Q. infectoria* galls levels that are realistic with respect to the final dosage form. Each excipient was thoroughly blended with drug extract to increase drug-excipients molecular contacts and also to accelerate the reaction if possible. extract- excipients blend was taken separately into

container and kept for one month study at room temperature and observe the changes. After one month storage of drug extract with excipients in various concentrations at room temperature, samples were observed for physical changes.

b. Chemical Compatibility Study

Assessment of possible incompatibilities between the *Q. infectoria* galls ethanolic extract and different excipients is an important part of pre-formulation. This involved assessing if there was irreversible degradation of the crude drug functional group to produce a therapeutically inactive or otherwise toxic compound/s.

i. FT-IR Spectroscopy Study

IR measurements were performed using FTIR Spectrophotometer (IR-470; Shimadzu, Japan) by the KBr disc method. The samples were ground, mixed thoroughly with KBr and compressed using IR compression machine and then scanned over the range of 4000 to 400 cm-1. Infrared spectroscopic analysis was done for the powder of *Q. infectoria* galls, polymers (Carbopol 940 and Xanthan gum), physical mixture of drug and polymers 1:1.

RESULTS AND DISCUSSION

Extraction Yield

The result indicated that *Q. infectoria* galls have high ethanolic extractive value 15.26 % in comparison to the water 9.24% extractive value (Table 1). The higher ethanolic extractive suggests that the ethanolic extract can be used for further investigations.

Table 1: *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	g46.2	9.24 %

These are also 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 and at the same time are best to determine the resin content of a drug.

Drug-Polymers Compatibility Assessment

Compatibility study performed between *Q. infectoria* galls ethanolic extract and excipients to assess any compatibility issues which will affect the physiochemical properties of the dosage form which interns alters *in-vivo* performance of dosage form.

a. Study of Interaction of The Extract With Polymers by Physical Compatibility Study

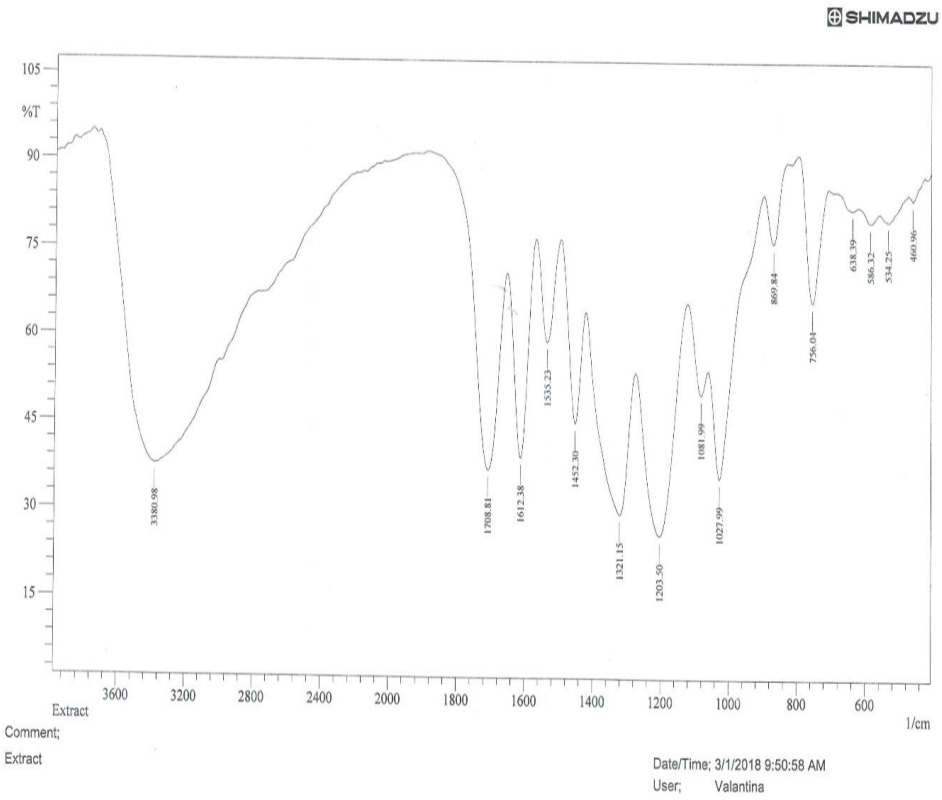
After 30 days storage of drug extract with excipients in various ratio at room temperature, samples were observed for physical changes but there is no physical changes observed in the mixture of extract and polymers combination.

b. Study of Interaction of The Extract With Polymers by Chemical Compatibility Study

i. Fourier Transform Infra-Red Spectroscopy (FTIR) Study

Fourier Transform Infra-Red Spectroscopy study was carried out to know any possible interference between the *Q. infectoria* galls extract and excipients. The present study was in wide usage from many a years in formulation development.

