Phytochemical study and thin layer chromatography of Ficusreligiosa leaves extract cultivated in Iraq

Noor S Jaafar*, Maha N Hamad, Duha A Alshammaa*, Zainab S Noori***
***Department of pharmacognosy and Medicinal Plants, College of Pharmacy, University of Baghdad, Baghdad, Iraq.**
****Department of pharmacognosy and Medicinal Plants, Baghdad College for medicinal sciencesBaghdad, Iraq**

Article Info:

Received 26 May 2021
Accepted 26 Jul 2021
Published Jul 2021

Corresponding Author email:
Zainab.nouri@copharm.uobaghdad.edu.iqiq
orcid: https://orcid.org/0000-0002-2697-882X

Abstract

Ficusreligiosa Linn, (Moraceae), is a large evergreen or deciduous, irregularly shaped tree. Traditionally the leaves are used for the treatment of constipation, vomiting, hiccup, and others. Leaves were extracted by two methods; maceration and soxhelt using hexane and 80% aqueous methanol, then subjected to preliminary phytochemical examination, fractionation with chloroform, ethyl acetate, and n.butanol, then TLC. Soxhelt was the suitable extraction method. Sterols, alkaloids, saponins, tannins, and flavonoids were identified in leaves. TLC examination demonstrates the possible presence of stigmasterol, chlorogenic acid, caffeic acid, rutin, and luteolin or apigenin.

Key words: *Ficusreligosa*, percentage yield, TLC, Stigmasterol, and chlorogenic acid.

دراسة كيميائية نباتية وكروماتوجرافيا الطبقة الرقيقة لمستخلص أوراقالتين المقدس المستزرع في العراق

نور صباح جعفر*، مها نوري حمد، ضحى عبد الصاحب الشماع*، زينب صفاء نوري***
***فرع العقاقير والنباتات الطبية، كلية الصيدلة، جامعة بغداد**
****فرع العقاقير والنباتات الطبية، كلية بغداد للعلوم الطبية**

الخلاصة:

ينتمي نبات التين المقدس للعائلة التوتية وتكون الشجرة دائمة الخضرة أو نفضية ، على شكل غير منتظم. تقليديا الأوراق تستخدم لعلاج الإمساك والقيء والفواق وغيرها. تم استخلاص الأوراق بطريقتين ؛ بالتنقيع وباستخدام جهازSoxheltباستخدام الهكسان والميثانول المائي 80 ٪ ، ثم خضع مستخلص الاوراق لفحص كيميائي أولي ، تجزئة مع الكلوروفورم ، أسيتات الإيثيل والبوتانول ثم TLC وكانSoxheltطريقة استخلاص مناسبة. تم تحديد الستيرويد والقلويدات والسابونين والفلافونيدات في الأوراق. يُظهر فحص TLC احتمال وجود ستيغماسترول ، وحمض الكلوروجينيك ، وحمض الكافييك، والروتين ، واللتيولين ، أو الأبجينين.

الكلمات المفتاحية: التين المقدس - النسبة المئوية للإنتاج- الستغماستيرول- حامض الكلوروجينيك.

Introduction

The medicinal value displayed by diverse curative plants arises from the phytochemicals existing in the plant, where some of these phytochemicals demonstrate therapeutic effects, and considered as lead compounds for providing pharmaceuticals having a crucial role in curing destructive diseases (1, 2). However in contemporary pharmacopeia not less than 25% of drugs are derived from plants and many others, synthetic analogs, based on lead molecules isolated from plants (3).

