LOW TEMPERATURE CRYSTAL STRUCTURE AND MAGNETIC BEHAVIOR OF BIS(2-AMINO-4-METHYLPYRIDINIUM) TETRACHLORIDOCUPRATE

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$(4\text{-MAPH})_2[CuCl_4]$ **(**4MAP = 2-amino-4-methylpyridine**) (1)** has been synthesized and characterized by single-crystal X-ray diffraction. The compound crystallizes in the monoclinic space group *C*2/c. The tetrachloridocuprate(II)(2-) ions pack in layers parallel to the *ab*-face of the crystal which are well separated by double layers of the 2-amino-4-methylpyridinium cations. The anions generate a square layer via short Cl…Cl interactions due to the C-centering. Variable temperature magnetic susceptibility measurements indicate the presence of weak antiferromagnetic interactions within the layers (J ~ -1 K).

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Introduction

Methyl-substituted 2-amino-pyridine molecules have been used as ligands, and their protonated forms as counterions, for a myriad of first-row transition metal halide complexes. 2-Amino-3-methylpyridine (3-MAP) complexes of the form ML_2X_2 are known for $CoCl_2$ and $CoBr_2$.[1] The corresponding salts, $(3\text{-MAPH})_nMX_m$ have been reported for Fe(III),[2] Co(II),[3] Cu(II),[4] and Zn(II)[5] as well. $(3\text{-MAPH})CuCl_3$, which forms a bichloride-bridged chain, has been extensively studied for its magnetic properties.[6] 2-Amino-5-methylpyridine (5-MAP) has been similarly studied. The neutral ML_2X_2 complexes have been described for cobalt(II) and zinc(II) chloride,[7] while the $(5\text{MAPH})_nMX_m$ salts are reported for Co(II),[3a,8] Cu(II),[3b,9] and Zn(II).[10] In the case of 2-amino-6-methylpyridine, (6MAP), the neutral compounds $M(6\text{MAP})_nX_2$ (n = 2,3) for Co, Ni, Cu, and Zn were prepared.[11] The related known $(6\text{MAPH})_nMX_m$ salts of first row transition metals include compounds of Co,[3b,12] Cu[11,13] and Zn.[11,14] Here also, similar to the 3MAP compound, detailed studies of the magnetic properties of $(6\text{-MAPH})CuCl_3$ have been reported.[6a,15]

The corresponding 4-methyl substituted pyridine moiety, 2-amino-4-methylpyridine (4MAP), has received similar attention. $Co(4\text{MAP})_2Cl_2$ has been reported[1a,3b] as have the corresponding copper(II)[16] and zinc(II) compounds.[17] The tetrachloridozincate salt[18] is known as well. We were particularly interested in the $(4\text{MAPH})_2CuCl_4$ complex. From the room temperature crystal structure,[19] it appeared that the compound could present a well isolated, two-dimensional magnetic lattice, but no magnetic data were reported. Thus, we undertook the synthesis, low-temperature X-ray crystal structure and temperature dependent magnetic study reported here.

Experimental

Copper(II) chloride dihydrate (dehydrated by storing in an oven at 130 C for 24 hours) and 2-amino-4-methylpyridine (4MAP) were purchased from Sigma Aldrich. Materials were used as received without further purification. IR spectra were recorded via ATR on a Perkin-Elmer Spectrum 100 spectrometer. X-Ray powder diffraction was carried out on a Bruker AXS-D8 X-ray Powder Diffractometer.

Synthesis

Bis(2-amino-4-methylpyridinium) tetrachloridocuprate (1).

4MAP hydrochloride (2.892 g, 20.0 mmol) was dissolved in 20 mL of isopropyl alcohol. Solid anhydrous $CuCl_2$ (1.345 g, 10.0 mmol) was added to the solution and stirred for 2 hours to form a light green precipitate (ppt. began to form within 10 minutes). The powder was isolated by vacuum filtration and recrystallized from 95 % ethanol to give yellow-green crystals of **1** (1.78 g, 42 %).

X-Ray structure analysis

Data for **1** were collected at 120(2) K using a Bruker/Siemens SMART APEX instrument (MoKα radiation, λ=0.71073 Å) equipped with a Cryocool NeverIce low temperature device. Data were measured using phi and omega scans; a full sphere of data was collected. Cell parameters were retrieved using SMART[20] software and refined using SAINTPlus[21] on all observed reflections. Data reduction and correction for L_p and decay were performed using SAINTPlus software. Absorption corrections were applied using SADABS.[22]

The structure was solved and refined using the SHELXS-97 program[23] and refined via least-squares analysis via SHELXL-2016.[24] Non-hydrogen atoms were refined using anisotropic thermal parameters. Hydrogen atoms bonded to

nitrogen atoms were located in the difference Fourier maps and their positions refined using fixed isotropic thermal parameters. The remaining hydrogen atoms were placed in geometrically calculated positions and refined using a riding model and fixed isotropic thermal parameters. Crystallographic information and details of the data collection can be found in Table 1.

Table 1. X-ray data of compound 1.

Empirical formula	**$C_{12}H_{18}N_4Cl_4Cu$**
Formula weight	423.64
Temperature	120(2) K
Wavelength	0.71073 Å
Crystal class	monoclinic
Space group	*C*2/c
a	11.2306(8) Å
b	12.3083(9) Å
c	13.8772(10) Å
β	111.805(2)°
Volume	1781.0(2) $Å^3$
Z	4
Density (calculated)	1.580 Mg m^{-3}
Absorption coefficient	1.824 mm^{-1}
F(000)	860
Crystal size	0.28 x 0.28 x 0.50 mm^3
θ range for data collection	2.56 to 33.706°
Index ranges	$-16 \le h \le 13$ $-18 \le k \le 16$ $-17 \le l \le 21$
Reflections collected	9819
Independent reflections	3259 [*R*(int) = 0.0543]
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.000 and 0.8038
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3259 / 0 / 106
Goodness-of-fit on F^2	1.056
Final *R* indices [$I>2\sigma(I)$]	R^1 = 0.0256, wR_2 = 0.0715
R indices (all data)	R^1 = 0.0272 wR_2 = 0.0725
Largest diff. peak and hole	0.575 and -0.702 e $Å^{-1}$

Magnetic susceptibility data collection

A Quantum Design MPMS-XL SQUID magnetometer was used to collect magnetization data for **1**. Powdered crystals were packed into a #3 gelatin capsule and mounted for data collection. Data were collected initially as a function of field from 0 to 50 kOe at 1.8 K. As the field returned to 0 kOe, several data points were recollected to check for hysteresis; none was observed. Magnetization was measured in a constant field of 1 kOe as a function of temperature from 1.8 to 310 K. The data collected were corrected for the background signal of the sample mount (measured independently), the temperature independent paramagnetism of the Cu(II) ion and for diamagnetic contributions of the constituent atoms which were estimated via Pascal's constants.[25] Data were fit using the $H = -J\sum S_1S_2$ Hamiltonian. Sample of **1** used for magnetic data collection was analyzed by powder X-ray diffraction and compared to the predicted powder pattern based on the single crystal structure. No impurities were observed.

Results

Crystal structure analysis

Compound **1** crystallizes in the monoclinic space group *C2/c*. The molecular unit is shown in Figure 1. The asymmetric unit comprises one half of the $CuCl_4^{2-}$ and one 4MAPH cation. The Cu(II) ions sits on a two-fold rotation axis. The structure has been reported previously (293 K),[19] selected bond lengths and angles for the two structures are shown in Table 2. A significant Jahn-Teller distortion results in a highly flattened tetrachloridocuprate ion with a mean trans angle[26] of 148.374(11)°. Comparison of the bond lengths and angles between 120(2) and 293 K shows only very slight changes.

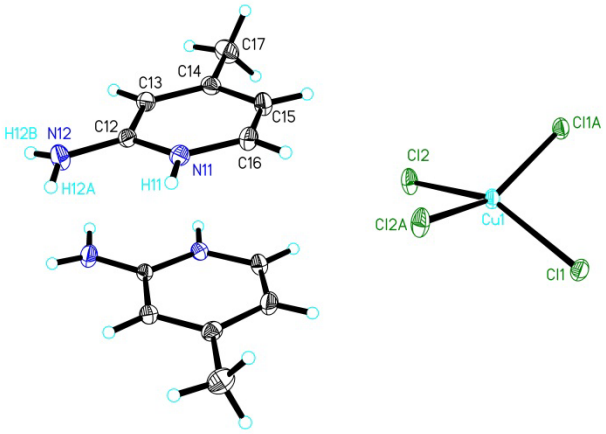


Figure 1. Thermal ellipsoid plot (50% probability) of the molecular unit of **1**. The asymmetric unit, copper coordination sphere and those H-atoms whose positions were refined are labelled. Symmetry operation for Cl1A and Cl2A (*-x, y, ½-z*).

Table 2. Selected bond lengths [Å] and angles [°] for **1** at 87 K (this work) and 295 K.[19]

Bond	**Distance (120 K)**	**Distance (295 K)**
Cu1-Cl1	2.2756(3)	2.261(2)
Cu1-Cl2	2.2705(3)	2.270(2)
Bond	**Angle (120 K)**	**Angle (295 K)**
Cl1-Cu1-Cl1A	94.183(15)	94.34(10)
Cl1-Cu1-Cl2	148.374(11)	146.17(8)
Cl1-Cu1-Cl2A	94.276(11)	95.14(7)
Cl2-Cu1-Cl2A	94.298(16)	94.80(10)

Symmetry operation for Cl1A and Cl2A (*-x, y, ½-z*)

Although there is some deviation from planarity of the NH_2 group, the sum of the angles is only 355.9(1)°, the short N12-C12 distance (1.3473(14) Å) indicates significant sp^2 character for the nitrogen atom due to conjugation with the pyridine ring. The amino substituent acts as an electron donating group, reducing its basicity while raising the basicity of the pyridine nitrogen atom. The pyridine is highly planar (mean deviation of constituent atoms = 0.0054 Å) and N12 lies only 0.0034 Å out of that plane.

The $CuCl_4^{2-}$ ions pack into layers parallel to the *ab*-plane via short Cl…Cl contacts (Figure 2). Adjacent ions are related via the *C*-centering operation. Parameters for the two-halide magnetic superexchange pathway are given in Table 3.

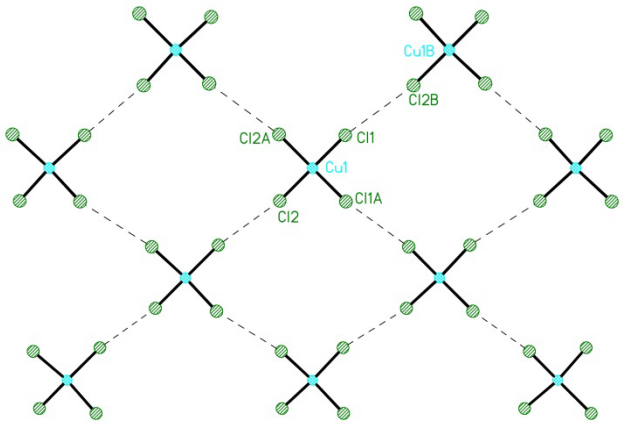
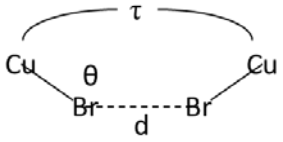


Figure 2. Layer formation in **1** via short Cl…Cl contacts. The short Cl…Cl contacts are represented as dashed lines.

Table 3. Two-halide superexchange pathway parameters for 1 at 120 K (this work) and 293 K (Ref. 20).



Bond	d (Å)	θ(°)[a]	τ (°)
Cu1-Cl1… Cl2B-Cu1B			
120 K	4.206	166.5/ 137.1	151.6
293 K	4.300	165.8/ 133.8	155.1

Layers of $CuCl_4^{2-}$ anions are separated by double layers of 4MAPH cations as seen in Figure 3. This motif is common in several $(BH)_2CuX_4$ complexes, where B is an organic base, such as the 5-methyl, 5-bromo and 5-chloro 2-aminopyridine compounds[9b,d] as well as other compounds in the *C*2/c space group such as (N-methyl-2-phenylethylammonium) tetrabromocuprate.[27] In all of those examples, the aromatic rings are ~ perpendicular to the *ab*-face of the crystals while in **1** the pyridine rings are nearly parallel to that plane. However, as the rings occur in a double layer (Fig. 3), the interlayer separation is still significant. The rings are nearly parallel (interplaner angle = 5.2°) and exhibit π-stacking with an average interring separation of ~3.45 Å, a distance between the ring centroids of 3.586 Å and a slip angle of 13.9°. The closest Cl…Cl contacts between layers are greater than 5.2 Å.

Table 4. Hydrogen bonding parameters for **1**.

