

ANTIMICROBIAL ACTIVITY OF ETHANOLIC CRUDE EXTRACTS OF
MEDICINAL PLANTS AND THEIR CYCLODEXTRIN-ENCAPSULATED FORMS¹Abdullaeva M.O., ²Gayibova S.N., ³Amirsaidova D.A., ⁴Aripov T.F.^{1,2,4}Institute of Bioorganic Chemistry of the Academy of sciences of the Republic of Uzbekistan³Microbiology Institute of the Academy of sciences of the Republic of Uzbekistan¹e-mail: Abdullayevamuyassar211@gmail.com<https://doi.org/10.5281/zenodo.8361877>

Аннотация. Ушбу мақола *Rhodiola heterodonta* L., *Ajuga turkestanica* L., *Rosa canina* L. (RC), *Petroselinum crispum* L., *Inula helenium* L., *Ginkgo biloba*, *Curcuma longa* L. экстрактлари ва уларнинг γ - ва 2-гидроксипропил- β - циклодекстринлар билан инкапсуляцияларини баъзи шартли-патоген микроорганизмларга қарши салоҳиятини баҳолашга қаратилган. *R.heterodonta*, *I.helenium* ва *G.biloba* максимал микробларга қарши фаоллиги борлиги аниқланди. Экстрактлар турли усуллар билан циклодекстринлар билан капсулаланганда микробларга қарши фаоллик *I.helenium*да камаяди, *R.heterodonta* ва *G.biloba* да эса ортади. Хусусан, циклодекстрин турининг аҳамияти ва инкапсуляция жараёни ҳам алоҳида аҳамиятга эга.

Аннотация. Целью данной работы является оценка антимикробиологического потенциала экстрактов *Rhodiola heterodonta* L., *Ajuga turkestanica* L., *Rosa canina* L. (RC), *Petroselinum crispum* L., *Inula helenium* L., *Ginkgo biloba*, *Curcuma longa* L. и их инкапсулированных циклодекстринами форм против некоторых условно-патогенных микроорганизмов. Было показано, что антимикробная активность экстракта *I.helenium* при инкапсулировании снижается, тогда как экстракты *R.heterodonta* и *G.biloba* проявляют повышенную антимикробную активность при инкапсулировании. В частности, особую роль при инкапсулировании играют тип циклодекстрина и метод инкапсулирования.

Abstract. This paper aims to evaluate the potential of *Rhodiola heterodonta* L., *Ajuga turkestanica* L., *Rosa canina* L. (RC), *Petroselinum crispum* L., *Inula helenium* L., *Ginkgo biloba*, *Curcuma longa* L. extracts and their cyclodextrin-encapsulated form against some opportunistic pathogenic microorganisms. It was found that *R.heterodonta*, *I.helenium* and *G.biloba* possess significant antimicrobial activity. When encapsulated with cyclodextrins by different methods antimicrobial activity reduced in case of *I.helenium* and increased in case of *R.heterodonta* and *G.biloba*. Importance of type of cyclodextrin and encapsulation procedure were observed to be specifically involved as well.

Калим сўзлари: биомойиллик, полифенол, гидрофоб, гидрофил, нанотехнология, микроорганизм.

Ключевые слова: биодоступность, полифенолы, гидрофобность, гидрофильность, нанотехнология, микроорганизм.

Keywords: bioavailability, polyphenol, hydrophobic, hydrophilic, nanotechnology, microorganism.

Introduction.

Medicinal herbs are considered as alternative to antibiotics due to broad-spectrum of secondary metabolites that prevent bacterial growth and/or kill bacterial cells. At the same time, most of secondary metabolites such as polyphenols, present low water solubility that leads to

