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Flavonoids Isolated from *Onosma chitralicum* of Antimicrobial Potential

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Research Article

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ABSTRACT

The *Onosma chitralicum* belonging to family Boraginaceous, has been acting as a source of traditional medicine and have cooled, laxative and anthelmintic effects. The objective of the current research work was to screen the biological potential of *Onosma chitralicum* and also to characterize the isolated chemical constituents of the plant. The crude extract of the plant and soluble fractions of different solvent like (n- C_6H_{14} hexane), ($CHCl_3$ chloroform), (EtoAc ethyl acetate), (n-BuOH butanol) and aqueous had been tested against various microbes (bacteria, fungi). Among them, the activity of (EtoAc ethyl acetate) fraction was determined by technique named column chromatography which was more potent as well as afforded compounds of 1 to 7. The results of these compounds activities showing that the activity of methanol, (EtoAc ethyl acetate) and (n-C_6H_{14} hexane) fraction of the plant was more than the fraction of (n- BuOH butanol), ($CHCl_3$ chloroform) and aqueous fractions against bacterial strains. The methanolic/crude extract activity against *A. flavus* (a fungal stain) were recorded as 65 mm that was excellent. The zone of inhibition of the compounds 1, 4, 5 and 6 were 7 mm, 9 mm, 3 mm, and 6 mm, respectively, so these compounds were more potent antibacterial than compound 2, 3 and 7. Against the tested fungal strain all the isolated compounds 1 to 7 were active. Compounds 2, 3 and 7 were less active than compounds 1, 4, 5 and 6 against the tested fungal strains. Fraction of (EtoAc ethyl acetate) was most active against the selected microbes and from (EtoAc ethyl acetate) fraction compounds 1 to 7 were isolated as well as act as an antimicrobial agent.

INTRODUCTION

Microorganisms, including viruses, bacteria and some others are potentially harmful to other living organisms mainly human. Bacteria largely gets resistant against antibiotics throughout the world as examined in many hospitals [1]. For that reason, invention of new and modified broad spectrum antibiotics is very important and essential in order to overcome such diseases. Antimicrobial agents have many sources, but plants are rich sources of them. Plants are used in different countries, medicinal usage of plants is due to its property of rich cause of many powerful and effective drugs [2]. Herbs are still used for the treatment of various diseases by the people due to their effectiveness and low cost, but doctors are often hesitant for their recommendation due to lack of knowledge on herbs, concerns about liability, product safety and the existence of pathogenic compounds that are harmful [3]. A large number of bacterial strains acquired resistance against various antibiotics due to changes in their structures, mutations in their genes, and most importantly due to excessively unselective intake of antibiotics for the treatment of various infectious diseases. All these modifications led towards the usefulness of most antibiotics. This generates a renewed interest in herbal medicines [4].

The name onosma was derived from “Osma” (Latin word) means smelly, was first time used by Linnaeus in modern botanical classification. In Asia, about 150 types of known species are included in this genus *Onosma* (Boraginaceae), including China (39 species), Turkey (95species), Pakistan (8 species) and 8 species are distributed in remaining Asian countries [5]. *Onosma chitralicum* is a well-known medicinal plant belonging to genus *Onosma*. *Onosma chitralicum* plant is mostly distributed in tropical, temperate, especially the Mediterranean regions. *Onosma chitralicum* plant is aperennial herb having multibranched stems. Leaves and stems are densely hairy having extensively growing hairs. The flowers of this plant are nearly actinomorphic and bisexual [6].

The properties *Onosma chtralicum* plants are having laxative, cooling and anthelmintic effects. This plant also shows effective treatment in derangements of blood, the diseases of the eye, abdominal pain, bronchitis, wound, fever and pile. This plant is locally used by the people of Chitral in order to treat the diseases of the eye and wounds [7].