	D-H(Å)	H…A(Å)	D…A(Å)	D-H…A(°)
N11-H11…Cl1	0.88(2)	2.653(19)	3.334(1)	136(1)
N11-H11…Cl2	0.88(2)	2.647(19)	3.412(1)	147(1)
N12-H12D...Cl1	0.86(2)	2.495(19)	3.348(1)	172(2)
N12-H12A...Cl2	0.82(2)	2.52(2)	3.293(1)	158(2)

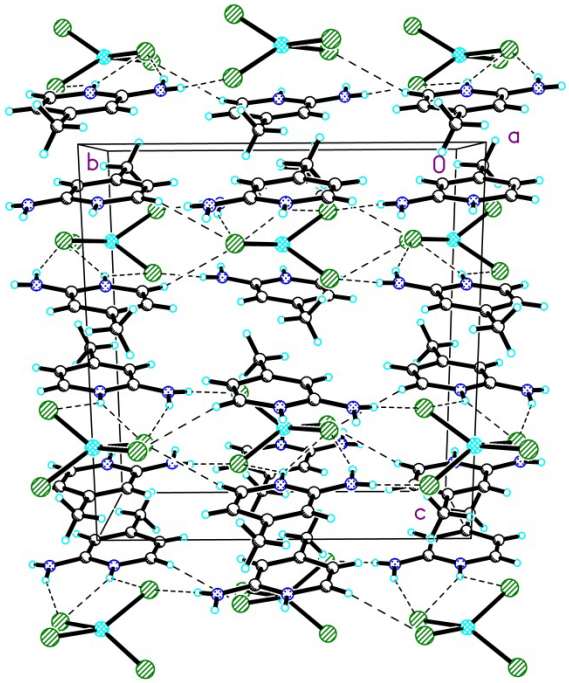


Figure 3. Packing of **1** viewed parallel to the *a*-axis showing the alternating layer structure.

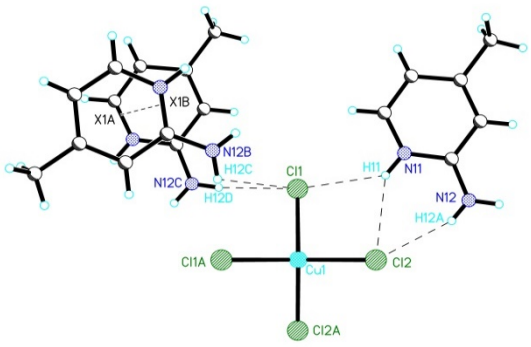


Figure 4. Hydrogen bonding observed in **1**.

The lattice is further stabilized through hydrogen bonds to both the amino and pyridinium hydrogen atoms as shown in Fig. 4. The hydrogen bonding parameters are given in Table 4.

Magnetic study

Magnetization data as a function of applied field show a linear response to ~ 20 kOe and then slight downward curvature to a maximum of ~ 5400 emu/mol at 50 kOe. This is in good agreement with the expected saturation magnetization of ~5,800 emu/mol for a $S = ½$ system with g near 2, indicating the presence of weak antiferromagnetic interactions, and suggest that saturation would be achieved at a slightly higher applied field.

Susceptibility data for **1** were collected as a function of temperature in a 1 kOe applied field from 1.8 K to 310 K. No maximum is visible in the susceptibility of **1** down to 1.8 K. However, a clear decrease in the χT product is seen at low temperatures (Figure 5). Based upon the crystal structure, the

data were fit to the *S*=½ uniform Heisenberg square layer model.[28] This resulted in a Curie constant (CC) of 0.4345(3) emu-K mol^{-1} Oe^{-1} and J = -1.02(6) K with a 7(4) % paramagnetic impurity. The $\chi(T)$ data were also fit to this model resulting in CC = 0.4359(1) emu-K mol^{-1} Oe^{-1} and J = -0.96(5) K with a 2(4) % paramagnetic impurity. The $\chi(T)$ fit emphasizes low temperature data, while the $\chi T(T)$ fit emhasizes high temperature data; the strong agreement between the two fits indicates the quality of the data. Attempts to fit the data to the 2D-square layer model with Curie-Weiss correction to account for interlayer interactions yielded θ values of zero within the error, indicating the good isolation of the layers.

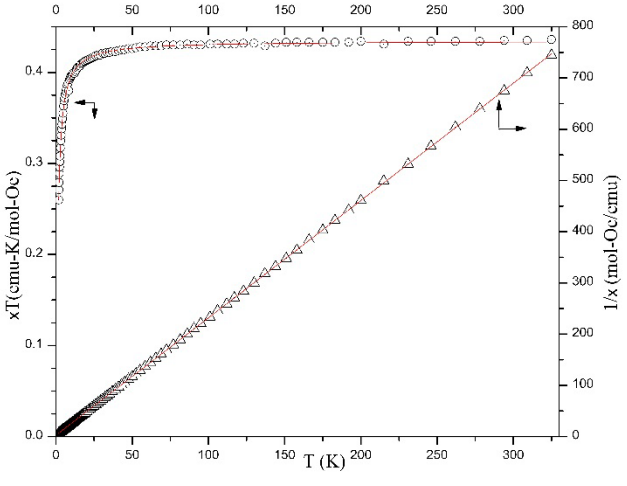


Figure 5. χT(T) (o) and 1/χ(T) (Δ) for **1**. The solid lines represent the best fits to the S = ½ uniform Heisenberg square layer model and the Curie-Weiss law, respectively.

Finally, the data above 5 K were fit to the Curie-Weiss law (Figure 5) resulting in *CC* = 0.4365(2) emu-K mol^{-1} Oe^{-1} and θ = -1.19(6) K in good agreement with the 2D-Heisenberg model. All are in agreement with very weak antiferromagnetic interactions in the compound.

Discussion

Compound **1** crystallizes as a well isolated 2D-layer which may be mapped onto the 2D-Heisenberg square. Although the Cu(II) ions actually form rhombi (the short and long axes are 11.23 Å and 12.31 Å), the Cl…Cl distances across the rhombi are all greater than 7.5 Å, much too great to propagate magnetic exchange. Similarly, although the interplaner Cl…Cl distance is much shorter (~ 5.2 Å) it is still greater than the range at which magnetic exchange is observed. Assuming that the exchange parameters for the two-halide pathway are similar to those reported between bromide ions,[27a] we can analyse the proposed exchange within the layers. The exchange coupling becomes stronger as the Cl…Cl distance shortens, as the θ angles approach 180° and at the τ torsion angle approaches either 0° or 180°. The Cl…Cl distance is within the range where weak antiferromagnetic interactions are typically observed.[3,4,10,27] However, only one of the θ angles is close to 180° and the torsion angle, while closer to 180° than 90°, is not particularly favourable. Thus, the weak exchange observed may be rationalized in terms of the two-halide superexchange pathway. Although the layers are indeed very well isolated, as suggested by the room temperature crystal structure, the magnetic exchange within the layers is too weak to warrant more detailed study.

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Supplementary data

CCDC 1917325 contains the supplementary crystallographic data for **1.** This data can be obtained free of charge via http://www.ccdc.cam.ac.uk/con-ts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or email: deposit@ccdc.cam.ac.uk.

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ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF PLANTS EXTRACTS OF ISRAEL AND PALESTINE. UNEXPLORED PARADISE

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Keywords: Antioxidant, anti-inflammatory, plant extracts, plant families, current research, future opportunities, systematic mapping.

Antioxidant and anti-inflammatory activities are among the most important properties of plant materials used by humans. For many medicinal plants and other natural products sources, there is clear relationship between these properties. Despite the fact that some approved, commercial drugs were developed from natural products that possess these properties, published literature scan reveals a disappointing image, in some geographical regions, with rich flora, the vast majority of these plants were never studied for antioxidant and/or anti-inflammatory activities. Expectedly, some plant families were extensively studied, while others, with some of the most common and widespread plant species, were almost totally ignored. In this review, we will introduce the current situation of studying medicinal properties of plants, especially antioxidant and anti-inflammatory activities, on the central part of the Eastern region of the Mediterranean basin. We will also present an overall view of future research opportunities and scientific collaborations. These opportunities and collaborations must be based on systematic mapping of current knowledge.

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INTRODUCTION

Antioxidant activity of various materials is one of most essential properties for prevention, inhibition and curing of many health disorders, as well as boosting healthy well being.[1] On the same level of importance or even higher, lies the anti-inflammatory activity of materials, and the search for anti-inflammatory agents is as old as humanity itself.[2] Many studies have indicated the interrelation between these two properties, especially for plant materials, such as extracts, essential oils, pure compounds, chemical modifications and approved drugs based on plant materials. This correlation is very clear when antioxidant activity results from the presence of polyphenolic compounds.[3] Expectedly, this clear relationship can be observed when testing various plant and fungi extracts, water and alcoholic extracts found most active.[4] This result can be understood on the basis of the high polarity of phenolic compounds that are soluble in polar solvents such as water and alcohols, more than in less polar solvents such as ethyl acetate, ether and hexane. But this correlation can be seen in wider view since it exists not only in plant matter, but also in other products derived from other living organisms, such as bee honey.[5] In this case also, it was evident that the polar (methanolic) extract and its fractions, contained the highest amount of active polyphenols.

The region on the Eastern side of the Mediterranean shores, namely Israel and Palestine, is one of the richest plant habitats. Plant diversity in this area, despite and because of the fact that wide parts of it are various types of arid lands, is unique and vast. This is because except for rain forests and Tundra climates, all other climates and plant habitats are found. This region is the junction of three continents: Asia, Africa and Europe. To give readers a sense of this richness, we can consider the area of this region (Israel and Palestine) 28292 km^2. The flora of this area consists 137 plant families that include 2652 identified, different plant species.[6] Compared to that, the area of Europe is 10180000 km^2 and it is home for 24700 different plant species.[7] This means that Europe is approximately 360 times Israel and Palestine are, and if the area index is applied, Europe should have 954720 plant species, while actually it is habitat for only less than 2.6 % of that theoretical number.

Literature and plant selection

Scanning the literature for published studies of antioxidant or anti-inflammatory activities of all 2652 plants of our region, was not a practical consideration, since this number is too large. So, we decided to search for publications about species that are included in plant families that consist of 10 or more plant species. At first look and thought, this might seem too few and arbitrary as plant families that consist of 10 or more species 44 out of 137, meaning around 32 %. But these families include 2355 species out of 2652, meaning around 89 %. Plant families and number of species they include are shown in Table 1.

Results of literature search

After the selection of plant families that we wanted to search for published studies of their antioxidant or anti-inflammatory activities, the selection of the plant species was a very interesting task. For some families, published studies emerged immediately, for many species and sometimes, many publications for each plant (see section 4, discussion). Some families were extensively studied. But these are in small in number, while the majority of families have been very partially studied and in some cases, not even that. Our findings are presented in Table 2.

DISCUSSION

Plants materials and their products, especially plant extracts are in use by humans since the dawn of humanity. Preparing drinks like tea, coffee or cacao, is actually one of the simplest and earliest methods of preparing plant extracts. In addition to nutritional uses, plants and their extracts were among the very first human remedies. Extractions were prepared using almost all available liquids: water, wine (hydroalcoholic extraction), different plant oils and vinegar (plant made acetic acid solution). Research efforts and financial support of drug discovery from plants, are influenced by many factors, and consequently, they vary over time. Many review articles were published about this topic. One of the most comprehensive was published by Pan *el al*[75] and other by Shen.[76]

Table 1. Plant families and species that grow in Israel and Palestine (Ref. 6)

Family	Species	Family	Species	Family	Species
Acanthaceae	2	Chenopodiaceae	73	Hypericaceae	9
Adoxaceae	3	Cistaceae	15	Iridaceae	34
Aizoaceae	11	Cleomaceae	4	Ixioliriaceae	1
Alismataceae	5	Colchicaceae	12	Juncaceae	9
Amaranthaceae	15	Convolvulaceae	35	Lamiaceae	115
Amaryllidaceae	51	Crassulaceae	13	Lauraceae	1
Anacardiaceae	8	Cucurbitaceae	7	Lentibulariaceae	2
Apiaceae	97	Cupressaceae	6	Liliaceae	17
Apocynaceae	17	Cymodoceaceae	2	Linaceae	8
Araceae	18	Cynomoriaceae	1	Loranthaceae	1
Araliaceae	1	Cyperaceae	40	Lythraceae	9
Arecaceae	3	Cytinaceae	1	Malvaceae	32
Aristolochiaceae	6	Dennstaedtiaceae	1	Marchantiophyta	20
Asparagaceae	48	Dioscoreaceae	2	Marsileaceae	1
Aspleniaceae	6	Dipsaceae	15	Meliaceae	1
Asteraceae	279	Dryopteridaceae	1	Menispermaceae	1
Berberidaceae	3	Elaeagnaceae	1	Molluginaceae	1
Biebersteiniaceae	1	Elatinaceae	3	Moraceae	8
Boraginaceae	70	Ephedraceae	3	Moringaceae	2
Brassicaceae	127	Equisetaceae	2	Myrtaceae	3
Bryophyta	67	Ericaceae	1	Neuradaceae	1
Butomaceae	1	Euphorbiaceae	45	Nitrariaceae	3
Cactaceae	1	Fabaceae	274	Nyctaginaceae	4
Campanulaceae	19	Fagaceae	6	Nymphaeaceae	3
Cannabaceae	1	Frankeniaceae	2	Oleaceae	4
Capparaceae	8	Fumariaceae	1	Onagraceae	8
Caprifoliaceae	2	Gentianaceae	5	Ophioglossaceae	2
Caryophyllaceae	117	Geraniaceae	27	Orchidaceae	31
Casuarinaceae	1	Haloragaceae	1	Orobanchaceae	16
Ceratophyllaceae	2	Hydrocharitaceae	4	Oxalidaceae	2
Paeoniaceae	1	Pteridaceae	5	Smilacaceae	1
Papaveraceae	36	Ranunculaceae	40	Solanaceae	24
Passifloraceae	1	Resedaceae	15	Styracaceae	1
Phytolaccaceae	1	Rhamnaceae	9	Tamaricaceae	10
Pinaceae	4	Rosaceae	27	Thelypteridiaceae	1
Plantaginaceae	63	Rubiaceae	45	Thymelaeaceae	2
Platanaceae	1	Ruppiaceae	1	Typhaceae	5
Plumbaginaceae	10	Rutaceae	5	Ulmaceae	1
Poaceae	235	Salicaceae	4	Urticaceae	8
Polygalaceae	2	Salvadoraceae	1	Valerianaceae	14
Polygonaceae	39	Salviniaceae	2	Verbenaceae	5
Polypodiaceae	1	Santalaceae	4	Violaceae	5
Pontederiaceae	1	Sapindaceae	4	Vitaceae	2
Portulacaceae	1	Saxifragaceae	2	Xanthorrhoeaceae	7
Potamogetonaceae	8	Scrophulariaceae	30	Zygophyllaceae	17
Primulaceae	8	Simaroubaceae	1		

Table 2. Selected published studies of antioxidant (AO) and anti-inflammatory (AI) of plants species extracts, from major plant families.