inadequate bioavailability and minimizes therapeutic effects. Such medicinal potentials of dietary polyphenols have been unlocked by advances in nanotechnology (liposomes, micelles, nanogels, dendrimers) which have increased bioavailability, reduced side-effect as well as enabled site-specific drug administration. Cyclodextrins (CD) (cyclic oligosaccharides) are also considered as promising encapsulating agents. Unique structural properties of CDs with internal hydrophobic cavity and external hydrophilic surface exhibit efficient inclusion properties. Thus, it was shown that encapsulation of an extract of high-calorie tea kudin, herbal tea obtained by brewing dried leaves of *Radum latifolia* with γ -CD, induced an increase in the levels of liver enzymes that metabolize xenobiotics, which include PPAR γ , CD36, CYP7A1, CYP3A, and GSTA1, increased liver weight and increased the accumulation of liver fat (Wüpper S. et al., 2019). Authors Miyoshi et al. (Miyoshi N. et al., 2011) noted increased bioavailability and physiological activity of tocotrienol-rich fractions from rice bran encapsulated with γ -CD. Brazilian green propolis extract in combination with γ -CD reduced hepatic tumor necrosis factor alpha and Sap mRNA levels and had significant anti-inflammatory properties (Rimbach G. et al., 2017). Escobar-Avello et al. encapsulated grapevine extract in hydroxypropyl-beta-cyclodextrin, thereby recycling viticultural by-products whose use had previously been limited due to their instability and low water solubility (Escobar-Avello D. et al., 2021). The results of the work of Hsu and co-authors show that when creating a complex of rhubarb extract with HP- β -CD (hydroxypropyl-beta-cyclodextrin), the solubility of rhubarb in water and its bioavailability increase, enhancing its effect on hepatoma cells - hepatic cell adenoma (Hsu C-M. et al., 2013). Despite numerous studies on the patterns and molecular mechanisms of action, interest in the possibility of encapsulating plant extracts with cyclodextrins is still relevant. In this work, the antimicrobial effects of cyclodextrin-encapsulated extracts of endemic plant of Uzbekistan (*Rhodiola heterodonta* L. and *Ajuga turkestanica* L.), as well as *Rosa canina* L., *Petroselinum crispum* L., *Inula helenium* L., *Gingko biloba* and *Curcuma longa* L. were investigated.

Materials and methods.

Plant crude extracts were obtained from two-hour in 40% ethanol extraction and spray drying, and generously provided by Ltd. BIOTON. CDs used were γ -CD and 2-hydroxypropyl- β -cyclodextrin (2-hp- β -CD) (Cyclolab, Hungary). CD-encapsulated forms of extracts were obtained in 1:1 (w:w proportion) through co-precipitation and freeze drying procedures, physical mixture was also prepared and used for comparison. Extracts abbreviations were as following: *Rhodiola heterodonta* L. (RH), *Ajuga turkestanica* L. (AT), *Rosa canina* L. (RC), *Petroselinum crispum* L. (PC), *Inula helenium* L. (IH), *Gingko biloba* (GB), *Curcuma longa* L. (CL) were investigated.

Antimicrobial activity was carried out by the agar diffusion scratch assay. Microbial cultures (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*) were from the collection of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan. The microbial cultures were washed from agar and diluted by sterile 0.9% isotonic NaCl until the number of cells reached 10^7 KOE/ml (according to McFarland). Crude and encapsulated extracts were dissolved at 100mg/ml by distilled water. Experiment was carried out according to (Gayibova et al., 2019) with minor modifications. Briefly for bacteria commercial nutritional agar (Himedia, India) and for *C.albicans* – commercial Sabouraud agar (Himedia, India) were used. Extract samples were added at 100 μ l, petri dishes kept at fridge 3-4 hours, then incubated at 37°C 20-24 hours for bacteria and 30°C 24-36 hours for *C.albicans*. Experiment was carried out triplicate.

Results

The antagonistic activity of extracts against 5 typical cultures of pathogenic and opportunistic microorganisms was studied. Among the studied samples, RH showed high antagonistic activity against all pathogens: *E.coli* (15 mm), *P. aeruginosa* (14 mm), *B. subtilis* (from 7 mm), *S. aureus* (15 mm), *C. albicans* (16 mm). GB also showed selective activity with maximum activity against *S.aureus* (from 26 mm), *B.subtilis* (from 18 mm), *C. albicans* (18 mm); IN with maximum activity against *B. subtilis* (from 25 mm), *S. aureus* (from 20 mm) and *Candida albicans* (14 mm); PC extract showed the highest selective activity against *S.aureus* compared to other extracts (from 27 mm). In the case of individual cyclodextrins, as well as extracts of AT, RC and CL no antimicrobial activity was observed under studied conditions (Table 1)

Table 1. Determination of antimicrobial activity of crude of extracts and CDs.