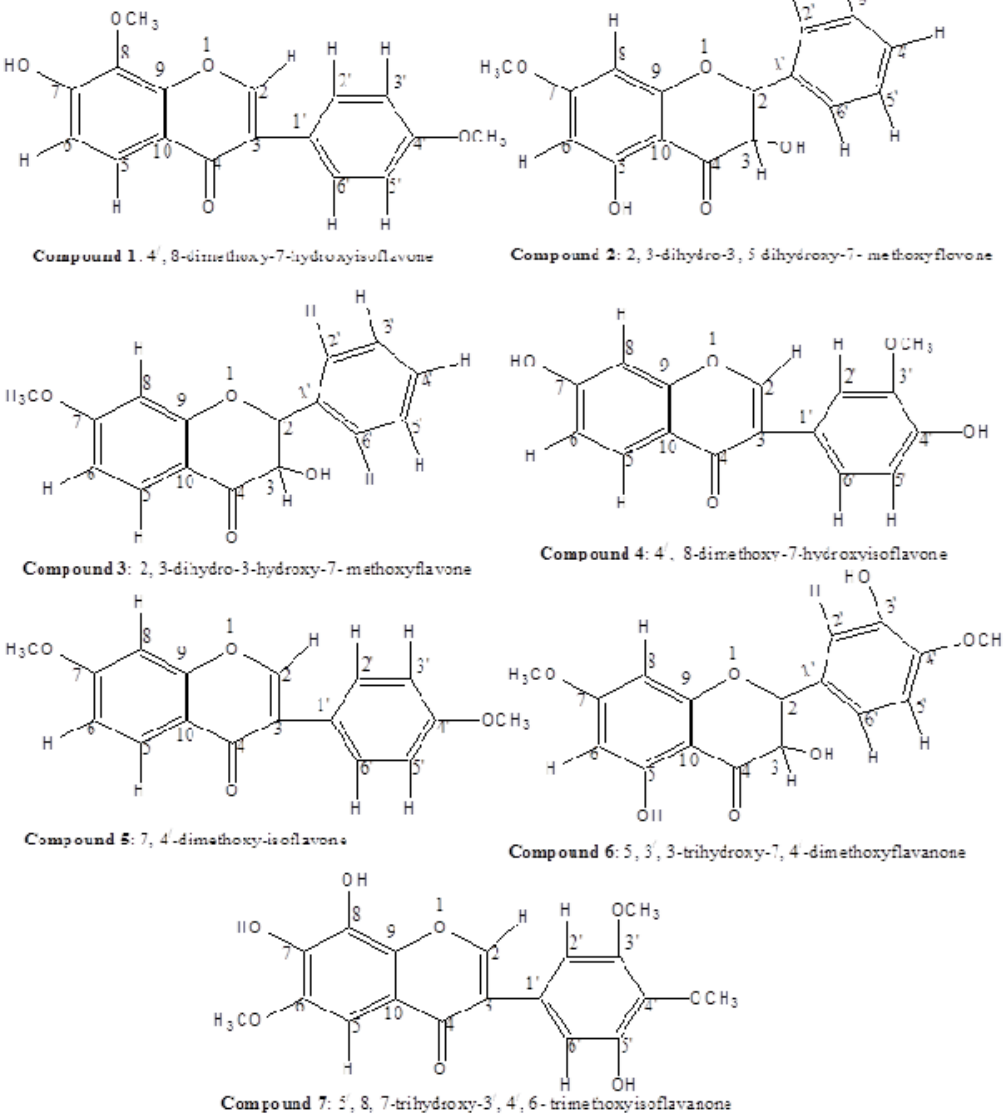
It is evident that *Onosma chitralicum* plant is a herbal medicines, it was confirmed by this literature survey but not examined in detail so far. The main objectives of this type of research were to calculate biological activities of crude extract as well as different fractions of the *Onosma chitralicum* (medicinal plant). It is also used for the isolation of the most active fraction of chemical constituents. The microbial activities of these constituents can also be evaluated. Here in this article, we report the antibacterial and antifungal activities of the crude extract, solvents soluble fractions and compounds (1-7) (Figure 1) isolated for the first time from *Onosma chitralicum* which were individualized by various techniques named 1D and 2D NMR.

MATERIALS AND METHODS

Collection of plant material

Chitral city is the source of the *Onosma chitralicum* plant. This plant was recognized by Dr. Nisar Ahmad, Department of Botany, KUST, Kohat, KP, Pakistan with (PSK 67) as a voucher specimen deposited there.

Figure 1. Structure of compounds 1-7.



Compound 1. 4′, 8-dimethoxy-7-hydroxyisoflavone

Compound 2: 2, 3-dihydro-3, 5 dihydroxy-7- methoxyflavone

Compound 3: 2, 3-dihydro-3-hydroxy-7- methoxyflavone

Compound 4: 4′, 8-dimethoxy-7-hydroxyisoflavone

Compound 5: 7, 4′-dimethoxy-isoflavone

Compound 6: 5, 3′, 3-trihydroxy-7, 4′-dimethoxyflavanone

Compound 7: 5′, 8, 7-trihydroxy-3′, 4′, 6- trimethoxyisoflavanone

Extraction, fractionation and bioassays

The shade and air dried plant *Onasma chitralicum* (2 Kg) was taken and ground in order to get small pieces, now these small pieces were soaked in (MeOH methanol) for 14 days and isolated its extract 3 times at room temperature. The resulting extract (methanolic) was evaporated at low pressure by using a rotary evaporator, ultimately dark greenish residue (80.0 g) was obtained. This residue was then suspended in (water H_2O) and separated successively with n-C_6H_{14} hexane, $CHCl_3$ choloroform, EtoAc ethyl acetate, n-BuOH butanol to obtain n-C_6H_{14} -soluble (15 g), $CHCl_3$-soluble (25 g), EtoAc-soluble (18 g), n-BuOH-soluble (12 g) and aqueous fraction (10 g), respectively. Antibacterial and antifungal activities of these fractions were studied. Agar diffusion method was used for antibacterial activity while for antifungal activities disk diffusion method was used [8].

