

**Screening of the antimicrobial potential of aqueous extracts of some plants collected from the city of Narafominsk, Moscow, Russia.**

**Abstract**

**Background:** The antimicrobial properties of medicinal plants, including those from Russian flora, can be a considerable asset in the fight against antibiotic resistance and the search for new antimicrobials. The present work aimed at assessing the antimicrobial properties of plants collected from the Narafominsk's flora, a city located on the outskirts of Moscow, Russia.

**Methods:** The plants were collected from June to August 2021, the extraction was carried out with water as a solvent and the antimicrobial activity of the extracts was tested on 3 referenced microorganisms from the American Type Culture Collection (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538 and *Candida albicans* ATCC 10231). We determined the inhibition diameters by the well diffusion method, the minimum inhibitory concentrations (MIC) & minimum bactericidal concentrations (MBC) by the microbroth dilution method.

**Results:** A total of 59 samples from 33 plants were extracted and included 44.1% of leaves (n=26), 32.2% of flowers (n=19), 8.5% of barks (n=5), 5.1% of whole plant (n =3) and 1 stem and 1 root (1.7%). Inhibition diameters ranged from 0 to 28 mm, MICs and MBCs from 0.5< to >128 mg/ml. 69.49% (n=41) of the plants showed an inhibition diameter greater than zero (>0 mm) against *S. aureus*, 42.37% (n=25) against *E. coli* and only 23.72% (n=14) against *C. albicans*. The plant parts ranked in decrease order of antimicrobial activity were as follows: flower < bark < leaf < whole plant < root < stem < fruit. The plants having shown a noteworthy antimicrobial (MIC  $\leq$  0.5 mg/ml) activity against at least 1/3 microorganisms tested and which deserve to be investigated in depth were: flowers of *Epilobium angustifolium*, *Spirea japonica*, *Heracleum mantegazzianum* and *Saponaria officinalis*, bark of *Picea abies* and the whole plant extract of *Rumex obtusifolius*. The second group ( $1 \leq$  MIC  $\leq$  4 mg/ml) of plants with no less worthy antimicrobial abilities were flowers of *Angelica sylvestris*,

*Arctium minus*, *Centaurea jacea*, *Convallaria majalis*, *Melampyrum nemorosum* and *Physocarpus opulifolius*, leaf of *Achillea millefolium* and *Heracleum mantegazzianum*, and bark of *Quercus robur*.

**Conclusion:** This study provides a strong baseline to consider some plants from Narafominsk in the search for new antimicrobials.

**Key words:** Russian flora, bioactive compounds, antimicrobial, extraction

## 1. Introduction

The systematic screening of the antimicrobial activity of biological materials and other substances of interest is an essential step in the search for new antimicrobials [1]. In recent years, many laboratories devoted to biomedical research have demonstrated the effectiveness of extracts from several plants against a variety of pathogens including resistant germs [1-8]. Studies on the search for new antimicrobials, especially those involving herbal medicines, has been on the rise since the beginning of the 21st century due to the alarming increase in the rate of infection by antibiotic-resistant microorganisms [1,3].

As part of a collaborative project dedicated to researching alternatives to conventional antimicrobials, many plants were recently collected from the flora of Narafominsk (a city on the outskirts of Moscow, Russia) and their aqueous extracts are being tested against 3 standard microorganisms from the American Type Culture Collection namely, *Staphylococcus aureus* ATCC 6538 (Gram + model), *Escherichia coli* ATCC 25922 (Gram – model) and *Candida albicans* ATCC 10231(fungi model).