Phytochemicals are bioactive constituents of plants' origin, naturally formed or synthesized in plants from primary metabolites, these chemicals not essential for plant growth or development but produced as a defensive agent against microorganisms, insects, and herbivores predation (4, 5). The fineness of the quality of herbal medicine and hence the secondary metabolites produced even in the same land or country are affected by various environmental factors like climate, height, rain full, and others conditions (4).
F. religiosa Linn, (Moraceae), local Arabic name teen mukadas, shajaratbebal (6). It is a large evergreen or deciduous, irregularly shaped, widely branched tree, up to 20-meter length (7, 8), considered as one of the longest living trees (9) distributed in most Asian countries (10) and cultivated as an ornamental tree (11). The shiny, thin leaves bear 5-7 vein when their first appear are red pinkish then change to cooper and finally at maturity turned dark green, these leaves grow to about 5-7 inches are ovate, cordate in shape, leathery with distinctive extended drip tip (12, 13). Different phytochemicals had been identified in leaves as tannins, flavonoids, sterols, and others. Traditionally the leaves alone are used for the treatment of constipation and show significant memory-enhancing effects, for vomiting, hiccup, dysentery, hemorrhoids, ulcer urological disorders, and another disease (14-16). Leaf's decoction and juice are used for toothache, asthma, hematuria, migraine, gastric problems, and scabies (17) leaves proven to have anti-diabetic effect (18). This work aimed to extract the leaves by two different methods, to investigate the secondary metabolites and to perform simple TLC analysis to give initial information concerning the type of sterols, phenols, flavonoids aglycones, and glycosides.

Materials and methods

Collection of plant material

Fresh leaves of F.religosa were obtained from the college of pharmacy / University of Baghdad in November (2017). The plant was authenticated by Dr. Sukaina Abbas in the college of science / Baghdad University. Leaves were washed thoroughly under running tap water, dried under shade for 10 days, ground in a mechanical grinder to a fine powder, and used for this study.

Preparation of plant extracts

For the preparation of crude extracts from leaves of F.religosa two different extraction methods were followed.

Maceration

Fifty grams of shade-dried pulverized plant material was macerated in 400mL of hexane with occasional stirring for one week for defatting, after defatting the marc was dried then macerated with 400 mL 80% methanol for one week, the extract was filtered, the solvent was evaporated to dryness using a rotary evaporator and the percentage yield was determined.

Soxhelt extraction

Fifty grams of shade-dried pulverized plant materials were extracted by maceration with 400 mL hexane for one week, after defatting the marc was dried, packed in a thimble of soxhelt, extracted with 80% aqueous methanol (methanol: water 80:20) for one week. The extract was filtered and the solvent was evaporated under reduced pressure using a rotary evaporator to get a dry extract. The percentage yield was calculated.

Fractionation

Crude methanolic extract (5 gm), obtained by soxhelt extractor was suspended in 50 ml distilled water and consequently fractionated by partitioning successively with chloroform, ethyl acetate, and finally by n.butanol using 50 mL x 3 for each fraction. The first two fractions were dried over anhydrous sodium sulfate, filtered, concentrated by rotary evaporator, air-dried, and then stored for further analysis.

Preliminary phytochemical investigation

The freshly prepared hydro-methanolic extract and powdered leaves were subjected to phytochemical analysis for flavonoids, phenols, alkaloids, phytosterols, and saponins (19-23).

2.4 TLC determination of phytochemicals

Thin-layer chromatography was used to verify and provide a prim prediction for constituents based on retardation factor value (Rf) by comparing the Rf of standard materials to the Rf of separated constituents. TLC for various fractions was accomplished on aluminum plates coated with 0.25mm silica gel 60 F254 as stationary phase. These plates were developed in different mobile phases according to the type of the tested phytochemicals. Detection of the separated spots was done by observation of the developed TLC plate under UV light (254 and 366nm) and after spraying with suitable spraying reagent. Table 1 illustrates the type of phytochemical, composition of the mobile phase, and spraying reagents used for theirid-entification (24).