Infrared spectroscopy was performed by KBr- supported over a frequency range of 4000 to 400 cm-1 using FTIR Spectrophotometer (IR-470; Shimadzu, Japan) facility provided at Khartoum University. The *Q. infectoria* galls extract and other formulation ingredients (polymers) were mixed at suitable dilution with KBr and pressed to result the KBr disc. Infrared spectroscopic analysis was done for the dry powder of *Q. infectoria* galls extract, polymers (Carbopol 940 and Xanthan gum), physical mixture of extract and polymers 1:1. FTIR produced by the software was evaluated to know the possible interactions. The infrared (FT-IR) spectra were obtained and performed according to method described previously. Figure 1; a-e, showed characteristic absorption bands obtained in the *Q. infectoria* galls extract alone and in a mixture of drug with excipients (polymers).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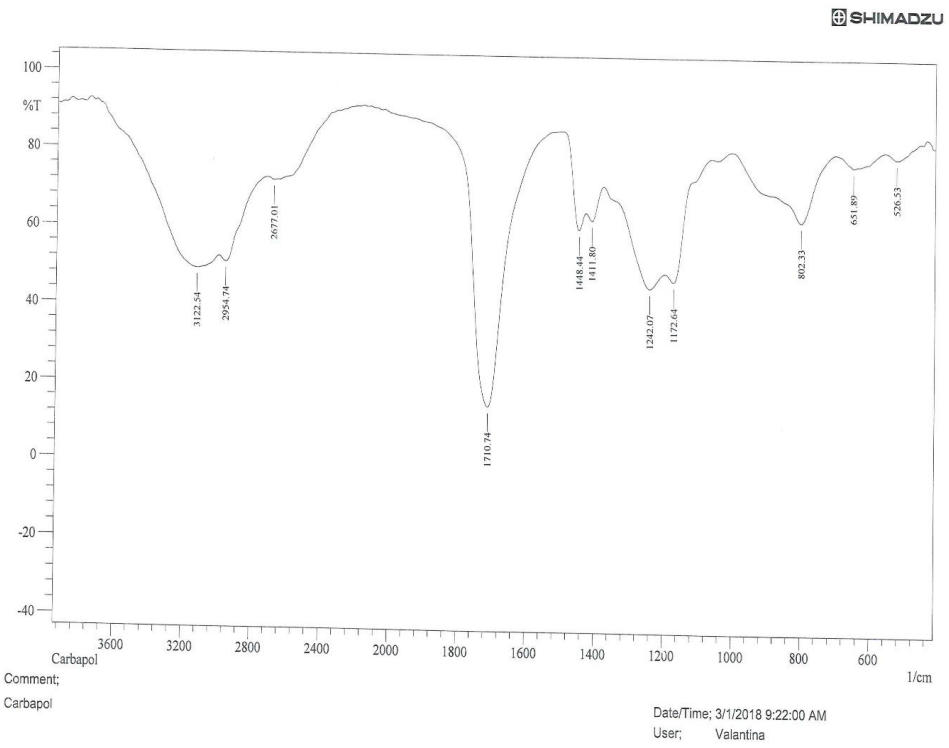
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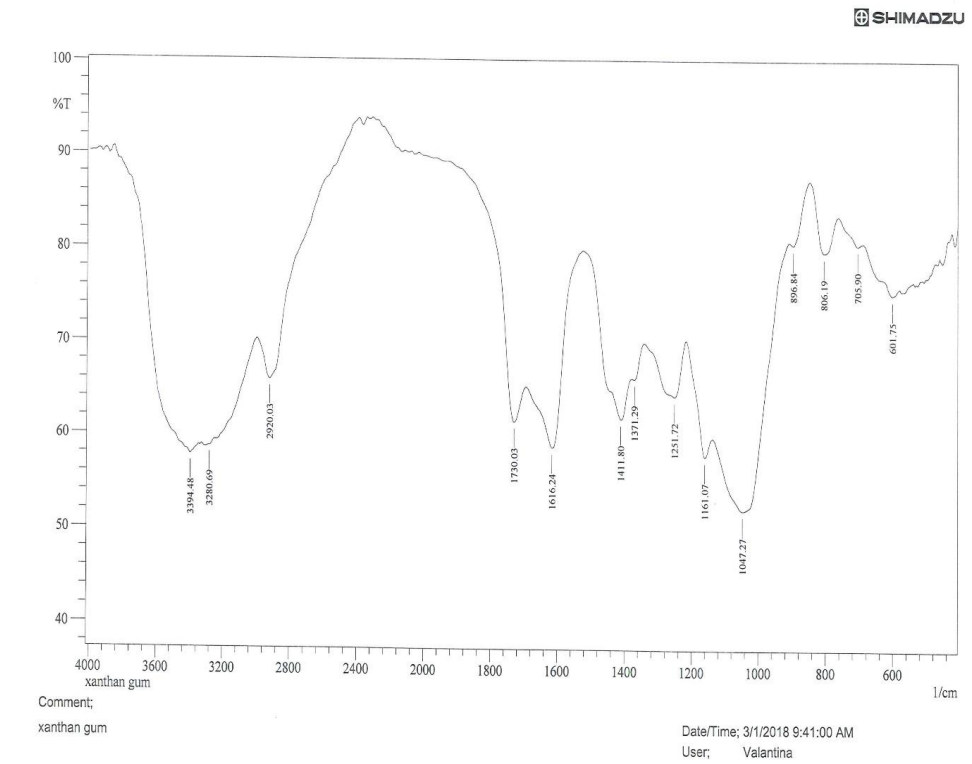
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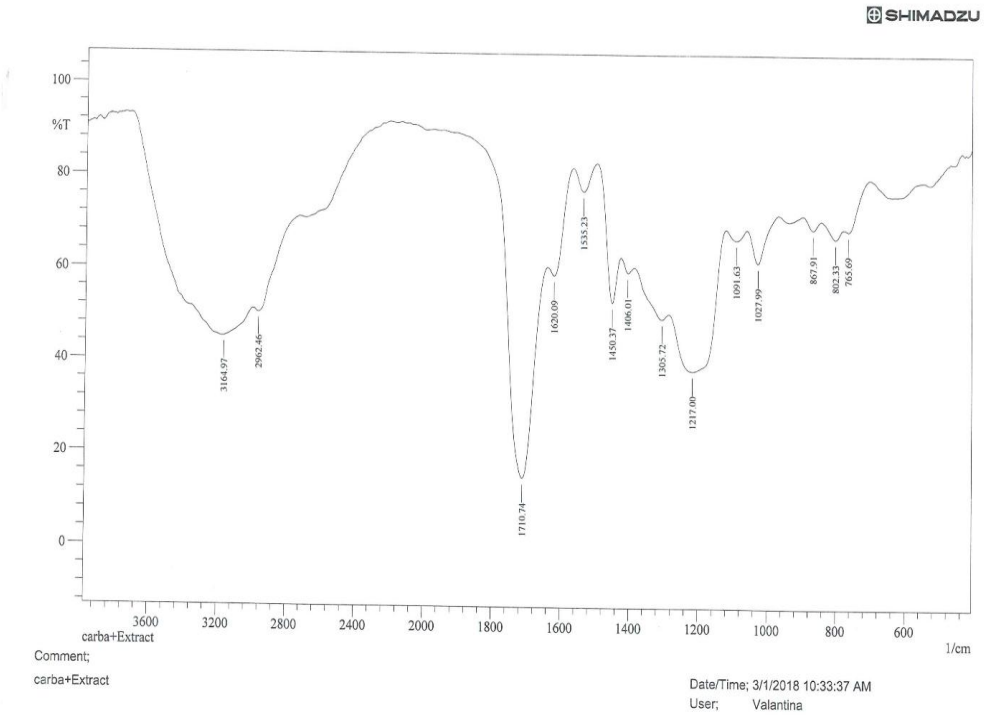
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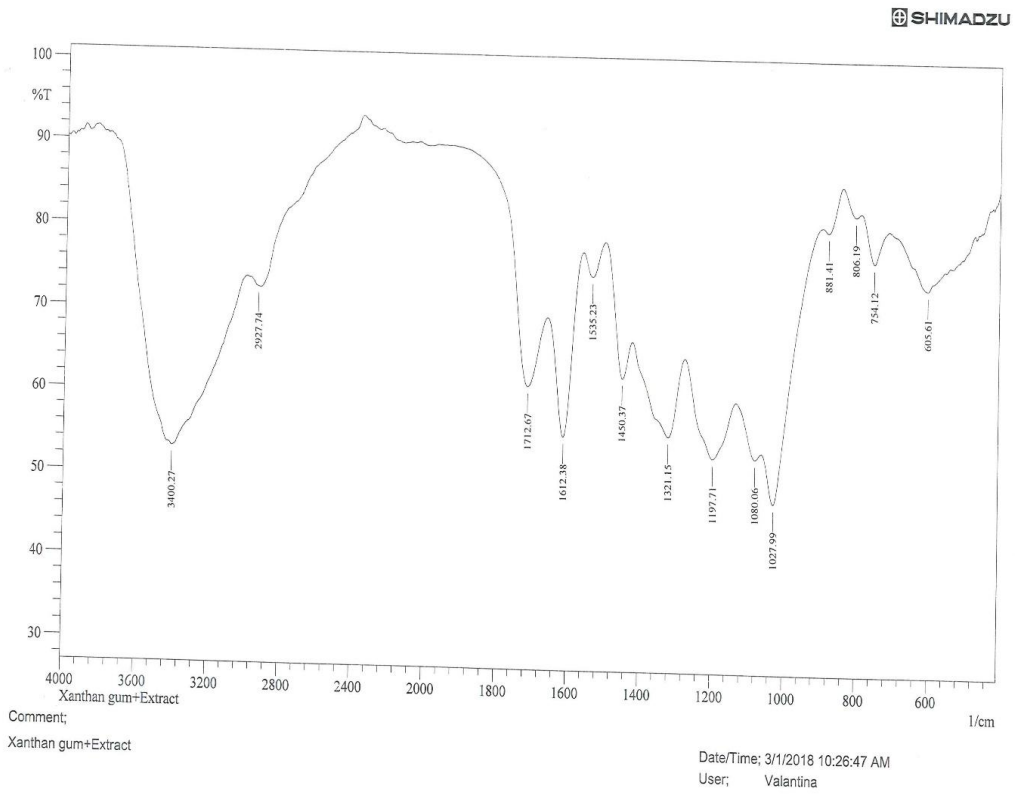