Isolation of chemical constituents

The most potent activity was observed in ethyl acetate fraction, so it was further subjected to a technique known as column chromatography. The elution through column chromatography was carried out on the basis of the gradient of polarity; solvent arranged on the basis of rising polarity as (n-C_6H_{14} → n- C_6H_{14}-EtoAc → pure- EtoAc. As a result four fractions A, fractions B, fractions C and fractions D were obtained by eluting n-C_6H_{14}-EtoAc of different fraction through column chromatography. Fraction A was obtained by using n- C_6H_{14}-EtoAc (8:2), consisted of two pure

compounds, compound 1 (4′,8-dimethoxy-7-hydroxyisoflavone) and compound 3 (2,3-dihydro-3-hydroxy-7 methoxyflavone) with ratio of (8.50:1.50 and 7.50:2.50), respectively. Fraction B was also obtained as discussed above with n- C_6H_{14}-EtoAc (6.0:4.0). Fraction B was consisted two compound, compound 2 (2, 3-dihydro-3, 5-dihydroxy-7- methoxyflavone) and compound 4 (7, 4′-dihydroxy-3′-methoxyisoflavone) with the ratio of (6.50:3.50 and 6.50:4.0), respectively. Fraction C was eluted by using n-C_6H_{14}-EtoAc by using the ratio of (9:1) was further subjected to column chromatography. Fraction C afforded only one pure compound, compound 5 (7, 4′-dimethoxy-isoflavone). Fraction D was eluted with n-C_6H_{14}-EtoAc by using the ratio of (5:5) subjected to column chromatography. This fraction consisted of 2 compounds, compound 6 (5, 3′, 3-trihydroxy-7, 4′-dimethoxyflavanone) and compound 7 (5′,7,8-trihydroxy-6, 3′,4′-trimethoxyflavanone) with the ratio of (2.50:7.50 and 4.50:5.50), respectively.

Characterizations techniques

The optical rotation of compounds was examined on a instrument named JASCO-DIP-360 digital polarimeter. Eyela (EF-10 model) flash chromatography a type of flash column chromatography technique was used by using silica gel (E. Merk Si 60, 230-400 mesh). The spectra of UV were reported on spectrophotometeric instruments like Shimadzu UV 240 and Hitachi UV- 3200. The Infrared (IR) spectra of compounds were measured with spectrophotometeric instrument named JASCO A- 302 and IRA- 1 infrared (IR). EI-MS and FAB-MS spectra of compounds were recorded on a spectrometer (MS-HX-110), using data system. At 300, 400 and 500 MegaHz, the spectra of 1H-NMR were observed on instrument named Bruker AM-300, AM-400 and 500 AMX spectrometers with respective frequency data system. TMS of compounds was used as a reference (mainly internally). The spectra of 13C-NMR (broad band and DEPT or GASPE) were accomplished on the same instruments at 75 MegaHz, 100 MegaHz and 125 MegaHz, respectively. The values of chemical shift were expressed as ppm (δ) and the coupling constants (J) values were expressed in Hz.

RESULTS AND DISCUSSION

The *Onosm chitralicum* plant crude extract, fractions of different solvents and compounds 1 to 7 were undergone to antimicrobial activities to study their biological importance. Different solvents either polar or non-polar (methanol, n-hexane, chloroform, ethyl acetate, n-butanol and aqueous) soluble fractions had been found to be active against the tested microbial specimen. For antibacterial assays 5 bacterial species i.e *Escherichia coli* strain, *Staphylococcus aureus* strain, *Pseudomonas aeruginosa* strain, *Bacillus subtilis* strain, *Salmonella typhi* strain and for antifungal assays 5 fungal species i.e *Aspergillus flavus* strain, *Aspergillus fumigates* strain, *Aspergillus niger* strain, *Fusarium solani* strain, *Canadida glabrata* strain were used.