Plant family	Plant species	Extract[a]	Activity	Reference
Aizoaceae	*Aizoon hispanicum*	aq,met	AO	8
	Trianthema portulacastrum	but	AI	9
Amaranthaceae	*Amaranthus graecizans*[b]	met	AO	10
	Amaranthus graecizans[b]	met	AI	11
Amaryllidaceae	*Allium ampeloprasum*	met	AO	12
	Allium ampeloprasum	met	AI	13
Apiaceae	*Ammi majus*	met	AO	14
	Ammi majus	hex,met	AI	15
Apocynaceae	*Calotropis procera*	aq	AO	16
	Calotropis procera	et,met	AI	17
Araceae	*Tanacetum vulgare*	EO	AO, AI	18
Asparagaceae	*Scilla autumnalis*	et	AO	19
	Agave americana	ac	AI	20
Asteraceae	*Chrysanthemum coronarium*	het	AO	21
	Chrysanthemum coronarium	met	AI	22
Boraginaceae	*Anchusa-undulata*	meaq	AO	23
	Anchusa-azurea	aq,met	AI	24
Brassicaceae	*Sinapis nigra*	het	AO	25
	Sinapis alba	het	AI	26
Bryophyta	None (see discussion)	None	None	None
Campanulaceae	*Campanula-retrorsa*	aq,dcm,met	AO,AI	27
Caryophyllaceae	*Silene aegyptiaca*	aq,met	AO	28
	Silene vulgaris	et	AI	29
Cistaceae	*Cistus salviifolius*	q,meaq	AO	30
	Cistus salviifolius	aq	AI	31
Colchicaceae	None (see discussion)	None	None	None
Convolvulaceae	*Convolvulus arvensis*	et	AO	32
	Convolvulus arvensis	et	AI	33
Crassulaceae	*Sedum sediforme*	aq,met,pet,ac	AO	34
	Sedum sediforme	met	AI	35
Dipsacaceae	*Knautia bidens*	aq,met	AO	36
	None (see discussion)	None	AI	None
Euphorbiaceae	*Euphorbia hirta*	met	AO	37
	Euphorbia hirta	et	AI	38
Fabaceae	*Ceratonia siliqua*	aq (honey)	AO	39
	Ceratonia siliqua	aq	AI	40
Geraniaceae	*Erodium laciniatum*	hex,het	AO	41
	Geranium robertianum	aq,hex	AO,AI	42
Iridaceae	None (see discussion)	None	None	None
Lamiaceae	*Salvia fruticosa*	ch,eta,met,but	AI,AO	43
	Salvia officinalis	aq,but	AI	44
Liliaceae	*Tulipa systola*	pet,et	AO	45
	None (see discussion)	none	AI	none
Malvaceae	*Alcea setosa*	dcm,met,aq	AO	46
	Malva sylvestris	et	AI	47
Marchantiophyta	None (see discussion)	None	None	None
Orchidaceae	None (see discussion)	None	None	None
Orobanchaceae	*Cistanche tubulosa*	aq	AO	48
	Cistanche tubulosa	aq	AI	49
Papaveraceae	*Papaver somniferum*	het	AO	50
	Fumaria capreolata	et	AI	51
Plantaginaceae	*Plantago coronopus*	hex	AO	52
	Veronica persica	het	AI	53
Poaceae	*Hordeum-vulgare*	et	AO	54
	Sorghum bicolor	het	AI	55
Polygonaceae	*Rumex crispus*	aq	AO	56
	Rumex crispus	aq	AI	57
Ranunculaceae	*Ranunculus arvensis*	aq, met,ac,ch	AO	58
	Ranunculus constantinapolitanus	met	AI	59

Resedaceae	*Reseda luteola*	chex, het, het, dcm	AO	60
	Reseda luteola	aq	AI	61
Rosaceae	*Crataegus aronia*	aq	AO	62
	Crataegus monogyna	het	AI	63
Rubiaceae	*Galium aparine*	met	AO	64
	None (see discussion)	None	AI	None
Scrophulariaceae	*Scrophularia hypericifolia*	meaq	AO	65
	Scrophularia hypericifolia	het	AI	66
Solanaceae	*Datura stramonium*	met	AO	67
	Solanum nigrum	Hex,meaq	AI	68
Tamaricaceae	*Tamarix aphylla*	het	AO	69
	Tamarix aphylla	et	AI	70
Valerianaceae	*Centranthus longiflorus*	met	AO	71
	Centranthus longiflorus	et	AI	72
Zygophyllaceae	*Zygophyllum album*	aq	AO	73
	Zygophyllum-simplex	aq,ch	AI,AO	74
Ranunculaceae	*Ranunculus arvensis*	aq,met,ac,ch	AO	58
	Ranunculus constantinapolitanus	met	AI	59
Resedaceae	*Reseda luteola*	chex,het,het,dcm	AO	60
	Reseda luteola	aq	AI	61
Rosaceae	*Crataegus aronia*	aq	AO	62
	Crataegus monogyna	het	AI	63
Rubiaceae	*Galium aparine*	met	AO	64
	None (see discussion)	None	AI	None
Scrophulariaceae	*Scrophularia hypericifolia*	meaq	AO	65
	Scrophularia hypericifolia	het	AI	66
Solanaceae	*Datura stramonium*	met	AO	67
	Solanum nigrum	hex,meaq	AI	68
Tamaricaceae	*Tamarix aphylla*	het	AO	69
	Tamarix aphylla	et	AI	70
Valerianaceae	*Centranthus longiflorus*	met	AO	71
	Centranthus longiflorus	et	AI	72
Zygophyllaceae	*Zygophyllum album*	aq	AO	73
	Zygophyllum simplex	aq,ch	AI,AO	74

a) aq, water; et, ethanol; eta, ethyl acetate; met, methnol; hex, hexane; dcm, dichloromethane; ac, acetone; ch, chloroform; but, *n*-butanol; het, hydroethanol; pet, petroleum ether; EO, essential oil; meaq, methanol-water; chex, cyclohexane.b) There is a mistake in the plant name it should be *sylvestris* not *silvestris*.

The second states that after scientists won the medicine Nobel Prize, for the development of approved drugs based of modifications of natural products, there is a "golden age" for drug discovery.[76] Both the articles agree that the basic, initial steps are as shown in Figure 1.

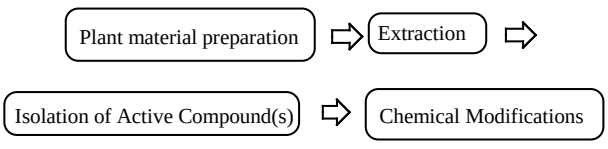


Figure 1. First steps of drug discovery from plant sources.

Clinical trials can start with the first step of "plant material preparation", but in most cases they will start after initial extraction, which in many reports, followed by additional fractionation of extracts.

This was presented in many of the publications we have cited. The published activity was linked to certain natural products. For example, Rodrigues Adao *et al*. reported the isolation the steroidal saponin presented in Figure 2. The biologocal/medicinal importance, especially their anti-inflammatory activity, of these compounds have been presented.[13]

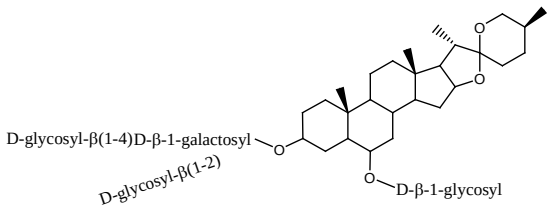


Figure 2. Steroidal saponin isolated from *Allium ampeloprasum.*[13]

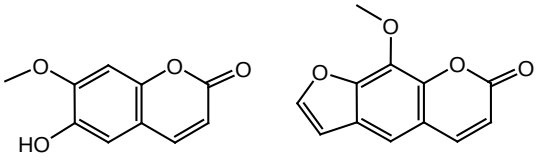


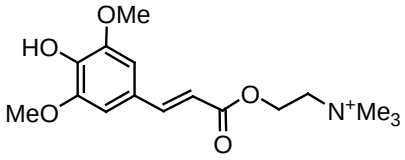
Figure 3. New anti-inflammatory coumarins isolated from *Ammi majus* extracts.[15]

The reported anti-inflammatory activity of *Ammi majus* that was reported by Selim and Ouf,[15] is actually not of the plant extract, which was prepared by extraction with *n*-hexane followed by methanol. Two new coumarins were isolated from the extract and were found as active anti-inflammatory agents (Figure 3).

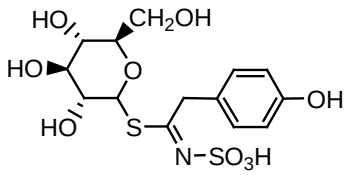
We found during preparing this review article that the vast majority of local plants of Israel and Palestine have never been studied for any biological activity, medicinal property or even for partial chemical composition. The number of examples is in thousands and some of them will be presented here. The presentation will be according to discussed plant family, not consecutively, but according to their appearance in Table 2.

Asparagaceae family includes 48 plants. Very few of them have been studied for antioxidant activity, and only the anti-inflammatory activity *ofAgave americana* has been published.[20] The major genera of this family (*Bellevalia*, 12 species *Ornithogalum*, 11 species) were never studied for either activity.

Yan-Fang *el al*.,[26] suggest that the anti-inflammatory activity of *Sinapis alba* results from the presence of sinapine, sinalbin (Figure 4) and the enzym myrosinase, that act synergistically.



Sinapine



Sinalbin

Figure 4. Structures of sinapine and sinalbin found in *Sinapis alba*[26]

This joint effect of natural products is well known and has gained greater interest over time.[77] But in recent years, there is a growing recognition of the fact that synergism between plant extracts plays an important role in many biological activities.[78,79]

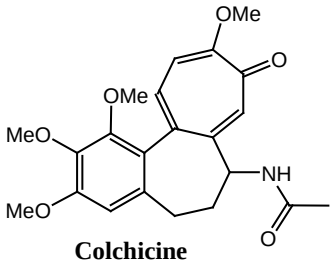
The Bryophyta family of non-vascular plants include 67 species in the area of interest of this review article. None of them has been studied for either anti-inflammatory or antioxidant activity. Moreover, most of them have not been studied for any biological activity. *Bryum argenteum* (*n*-butanol extract, among four extracts) has considerable antibacterial activity.[80] Some non-local species have been studied for their antioxidant[81] and anti-inflammatory activities.[82]

The case of Colchicacea family is one of the strangest and most interesting. Israel and Palestine is home to 12 very beautiful plant species of this family, but none of them was studied for anti-inflammatory or antioxidant activity. Actually, some of them have been studied but most of the interest of researchers was focused on alkaloid content, identification and some biological properties of these alkaloids. Summary of these published studies is shown in Table 3.

Table 3. Summary of published medicinal and phytochemical research of plants of Colchicacea family, native of Israel and Palestine.