## 2. Materials and Methods

### 2.1. Plants collection and extraction procedure

The plants were collected from June to August 2021 mainly at two points in the city of Narafominsk in Russia. The geolocation of the first point was 8PG3+9H Shchekutino, Moscow Oblast, Russian Federation while the second point was 9G7R8PH2+MP Shchekutino, Moscow Oblast, Russian Federation. After identifying the collected plants using the mobile professional version of PictureThis-Plant Identifier (Glory LLC, 2021), they were kept fresh in plastic bags and transported without special treatment to the microbiology laboratory of the People's Friendship University of Russia (RUDN University). Once in the laboratory, the extraction procedure was performed as in our

previous study [9], with slight modifications. Briefly, 20 g of each fresh sample previously crushed using a blender were introduced into a conical flask. After adding 180 ml of distilled water, the flasks were covered tightly and were shaken at 300 rpm for 2 hours and 25°C in a shaker incubator (Heidolph Inkubator 1000 coupled with Heidolph Unimax 1010, Germany). The mixtures were then filtered using Whatman filter paper № 1, but instead of rotary evaporator, water was evaporated at 40°C in Petri dishes using the above-mentioned shaker incubator as show in figure 1.

After de drying process, each crude extract was dissolved in the required volume of Dimethyl sulfoxide 5%, v/v (DMSO, BDH Laboratories, VWR International Ltd., USA) to achieve a concentration of 128 mg/ml. The extracts were further sterilized by microfiltration (0.22 µm) and the solution obtained was used in the microdilution process and to prepare the solution used for the well diffusion method (50mg/ml).



**Figure 1.** Drying process of aqueous plant extracts using open petri dishes in an incubator shaker at 40°C.

## 2.2. Bacterial strains, culture conditions and inoculum preparation

The microorganisms used for the screening of antimicrobial activity consisted of three standard strains from the American Type Culture Collection. *Staphylococcus aureus* ATCC 6538 was used as Gram + model, *Escherichia coli* ATCC 25922 as Gram - model and *Candida albicans* ATCC 10231 as

fungi model.

Bacteria were cultured for 24 h at 37°C in 5 ml of Brain Heart Infusion Broth (BHIB; HiMedia™ Laboratories Pvt. Ltd., India), while the yeast (*C. albicans* ATCC 10231) was cultured in the same volume of Sabouraud Dextrose Broth (SDB, HiMedia™ Laboratories Pvt. Ltd., India) and the same conditions. After incubation, 1.5 ml of the media containing the microorganisms was centrifuged at 3000 rpm for 15 minutes in a 1.5 microcentrifuge tubes, the pellet was recovered, washed twice with sterile Phosphate Buffer Saline (PBS), stirred in 250µL of PBS and the right volume was resuspended in 5 ml of PBS to obtain a concentration equivalent to McFarland 0.5 using DEN-1 McFarland Densitometer (Grant-bio).

### 2.3. Screening of antimicrobial activity

The antimicrobial activity of the extracts was first assessed by determining the inhibition diameters using the well diffusion method as we described in our previous study [9]. Briefly, 100 µl of each microorganism were spread on 15 ml of sterile Muller Hinton Agar (MHA HiMedia™ Laboratories Pvt. Ltd., India) or Sabouraud Dextrose Agar (for *C. albicans*) previously poured and cooled into Petri dishes. Wells with a capacity of 20 µl were drilled on the culture medium and 20 µl (at 50 mg/ml) of each extract was added. The sterile DMSO 5% used to prepare the extracts was used as negative control while nitrofurantoin and nystatin were used as positive controls. All the trials were done in triplicate and after overnight incubation at 37 °C, the inhibition diameters were measured.

We further determined the minimum inhibitory concentrations (MIC) and the minimum bactericidal concentrations (MBC) of each extract. MIC is the lowest concentration of antibacterial that completely inhibits the visible growth of the microorganism while the MBC is the lowest concentration which kills 99.9% of the microbial population tested [10]. The MIC of the extracts was determined using the microbroth dilution assay described by D'Aquila et al [11]. Briefly, after introducing 100 µL of sterile broth (BHIIB or SDB) in each well of sterile U-bottom 96-well microplates, 100 µL of each extract (100 mg/ml) was subjected to serial twofold dilution and DMSO 5% was used as negative control. Each column corresponding to only one extract was seeded with 10 µL of a single microorganism prepared as described above. Finally, after overnight incubation at 37°C, MIC was considered the lowest concentration of the tested material that inhibited the visible growth of the bacteria. We then determined the MBCs by subculturing the wells without visible growth (with concentrations  $\geq$  MIC) on MHA or SDA plates. Inoculated agar plates were incubated at 37°C for 48h and MBC was considered the lowest concentration that did not yield any microbial

growth on agar. The tolerance level of microorganisms against each extract was finally determined as suggested by Mondal et al. [12]. by the formula: Tolerance = MBC/MIC. When the ratio of MBC/MIC is  $\geq 16$ , the antibacterial efficacy of the test agent was considered as bacteriostatic, whereas MBC/MIC  $\leq 4$  indicated bactericidal activity [12].