Table (1): Phytochemicals, the composition of the mobile phase, and spraying reagents used for their detection.

phytochemicals	Mobile phase used	Composition	Spray reagent used
Phytosterols	chloroform: ethyl acetate	6:4	5% H_2SO_4
Phenolic compounds	ethyl acetate: toluene: formic acid	3:3:1	5% ferric chloride solution
Flavonoid's glycosides	n.butanol: acetic acid: water	14:3:3	5% alcoholic KOH
Flavonoids aglycone	toluene: ethyl acetate: formic acid	10:9:1	5% alcoholic KOH

Result and discussion

Before analyzing, separating, and isolating the phytochemical constituents of the herbs, extraction must be performed. Extraction is an essential step in the analysis of medicinal plants since it is fundamental to acquire and purify the desired chemical components from the plant for further characterization (25), two extraction methods were used; cold maceration and soxhelt extraction. The effect of extraction methods is determined based on percentage yield. Percentage or extraction yield is (the weight or mass of extract / the weight or mass of dry matter). Different factors affect the extraction process, the effect of these factors can be compared through the measurement of percentage yield (26).

% yield = (weight of dry extract / weight of dry powder) x 100

Soxhelt extraction gave a higher percentage yield than maceration, this may be attributed to the fact that the plant powder is continuously extracted, i.e., the solvent saturated in dissolvable components or metabolites siphoned into the flask, fresh re-condensed solvent re-extracts the plant material in the thimble (27, 28). Extraction by soxhelt is preferred over maceration since no filtration, and less amount of solvent is required (29, 30). The result of the percentage yield is summarized in table 2.

Table (2): Percentage yield of the extraction method

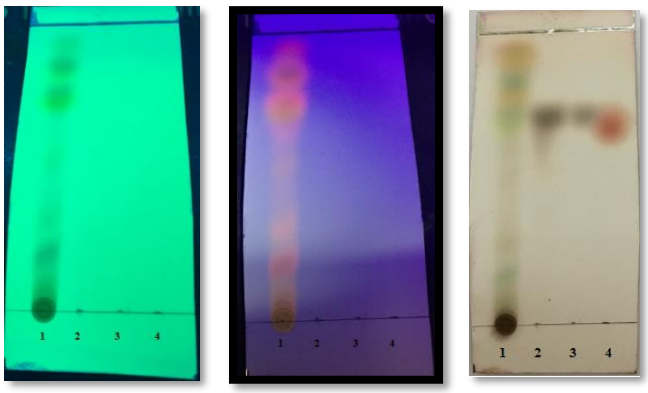
Extraction method	Maceration	Soxhelt
Percentage yield	16.68	22.23

Preliminary phytochemical screening tests are useful in the recognition of bioactive principles and consequently may lead to drug or medicine discovery and development. In this study, several phytochemical constituents have been identified, these include flavonoids, tannins, saponins, alkaloids, and sterols (6, 31, 32). The result of the phytochemical analysis is shown in table 3

Table (3): Qualitative profile of the phytochemicals found in leaves of the plant.

Extract	Flavonoids	Alkaloids	Phenols	Saponins	Sterols
Leaves	+	+	+	+	+

Regarding TLC analysis for sterols, chloroform fraction shows 7 spots with R_f values 0.19, 0.3, 0.53, 0.71, 0.79, 0.85 and 0.94 (table 4) Phytosterol (R_f 0.71) could be stigmasterol(33)or β-sitosterol. These two phytosterols have similar $R_f$value which could be attributed to structural similarity and approximate molecular weight (34). Stigmasterol was identified previously in *F. religosa* leaves(33).



(a) (b) (c)

Figure (1): Chloroform fraction, 2: β-sitosterol, 3: Stigmasterol, 4: Cholesterol. a: Under UV light 254nm, b: Under UV light 366nm, c: Ufter spraying with 5% H_2SO_4.

