Received: 25 June 2020 | Revised: 10 August 2020 | Accepted: 12 August 2020
DOI: 10.22034/ecc.2020.113247
Eurasian Chemical Communications
SAMI

FULL PAPER

Comparing different extraction methods for oral syrup formulation of major bioactive compounds from *Cordia Myxa* fruit

Arash Larki[a] |Nader Shakiba Maram[b] |Amanollah Zarei Ahmady[c,*] |Neda Mohtasham[d] |Ladan Mafakher[e] |Nooshin Khelghati[e] |Ehsan Hedayati[f]

[a]*Department of Marine Chemistry, Faculty of Marine Science, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran*

[b]*Nanotechnology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[c]*Medicinal Plant Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[d]*Department of Clinical Toxicology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[e]*Medicinal Plant Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[f]*Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

***Corresponding Author:**
Amanollah Zarei Ahmady
Email: zarei-a@ajums.ac.ir
Tel.: +98-613-373-8378

The fruit of *Cordia myxa (C. myxa)* is widely used for the treatment of respiratory and urinary infections, and as a diuretic, astringent, demulcent and expectorant agent. Other pharmacological effects such as anti-inflammatory, antibacterial, antiviral, anti-allergic, antitumor and antioxidants activity have also been reported for C. myxa by other studies. This study aimed to compare different extraction methods and offer a way to produce an easy-to-use formulation. In this study, extracts from the fresh and dried fruits of *C. myxa* were obtained using four extraction methods including soxhlet, maceration, percolation and digestion. Extraction from the dried fruits showed better results compared with that of the fresh fruits. Also, the soxhlet method of extraction using dry powdered fruits was the most efficient for extraction of active components of *C. myxa* fruits. Preliminary phytochemical screening showed the aqueous extract of fruits to be full of active ingredients such as alkaloids, flavonoids, tannins, phenolic compounds, Steroids, carbohydrates, and saponins. Finally, an oral syrup formulation from *C. myxa* fruits extract was prepared for the first time and its physicochemical properties such as light transmittance, visual inspection, pH measurement, sucrose concentration, viscosity, and antimicrobial tests were evaluated. The applied method is a quite easy, simple, environmental friendly and convenient way for extraction and oral syrup preparation of *C. myxa* fruits.

KEYWORDS

Medicinal plants; *cordia myxa*; extraction; oral syrup; Rutin; soxhlet.

Introduction

According to World Health Organization, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be applied for therapeutic purposes (1). Containing a multitude of chemical compounds like alkaloids, flavonoids, glycosides, saponins, tannins, resins, sesquiterpene, and oils, these phytochemicals have been used for centuries in treatment and prevention of diseases (2). These active constituents are usually secondary metabolites, derived from biosynthetic pathways present within plant tissue. Currently, there is an almost obsession on Medicinal plants as a huge source of therapeutic phytochemicals which could be a enormous source of developing of novel drugs. Herbal medicines have the ability to affect

body systems variably depending on the chemical constituents present in the used plant (3). Most of the phytochemicals from plant sources such as phenolics and flavonoids have been reported to have a positive impact on health and cancer prevention (4). Also, high content of phenolic and flavonoids in medicinal plants have been associated with their antioxidant activities that play a role in the prevention of the development of the age-related disease, particularly caused by oxidative stress (5).

While the use of chemical and synthetic drugs has been highly promoted over the past half-century, the use of medicinal plants and herbal drugs have become increasingly popular in recent years due to their side effects. Interest in utilizing natural sources in the development and formulation of oral products, as an alternative to conventional drugs and synthetic products, contributes to increasing interest in research and industrial application of medicinal plants (6).

Extraction is a commonly used technique for obtaining active substance, an important step in processing of bioactive components from medicinal plants (7). Extraction methods such as maceration, soxhlet, percolation, decoction, and digestion are widely used in medicinal plants research, each having their own advantages and disadvantages. Both fresh and dried samples are applicable in the study of medicinal plants. In most cases, the dried sample is preferred considering the time needed for experiment design. Sulaiman (8) limited the interval between harvest and laboratory preparation at the maximum period of 3 hours to maintain the freshness of samples, making them fragile and tend to deteriorate faster than dried samples. Comparing fresh and dried *Moringa oliefera* leaves indicated no difference in total phenolics, but flavonoids content was higher in the dried sample (7).