Plant Species	Research Interest and Findings	Ref.
Androcymbium palaestinum	Alkaloids. Two new	83
Colchicum ritchii	Alkaloids: demecolcine, colchicine	84
C. tunicatum *C. hierosolymitanum*	Chemical composition, cytotoxicity	85
C. tauri *C. stevenii*	Application of liquid chromatography methods. 18	86
C. tunicatum	Alkaloids were identified and their structures are presented	
C. hierosolymitanum	Increasing production of colchicine	87
C. hierosolymitanum *C. tunicatum*	Effect of NPK fertilizer of the production of colchicine	88
C. hierosolymitanum *C. tunicatum*	Effect of NPK fertilizer of the production of colchicine	89
C. hierosolymitanum *C. stevenii*	Determination of colchicine using various analytical methods	90
C. ritchii	Five alkaloids. Two new.	91
C. stevenii	Cytotoxicity study of active compounds isolated using various methods	92
C. tauri	Isolation of nine alkaloids. One new.	93
C. svovitsii	Alkaloids. Structure of szovitsamine	94
C. svovitsii	Alkaloids. *O*-methylkreysigine	95
C. svovitsii	Alkaloids. Two phenethylisoquinolines	96
C. svovitsii	Analgesic activity of methanolic extract	97

The alkaloid that attracted most research interest is colchicine and most of the isolated alkaloids from *Colchicum* species are its derivatives. Interestingly enough, this compound was tested for anti-inflammatory activity and found active.[98,99]



Colchicine

Figure 5. Structure of colchicine found in plants of Colchicacea family.[84]

Finally, it is important to mention that some non-local Colchicacea species have been studied for anti-inflammatory and antioxidant activities.[100,101]

Even though Dipsacaceae family includes only15 local plant species, only *Knautia bidens* was tested for antioxidant activity,[36] before we started our experimental research. None of these plants was studied for anti-inflammatory activity despite two very important facts. First, some of the plants of this family (e.g. *Cephalaria joppensis*) are very common in the non-arid areas of Israel and Palestine. Secondly, some non-local species, we studied showed high activity, and these studies were conducted a decade ago.[102]

Local plants of Iridaceae family belong to two major genera, *Crocus* and *Iris*. All plants of this family have very beautiful flowers, and some species are relatively very common. The genus *Iris* have some cultural and national aspects (*I. regis-uzziae* and *I. palaestina*). Despite all this, none of these plants, 34 total of the Iridaceae family, was inveatigated for antioxidant or anti-inflammatory activities, while some of the non-local species have been studied for both properties.[103,104]

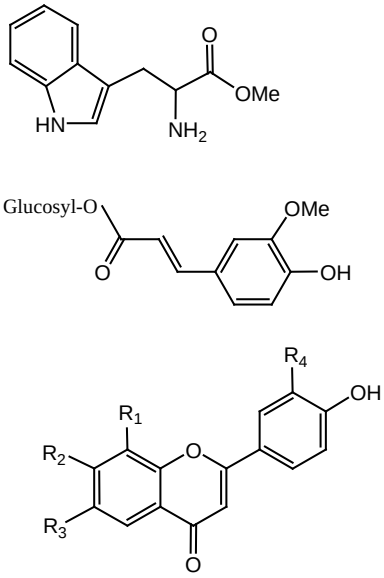
Three small genera consist the family of Liliaceae, which include a total number of 17 plants. As we have shown in Table 2, only *Tulipa systola* has been studied for antioxidant activity, while none of these species has been studied for anti-inflammatory activity. Non-local plants such as *Fritillaria cirrhosa* have been recently investigated for anti-inflammatory activity.[105]

Local liverworts of the Marchantiophyta family belong mainly to the *Riccia* genus, 10 out of 20 species of the whole family. But none of the plants of this genus or the other Marchantiophyta family plants has been inveatigated for antioxidant or anti-inflammatory activities. As for non-local plants, *Riccia fluitans* and some others, have been extensively studied.[106,107] Contrary to the ordinary look of Marchantiophyta, the flowers of the Orchidaceae family are among the most spectacular in nature. Yet, the local plants of this family (31) have been totally ignored in terms of antioxidant and anti-inflammatory research, so far. This is not the situation for non-local species.[108,109]

The genus *Rumex* (Polygonaceae) includes 15 local plants. One of them, *Rumex pulcher* is one of the most important winter delicacies in the Palestinian society. In addition to its nutritional value, it has many uses in traditional medicine. In Lebanon and Syria, the most edible species is *R. acetosa*, and local communities use it as a medicinal plant also.[110] New studies of non-local species reveal the health promoting phytochemicals that they contain.[111] The structures of these compounds are shown in Figure 6.

Strangely enough, the most common and edible local species (*R. pulcher*) has never been investigated for any medicinal, nutritional, phytochemical or any other related property.

Galium (not Gallium) is the genus that includes the largest number of local plants of the Rubiaceae family. As we presented in Table 2, none of the plants of this family was studied for anti-inflammatory activity. *Galium aparine* is one of the most studied plants of this genus, and some publications claim that its anti-inflammatory activity has been published,[112] but we found no reliable support of these claims. Interestingly, asperulosidic acid (Figure 7), was isolated from this plant,[113] and this phytochemical is reported to possess anti-inflammatory activity.[114]



(1) R_1= Glucosyl R_2= OH R_3= H R_4= H
(2) R_1= H R_2= OH R_3= Glucosyl R_4= H
(3) R_1= H R_2= Glucosyl R_3= H R_4= OH

Figure 6. Phytochemicals isolated from *Rumex cyprius*.[111]

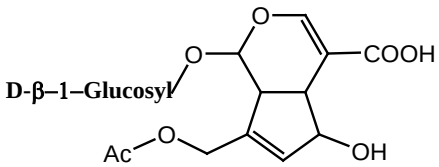


Figure 7. Asperulosidic acid from *Galium aparine* and has anti-inflammatory activity.[113]

But it is important to mention that while local species such as *Oldenlandia capensis* were not studied for anti-inflammatory activity, non-local species like *Oldenlandia diffusa* has been extensively studied for many medicinal properties.[115]

From traditional medicine to methodical science

The findings of this work are very important for us, despite the fact that the method of selecting plant families for our search can be considered inaccurate. We searched for published reports about antioxidant and anti-inflammatory properties of species included in local plant families that have 10 or more plant species. We explained the statistical relevance of our method in the section of Literature and Plant Selection. However, we know that this method suffers two major weaknesses

(1) It produces the impression of equality between families having published works about antioxidant and anti-inflammatory effects. Readers who are not familiar with

plants of the selected area, can not distinguish between the scarcity of studies of Scrophulariaceae on one hand, and Lamiaceae on the other hand, since both appeared in Table 2 as reported.

While reports that we cited about Scrophulariaceae (30 species) are the only ones we found, the Lamiaceae family (115 species) has been very extensively investigated. The difference is not a matter of plant species number only. It is mainly because the Lamiaceae family includes some of the most aromatic and/or medicinal and/or edible, local plant genera namely *Lamium*, *Lavandula*, *Majorana*, *Marrubium*, *Micromeria*, *Nepeta*, *Origanum*, *Phlomis Salvia*, *Stachys*, *Teucrium* and *Ziziphra*. For this reason, there are dozens of reports about the properties and activities of this family.

The Solanaceae family is even a better example. It is consisted of 24 plant species, less than the Scrophulariaceae family, but the medicinal activities of the Solanaceae family have been published in numerous studies. In this case, the main reason is that plants of the family are among those that contain the highest concentrations and types of alkaloids, some are very toxic. These plants were used since antiquity as powerful medicinal plants for very wide variety of purposes, including few spiritual applications.

(2) This selection method, on the contrary, ignores some very important medicinal plant families and plant species. Some families that include fewer than 10 plant species are among the most important and most studied. One of these is Oleaceae, which includes *Olea europaea*, common olive tree. Not only olive oil with its superb nutritional and medicinal properties, but all other parts of the tree, including wild species, rae vastly researched for antioxidant and anti-inflammatory activities.[116,117] In the first work, leaves were extracted with aqueous methanol after removing lipophillic fraction with *n*-hexane, and in the second work, anti-inflammatory activity was tested for methanolic and chloroform extracts.

In some cases, the omission is even more apparent. The Portulacaceae family, is locally presented by just one species, *Portulaca oleracea*. This plant has been extensively studied for almost all biological, medicinal and nutritional (widely edible) properties, including antioxidant (aqueous extract),[118] and anti-inflammatory (hydroalcoholic extract).[119]

As we mentioned above, our results of literature search are important. This was revealed to us while preparing and writing our previous works. To link the knowledge of traditional medicine and herbalism of local communities, with modern systematic research, we initiated in 2016 a series of review articles. Each one of these articles reviewed a plant genus of the most known and useful in local and regional traditional medicine and herbalism. In each one of these article we highlighted the ethnobotanical knowledge and uses of the plants, along with presenting the latest discoveries of medicinal and biological properties. These review articles are shown in Table 4.

But the impression of literature abundance about medicinal plants that these review articles might produce is misleading. While preparing a review article titled, "Anti-inflammatory Activity of Natural Products",[125] we discovered the first fault lines of this assumption. Strangely enough, we found that some of the most commonly used plants by local populations, have never been studied for many biological activities. For example, *Eminium spiculatum* (Araceae) is closely related to *Arum* plants,[122] and like *Arum palaestinum*, it is eaten by local populations and used for cancer treatment in ethnomedicine. We discovered that it was studied for some medicinal activities, such as antioxidant,[126] but to best of our knowledge, not researched for anti-inflammatory activity up to the time of writing this review.

Table 4. Our published plant-genus-specific review articles.

Genus	Major Species of Interest	Reference
Micromeria	*M. fruticosa*	120
Alcea	*A. setosa*	121
Arum	*A. palaestinum*	122
Malva	*M. Sylvestris*	123
Ceratonia	*C. siliqua*	124

The Malvaceae family include two of the plant genera that presented in table 4, *Alcea* and *Malva*. Many of the plants of this family are edible and almost all of them are used as medicinal plants. But while some species were very widely studied (*Alcea setosa* and *Malva sylvestris*, see table 2) and such as *Corchorus olitorius* (Molokhia),[127,128] other have been almost completely ignored. For example, *Malva sylvestris* is most common among the *Malva* species, and it is the largest (by size), it is also the major component of some traditional foods. *Malva nicaeensis* is slightly less common and it grows in hilly landscapes rather than planes like *M. sylvestris*. But *M. nicaeensis* has softer texture and it is considered more delicious. Despite this, while dozens of articles for almost every possible biological or medicinal property of *M. sylvestris* have been published, *M. nicaeensis* was never studied for anti-inflammatory or antioxidant activity. In fact, there are only few reports about heavy metal accumulation in it[129] and some reports of lipase inhibition.

The great diversity and quantities of local plants sometime results in the neglect of some remarkable species even in traditional medicine practice. One of such ignored plants in local ethnomedicine is *Lotus angustissimus*, a member of the Fabaceae family (274 local species). It is very common in most habitats of Israel and Palestine, with beautiful yellow flowers and a very distinctive smell. But the main reason that makes it potentially interesting for both traditional and modern medicinal research, is the fact that grazing livestock avoid it completely. This is an indication that it might contain toxic natural products, similar to most plant species of the Solanaceae family that have been extensively studied. Grazing animals naturally identify toxic plants and avoid them, and most poisoning cases occur when these plants are dried and mixed with other foods.[130,131]

The case of *Notobasis syriaca* is even stranger. Locally, it is the only plant of the genus *Notobasis*, but this genus is one of the vast Asteraceae family (279 local plants). Except in desert parts of the reported area, *N. syriaca* is fairly widespread, high with pretty, unmistakable flowers, edible

(peeled, young, fresh stems) and its seeds are used as substitute of coffee beans. In traditional medicine, it is known for its anti-inflammatory use. While preparing the aritcle on anti-inflammatory Activity of natural products",[125] we found that this property has never been investigated. As a result, we conducted a research that expectedly yielded positive results.[132]

At this stage we prepared a list of locally very widespread plants that we planned to study, because each one of them has either never been studied before for various medicinal activities or the published studies are very incomplete or inconsistent. The major two properties that we investigated as a start are total phenolic content and antioxidant activity, but we also studied other activities of these plants extracts. A summary of these studies is presented in Table 5.

Table 5. Summary selected properties we studied of local medicinal plants.

Plant Species	Extracts[a]	Studied Proprties	Reference
Notobasis syriaca	aq	Anti-inflammatory	132
Carthamus tenuis *Cephalaria joppensis*	aq, et, etac	Total penolic content, antioxidant, antifungal	133
Notobasis syriaca *Scolymus maculatus*	aq, et, etac	Total penolic content, antioxidant, antifungal, alkaloid content	134
Prosopis fatcta	aq, et, etac,hex	Total penolic content, antifungal, anti-termite	135

[a]aq, aqueous; et, ethanol; etac, ethyl acetate; hex, *n*- hexane

It is important to indicate that anti-inflammatory tests of some extracts mentioned in Table 5, are currently being conducted and others are planned to be performed in the future. It is also important to indicate that our list hughly exceeds this small number of partially studied plants.

CONCLUSIOS AND RECOMMENDATIONS

(1) Significant majority of the plant species of Israel and Palestine have never been studied for any biological or medicinal properties.

(2) Some of the plant families have been completely ignored by researcher of anti-inflammatory or antioxidant activities.

(3) Some of these ignored plants and/or families have known properties in traditional medicines.

(4) Researchers should collaborate to plan comprehensive studies of these plant species.

(5) As a start, there is a need for an immediate mapping and documenting published studies of biological and medicinal properties.

(6) There is a need for database of plants/properties that have not been studied yet.

(7) A very comprehensive effort is needed to study the properties of these plants.

(8) Collaboration between researchers from different disciplines in crucial.

(9) Collaboration between researchers from different countries in vital.

(10) Governments of Europe and the Middle East should support this joint effort, since this can bring up some breakthroughs in drug discovery and development.