### 3. Results and discussion

In this study, we only used water as solvent because all the research focused on the antimicrobial activity of plant extracts using different solvents showed that the aqueous extracts were less active than the others [9,10]. Therefore, by testing the aqueous extracts we hypothesized that if the latter presented an antimicrobial activity, the extracts with the other solvents would present a greater activity.

The complete list of the 33 plant species tested in this work is shown in Table 1. As indicated, in some cases we performed extraction of different parts of the plants. A total of 59 samples were extracted, including 44.1% of leaves (n=26), 32.2% of flowers (n=19), 8.5% of barks (n=5), 5.1% of whole plant (n=3) and 1 stem and 1 root (1.7%). As shown in Table 1, the antimicrobial activity of the extracts was assessed by determining the inhibition diameters, the minimum inhibitory concentrations (MIC), the minimum bactericidal concentrations (MBC) and the tolerance level. The inhibition diameters ranged from 0 to 28 mm, the MICs and MBCs from 0.5 to  $>128$  mg/ml, demonstrating the great disparity between the antimicrobial potentials of the extracts. Regarding the inhibition diameters, strain *S. aureus* ATCC 6538 was the most sensitive, followed by *E. coli* ATCC 25922 while very few plants showed antimicrobial activity against *C. albicans* ATCC 10231 (Figure 2). Indeed, independently of the plants or their parts used, 69.49% (n=41) of the plants showed an inhibition diameter greater than zero ( $>0$ ) against *S. aureus*, 42.37% (n=25) against *E. coli* and only 23.72% (n=14) against *C. albicans*. This first observation corroborates with data in the literature which suggests that medicinal plants are more often active on Gram + bacteria than on Gram – bacteria [13]. For example, in a study by Cock [14] where he assessed the antibacterial activity of 39 methanol extracts of 25 Australian plants against *Bacillus cereus*, *Bacillus subtilis* (2 Gram +) and *Pseudomonas aeruginosa* and *Aeromonas hydrophila* (2 Gram -), the author found that the Gram-positive bacteria were more sensitive than the Gram-negative ones. Similar observations have also been reported by Koohsari et al. [13] and more recently by Mouafo et al.[10]. This difference in sensitivity can obviously be explained by the difference in composition and nature between both cell wall and

membranes of Gram + and Gram - bacteria. Indeed, Lipopolysaccharide's layer and periplasmic space of Gram - bacteria act as a barrier against the permeability of antimicrobials and, therefore, make them less sensitive to antimicrobials, chemical compounds and even herbal drugs compared to Gram + bacteria [13]. In addition, we observed that very few plants were active against *C. albicans* ATCC 10321. This observation is quite normal because it is well known that eukaryotic cells are more able to resist to several antimicrobials unlike prokaryotic ones, because they possess less phospholipids in their membranes [15]. Phospholipids are mostly involved in the preliminary interaction with antimicrobial and their anionic nature will ease the penetration of the antimicrobials into cells [15].