The genus *Cordia* has 300 identified species, one of which is *C. myxa* that belongs to the *Boraginaceae* family and grows in tropical and subtropical regions of the world, such as Iran (9). The fruits of this herbal plant are locally known as "Sepestan" in some regions of Iran (10) and contain phenolic compounds. The fruit of *C. myxa* is widely used for the treatment of respiratory and urinary infections, and also as diuretic, astringent, demulcent and expectorant agent. In some studies, it has been reported that *C. myxa* fruit has some pharmacological effects such as anti-inflammatory, antibacterial, antiviral, anti-allergic, antitumor and antioxidants activity (9). Because of the mentioned effects, an-easy-to-use method of preparation must be produced, so oral syrup form was chosen because of its ease of administration and the ability to disguise the bad taste of medications (11). We designed and conducted this study to determine the best way for the extraction of *C. myxa* and propose an easy way to use oral formulation of *C. myxa* extract.

Methods and materials

Apparatus and reagents

Heidolph rotary evaporator (model Laboratory 4000, Germany) was used for the removal of solvent. Absorption spectra and absorbance measurements were obtained with a GBC UV-visible spectrophotometer model Cintra 101 (Sidney, Australia) operating at 359 nanometer (nm) using quartz cells. pH measurements were carried out by a digital pH-Meter model 632, Metrohm(Herisau, Switzerland) with a combined glass electrode. Extracts were filtered through Whatman (No. 1) filter paper. Each extract was prepared freshly for the analysis to prevent any degradation. All chemicals were analytical grade and doubled distilled water was used throughout. Rutin was purchased from Sigma–Aldrich (St. Louis, MO, USA). The ripe fruits of *C. myxa* were purchased from a local market in Ahvaz, Iran during spring 2018.

Sample preparation and extraction procedures

The ripe fruits were cleaned carefully, washed several times with deionized water. Half of the fruits were left to dry in the oven at 50 Centigrad degrees (ºC) for 2 weeks and other half were sliced. A total amount of 2 kilograms (Kg) of dried fruits was obtained, and then grinded, powdered and homogenized in a blender after removal of the seeds.

Soxhlet

In this method (12), 100 g of the powdered dried fruits and 200 g of the sliced fresh fruits were weighed separately and each of them placed into the thimble. Then 250 mililiters (ml) distilled water was poured into a flask and heated to 100 °C. After that, the soxhlet operation was started and when the soxhlet chamber was almost full, the chamber was emptied by the siphon and the solvent was returned to the distillation flask, several times for 8 hours. Finally, a clear cherry red solution was obtained. The aqueous extract was concentrated under vacuum in a rotary evaporator at 50 °C in order to ensure the complete removal of water until a viscous dark residue remained. The procedures was repeated three times and the extract was stored in a non-transparent bottle in refrigerator to prevent decomposition.

Maceration

100 g of powdered dried fruits and 200 g of sliced fresh fruits were weighed separately and each of them soaked in 500 ml of distilled water. The two mixtures were then stirred on a magnetic stirrer frequently at room temperature and incubated for two days by the maceration method to dissolve their components(12). The mixture was then filtered using a filter paper and obtained solution centrifuged for 20 min until the clear solution was obtained. Then the aqueous extract was concentrated under vacuum in a rotary evaporator at 50 ° C in order to ensure the removal of water until a viscous dark residue remained. The above procedures were repeated three times and the extract was stored in a non-transparent bottle in the refrigerator.