№	Samples Test culture	AT	PC	IH	RH	GB	RC	CL	γ -CD	2-hp- β -CD
		Zone, mm								
1	<i>E.coli</i>	-	-	-	15	-	-	-	-	-
2	<i>P.aeruginosa</i>	-	-	-	14	-	-	-	-	-
3	<i>S.aureus</i>	-	27	20	15	26	-	-	-	-
4	<i>B.subtilis</i>	-	-	25 (b)	7	18	-	-	-	-
5	<i>C.albicans</i>	-	-	14	16	18	-	-	-	-

In the presence of cyclodextrins most of samples lost their antimicrobial activity. Antimicrobial activity demonstrated only RH, IH and GB encapsulated forms against *B.subtilis*. Thus IH showed bactericidal activity only in the presence of 2-hp- β -CD encapsulated both by freeze drying and co-precipitation methods, and the inhibition zone reduced to 15-19 mm. GB encapsulated by both CDs demonstrated slightly higher inhibiting activity only when encapsulation was performed by co-precipitation method. On the contrary RH encapsulated forms performed higher antimicrobial effect in comparison to crude extract. The highest inhibition effect showed encapsulated forms with γ -CD by freeze drying and co-precipitation methods (17-19 mm). The physical mixture of RH with both CDs revealed significant inhibition against *P.aeruginosa* (17-18 mm). The obtained results are presented in table 2.

Table 2. Determination of antimicrobial activity of γ -CD and 2-hp- β -CD encapsulated forms of extracts (b – bactericidal effect)

Test culture Samples	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>B.subtilis</i>	<i>C.albicans</i>
	Zone, mm				
IH/2-hp- β -CD (co-precipitation)	-	-	-	15 (b)	-

IH/2-hp- β -CD (freeze drying)	-	-	-	19 (b)	-
RH/ γ -CD (co-precipitation)	-	-	19 (b)	17	-
RH/2-hp- β -CD (co-precipitation)	-	-	-	16	-
RH/ γ -CD (freeze drying)	-	-	-	17 (b)	-
RH/2-hp- β -CD (freeze drying)	-	15	-	10	-
RH/ γ -CD (physical mixture)	-	17	-	10	-
RH/2-hp- β -CD (physical mixture)	-	18	-	9	-
GB/2-hp- β -CD (co-precipitation)	-	-	-	23	-
GB γ -CD (co-precipitation)	-	-	-	18 (b)	-

Discussion.

Medicinal plants are still important source of natural compounds with strong antibacterial effect. Cyclodextrins application removes the restrictions imposed by low bioavailability of plant secondary metabolites. Recently Moriwaki M. et al (2023) showed improved bioavailability of GB extract in the presence of γ -CD. They observed 4, 6.1, and 10.4-folds enhanced bioavailability of components quercetin, kaempferol, and isorhamnetin in plasma when administrated GB/ γ -CD in comparison to GB. IH was mostly reported as anti-inflammatory agent. Though Diguta C. et al (2014) demonstrated antimicrobial activity of IH and declared that the higher ethanol percentage used for extraction the stronger inhibition activity of IH. RH is endemic plant of Uzbekistan and appeals significant interest as adaptogenic remedy. In our work RH, GB and IH crude extracts possessed significant antimicrobial activity while encapsulating with cyclodextrins reduced (in case of IH) and increased (in case of RH and GB) inhibition zones. The results obtained demonstrate the importance of cyclodextrin type, methods used for encapsulation and proportion rate might be important as well. Thus the studies of optimal proportions of encapsulated substance and encapsulating cyclodextrins and optimal encapsulating procedures require further research.

Conclusion. The obtained results show that PC, IH and GB show inhibitory effect against *S.aureus*. IH and GB also demonstrated activity against *C.albicans*. RH was found to be effective against *E.coli*, *P.aeruginosa*, *S.aureus*, *B.subtilis*, *C.albicans*. In the presence of cyclodextrins inhibitory activity of PC, IH and GB varies.

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