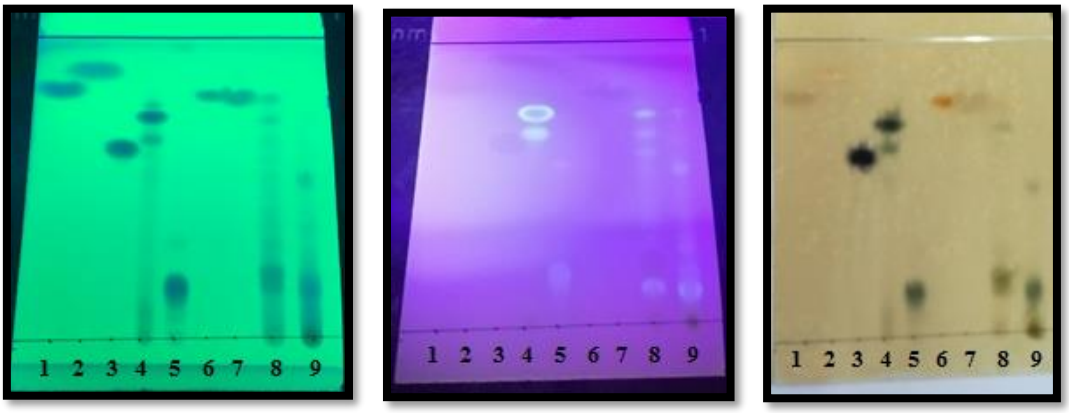
Table (4): $R_f$valuesof sample and standards

Sample	No. of spots after spraying	R_f values
Chloroform fraction	7	0.19, 0.3, 0.53, 0.71, 0.79, 0.85, 0.94
β-sitosterol	1	0.71 gray spot
Stigmasterol	1	0.71 green spot
Cholesterol	1	0.7 pink spot

Concerning phenolic compounds, after derivatization, the ethyl acetate revealed the presence of three compounds with $R_f$values (0.26, 0.33, 0.75), while n.butanol fraction shows the presence of two compounds with R_f values (0.17, 0.53) (table 5). By comparing the $R_f$value of separated compounds with R_f of reference compounds, caffeic acid was detected in ethyl acetate fraction, while chlorogenic acid was detected in n.butanol fraction. chlorogenic acid is an ester of caffeic acid and quinic acid (35) Chlorogenic acid was detected in fruits, leaves, and bark of *F.religosa* in previous phytochemical studies(36).

Table (5): R_f values of samples and standards

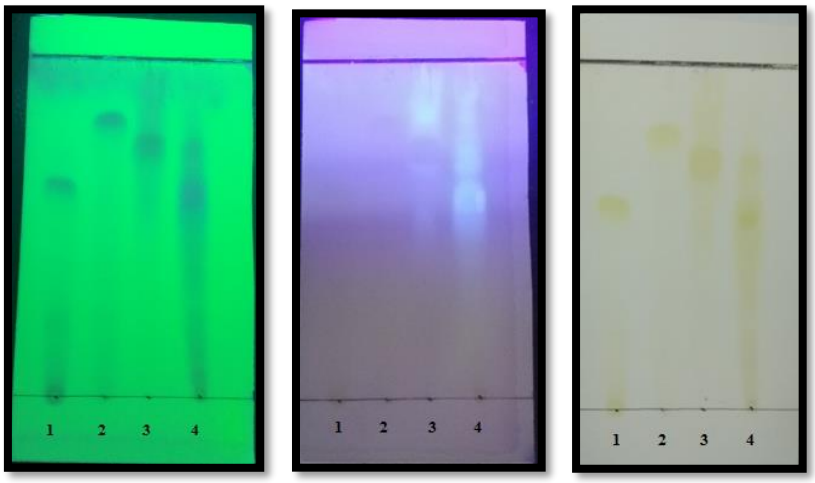
Compound	No. of spots after spraying	R_f values
Ethyl acetate fraction	3	0.26, 0.33, 0.75
n.butanol fraction	2	0.17, 0.53
Vanillin	1	0.82 beige
Benzoic acid	1	0.89 light orange
Gallic acid	1	0.6 dark blue
Caffeic acid	2	0.75 dark blue
Chlorogenic acid	1	0.17 olive green
p. coumaric acid	1	0.78 orange
Resorcinol	1	0.78 beige



(a) (b) (c)

Figure (2): TLC chromatogram of standard material and tested fractions. Detection (a) under UV light 254nm, (b): under UV light 366nm and (c): after spraying with 5% $FeCl_3$ solution. 1: Vanillin, 2: Benzoic acid, 3: Gallic acid, 4: Caffeic acid, 5: Chlorogenic acid, 6: p. coumaric acid, 7: Resorcinol, 8: Ethyl acetate fraction, 9: n.butanol fraction.