Percolation

100 g of the powdered dried fruits and 200 g of the sliced fresh fruits were weighed separately and each of them poured into a percolator with 500 ml distilled water and allowed to stand for 2 hours after that 500 ml distilled water was added to the mixtures and allowed them to macerated into closed percolator for 48 hours at room temperature(12). Then the stopcock was opened and liquid extract collected in an Erlen drop by drop. Additional distilled water added to the percolator until output droplets became colorless. Aqueous extract was concentrated under vacuum in a rotary evaporator at 50°C until a viscous dark residue remained. The above procedures were repeated three times and the extract was stored in a non-transparent bottle in the refrigerator.

Digestion

100 g of the powdered dried material and 200 g of the sliced fresh fruits were weighed separately. Then 50 ml concentrated HNO_3 added to each of them and heated in a water bath at 80 °C for 20 minutes(12). After that, the mixtures were cooled, filtered by a filter paper and 500 ml distilled water was added to them and heated at 80 ° C for 20 minutes. The obtained aqueous extract was concentrated under vacuum in a rotary evaporator at 50° C until a viscous dark residue remained. The above procedures were repeated three times and the extract was stored in a non-transparent bottle in the refrigerator.

Rutin percent determination

To determine the amount of Rutin, after dissolution of 50 miligrams Rutin in water, its

various concentrations prepared and their UV absorption in 359 nm was read and a calibration curve was drawn up (Figure 1) and used to determine the amount of extracted Rutin in each extraction methods.

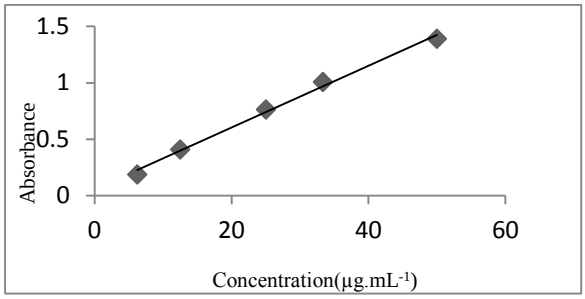

FIGURE 1 Rutin calibration curve

Phytochemical screening of the extract

The crude *C. myxa* fruit extract was analyzed for alkaloids, flavonoids, steroids, saponins, triterpenoids, tannins, proteins, vitamin C and phenolic compounds using standard procedures of analysis(13). Following qualitative tests were done on the dried soxhlet extract and the results are shown in Table 2.

Alkaloid screening

According to Wagner's test, 100 mg of extract was taken and few drops of Wagner's reagent were added and the formation of a reddish-brown precipitate indicates the presence of alkaloids. Also, according to Mayer's test, 100 mg of extract was taken and a few drops of Mayer's reagent were added and the formation of a yellow precipitate indicates the presence of alkaloids(14).

Flavonoids screening

Flavonoids screening was done according to Shinoda test and alkaline reagent test. The fruit extract (100 mg) was added to the pinch of magnesium turnings and 1 ml concentrated hydrochloric acid was added. The formation of pink color indicated the presence of flavonoids(15). As well as, 10 mg of extract was added to 1 ml sodium hydroxide solution 10%, showed an increase in the intensity of yellow color which would become colorless on the addition of 1 ml concentrated hydrochloric acid, indicated the presence of flavonoids(16).

Phenolic compounds screening

Pursuant to Lead acetate test, 100 mg of fruit extract was taken and 5 ml of 1% lead acetate solution was added and the formation of precipitate indicated the presence of phenolic compounds(17).

Tannins screening

Based on the Ferric chloride test, the development of dark bluish-black color by adding 5 ml of 5% ferric chloride to 100 mg of fruit extract confirms the presence of tannins in this extract(18).

Steroids screening

Salkowski's test has done by adding 10 ml concentrated sulfuric acid along the sides of the test tube containing 100 mg of fruit extract dissolved in 10 ml of chloroform. The upper layer turns red and the lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterols compound, in the fruit extract(19).

Carbohydrates screening

Fehling's and Benedict's tests have done by adding 10 ml of their solution to two test tubes containing 100 mg of fruit extract separately and boiled in a water bath. The formation of yellow or red and red or yellow or green precipitate indicated the presence of reducing sugars(20).