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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF NEW TETRAHYDROQUINOLINE AND PYRIMIDINE DERIVATIVES

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A series of new tetrahydroquinoline derivatives **(4a-j)** were prepared by one-pot multicomponent, [4+2] cycloaddition route from 4-aminonaphthalene, aromatic aldehydes and dihydrofurane (DHF) by using $InCl_3$ catalyst under reflux temperature and also, pyrimidine derivatives **(5a-n)** were prepared by the same route from benzimidazole, aromatic aldehydes and maleic anhydride by using piperidine catalyst under ultrasonic irradiations. The synthesized tetrahydroquinoline and pyrimidine derivatives were characterized (IR, ^{1}H NMR, ^{13}C NMR). The synthesized tetrahydroquinoline and pyrimidine derivatives have been evaluated for antimicrobial, anti-tuberculosis and anti-inflammatory activities.

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INTRODUCTION

Now a day, a huge number of a heterocyclic compound discovered through various eco-friendly methods like one-pot multicomponent reaction, ultrasonic irradiations technique or Diels-Alder reactions.[1-3] Tetrahydroquinoline and pyrimidine derivatives are broadly used in medicinal chemistry. They showed a huge number of important biological properties such as antimicrobial,[4-7] antimalarial,[8-10]analgesic,[11] anthelmintic,[12] antitumor,[13] anti-inflammatory,[14] antiviral[15-17]and anticancer[18] activity. Keeping the view of the biological importance of heterocyclic compounds,[19] we studied the biological activity of tetrahydroquinoline[20] pyrimidine,[21] thiazolone,[22] and benzenesulfonamide[23] derivatives.

EXPERIMENTAL

The starting materials and various solvents were commercially available (Sigma-Aldrich and Avra labs). Reaction courses were monitored by TLC on silica gel precoated F254 Merck plates. Developed plates were examined with UV lamps (254 nm). Melting points were recorded on SRS Optimelt, melting point apparatus and are uncorrected. IR spectra were recorded on an FT-IR (Bruker). ^{1}H NMR spectra were recorded on a 400 MHz Bruker spectrometer and were recorded in DMSO-d_6 solvent ^{13}C NMR spectra were recorded in DMSO-d_6 solvent on a 100 MHz Bruker spectrometer. Chemical shifts are reported as δ ppm units (TMS).

The following abbreviations are used; singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). Mass spectra were taken with Micromass-QUATTRO-II of WATER mass spectrometer.

General procedure for synthesis of tetrahydroquinoline derivatives

In around bottom flask, 4-aminonaphthalene (0.1 mol), aromatic aldehyde (0.1 mol), dihydrofurane (DHF) (0.1 mol) and catalyst - $InCl_3$ (20 mol %) in EtOH as solvent (5 mL) were refluxed at for 7 h. The reaction condition was checked by employing TLC technique, using ethyl acetate: hexane (5:5) as solvents. After completing reaction, reaction mixture was cooled at room temperature. For crystallization, 10 mL methanol was added to the reaction mixture and then cooled at 22 °C, following stirring for 20 minutes. Products were filtered using G_1 sintered crucible. Products were recrystallized from ethyl alcohol.

11-(2,5-Dimethoxyphenyl)-2,3,3a,10,11,11a-hexahydrobenzo[*h*]furo[2,3-*c*]quinoline (4a)

Blackish grey crystal, m.p. 215-218 °C. FT-IR (KBr cm^{-1}): 3324 (-NH), 2910 (-CH), 1568 (-C=C- aromatic), 1210 (ether), ^{1}H NMR 400 MHz, DMSO-d_6) 9.96 (s, 1H, D_2O exchangeable -NH), 8.32-7.45 (m, 6H, Ar), 6.9-7.10 (m, 2H,Ar), 6.75 (s, 1H, Ar),4.8 (t, 1H, -CH), 4.38 (d, 1H, -CH), 3.77-3.85 (m, 2H, -CH_2), 3.8-(s, 6H, -CH_3), 2.43 (m, 1H, -CH), 1.1-1.7(m, 2H, CH_2).^{13}C NMR (100 MHz, DMSO-d_6): 189.51, 156.24, 153.76, 151.34, 127.72, 125.26, 124.96, 112.90, 111.70, 111.34, 110.34, 104.37, 93.19, 78.0, 76.0, 56.10, 40.0, 38.0, Mass (m/z): $[M+1]^+$: 361.16

11-(4-Cyanophenyl)-2,3,3a,10,11,11a-hexahydrobenzo[*h*]furo[2,3-*c*]quinoline (4b)

Light yellow crystals, m.p 250-253 °C. FTIR (KBr cm^{-1}): 3372 (-NH), 2965 (-CH), 2183 (-CN), 1559 (-C=C- aromatic), 1215 (ether).^{1}H NMR 400 MHz, DMSO-d_6) δ 10.14 (s, 1H, D_2O exchangeable -NH), 8.33- 7.45(m, 6H,

Ar), 7.64 (d, 2H, Ar), 7.34 (d, 2H, Ar), 4.82 (t, 1H, CH), 4.37 (d, 1H, CH), 3.77-3.85 (m, 2H, CH_2), 2.43 (m, 1H, CH), 1.1-1.7(m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 188.51, 156.14, 153.71, 150.92, 127.69, 125.36, 125.05, 112.84, 111.66, 118.0, 111.3, 110.41, 104.39, 93.19, 78.0, 76.0, 40.0, 38.0, Mass (m/z): $[M+1]^+$326.13

11-(3-Hydroxy-4-methoxyphenyl)-2,3,3a,10,11,11a-hexahydrobenzo[*h*]furo[2,3-*c*]quinoline (4c)

Brown cystals, m.p. 210-212 °C. FTIR (KBr cm^{-1}): 3377 (-OH), 3338 (-NH), 2940 (-CH), 1556 (-C=C-aromatic), 1213(ether); ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.95 (s, 1H, D_2O exchangeable -NH), 8.32-7.44 (m, 6H, Ar),7.12-6.92 (d, 2H,Ar), 7.02 (s, 1H, Ar, 4.9 (s, 1H,-OH), 4.8 (t, 1H, -CH), 4.39 (d, 1H, -CH), 3.67-3.88 (m, 2H, -CH_2), 3.8 (s, 3H, -CH_3), 2.43 (m, 1H, -CH_2), 1.1-1.8 (m, 2H, -CH_2), ^{13}C NMR (100 MHz, DMSO-d_6): 192.60, 157, 153.98, 152.44, 128.02, 126.26, 125.16, 113.10, 112.20, 111.53, 110.14, 105.01, 93.20, 78.0, 76.0, 56.11, 40.10, 38.0, Mass (m/z): $[M+1]^+$347.15.

11-(Phenyl)-2,3,3a,10,11,11a-hexahydrobenzo[*h*]furo[2,3-*c*]quinoline (4d)

White crystals, m.p. 200-203 °C. FTIR (KBr cm^{-1}): 3357 (-NH), 2922 (-CH), 1578 (-C=C- aromatic), 1216 (ether), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.92 (s, 1H, D_2O exchangeable -NH), 8.32-7.47 (m, 6H,Ar),7.62-7.10 (m, 5H,Ar), 4.8 (t, 1H, -CH), 4.39 (d, 1H, -CH), 3.68-3.86 (m, 2H, -CH_2), 2.42 (m, 1H, -CH_2), 1.1-1.7 (m, 2H, -CH_2), ^{13}C NMR (100 MHz, DMSO-d_6): 191.98, 157.45, 153.97, 153.64, 127.92, 126.23, 125.36, 113.12, 112.20, 111.53, 110.14, 105.25, 92.23, 78.08, 76.11, 40.02, 38.13, Mass (m/z): $[M+1]^+$301.14.

11-(3-Bromophenyl)-2,3,3a,10,11,11a-hexahydrobenzo[*h*]furo[2,3-*c*]quinoline (4e)

Brown crystals, m.p. 195-197°C, FTIR (KBr cm^{-1}): 3372 (-NH), 2930 (-CH), 1581 (-C=C-aromatic), 1237 (ether), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.93 (s, 1H, D_2O exchangeable -NH), 8.33-7.47 (m, 6H, Ar),7.46 (s, 1H, Ar), 7.52-7.26(m, 3H, Ar), 4.8 (t, 1H, -CH), 4.39 (d, 1H, -CH), 3.67-3.88 (m, 1H, -CH), 2.44 (m, 2H, -CH_2), 1.1-1.79 (m, 2H, -CH_2), ^{13}C NMR (100 MHz, DMSO-d_6): 192.63, 148.25, 154.18, 152.10, 127.87, 126.12, 124.96, 114.00, 112.21, 111.62, 110.04, 105.11, 93.31, 78.12, 76.06, 40.25, 38.07, Mass (m/z): $[M+1]^+$381.00.

11-(4-Methoxyphenyl)-2,3,3a,10,11,11a-hexahydrobenzo[*h*]furo[2,3-*c*]quinoline (4f)

Whitecrystals, m.p. 216-218 °C, FTIR (KBr cm^{-1}): 3377 (-NH), 2931 (-CH), 1562 (-C=C- aromatic), 1234 (ether), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.94 (s, 1H, D_2O exchangeable –NH), 8.31-7.43 (m, 6H, Ar),7.23 (d, 2H, Ar), 7.04 (d, 2H, Ar), 4.81 (t, 1H, -CH), 4.40 (d, 1H, -CH), 3.67-3.86 (m, 2H, -CH_2), 3.82 (s, 3H, -CH_3), 2.44 (m, 1H, -CH_2), 1.13-1.80 (m, 2H, -CH_2),^{13}C NMR (100 MHz, DMSO-d_6): 189.63, 157.25, 154.15, 152.54, 128.22, 126.46, 125.12, 113.08, 112.15, 111.51, 111.01, 105.31, 92.96, 78.0.1, 76.22, 56.21, 40.30, 38.06, Mass (m/z): $[M+1]^+$331.16

11-(4-Chlorophenyl)-2,3,3a,10,11,11a-hexahydrobenzo[*h*]furo[2,3-*c*]quinoline (4g)

Olive green crystals,m.p 160-164 °C. FTIR (KBr cm^{-1}): 3382 (-NH), 2917 (-CH), 1563 (-C=C-aromatic), 1209 (ether), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.96 (s, 1H, D_2O exchangeable -NH), 8.31-7.45 (m, 6H, Ar), 7.66 (d, 2H, Ar), 7.52 (d, 2H, Ar), 4.80 (t, 1H, -CH), 4.41 (d, 1H, -CH), 3.67-3.85 (m, 2H, -CH_2), 2.44 (m, 1H, -CH_2), 1.13-1.78 (m, 2H, -CH_2), ^{13}C NMR (100 MHz, DMSO-d_6): 186.41, 148.60, 148.40, 148.11, 139.99, 136.80, 131.14, 129.79, 131.72, 127.02, 126.81, 126.44, 126.57, 123.97, 123.91, 113.30, 93.00, 79.12, 78.00, 40.12, 38.20, Mass (m/z): $[M+1]^+$335.12.

11-(2,4-Dicyanophenyl)-2,3,3a,10,11,11a-hexahydrobenzo[*h*]furo[2,3-*c*]quinoline (4h)

White crystals, m.p. 220-222 °C. FTIR (KBr cm^{-1}): 3384 (-NH), 2932 (-CH), 1545 (-C=C-aromatic), 1218 (ether), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.98 (s, 1H, D_2O exchangeable -NH), 8.32-7.47 (m, 6H, Ar),7.78 (s, 1H, Ar), 7.46 (d, 1H, Ar), 7.11 (d, 1H, Ar), 4.82 (t, 1H, -CH), 4.40 (d, 1H, -CH), 3.67-3.86 (m, 2H, -CH_2), 2.45 (m, 1H, -CH_2), 1.14-1.79 (m, 2H, -CH_2), ^{13}C NMR (100 MHz, DMSO-d_6): 190.61, 156.00, 153.88, 12.44, 127.92, 126.16, 125.10, 113.13, 112.21, 121.93, 110.09, 105.11, 93.19, 78.05, 76.11, 40.22, 38.12, Mass (m/z): $[M+1]^+$369.07.

11-(4-Nitrophenyl)-2,3,3a,10,11,11a-hexahydrobenzo[*h*]furo[2,3-*c*]quinoline(4i)-

Yellow crystals, m.p 222-223 °C, FTIR (KBr cm^{-1}): 3300 (-NH), 2921 (-CH), 1550 (-C=C-aromatic), 1217 (ether), ^{1}H NMR 400 MHz, DMSO-d_6) δ 10.00 (s, 1H, D_2O exchangeable -NH), 8.77-7.10 (m, 6H,Ar),8.11 (d, 2H, Ar), 7.61 (d, 2H, Ar), 4.80 (t, 1H, -CH), 4.37 (d, 1H, -CH), 3.68-3.33 (m, 2H, -CH_2), 2.44 (m, 1H, -CH_2), 1.1-1.45 (m, 2H, -CH_2), ^{13}C NMR (100 MHz, DMSO-d_6): 187.51, 158.69, 149.30, 147.51, 141.90, 133.90, 130.04, 128.89, 127.92, 126.96, 126.80, 126.34, 126.25, 124.17, 123.81, 113.20, 93.00, 79.10, 78.00, 40.02, 38.23,Mass (m/z): $[M+1]^+$346.15.