Antibacterial activity of fractions

The antibacterial activities of *Onasma chitralicum* against five bacterial strains as shown in Table 1 were found active against all of them. According to result, fraction of (EtoAc ethyl acetate), crude, and (n-C6H14 hexane) of *Onasma chitralicum* revealed more potent activity than ($CHCl_3$ choloroform), (n-butanol BuOH), and aqueous fractions against various strains. The activity of ethyl acetate fraction (16.0 mm) was shown against *S. typhi*, while against *E. coli* and *S. aureus* with (12 mm) inhibition zone for each. The activity of Methanol/crude fraction against *S. typhi* strain (17 mm), *E. coli* strain (13 mm) and *S. aureus* strain (13 mm), activity of n-hexane fraction against *E. coli* (11 mm), *B. subtilis* strain (10 mm), *S. aureus* strain (8 mm) and *S. typhi* strain (7mm). *S. aureus* and *S. typhi* strain are excellently effected by all fractions.

Table 1. Antibacterial activities (mm) of various fractions of *Onasma chitralicum.*

Sl no.	Fractions	*E. coli*	*P.aeruginosa*	*S. aureus*	*S. typhi*	*B. subtilis*
1	Crude	13	8	13	17	12
2	n-hexane (C_6H_{14})	11	2	8	7	10
3	Chloroform ($CHCl_3$)	4	6	6	3	2
4	Ethyl acetate (EtoAc)	12	3	12	16	11
5	n-butanol (BuOH)	1	4	5	3	2
6	Aqueous	3	1	3	5	2
7	Doxycycline (Standard)	16	1.09	20	24	22

Antifungal activity of fractions

The antifungal activities of *Onasma chitralicum* were subjected to five fungal strains i.e *Aspergillus flavus* strain, *Aspergillus fumigates* strain, *Fusarium solani* strain, *Canadida glabrata* strain and *Aspergillus niger* strain. According to result, the activity of (EtoAc ethyl acetate) and methanol/crude fractions of *Onasma chitralicum* was not only excellent, but also active against all fungal strains as compared to ($CHCl_3$ chloroform), n-hexane, (n-BuOH butanol) and aqueous fractions. Methanolic fraction's activity against *A. flavus* strain was (65 mm), *F. solani* was (63 mm) and *A. niger* was (58mm) whereas the ethyl acetate fraction's activity against, *A. flavus* strain was (60 mm), *F. solni* strain (57 mm), *A. niger* strain (48 mm) and *F. fumigatus* strains were (40 mm). The activity of n-hexane fraction recorded against *A. flavus* strain was (50 mm), *F. solani* strain (40 mm), *A. niger* strain (54 mm) and *C. glabrata* strain (36 mm). Similarly fraction of (n-butanol BuOH), ($CHCl_3$ chloroform), and aqueous fractions were also active but degree of activity is less than other fraction.

Bioscreening of compound 1-7

Antifungal and antibacterial activities of compounds 1 to 7 were subjected by using 28 µg/ml of the compounds. The zone of inhibition of isolated compounds 1 to 7. Compounds 1, 4, 5 and 6 were anti-bacterially active against all the tested range of bacteria while the compound 2, 3 and 7 were less diverse in their activity against some bacteria. In antifungal activities the degree of activity of compounds 1, 3, 4, 5 and 6 were more than compounds 2 and 7, but all compounds 1 to 7 were antifungal active.

Antibacterial Activity of compounds

The zone of inhibition of the compounds 1, 4, 5 and 6 were 7 mm, 9 mm, 3 mm, and 6 mm, respectively, so these compounds were more potent antibacterial than compound 2, 3 and 7 against the bacterial strains i.e. *E. coli* and *S. aureus*, only compound 2 were antibacterial active with inhibition zone 8 mm and 5 mm, respectively. Compounds 3 and 7 with inhibitions zone 7 mm and 6 mm, respectively, were potent against only three strains of bacteria.

Antifungal activity of compounds

Against the tested fungal strains i.e. *Fusarium solani*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Canadida glabrata* and *Aspergillus niger* all the isolated compounds 1 to 7 were active. Compounds 2, 3 and 7 were less active than compounds 1, 4, 5 and 6 against the tested fungal strains.

CONCLUSION

The current research work showed that the active fraction against the tested microbes were the solvent fractions i.e. ($CHCl_3$ chloroform), n-hexane, (n-butanol BuOH) (EtoAc ethyl acetate) and the crude fraction of Onosma chitralicum were found active against the tested microbes. But the most effective fraction was of the Ethyl acetate. The isolation from ethyl acetate soluble fraction resulted in seven compounds 1 to 7, as potential agents by which complications

resulting from microbes are treated. However, a further study (*in vivo*) would help in supporting further insight into the pharmacological properties of these isolated compounds.

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