Saponins screening

The formation of foam to a length of 1cm indicated the presence of saponins when 100 mg of fruit extract was diluted with 20 ml

distilled water and shaken well in a cylinder for 15 min according to Foam test(15).

Proteins screening

Pursuant to the Biuret test, 100 mg of fruit extract, 5 ml 40% sodium hydroxide solution and 5 drops of 1% copper sulfate solution were added. The nonexistence(absence) of a violet color indicates the non-presence of protein (20). It confirmed by the Ninhydrin test, in that, 100 mg of extract was taken and 5 drops of freshly prepared 0.2% ninhydrin reagent were added and heated. The non-appearance of pink or purple color indicated the inexistence of proteins, peptides or amino acids(15).

Vitamin C screening

Lack of yellow precipitate formation would suggest the lack of vitamin C when 100 mg of extract was treated with a few drops 2,4-dinitrophenyl hydrazine solution and dissolved in 5 ml concentrated sulfuric acid (DNPH Test) (21).

Triterpenoids screening

The amount of 100 mg crude extract was mixed with 5 ml concentrated acetic anhydride, boiled and cooled. Then 2.5 ml concentrated sulfuric acid was added to the test tube. According to the Liebermann-Burchard test, not being observed green color of the upper layer and the formation of a dark red color in the lower layer indicated a negative test for triterpenoids (22).

Preparation of oral syrup

Preservatives were added to distilled water and warmed up to 70 ° C, sucrose was then added and mixed until complete dissolution and was left for cooling. 3 grams of fruit extract was added to diluted Citric acid and added to the solution after cooling. The mix was finalized By adding 70% sorbitol solution, glycerin and grape oil, distilled water in a volume of 100 ml. physicochemical properties such as the amount of active ingredient and the viscosity of prepared syrup was determined, then it was tested for microbial activity.

Results

Soxhlet method of dried sample was the most efficient method. The amount of Rutin was equal to 24 microgram per milliliter. The apparent color was evaluated using the light intensity of the incoming and outgoing light was transparent and after 6 months, the color stability was acceptable. At the time of syrup preparation and after 6 months, visual inspection did not show any solid particles and the pH of the product is about 5.5, and after a year there was no significant change. Viscosity was 900cp and specific gravity of syrup was 1.24 g/ml. Microbial survey revealed that one gram of syrup had 10 nonpathogenic microorganisms and bacteria such as *E. coli*, *S. typhi*, *P. aeuriginosa,* and *S. aureus* were absent. Expiration of the product is estimated at two years using stability accelerated tests, after which the amount of Rutin is reduced to 90% of initial amount. A ranked comparison of Rutin extraction methods is shown in Table 1, and the Results of preliminary phytochemical screening of aqueous *C. myxa* extract is shown in Table 2.

TABLE 1. Efficiency and Rutin percentage of extraction methods

Rank	Extraction Method	Extraction Types	Rutin (% W/W ± SD)	Efficiency (% ± SD)[1]
1	Soxhlet	Dry powdered fruits	0.80 ± 0.008	15 ± 0.16
2		Fresh fruits	0.74 ± 0.008	14 ± 0.12
3	Maceration	Dry powdered fruits	0.76 ± 0.011	12 ± 0.15
4		Fresh fruits	0.73 ± 0.007	10 ± 0.08

5	Percolation	Dry powdered fruits	0.74 ± 0.006	11 ± 0.09
6		Fresh fruits	0.72 ± 0.009	6 ± 0.11
7	Digestion	Dry powdered fruits	0.62 ± 0.006	9 ± 0.10
8		Fresh fruits	0.55 ± 0.004	7 ± 0.04

[1]Water content in fresh fruits is calculated in the extract content

This shows a clear advantage in using dry powdered fruits over the use of fresh fruits in all extraction methods, and also shows the soxhlet method to be the most efficient method of extraction.