11-(4-Flurophenyl)-2,3,3a,10,11,11a-hexahydrobenzo[*h*]furo[2,3-*c*]quinoline (4j)

White crystals, m.p 211-213 °C. FTIR (KBr cm^{-1}): 3321 (-NH), 2920 (-CH), 1536 (-C=C-aromatic), 1205 (ether), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.94 (s, 1H, D_2O exchangeable -NH), 8.31-7.44 (m, 6H, Ar),7.32 (d, 2H, Ar), 7.21 (d, 2H, Ar), 4.80 (t, 1H, -CH), 4.39 (d, 1H, -CH), 3.66-3.80 (m, 2H, -CH_2), 2.43 (m, 1H, -CH_2), 1.13-1.70 (m, 2H, -CH_2), ^{13}C NMR (100 MHz, DMSO-d_6): 179.21, 152.49, 148.30, 147.33, 141.84, 133.72, 131.14, 127.89, 127.88, 127.16, 126.65, 126.96, 125.83, 124.10, 123.41, 113.90, 92.90, 78.93, 78.00, 40.00, 38.13,Mass (m/z): $[M+1]^+$319.14.

General procedure for pyrimidine derivatives

In the ultrasound-assisted method, a mixture of piperidine (10 mol %), isoniazide (0.1 mol), aldehyde (0.1 mol) and maleic anhydride (0.1 mol) in dichloroethane (DCE) as solvent (5 mL) was irradiated with ultrasound (with a frequency of 50 Hz and power of 250 V AC) at 70 °C for 2 h. The reaction progress was checked on TLC using ethyl acetate:hexane (5:5) as solvents. After the completion of reaction, the mixture was cooled at room temperature. Charged methanol (10 mL) was used for crystallization and then the mixture was cooled to 22 °C and stirred for 30 min. The product was filzterd with G_1 sintered crucible and recrystallized from ethyl alcohol.

4-(4-Methoxyphenyl)-5a,6-dicyclobenzo[4,5]imidazo[1,2-a]furo[3,4-*e*]pyrimidine-1,3(3a*H*,11a*H*)-dione (5a)

White crystals, m.p. 250-252 °C. FTIR (KBr cm^{-1}): 3250 (-NH), 2803 (-CH), 1727 (C=O), 1612 (C=N), 1660 (C=C aromatic), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.8 (s, 1H, D_2O exchangeable NH), 6.13-7.95 (m, 8H, Ar), 3.86 (s, 1H, -CH), 3.1-3.2 (s, 3H, -CH_3), 2.6 (d, 1H, -CH), 2.3 (d, 1H, -CH), ^{13}C NMR (100 MHz, DMSO-d_6): 168.27, 168.12, 166.49, 151.40, 149.09, 137.05, 132.72, 130.22, 129.66, 129.51, 129.09, 123.10, 121.14, 113.76, 112.03, 80.13, 57.0, 47.10, 40.0-39.1, Mass (m/z): $[M + 1]^+$349.12.

4-(2,5-Dimethoxyphenyl)-5a,6-dicyclobenzo[4,5]imidazo[1,2-*a*]-furo[3,4-*e*]pyrimidine-1,3(3a*H*,11a*H*)-dione (5b)

White crystals, m.p. 168-170 °C. FTIR (KBr cm^{-1}): 3249 (-NH), 2826 (-CH), 1723 (C=O), 1618 (-C=N), 1568 (C=C aromatic), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.86 (s, 1H, D_2O exchangeable NH), 6.16-7.97 (m, 7H, Ar), 3.82 (s, 1H -CH), 3.2 (d, 1H, -CH), 2.7 (s, 6H, 2-CH_3), 2.4 (d, 1H, -CH), ^{13}C NMR (100 MHz, DMSO-d_6): 170.00, 169.02, 160.19, 151.45, 150.09, 139.15, 132.13, 131.32, 130.22, 130.04, 129.96,a 129.74, 129.09, 123.09, 121.94, 113.76, 111.57, 79.0, 56.90, 46.97, 38.91- 40.0, Mass (m/z): $[M+1]^+$379.12.

4-(3,4-Dihydroxyphenyl)-5a,6-dicyclobenzo[4,5]imidazo[1,2-*a*]furo[3,4-*e*]pyrimidine-1,3(3a*H*,11a*H*)-dione (5c)

White crystals, m.p. 223-225 °C. FTIR (KBr cm^{-1}): 3282 (-OH), 3239 (-NH), 2825 (-CH), 1731 (-C=O), 1620 (-C=N), 1587 (C=C aromatic), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.8 (s, 1H, D_2O exchangeable NH), 6.38-7.95 (m, 7H, Ar), 4.82 (s, 2H, CH_2), 3.1 (m, 1H, CH), 2.6 (d, 1H, CH), 2.3 (d, 1H, CH), ^{13}C NMR (100 MHz, DMSO-d_6): 170.10, 168.53, 165.19, 151.61, 148.84, 142.56, 136.01, 130.85, 122.79, 120.94, 118.76, 116.45, 117.35, 114.98, 111.52, 101.12, 79.0, 44.53,Mass (m/z): $[M+1]^+$351.09.

4-(3-Hydroxyphenyl)-5a,6-dicyclobenzo[4,5]imidazo[1,2-*a*]furo[3,4-*e*]pyrimidine-1,3(3a*H*,11a*H*)-dione (5d)

White crystals, m.p.201-202 °C. FTIR (KBr cm^{-1}): 3278 (-OH), 3229 (-NH), 2839 (-CH), 1722 (-C=O), 1628 (C=N), 1568 (-C=C-aromatic), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.87 (s, 1H, D_2O exchangeable NH), 6.68-7.71 (m, 8H,Ar), 4.82 (s, 1H,-OH), 3.82 (s, 1H, -CH), 3.2 (d, 1H, -CH), 2.4 (d, 1H, -CH), ^{13}C NMR (100 MHz, DMSO-d_6): 170.30, 169.12, 165.00, 157.19, 141.35, 140.29, 138.14, 135.15, 133.13, 131.32, 130.04, 129.09, 121.94, 120.21, 119.23, 118.34, 118.12, 115.41, 114.76, 111.57, 96.5, 75.0, 40.0,Mass (m/z): $[M+1]^+$335.08.

4-(4-Cyanophenyl)-5a,6-dicyclobenzo[4,5]imidazo[1,2-*a*]furo[3,4-*e*]pyrimidine-1,3(3a*H*,11a*H*)-dione (5e)

Yellow crystals, m.p. 194-196 °C. FTIR (KBr cm^{-1}): 3262 (-NH), 2845 (-CH), 2184 (-CN), 1727 (-C=O), 1618 (C=N aromatic), 1577 (-C=C-aromatic), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.9(s, 1H, D_2O exchangeable NH), 6.16-8.00 (m, 8H,Ar), 3.93 (s, 1H, -CH), 3.2 (d, 1H, -CH), 2.4 (d, 1H, -CH), ^{13}C NMR (100 MHz, DMSO): 170.50, 168.52, 164.19, 151.45, 142.23, 139.75, 132.13, 131.92, 130.02, 129.66, 129.74, 129.09, 119.09, 118.94, 118.56, 113.76, 115.08, 111.07, 102.00, 77.10, 41.97,Mass (m/z): $[M+1]^+$344.09.

4-(3-Hydroxy,4-methoxyphenyl)-5a,6-dicyclobenzo[4,5]imidazo[1,2-*a*]furo[3,4-*e*]pyrimidine-1,3(3a*H*,11a*H*)-dione (5f)

Yellow crystals, m.p. 210-212 °C. FTIR (KBr cm^{-1}): 3289 (-OH), 3239 (-NH), 2819 (-CH), 1741 (-C=O), 1612 (C=N), 1582 (C=C aromatic), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.88 (s, 1H, D_2O exchangeable NH), 6.31-7.46 (m, 7H, Ar), 4.15 (s, 1H, -OH), 3.91 (s, 1H, -CH), 3.2 (d, 1H, -CH), 3.0 (s, 3H, -CH_3), 2.2 (d, 1H, -CH), ^{13}C NMR (100 MHz, DMSO-d_6): 170.20, 168.32, 163.19, 151.85, 148.09, 142.32, 138.35, 133.13, 121.94, 119.54, 118.21, 115.30, 112.35, 111.17, 76.0, 56.90, 42.17, Mass (m/z): $[M + 1]^+$365.09

4-(Phenyl)-5a,6-dicyclobenzo[4,5]imidazo[1,2-*a*]furo[3,4-*e*]pyrimidine-1,3(3a*H*,11a*H*)-dione (5g)

White crystals, m.p. 245-248 °C. FTIR (KBr cm^{-1}): 3250 (-NH), 2812 (-CH), 1717 (-C=O), 1620 (C=N), 1592 (-C=C-aromatic), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.94 (s, 1H, D_2O exchangeable NH), 6.08-7.97 (m, 9H, Ar), 3.76 (s, 1H, -CH), 3.17 (d, 1H, -CH), 2.54 (d, 1H, -CH),^{13}C NMR (100 MHz, DMSO-d_6): 170.35, 168.52, 164.75, 151.95, 147.46, 138.37, 132.12, 131.13, 130.65, 129.71, 129.57, 129.22, 128.29, 122.80, 121.60, 114.21, 111.54, 79.0, 46.97, 38.9, 40.10**,** Mass (m/z): $[M+1]^+$319.11.

4-(3-Bromophenyl)-5a,6-dicyclobenzo[4,5]imidazo[1,2-a]furo[3,4-*e*]pyrimidine 1,3(3a*H*,11a*H*)-dione (5h)

Brown crystals, m.p. 238-240 °C. FTIR (KBr cm^{-1}): 3259 (-NH), 2829 (-CH), 1719 (-C=O), 1628 (C=N), 1584 (-C=C-aromatic), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.8 (s, 1H, D_2O exchangeable NH), 6.20-7.86 (m, 8H, Ar), 3.90 (s, 1H,-CH), 3.1 (d, 1H, -CH), 2.4 (d, 1H, -CH), ^{13}C NMR (100 MHz, DMSO-d_6): 170.25, 168.02, 163.19, 142.22, 142.09, 133.20, 130.14, 126.96, 126.12, 123.19, 119.02, 118.14, 115.46, 111.07, 99.65, 76.0, 41.68,Mass (m/z): $[M+1]^+$397.01

4-(4-Hydroxyphenyl)-5a,6-dicyclobenzo[4,5]imidazo[1,2-*a*]furo[3,4-*e*]pyrimidine 1,3(3a*H*,11a*H*)-dione (5i)

White crystals, m.p. 190-192 °C. FTIR (KBr cm^{-1}): 3279 (-OH), 3243 (-NH), 2829 (-CH), 1713 (C=O), 1621 (C=N), 1588 (-C=C-aromatic), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.83 (s, 1H, D_2O exchangeable NH), 6.20-7.91 (m, 8H, Ar), 3.93 (s, 1H, -OH), 3.78 (s, 1H, -CH), 2.9 (d, 1H, -CH), 2.43 (d, 1H, -CH), ^{13}C NMR (100 MHz, DMSO-d_6): 170.50, 168.22, 163.69, 160.00, 142.65, 140.11, 139.05, 130.00, 130.04, 129.19, 119.09, 118.94, 115.66, 111.47, 75.0, 99.90, 41.97, Mass (m/z): $[M+1]^+$335.08.

4-(4-Chlorophenyl)-5a,6-dicyclobenzo[4,5]imidazo[1,2-*a*]furo[3,4-*e*]pyrimidine 1,3(3a*H*,11a*H*)-dione (5j)

White crystal, m.p. 215-218 °C. FTIR (KBr cm^{-1}): 3268 (-NH), 2855 (-CH), 1717 (-C=O), 1630 (-C=N), 1576 (C=C aromatic), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.95 (s, 1H, D_2O exchangeable NH), 6.14-7.92 (m, 8H,Ar), 3.8 (s, 1H, -CH), 3.2-3.0 (d, 1H, -CH), 2.5 (d, 1H, -CH),^{13}C NMR (100 MHz, DMSO-d_6): 170.35, 168.78, 164.60, 151.66, 147.46, 136.02, 131.16, 131.03, 130.02, 129.82, 129.60, 128.92, 128.42, 123.01, 121.45, 113.79, 111.42, 79.0, 44.40, 38.0-40.00,Mass (m/z): $[M+1]^+$353.00.

4-(2,4-Dichlorophenyl)-5a,6-dicyclobenzo[4,5]imidazo[1,2-*a*]furo[3,4-*e*]pyrimidine-1,3(3a*H*,11a*H*)-dione (5k)

Brownish crystals, m.p. 180-184 °C. FTIR (KBr cm^{-1}): 3168, 2665, 2130, 1658, 1615, 1591, 1536; ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.90 (s, 1H, D_2O exchangeable NH), 6.23-7.9 (m, 7H, Ar), 3.7 (s, 1H), 3.2-3.2 (d, 1H), 2.5 (d, 1H), ^{13}C NMR (100 MHz, DMSO-d_6): 170.30, 168.68, 163.90, 151.56, 146.40, 135.02, 131.26, 131.23, 131.02, 129.72, 129.60, 128.49, 128.43, 123.11, 121.53, 114.89, 112.00, 79.00, 44.35, 38.0-40.05,Mass (m/z): $[M+1]^+$387.02.