Furthermore, considering the parts of the plants used and independently of the plants themselves, this study revealed that the parts of the plants ranked in decrease order of antimicrobial activity were as follows: flower < bark < leaf < whole plant < root < stem < fruit. Indeed, as shown in Table 1, regardless of the microorganism, the lowest minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were observed with flower extracts. For example, MIC < 0.5 mg/ml and inhibition diameters > 20 mm were simultaneously observed with flower extracts of *Epilobium angustifolium*, *Spirea japonica*, *Heracleum mantegazzianum*, *Saponaria officinalis* against *E. coli* and *S. aureus*. Interestingly, the leaves or other parts of these same plants showed almost 10 times lower antimicrobial activity than those of the flowers. This is the case of extracts from the leaves of *Spirea japonica* which was completely inactive against all 3 microorganisms tested (D=0 mm; MIC and MBC > 128 mg/ml) while the flowers exhibited inhibition diameters ranging from 16-25 mm and MICs of 0.5 and 4 mg/ml against *S. aureus* and *E. coli* respectively. The explanation of these observations is very mixed in the literature. Some authors explain it by a difference in composition and non-uniform distribution of active compounds throughout the plant [16] while others, contrary to our findings, obtained results refuting this hypothesis and showing a similar antimicrobial activity between leaves and flowers [17]. Our findings were all the same surprising and contrary to the logic which would like the leaves extract to be more active because they are the centers of intermediary metabolism leading to biologically active secondary metabolites [16]. Notwithstanding all this, it would be advisable to study the chemical composition of the extracts in order to definitively shed light on these differences.

Moreover, against all expectations, some plants whose antimicrobial activity has already been reported in the literature were ineffective against all the microorganisms tested. These include, for example, the leaves of *Cirsium heterophyllum*, *Cirsium oleraceum*, *Pleurozium schreberi*, *Salix caprea*,

*Sorbus aucuparia*, *Spirea japonica* and *Stachys palustris* [18-21]. This observation can be explained by the difference in climate, area, and harvest time as it has been reported that different agro-climatic conditions have effects on the phytochemical compounds such as Alkaloids, phenols, flavonoids, saponins, and terpenes [22]. Kumar et al. [22]. in his study where he collected Aloe vera from different climatic zones of India and analyzed the effect of climate change on phytochemicals, total phenolic content, and antioxidant potential, concluded that extracts from highland and semi-arid zones possessed maximum antioxidant potential while those from tropical zones showed the least antioxidant activity in all assays.

Finally, Kuete et al. [23] reported that extracts with MIC lower than 0.625 mg/mg can be considered as deserving strong antimicrobial activity while those with MIC higher than this value deserve weak antimicrobial activity. Therefore, and overall, referring to this classification, the flowers of *Epilobium angustifolium*, *Spirea japonica*, *Heracleum mantegazzianum* and *Saponaria officinalis*, bark of *Picea abies* and the whole plant extract of *Rumex obtusifolius* have strong antimicrobial activity with MIC scores  $\leq 0.5$  mg/ml. However, starting from our initial hypothesis which assumed that a plant demonstrating a weak antimicrobial activity with the aqueous extract could demonstrate greater activity with other solvents (i.e., ethanol, methanol, acetone, hexane, etc.), other plants may also be considered for further study. These include those that have shown MICs between 1 and 4 mg/ml and high inhibition diameters against at least one microorganism such as flowers of *Angelica sylvestris*, *Arctium minus*, *Centaurea jacea*, *Convallaria majalis*, *Melampyrum nemorosum* and *Physocarpus opulifolius*, leaf of *Achillea millefolium* and *Heracleum mantegazzianum*, and bark of *Quercus robur*.