TABLE 2 Results of preliminary phytochemical screening of aqueous *C. myxa* extract

Row	Phytochemical Testes	Results
1	Alkaloids	+
2	Tannins	+
3	Phenolic compounds	+
4	Flavonoids	+
5	Saponins	+
6	Steroids	+
7	Carbohydrates	+
8	Vitamin C	-
9	Triterpenoids	-
10	Proteins	-

Discussion

There are many studies counting several properties for *C. myxa*, such as anti-inflammatory and analgesic (3, 23), wound healing (24), protective role against liver fibrosis (25), Gastroprotective and antiulcer effect (9, 26, 27), Anti-leishmanial effects(28) , among many others. Phytochemical studies have also showed *C. myxa* as a considerable reservoir of rare elements (such as selenium, copper, zinc, iron, and manganese), phenolic, and flavonoid compounds (Robinin, Datiscoside, Rutin, Hesperidin, Dihydrorobinetin, Caffeic acid and Chlorogenic Acid) (7). The anti-leishmanial activity of *C. myxa* may be because of phenolic and flavonoid compounds (Rutin and Caffeic acid) and some trace element content (selenium) (28).

Rutin, a well-known natural antioxidant, is one of the medicinally important flavonoids found in *C. myxa*. It makes up to 1% of the whole dried *C. myxa* plant (3). Rutin can reduce capillary fragility, swelling and bruising and has been used in the treatment of venous insufficiency (varicose veins, hemorrhoids, diabetic vascular disease, and diabetic retinopathy), and for improving microvascular blood flow (pain, tired legs, night cramps, and restless legs) (2, 5). Rutin has a role in stimulating cyclic AMP synthesis(7), preventing phospholipase(29) and superoxidase (30), secretion inhibition and aggregation of platelets, mild vasoconstrictor and increasing the resistance of small blood vessels through inhibition of Catechol-O-methyltransferase (9), anti-tumour (10), and analgesic with non-opiod mechanism(12).

In this study we showed the presence of Alkaloids, Tannins, Phenolic compounds, Flavonoids, Saponins and Steroids in *C. myxa*. We also showed that using dried fruits shows a clear advantage over the use of fresh fruits in all extraction methods, and that the soxhlet method was the most efficient method of extraction.

Conclusion

C. myxa, or Sepestan as it is known in Iran, has been used as a traditional medicine for various

purposes. Here we showed the presence of several bioactive compounds in its extract, and proposed a way to produce an easy to use oral formulation. While using *C. myxa* as it has been used by traditional medicine may be beneficial, more studies should be done to evaluate the exact content of bioactive compounds in its extract and whether or not it can be used to produce an alternative medicine.

Acknowledgments

This study was made possible by grant number MPRC-9414 by Vice Chancellery for Research and technology at Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Authors would like to declare that no conflict of interest exists.

Orcid:

Arash Larki: https://orcid.org/0000-0002-9062-2465
Amanollah Zarei Ahmady: https://orcid.org/0000-0001-9315-9149
Neda Mohtasham: https://orcid.org/0000-0001-8187-1518
Ladan Mafakher: https://orcid.org/0000-0003-3767-9769
Nooshin Khelghati: https://orcid.org/0000-0002-3323-532X
Ehsan Hedayati: https://orcid.org/0000-0002-1030-5209