4-(4-Nitrophenyl)-5a,6-dicyclobenzo[4,5]imidazo[1,2-*a*]furo[3,4-*e*]pyrimidine-1,3(3a*H*,11a*H*)-dione (5l)

Yellow crystals, m.p. 210-212 °C. FTIR (KBr cm^{-1}): 3261 (-NH), 2831 (-CH), 1734 (-C=O), 1735 (C=N), 1572 (C=C aromatic), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.9 (s, 1H, D_2O exchangeable NH), 6.16-8.40 (m, 8H, Ar), 3.82 (s, 1H, -CH), 3.3 (d, 1H, -CH), 2.3 (d, 1H, -CH), ^{13}C NMR (100 MHz, DMSO-d_6): 170.30, 168.12, 164.19, 151.05, 142.25, 139.05, 137.13, 127.49, 127.90, 129.54, 118.76, 115.23, 111.07, 76.0, 41.04, Mass (m/z): $[M+1]^+$364.09.

4-(4-Fluorophenyl)-5a,6-dicyclobenzo[4,5]imidazo[1,2-*a*]furo[3,4-*e*]pyrimidine-1,3(3a*H*,11a*H*)-dione (5m)

Greenish crystals, m.p. 220-222 °C. FTIR (KBr cm^{-1}): 3246 (-NH), 2802 (-CH), 1710 (-C=O), 1630 (-C=N) 1594 (C=C aromatic),^{1}H NMR 400 MHz, DMSO-d_6) δ 9.0 (s, 1H, D_2O exchangeable NH), 6.10-7.95 (m, 8H, Ar), 3.7 (s, 1H, -CH), 3.2-3.0 (d, 1H, -CH), 2.5 (d, 1H, -CH), ^{13}C NMR (100 MHz, DMSO-d_6): 170.20, 168.53, 164.30, 151.61, 148.00, 138.00, 136.14, 131.00, 122.97, 122.00, 114.00, 111.52, 79.00, 44.53, 39.00-40.00,Mass (m/z): $[M + 1]^+$337.08.

4-(2,3-Dihydroxyphenyl)-5a,6-dicyclobenzo[4,5]imidazo[1,2-*a*]furo[3,4-*e*]pyrimidine-1,3(3a*H*,11a*H*)-dione (5n)

Milky white crystals, m.p. 198-200 °C. FTIR (KBr cm^{-1}): 3266 (-OH), 3225 (-NH), 2869 (-CH), 1725 (-C=O), 1624 (-C=N), 1542 (-C=C- aromatic), ^{1}H NMR 400 MHz, DMSO-d_6) δ 9.88 (s, 1H, D_2O exchangeable NH), 6.16-7.68 (m, 7H, Ar), 4.7(s, 2H, -OH), 3.82 (s, 1H, -CH), 3.0 (d, 1H,-CH), 2.4 (d, 1H, -CH), ^{13}C NMR (100 MHz, DMSO-d_6): 169.90, 169.02, 160.25, 151.45, 150.29, 149.15, 132.43, 131.32, 130.02, 130.04, 129.96, 129.74, 129.09, 123.09, 121.94, 113.76, 111.77, 101.00, 78.00,41.00., Mass (m/z): $[M+1]^+$351.08.

Biological activity

The in-vitro antimicrobial activity has been studied by a disc diffusion method or Kirby-Bauer method[24] with different strains of bacteria and fungi. Gentamicin and amphotericin B were used as positive controls for bacteria and fungi, respectively. The compounds were screened for antibacterial activity against *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcusaureus*ATCC 25923 in Mueller-Hinton agar (M173) medium, and for antifungal activity against *Candida sp.* in Sabouraud‘s dextrose agar medium. The plates were incubated at 37 °C for 24 h for both antibacterial and antifungal activities.

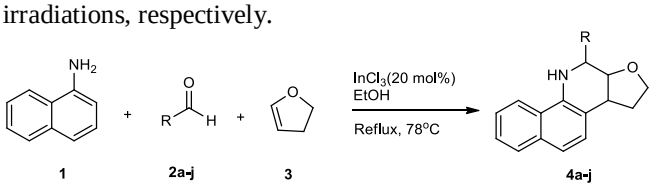
The in-vitro antituberculosis activity has been carried out by CLAIRO COMBI method[25]with tuberculosis bacteria and Streptomycin was used as a positive control. Liquefied sterile Lowenstein-Jensen agar is poured into Petri dishes kept on the level surface. Media depth was 4 mm. After solidification, the dishes were dried for 30 min in an incubator at 37 °C to remove excess moisture from the surface. While pouring into the plates, 5 % defibrinated sterile blood was added to the test organism. The plates were incubated at 37 °C for 4 days.

In vitro anti-inflammatory activity measurement (human red-blood-cell (HRBC) membrane stabilization method): Fresh whole human blood (5mL) are collected and transferred to the centrifuged tubes containing Heparin or EDTA or sodium citrate to prevent clotting. The tubes are centrifuged at 3000 rpm for 10 min and are washed three times with equal volume of normal saline. The volume of the blood is measured and reconstituted as 10% v/v suspension with normal saline. The reaction mixture consists of 1.0mL of a test sample of different concentrations in normal saline and 0.5mL of 10% HRBC suspension, 1 mL of 0.2 M phosphate buffer, 1 ml hypo saline were incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 30 mins. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac was used as a control.[26-29]

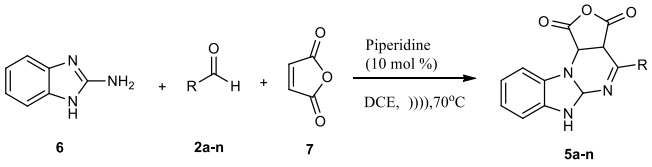
RESULTS AND DISCUSSION

We have synthesized (11-(*R*)-2,3,3a,10,11,11a-hexahydrobenzo[*h*]furo[2,3-*c*]quinoline(**4a-j**) (Scheme 1 and Table 1) and 4-(*R*)-5a,6-dicyclobenzo[4,5]imidazo[1,2-*a*]furo[3,4-*e*]pyrimidine-1,3(3a*H*,11a*H*)-dione derivatives

(**5a-n**) (Scheme 2 and Table 2) via one-pot multi-component [4+2]cycloaddition reactions of dienes and a dienophiles using $InCl_3$ or piperidine as Lewis base catalyst in EtOH under reflux or in dichloroethane under ultrasound irradiations, respectively.



Scheme 1. Synthesis of tetrahydroquinoline derivatives.



Scheme 2. Synthesis of pyrimidine derivatives

First, imines (Schiff bases) form from the 4-aminonaphthalene (**1**) or 2-aminobenzimidazole (**6**) and the aromatic aldehydes (**2a-j**) and these are cyclized with dihydrofurane (**3**) or maleic anhydride (**7**) into the appropriate condensed heterocycles (**6** and **7**, respectively). The synthesized tetrahydroquinoline (**4a-j**) and pyrimidine (5**a-n**) derivatives were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy techniques.

The prepared compounds have been screened for antibacterial, antifungal, antituberculosis[32] and anti-inflammatory activities and measured the zones showing complete inhabitation and record the diameters of zones to the nearest millimeter. The results are summarized in Tables 3 and 4.

All tetrahydroquinoline derivatives (**4a-j**) showed antibacterial activity only against *Staphylococcus aureus* ATCC 25923 bacteria but did not show any activity against *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC27853 or antifungal activity against *Candida sp.*

Table 1. Tetrahydroquinoline derivatives synthesized using $InCl_3$ as catalyst via [4+2] cycloaddition route

Entry	R	Product	Time, h	Yield, %	M.p.,^{o}C
1	2,5-$(MeO)_2C_6H_3$	**4a**	3.15	88	218
2	4-CNC_6H_4	**4b**	3.50	81	253
3	4-HO-3-$MeOC_6H_3$	**4c**	3.40	77	212
4	Ph	**4d**	2.45	80	203
5	3-BrC_6H_4	**4e**	3.20	81	197
6	4-$MeOC_6H_4$	**4f**	3.48	80	218
7	4-ClC_6H_4	**4g**	3.30	79	164
8	2,4-$(Cl)_2C_6H_3$	**4h**	4.00	76	222
9	4-$NO_2C_6H_4$	**4i**	2.15	74	223
10	4-FC_6H_4	**4j**	3.27	82	213

Table 2. Pyrimidine derivatives synthesized using piperidine as catalyst via [4+2] cycloaddition route

Entry	R	Product	Time, min	Yield, %	M.P.,^{o}C
1	4-$MeOC_6H_4$	**5a**	68	80	252
2	2,5- $MeO_2C_6H_3$	**5b**	30	74	170
3	3,4-$HO_2C_6H_3$	**5c**	60	77	225
4	3-HOC_6H_4	**5d**	32	78	202
5	4-CNC_6H_4	**5e**	52	73	196
6	4-HO-3-$MeOC_6H_3$	**5f**	48	71	212
7	Ph	**5g**	53	75	248
8	3-BrC_6H_4	**5h**	63	79	240
9	4-HOC_6H_4	**5i**	72	69	192
10	4-ClC_6H_4	**5j**	69	78	218
11	2,4-$Cl_2C_6H_3$	**5k**	74	76	184
12	4-$NO_2C_6H_4$	**5l**	64	71	212
13	4-FC_6H_4	**5m**	79	77	222
14	2,3-$HO_2C_6H_3$	**5n**	50	74	200

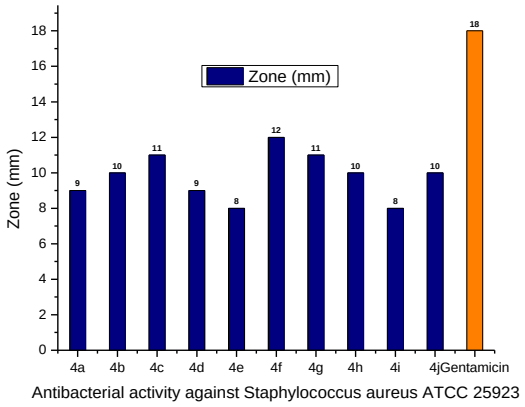


Figure 1. Anti-bacterial activity of tetrahydroquinoline derivatives against *Staphylococcus aureus* ATCC 25923

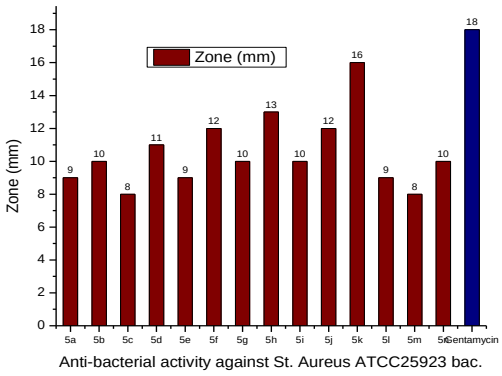


Figure 2. Anti-bacterial activity of pyrimidine derivatives against *Staphylococcus aureus* ATCC 25923

All pyrimidine derivatives (**5a-n**) showed antibacterial activity only against *Staphylococcus aureus* ATCC 25923 bacteria, but not showed against *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC27853. Two pyrimidine derivatives samples (**5j** and **5k**) showed antifungal activity against *Candida sp.*

Antituberculosis activity

The tetrahydroquinoline derivatives (**4a-j**) do not show any antituberculosis activity, but all pyrimidine derivatives (**5a-n**) hadantituberculosisactivity. The compound **5k** showed activity in the higher while **5a, 5h** and **5i** in the lower zone.

The percentage of HRBC hemolysis and membrane stabilization or protection was calculated by using the standard formula.

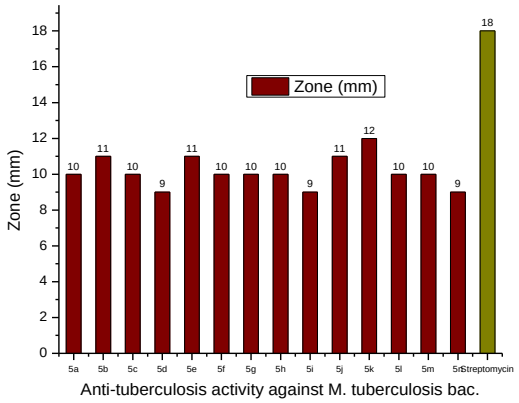


Figure 3. The anti-tuberculosis activity of pyrimidine derivatives

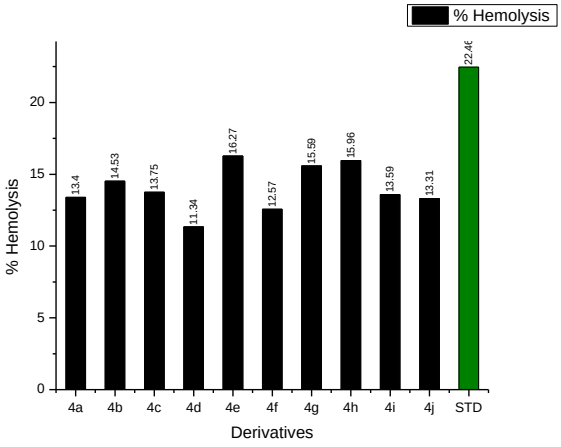


Figure 4. Anti-inflammatory activity of tetrahydroquinoline derivatives

The results can be seen in Tables **3** and **4.** In the tetrahydroquinoline-series, sample (**4d**) showed the highest percentage of HRBC membrane stabilization and sample (**4h**) showed the lowest rate of HRBC membrane stabilization.