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Figure 1: FT-IR spectra performed using FTIR Spectrophotometer (IR-470; Shimadzu, Japan), scanned over the range of 4000 to 400 cm-1, Infrared spectroscopic analysis was done for the powder of *Q. infectoria* galls, polymers (Carbopol 940 and Xanthan gum), physical mixture of drug and polymers 1:1.

a) FT-IR spectra of Extract.

b) FT-IR spectra of Carbopol 940 polymer.

c) FT-IR spectra of Xanthan gum.

d) FT-IR spectra of physical mixture of Extract and Carbopol 1:1.

e) FT-IR spectra of physical mixture of Extract and Xanthan gum 1:1.

FTIR spectroscopy has been successfully used for exploring the differences in molecular conformations, crystal packing and hydrogen bonding arrangements for different solid state forms of an organic compound. Spectral variations originates due to alteration in bonds that exhibit characteristic vibrational frequencies, leading to frequency shifts and splitting in absorption peaks.[33] The FTIR spectrum of *Q. infectoria* galls extract (Figure 1 a) showed characteristic absorption bands, when the extract were incorporated with carbopol 940

polymer, the observation from the prominent peaks obtained from carbopol 940 alone and the mixture of both the extract with carbopol (figure 1 b and d) showed that there was no major change in the position of peak obtained in the extract alone and in a mixture of extract with Carbopol 940, indicated no distinct interaction. Similarly this was repeated with xanthan gum as showed in (figure 1 c and e) prominent peaks were observed indicating no interference and no incompatibility in the mixture. FT-IR study showed that there was no major change in the position of peak obtained in the drug alone and in a mixture of drug with excipients, which shows that were no chemical instabilities in drug – excipient combinations, suggesting no evidence of interactions between the functional groups of these components. From the results of FTIR method, it is proven that FTIR as fast screening tools to check compatibility in early stages of a pre-formulation process. Based on our results, all mentioned excipients were found to be compatible with *Q. infectoria* galls ethanolic extract.

CONCLUSION

The use of instrumental analytical techniques is a valuable tool for the pharmaceutical industry. The active substance/excipients compatibility studies play the role of identifying in an as short as possible time interactions between potential formulation excipients and the active substance. The FTIR method, it is proven that FTIR as fast screening tools to check compatibility in early stages of a pre-formulation process. With FTIR spectroscopy, it was possible to indicate excipients that may facilitate formulation of the dry extract from *Q. infectoria* galls, outlining a pharmaceutical form for this plant whose pharmacological activities have long been known by people from different regions of the world and proven by several studies including our previous study.

Based on our results, all mentioned excipients were found to be compatible with *Q. infectoria* galls ethanolic extract. This study also demonstrates the importance of using instrumental techniques in the early stages of development of herbal medicine, selecting excipients that can optimize the activity of the extract of from *Q. infectoria* galls. Thus, this holds great promise for future research for the formulation of potent antifungal drug for the present plant.

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