Table 1: Inhibition diameter, MICs and MBCs of the plant samples collected

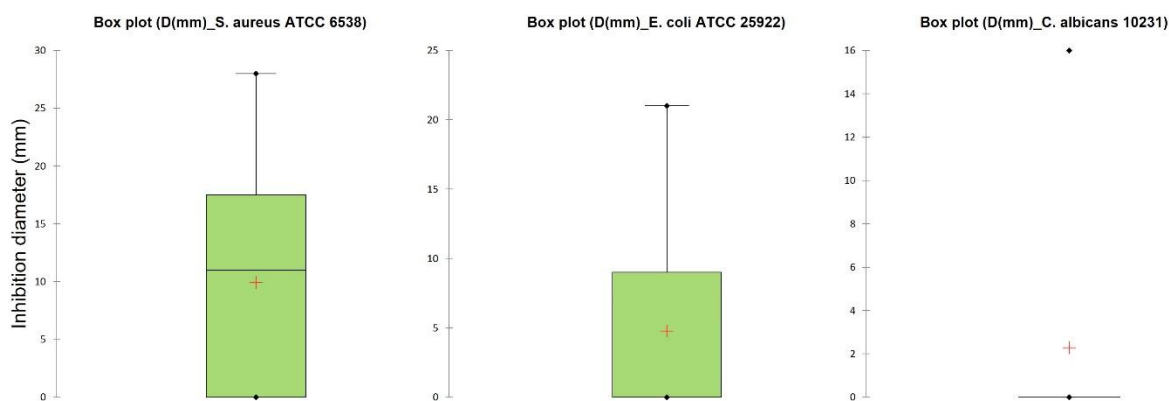
Plants	Parts	<i>S. aureus</i> ATCC 6538				<i>E. coli</i> ATCC 25922				<i>C. albicans</i> 10231			
		D (mm)	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC	D(mm)	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC	D(mm)	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC
<i>Achillea millefolium</i>	Leaf	18 ±1	4	8	2	9 ±0	16	64	4	0±0	>128	>128	-
<i>Angelica sylvestris</i>	Root	9±1	32	32	1	0±0	>128	>128	-	0±0	>128	>128	-
	Leaf	11±0	16	32	2	7±1	64	128	2	0±0	>128	>128	-
	Flower	19±1	2	2	1	13±1	8	32	4	9±0	64	128	2
<i>Arctium minus</i>	Leaf	5±0	128	128	1	0±0	>128	>128	-	0±0	>128	>128	-
	Flower	21±3	4	4	1	6±0	64	>128	-	0±0	>128	>128	-
<i>Bidens tripartita</i>	Leaf	6±0	128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
	Flower	14±2	16	64	4	0±0	>128	>128	-	0±0	>128	>128	-
<i>Centaurea jacea</i>	Flower	18±3	4	32	8	6±0	128	>128	-	0±0	>128	>128	-
<i>Cirsium heterophyllum</i>	Leaf	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
	Flower	7±0	64	>128	-	4±0	128	>128	-	0±0	>128	>128	-
<i>Cirsium oleraceum</i>	Leaf	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
	Flower	11±2	32	128	4	9±0	64	>128	-	0±0	128	>128	-
<i>Convallaria majalis</i>	Leaf	12±3	16	64	4	9±1	64	64	1	0±0	>128	>128	-
	Flower	18±2	2	4	2	13±2	16	64	4	15±2	8	32	4

<i>Epilobium angustifolium</i>	Flower	24±1	0.5	0.5	1	21±3	1	2	2	16±1	4	4	1
<i>Equisetum sylvaticum</i>	Flower	14±2	8	32	4	7±0	64	>128	-	0±0	>128	>128	-
	Leaf	7±1	64	64	1	0±0	>128	>128	-	0±0	>128	>128	-
<i>Frangula alnus</i>	Fruits	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
	Leaf	6±0	128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
<i>Heracleum mantegazzianum</i>	Leaf	20±2	4	8	2	0±0	128	>128	-	0±0	>128	>128	-
	Stem	6±0	128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
	Flower	24±2	0.5	1	2	17±1	0.5	4	8	12±2	8	16	2
<i>Humulus lupulus</i>	Leaf	11±0	64	128	2	8±0	64	>128	-	8±0	64	>128	-
<i>Hylotelephium telephium</i>	Flower	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
	Leaf	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
<i>Melampyrum arvense</i>	Flower	11±1	8	8	1	5±0	128	128	1	5±0	64	>128	-
<i>Melampyrum nemorosum</i>	Leaf	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
	Flower	19±0	2	4	2	14±0	8	32	4	6±1	64	64	1
<i>Phlox paniculata</i>	Leaf	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
	Flower	12±1	64	128	2	8±0	64	>128	-	9±1	64	128	2
<i>Physocarpus opulifolius</i>	Flower	16±3	4	32	8	11±0	32	64	2	0±0	>128	>128	-
<i>Picea abies</i>	Bark	20±2	0.5	1	2	15±1	1	4	4	9±0	16	16	1
	Leaf	15±0	8	16	2	0±0	>128	>128	-	0±0	>128	>128	-