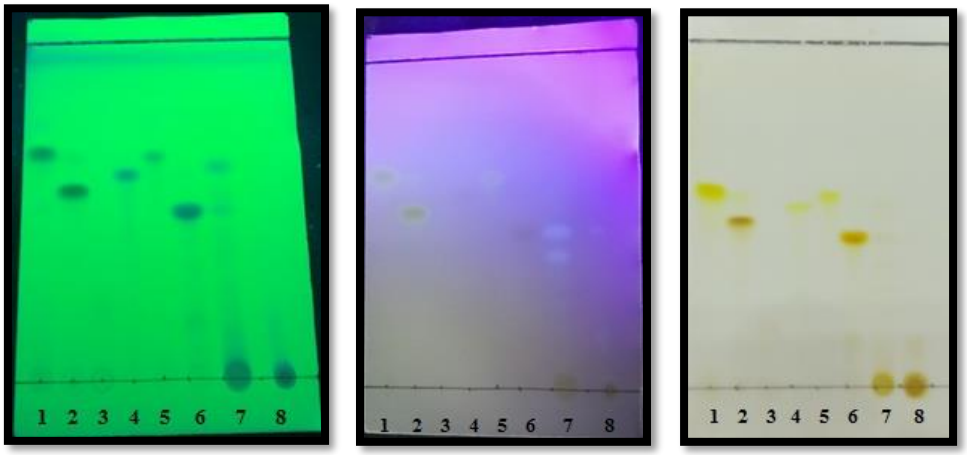
For flavonoid glycosides, the ethyl acetate fraction after spraying with 5% alcoholic KOH, showed the presence of a single yellow spot (Rf value = 0.68). n.butanol fraction showed the presence of two yellow spots (Rf values= 0.56, 0.67) respectively. One of the separated compounds in n.butanol fraction might be rutin, (rutin standard Rf value= 0.57). Previous phytochemical studies verified the presence of rutin in leaves of *F.religosa* and other species (36, 37).



(a) (b) (c)

Figure(3): TLC chromatogram of standard material and tested fractions. Detection (a) under UV light 254nm, (b): under UV light 366nm and (c): after spraying with 5% alcoholic KOH solution. 1: Rutin, 2: Quercetrin, 3: Ethyl acetate fraction, 4: n.butanol fraction.

For flavonoids aglycons, in ethyl acetate and n.butanol fractions, four compounds (R_f values = 0.11, 0.36, 0.45, 0.54) and one compound (R_f value 0.11) were visualized respectively after derivatization. Based on $R_f$values of standards, compounds ($R_f$values = 0.45, 0.54) in ethyl acetate fraction could be luteolin and apigenin respectively.



(a) (b) (c)

Figure (4): TLC chromatogram of standard materials and tested fractions. Detection (a) under UV light 254nm, (b): under UV light 366nm and (c): after spraying with 5% alcoholic KOH solution. 1: Kaempferol, 2: Quercetin, 3: Myricetin, 4: Apigenin, 5: Isorhamnetin, 6: Luteolin, 7: Ethyl acetate fraction, 8: n.butanol fraction.

Table (6): R-values of samples and standards

Compound	No. of spots after spraying	R_f values
Ethyl acetate fraction	4	0.11, 0.36, 0.45, 0.54 faint yellow
n.butanol fraction	1	0.11 faint yellow
Kaempferol	1	0.58 yellow
Quercetin	1	0.47 yellow
Myricetin	1	0.16 faint yellow
Apigenin	1	0.54 yellow
Isorhamnetin	1	0.56 yellow
Luteolin	1	0.45 yellow

Conclusion

The soxhlet extraction method is an appropriate tool to extract phytochemicals from plant materials. Soxhelt was the suitable extraction method based on percentage yield.

From the results of the phytochemical screening, it can be proved that *F.religosa* is a valuable source of phytochemicals like flavonoids, tannins, and sterols which were initially detected through thin layer chromatography examination by matching Rf values of separated constituents with reference standards. Further studies are required to confirm the presence of the detected compounds and to identify the unknown separated spots and elucidation their structures.