References

[1] S.K. Wong, Y.Y. Lim, N.R. Abdullah, F.J. Nordin, *Pharmacognosy Res.*, **2011**, *3*, 100-106.
[2] V.Y. Waisundara, Traditional Herbal Remedies of Sri Lanka: CRC Press, **2019**.
[3] K. Kokila, S.D. Priyadharshini, V. Sujatha, *Int J Pharm Pharm Sci.*, **2013**, *5*, 70-73.
[4] M. Alasvand, V. Assadollahi, R. Ambra, E. Hedayati, W. Kooti, I. Peluso, *Oxid Med Cell Longev*, **2019**, *2019*, 9475908.
[5] J.D. Phillipson, *Phytochemistry*, **2001**, *56*, 237-243.
[6] I. Muhallilin, S.I. Aisyah, D. Sukma, *Biodiversitas.*, **2019**, *20*, 862-866.
[7] N. Azwanida, *Med Aromat Plants.*, **2015**, *4*, 2167-0412.1000196.
[8] S.F. Sulaiman, A.A.B. Sajak, K.L. Ooi, E.M. Seow, *J Food Compost Anal.*, **2011**, *24*, 506-515.
[9] Z. Inas, A. Hala, H.H. Gehan, *Life Sci J.*, **2011**, *8*, 433-445.
[10] M. Ranjbar, H.N. Varzi, A. Sabbagh, A. Bolooki, A. Sazmand, *Pak J Biol Sci.*, **2013**, *16*, 2066-2069.
[11] F.L. Lopez, T.B. Ernest, C. Tuleu, M.O. Gul, *Expert Opin Drug Delivery.*, **2015**, *12*, 1727-1740.
[12] S.S. Handa, S.P.S. Khanuja, G. Longo, D.D. Rakesh, Organization UNID. Extraction technologies for medicinal and aromatic plants: Earth, Environmental and Marine Sciences and Technologies, **2008**, 260.
[13] S.F. Zohra, B. Meriem, S. Samira, M.A. J. Muneer, *Nat Prod Plant Resour.*, **2012**, *2*, 512-516.
[14] V. Arya, N. Thakur, C. Kashyap, *J Pharmacogn Phytochem.*, **2012**, *1*, 1-5.
[15] T. Geetha, N. Geetha, *Int J PharmTech Res.*, **2014**, *6*, 521-529.
[16] R. Uc, V.M.G. Nair, *Int J Pharm Pharm Sci.*, **2013**, *5*, 629-34.
[17] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, *Methods Enzymol.*, **1999**, *299*, 152-178.
[18] R. Kujur, V. Singh, M. Ram, H.N. Yadava, K. Singh, S. Kumari, B. Roy, *Pharmacognosy Res.*, **2010**, *2*, 258-263.
[19] K. Mukunthan, S. Balaji, *Int J Green Nanotechnol.*, **2012**, *4*, 71-79.
[20] S. Sadasivam, Biochemical methods: New age international, **1996**, 256.
[21] G. Büchi, N. Yang, *J Am Chem Soc.*, **1957**, *79*, 2318-2323.
[22] E. Iqbal, K.A. Salim, L.B. Lim, *J King Saud Univ, Sci.*, **2015**, *27*, 224-232.
[23] M. Ranjbar, H.N. Varzi, A. Sabbagh, A. Bolooki, A. Sazmand, *Pak J Biol Sci.*, **2013**, *16*, 2066-2069.

[24] A.A. Hemmati, H. Kalantari, A. Jalali, S. Rezai, H. Haghighi Zadeh, *Exp Toxicol Pathol*., **2012**, *64*, 181-186.
[25] K. Thirupathi, S.S. Kumar, P. Goverdhan, B. Ravikumar, D. Krishna, G.K. Mohan, *Niger J Nat Prod Med*., **2007**, *11*, 37-40.
[26] S. Palle, A. Kanakalatha, C.N. Kavitha, *J Diet Suppl*., **2018**, *15*, 373-385.
[27] F.M. Al-Awadi, T. Srikumar, J. Anim, I. Khan, *Nutrition*, **2001**, *17*, 391-396.
[28] J. Saki, S. Khademvatan, N. Pazyar, A. Eskandari, A. Tamoradi, P. Nazari, *Jundishapur J Microbiol*., **2015**, *8*, e59827.
[29] B.K. Tiwari, N.P. Brunton, C. Brennan, Handbook of plant food phytochemicals: sources, stability and extraction: John Wiley & Sons, **2013**.
[30] A. Malik, A.R. Ahmad, *Int J PharmTech Res*., **2015**, *7*, 243-246.

How to cite this article: Arash Larki, Nader Shakiba Maram, Amanollah Zarei Ahmady*, Neda Mohtasham, Ladan Mafakher, Nooshin Khelghati, Ehsan Hedayati. Comparing different extraction methods for oral syrup formulation of major bioactive compounds from *cordia myxa* fruit**.** *Eurasian Chemical Communications*, 2020, 2(9), 953-960.
Link:
http://www.echemcom.com/article_113247.html