<i>Pleurozium schreberi</i>	WP	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
<i>Polygonum aviculare</i>	WP	6±0	64	128	2	0±0	>128	>128	-	0±0	>128	>128	-
<i>Polytrichum juniperinum</i>	Leaf	7±1	64	64	1	0±0	>128	>128	-	0±0	>128	>128	-
<i>Prunus mexicana</i>	Leaf	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
	Fruits	0±0	>128	>128	-	0±0	>128	>128	-	9±1	64	64	1
	Bark	17±1	32	32	1	11±1	4	32	8	9±0	64	128	2
<i>Quercus robur</i>	Bark	20±4	1	1	1	19±1	2	8	4	10±1	8	32	4
	Leaf	6±0	64	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
<i>Robus saxalis</i>	Leaf	12±1	32	64	2	0±0	>128	>128	-	0±0	>128	>128	-
	Fruits	5±0	128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
<i>Rumex obtusifolius</i>	WP	22±3	0.5	0.5	1	18±1	1	2	2	0±0	>128	>128	-
<i>Salix caprea</i>	Bark	13±1	16	16		6±0	64	>128	-	0±0	>128	>128	-
	Leaf	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
<i>Saponaria officinalis</i>	Leaf	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
	Flower	28±4	0.5<	1	-	21±2	0.5	0.5	1	13±0	8	16	2
<i>Solidago virgaurea</i>	Flower	15±3	32	64	2	9±1	16	64	4	5±0	64	>128	-
	Leaf	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
<i>Sorbus aucuparia</i>	Bark	16±1	64	64	1	0±0	>128	>128	-	0±0	>128	>128	-
	Fruit	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-

	Leaf	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
<i>Spirea japonica</i>	Flower	25±2	0.5<	0.5<	-	16±1	4	8	2	0±0	>128	>128	-
	Leaf	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
<i>Stachys palustris</i>	Leaf	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
	Flower	19±2	8	32	4	0±0	>128	>128	-	0±0	>128	>128	-
<i>Urtica dioica</i>	Leaf	0±0	>128	>128	-	0±0	>128	>128	-	0±0	>128	>128	-
<i>Control 1 (DMSO 5%)</i>	-	0±0	-	-	-	0±0	-	-	-	0±0	-	-	-
<i>Control 2 (Nitrofurantoin)</i>	-	32±3	16*	32*	2	28±1	32*	32*	1	-	-	-	-
<i>Control 3 (Nystatin 50 µg)</i>	-	-	-	-	-	-	-	-	-	23	8	8	1

WP=Whole plant, \*: concentrations are expressed in µg/ml instead of mg/ml. The results of the inhibition diameter are the mean value of each triplicate.



**Figure 2.** Box plot showing the overall trend of inhibition diameters of the extracts against *S. aureus* ATCC 6538, *E. coli* ATCC 25922 and *C. albicans* ATCC 102131

## Conclusion

The aim of this study was to assess the antimicrobial properties of plants collected from the Narafominsk's flora, a city located on the outskirts of Moscow, Russia. A total of 59 samples from 33 plants were extracted and included 44.1% of leaves (n=26), 32.2% of flowers (n=19), 8.5% of barks (n=5), 5.1% of whole plant (n =3) and 1 stem and 1 root (1.7%). After having evaluated the antibacterial activity of these plants using disc diffusion method and microbroth dilution method, the plants having shown a noteworthy antimicrobial activity against at least 1 of the 3 microorganisms tested and which deserve to be investigated in depth are: flowers of *Epilobium angustifolium*, *Spiraea japonica*, *Heracleum mantegazzianum* and *Saponaria officinalis*, bark of *Picea abies* and the whole plant extract of *Rumex obtusifolius*. The second group of plants with no less worthy antimicrobial abilities were flowers of *Angelica sylvestris*, *Arctium minus*, *Centaurea jacea*, *Convallaria majalis*, *Melampyrum nemorosum* and *Physocarpus opulifolius*, leaf of *Achillea millefolium* and *Heracleum mantegazzianum*, and bark of *Quercus robur*.

## COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the

research was not funded by the producing company rather it was funded by personal efforts of the authors.

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