Acknowledgment

The authors thankful to the college of pharmacy/ Baghdad University for their valuable support this work.

References

1- Khalid S, Adil Shahzad N, Muhammad A, Anwar P. Phytochemical screening and analysis of selected medicinal plants in Gujrat. Journal of Phytochemistry and Biochemistry. 2018;2:108.

2- Segneanu A, Grozescu I, Sfirloaga P. The influence of extraction process parameters of some biomaterials precursors from Helianthus annuus. Digest Journal of Nanomaterials & Biostructures (DJNB). 2013;8(4).

3- Madhu M, Sailaja V, Satyadev T, Satyanarayana M. Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. Journal of Pharmacognosy and Phytochemistry. 2016;5(2):25.

4- Santhi K, Sengottuvel R. Qualitative and quantitative phytochemical analysis of Moringa concanensis Nimmo. International Journal of Current Microbiology and Applied Sciences. 2016;5(1):633-40.

5- Figueiredo AC, Barroso JG, Pedro LG, Scheffer JJ. Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour and Fragrance journal. 2008;23(4):213-26.

6- Al-Snafi AE. Pharmacology ofF. religiosa-A review. IOSR Journal of Pharmacy. 2017;7(3):49-60.

7- Makhija IK, Sharma IP, Khamar D. Phytochemistry and Pharmacological properties of F. religiosa: an overview. Annals of Biological Research. 2010;1(4):171-80.

8- Rutuja RS, Shivsharan U, Shruti AM. F. religiosa (Peepal): A Phytochemical and pharmacological review. Interantional Journal of Pharmaceutical and Chemical Sciences. 2015;4:360-70.

9- Paliwal D. Prelimi ary ad pharmacological profile of F. religiosa l.: a overview. 2011.

10- Melinda KP, Rathinam X, Marimuthu K, Diwakar A, Ramanathan S, Kathiresan S, et al. A comparative study on the antioxidant activity of methanolic leaf extracts of F. religiosa L, Chromolaena odorata (L.) Cynodon dactylon (L.) Pers. and Tridax procumbens L. Asian Pacific Journal of Tropical Medicine. 2010;3(5):348-50.
11- Bhalerao SA, Sharma AS. Ethenomedicinal, phytochemical and pharmacological profile of F. religiosa Roxb. Int J Curr Microbiol App Sci. 2014;3(11):528-38.
12- Gautam S, Meshram A, Bhagyawant SS, Srivastava N. F. religiosa-potential role in pharmaceuticals. International journal of pharmaceutical sciences and research. 2014;5(5):1616.
13- Jangde RK. Plant Profile of Ficus religosa: A Review. Research Journal of Science and Technology. 2015;7(4):193.
14- Kumar A, Sandeep D, Tomer V, Gat Y, Kumar V. F. religiosa: A wholesome medicinal tree. Journal of Pharmacognosy and Phytochemistry. 2018;7(4):32-7.
15- Kaur A, Rana A, Tiwari V, Sharma R, Kumar S. Review on ethanomedicinal and pharmacological properties of F. religiosa. Journal of applied pharmaceutical science. 2011;1(8):6-11.
16- Khan MSA, Hussain SA, Jais AMM, Zakaria ZA, Khan M. Anti-ulcer activity of F. religiosastem bark ethanolic extract in rats. J Med Plants Res. 2011;5(3):354-9.
17- Gulecha V, Sivakumar T, Upaganlawar A, Mahajan M, Upasani C. Screening ofF. religiosaleaves fractions for analgesic and anti-inflammatory activities. Indian journal of pharmacology. 2011;43(6):662.
18- Choudhary S, Pathak AK, Khare S, Kushwah S. Evaluation of antidiabetic activity of leaves and fruits ofF. religiosa Linn. International Journal of Pharmacy & Life Sciences. 2011;2(12).
19- Ahmed OH, Hamad MN, Jaafar NS. Phytochemical investigation of Chenopodium murale (Family: Chenopodiaceae) cultivated in Iraq, isolation and identification of scopoletin and gallic acid. Asian J Pharm Clin Res. 2017;10(11):70-7.
20- Iqbal E, Salim KA, Lim LB. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of Goniothalamus velutinus (Airy Shaw) from Brunei Darussalam. Journal of King Saud University-Science. 2015;27(3):224-32.
21- Ramamurthy V, Sathiyadevi M. Preliminary phytochemical screening of methanol extract of Indigofera trita Linn. J Mol Histol Med Physiol. 2017;2(1):100011.
22- UC R, NAIR VMG. Phytochemical analysis of successive reextracts of the leaves of Moringa oleifera Lam. International Journal of Pharmacy and pharmaceutical sciences. 2013;5:629-34.
23- Alabri THA, Al Musalami AHS, Hossain MA, Weli AM, Al-Riyami Q. Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of Datura metel L. Journal of King Saud University-Science. 2014;26(3):237-43.
24- El Yahyaoui O, Ouaaziz NA, Guinda I, Sammama A, Kerrouri S, Bouabid B, et al. Phytochemical screening and thin layer chromatography of two medicinal plants: Adansonia digitata (Bombacaceae) and Acacia raddiana (Fabaceae). Journal of Pharmacognosy and Phytochemistry. 2017;6(1):10-5.
25- Soares MO, Alves RC, Pires PC, Oliveira MBP, Vinha AF. Angolan Cymbopogon citratus used for

therapeutic benefits: Nutritional composition and influence of solvents in phytochemicals content and antioxidant activity of leaf extracts. Food and chemical toxicology. 2013;60:413-8.

26- Dhanani T, Shah S, Gajbhiye N, Kumar S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. Arabian Journal of Chemistry. 2017;10:S1193-S9.

27- Jansirani D, Saradha R, Salomideborani N, Selvapriyadharshini J. Comparative evaluation of various extraction methods of curcuminoids from Curcuma longa. Journal of Chemical and Pharmaceutical Science. 2014:286-8.

28- Sarker SD, Nahar L. An introduction to natural products isolation. Natural products isolation. 2012:1-25.

29- Sharifi N, Mahernia S, Amanlou M. Comparison of different methods in quercetin extraction from leaves of Raphanus sativus L. Pharmaceutical Sciences. 2016;23(1):59-65.

30- Azwanida N. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants. 2015;4(196):2167-0412.

31- Prakash V, Gandotra S, Kumar P, Singh N. Phytochemical Screening and Antimicrobial Activity of F. religiosa. Journal of Pharmaceutical Sciences and Research. 2017;9(2):100.

32- Saha S, Goswami G. Study of anti ulcer activity of F. religiosa L. on experimentally induced gastric ulcers in rats. Asian Pacific Journal of Tropical Medicine. 2010;3(10):791-3.

33- Chitra Gupta S. Taxonomy, phytochemical composition and pharmacological prospectus of F. religiosaLinn.(Moraceae)—A review. The Journal of Hytopharmacology. 2012;1(1):57-70.

34- Jaber BM, Jasim SF. Phytochemical study of stigmasterol and β-sitosterol in Viola odorata plant cultivated in Iraq. Iraqi journal of biotechnology. 2014;13(2).

35- Jaafar NS, Hamad MN, Alshammaa DA, Abd MR. Preliminary phytochemical screening and high performance thin layer chromatography [HPTLC] detection of phenolic acids in Lanatacamara leaves cultivated in Iraq International research journal of pharmacy. 2018;9(7):59-64.

36- Kumar S, Singh A, Kushwaha AK, Tiwari R, Chaudhary LB, Srivastava M, et al. The UPLC–ESI–QqQLIT–MS/MS method for quantitative determination of phytochemicals in ethanolic extracts of different parts of eight Ficus species: Development and validation. International Journal of Food Properties. 2018;21(1):328-44.

37- Trifunschi SI, Ardelean DG. Flavonoid extraction from Ficus carica leaves using different techniques and solvents. Zbornik Matice srpske za prirodne nauke. 2013(125):81-6.