In pyrimidine derivatives case, sample (**5a**) showed the highest percentage of HRBC membrane stabilization and sample (**5c**) showed the lowest rate of HRBC membrane stabilization.

Table 3. Anti-inflammatory activity of tetrahydroquinoline derivatives (**4a-j**)

Product	% Hemolysis	% Protection	Product	% Hemolysis	% Protection
4a	13.40	86.59	**4g**	15.59	84.40
4b	14.53	85.46	**4h**	15.96	84.03
4c	13.75	86.24	**4i**	13.59	86.40
4d	11.34	88.65	**4j**	13.31	86.68
4e	16.27	83.72	**Ref.**	22.46	77.53
4f	12.57	87.42			

Table 4. Anti-inflammatory activity of pyrimidine derivatives (**5a-5n**)

Product	% Hemolysis	% Protection	Product	% Hemolysis	% Protection
5a	15.52	84.47	**5h**	16.03	83.96
5b	18.07	81.92	**5i**	16.38	83.61
5c	20.94	79.07	**5j**	17.62	82.37
5d	19.67	80.32	**5k**	17.74	82.25
5e	17.35	82.62	**5l**	16.87	83.12
5f	17.48	82.51	**5m**	20.18	79.81
5g	19.02	80.97	**5n**	19.11	80.88
			Ref.	22.46	77.53

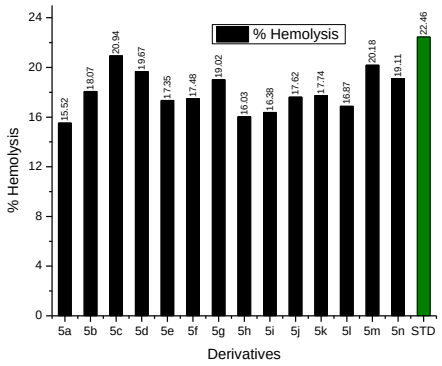


Figure 5. Anti-inflammatory activity of pyrimidine derivatives (**5a-5n**)

CONCLUSION

We have used an eco-friendly route for the preparation of new tetrahydroquinoline and pyrimidine derivatives and screened for their antibacterial against *E. coli, Pseudomonas aeruginosa and Staphylococcus aureus*, antifungal activity against *Candida sp.*, anti-tuberculosis activity against *tuberculosis bacteria* and in vitro anti-inflammatory activity. We have concluded that these series of compounds certainly hold great promise toward good active leads in medicinal chemistry.

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GREEN CHEMISTRY APPROACH FOR THE SYNTHESIS OF NOVEL TETRAZOLE DERIVATIVES AND EVALUATION OF ANTIFUNGAL ACTIVITY

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Keywords: chalcones; tetrazoles; antifungal activity; 2-substituted 4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepines

New 2-substituted-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine derivatives were synthesized by conventional as well as microwave method. Benzonitrile and sodium azide in the presence of ammonium chloride and DMF produces 5-phenyltetrazole; this on reaction with acetic anhydride forms 5-phenyl-1-acetyl tetrazole which reacted with different aromatic aldehydes in the presence of the alkaline medium, to yield corresponding chalcones. Chalcones on further reaction with o-phenylenediamine yield 2-substituted-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepines (**4a-4j**). The structures of newly synthesized compounds were characterized by physical and spectral characteristics by FT-IR and ^{1}H NMR spectroscopy. All synthesized compounds were evaluated for their antifungal activity by MIC (minimal inhibitory concentration, broth dilution method) against *A. niger* and C. *albicans*. All synthesized compounds show moderate to good antifungal activity.

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Introduction

Tetrazoles have been attracted as an important class of heterocyclic compounds in the field of clinical research and medicinal chemistry. Tetrazoles have not been found in nature, but they are resistant to biological degradation. This property makes it possible to use tetrazoles as isosteric substituents of various functional groups in the development of biologically active substances.[1] Tetrazole and their derivatives have great importance in pharmaceutical chemistry due to their diverse biological activity such as antifungal,[2] antibacterial,[3] antiinflammatory,[4] antituberculous,[5] antihypertensive agents,[6] anticancer,[7] antibiotic[8] and anticonvulsant.[9]

Development of the tetrazole chemistry has mainly been associated with the wide-scale application of these compounds in medicine, biochemistry and agriculture. The tetrazole functionality plays a vital role in medicinal chemistry, primarily due to its ability to serve as the bioequivalent (bioisoster) of the carboxylic acid group. In particular, 1-substituted tetrazoles and 5-thio-substituted tetrazoles have been used in the synthesis of pharmacologically active drugs.[3]

The 1,5–benzodiazepines moiety is a privileged class of pharmacophore, as compounds bearing this structural unit possess a broad spectrum of biological activities, as antimicrobial,[10] anti-inflammatory[11], anticancer[12] and anticonvulsant activities.[13] The synthesis of the 1,5–benzodiazepines moiety involves the reaction of chalcones with o-phenylenediamine.[14] Tetrazoles clubbed with benzodiazepines will help to improve the antifungal properties of the pharmacophore leading to more potent compounds.

In recent years, organic reactions involving a green chemistry approach have received considerable attention in organic synthesis because of their ease handling, enhanced reaction rates, more excellent selectivity, simple workup and recoverability of the products.[15] The synthesis of novel tetrazole based benzodiazepines derivatives and investigation of their chemical and biological behavior has gained more importance in recent decades for biological and pharmaceutical reasons.

In continuation of research in the field of green chemistry, an attempt is made to synthesize tetrazole containing benzodiazepine and the compounds have been evaluated for antifungal activity, which has not been reported yet.

Experimental

Melting points were determined with open capillary and were uncorrected. FT-IR spectra were recorded on a 'JASCO FT-IR-4600' spectrophotometer, ^{1}H-NMR spectra were recorded in BRUKER AVANCE II400'NMR spectrometer at 400 MHz frequency in DMSO using TMS as an internal standard.

Synthesis of 5-phenyltetrazole (1)

A mixture of benzonitrile (3.3 g, 0.10 mol), sodium azide (0.65 g, 0.10 mol) dimethylformamide (10 mL) and ammonium chloride (5.3 g, 0.10 mol) was heated in an oil bath for 7 h at 125 °C. The solvent was removed under reduced pressure. The residue was dissolved in 100 mL of

water and carefully acidified with concentrated hydrochloric acid to pH 2. The solution was cooled to 5 °C in an ice bath. Compound **1** has been recrystallized from aqueous methanol.

Synthesis of 5-phenyl-1-acetyltetrazole (2)

A solution of 5-phenyl tetrazole (12.8 g, 0.08 mol), acetic anhydride (0.08 mol) and 2-3 drops of concentrated sulphuric acid were heated for 15-20 min on a water bath, then cooled and poured into ice-cold water. The product was filtered and dried and recrystallized from ethanol.

General procedure for the preparation of chalcones (3a-3j): Method 1. Conventional synthesis

A solution of 5-phenyl-1-acetyltetrazole (8.5g, 0.005 mol) and the aromatic aldehyde (0.005 mol) in ethanol (12 mL) was cooled to 5 to 10 °C in an ice bath. The cooled solution was treated with dropwise addition of aqueous potassium hydroxide (2.5 mL, 50 %). The reaction mixture was stirred for 30 min and then left overnight. The resulting dark solution was diluted with ice water and carefully acidified using diluted hydrochloric acid. The chalcone was collected by filtration and washed with aqueous sodium bicarbonate and water then recrystallized from ethanol.

Method 2. Microwave-assisted synthesis

A mixture of 0.01 mol 5-phenyl-1-acetyltetrazoles, 0.01 mol of aromatic aldehydes, ethanol (5 mL) and 2.5 mL of NaOH (6 M) was kept in a microwave oven at level 2 and time for 2 min. The removed mixture was cooled in an ice-bath and acidified with concd. HCl. The chalcone was collected by filtration.

Synthesis of substituted tetrazoles derivatives (4a-4j) - Method 1. Conventional synthesis

A mixture of chalcone (**3a-h**) (0.01 mol) and o-phenylenediamine (0.01 mol) was dissolved in absolute ethanol (30 mL) in the presence of 20 % aq. NaOH, and the reaction mixture was refluxed for about 5 h. After completion of the reaction, the reaction mixture was poured into crushed ice. The product obtained was filtered, washed with cold water. The compounds were obtained as yellow, brown, or dark brown crystals and they were recrystallized from ethanol

Method 2. Microwave-assisted synthesis

A mixture of 0.1 mol of chalcone (**3a-j**), 0.1 mol of o-phenylenediamine, ethanol (5 mL) and 3 ml of NaOH solution (6 M) was kept in a microwave oven at level 3 and time for 3 min. The removed mixture was cooled in an ice-bath and acidified with concd. HCl. The synthesized tetrazole derivatives were collected by filtration.

Spectral data of compounds: [IR (KBr), ν, cm^{-1} and ^{1}H-NMR (DMSO), δ ppm]

2-(4-Chlorophenyl)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4a)

FT-IR: 1228 (N-N=N-), 1140 (tetrazole), 3565 (N-H), 1652 (C=C), 2929 (Ar-CH), 1521 (C=N). ^{1}H-NMR: 3.2-5.3 (5H, m, benzodiazepine), 6.5-7.9 (13H, m, Ar-H).

4-[4-(5-Phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepin-2-yl]phenol (4b)

FT-IR: 1338 (N-N=N-), 1108 (tetrazole), 3586 (N-H), 1646 (C=C), 2969 (Ar-CH), 1507 (C=N). ^{1}H-NMR: 3.1-5.2 (5H, m, benzodiazepine), 5.4(1H, s, OH), 6.6-7.8 (13H, m, Ar-H).

2-(4-Bromophenyl)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4c)

FT-IR:1216 (N-N=N-), 1116 (tetrazole), 3637 (N-H), 1638 (C=C), 2969 (Ar-CH), 1589 (C=N). ^{1}H-NMR: 3.2-5.3 (5H, m, benzodiazepine), 6.6-7.8(13H, m, Ar-H).

2-(2,4-Dimethoxy)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4d)

FT-IR:1216 (N-N=N-), 1123 (tetrazole), 3637 (N-H), 1635 (C=C), 2956 (Ar-CH), 1540 (C=N). ^{1}H-NMR: 3.2-5.3 (5H, m, benzodiazepine), 6.6-7.8 (12H, m, Ar-H), 2.27 (6H, s, OCH_3)

2-(4-Nitrophenyl)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4e)

FT-IR:1216 (N-N=N-), 1092 (tetrazole), 3446 (N-H), 1683 (C=C), 2959 (Ar-CH), 1558 (C=N). ^{1}H-NMR: 3.2-5.4(5H, m, benzodiazepine),6.4-7.9(13H, m, Ar-H).

2-(2-Chlorophenyl)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4f)

FT-IR: 1228 (N-N=N-), 1116 (tetrazole), 3565 (N-H), 1652 (C=C), 2929 (Ar-CH), 1521 (C=N). ^{1}H-NMR: 3.2-5.3 (5H, m, benzodiazepine), 6.5-7.9 (13H, m, Ar-H).

2-(3-Nitrophenyl)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4g)

FT-IR:1216 (N-N=N-), 1183 (tetrazole), 3446 (N-H), 1683 (C=C), 2959 (Ar-CH), 1558 (C=N). ^{1}H-NMR: 3.4-5.3(5H, m, benzodiazepine), 6.3-7.9(13H, m, Ar-H).

2-(Furoyl-2-yl)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4h)

FT-IR: 1215 (N-N=N-), 1120(tetrazole), 3524 (N-H), 1636 (C=C), 3013 (Ar-CH), 1540 (C=N). ^{1}H-NMR: 3.2-5.3 (5H, m, benzodiazepine), 6.5-7.9 (12H, m, Ar-H).

2-(4-Dimethylamino)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine (4i)

FT-IR: 1218 (N-N=N-), 1133 (tetrazole), 3565 (N-H), 1652 (C=C), 2968 (Ar-CH), 1540 (C=N). ^{1}H-NMR: 3.2-5.3 (5H, m, benzodiazepine), 6.5-7.9 (13H, m, Ar-H), 2.7 (6H, s, $(CH3)_2$)

2-(4-Dimethylamino)-4-(5-phenyl-1H-tetrazol-1-yl)-2,3,5a,9a-tetrahydro-1H-1,5-benzodiazepine.

FT-IR: 1224 (N-N=N-), 1119 (tetrazole), 3545 (N-H), 1652 (C=C), 2924 (Ar-CH), 1507 (C=N). ^{1}H-NMR: 3.2-5.6 (5H, m, benzodiazepine), 6.7-8.1(14H, m, Ar-H), 6.3-6.5 (2H, s, CH=CH) **(4j)**

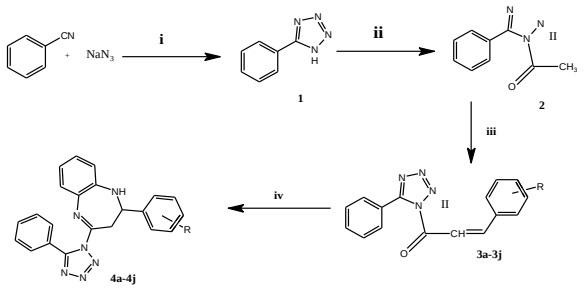
Antifungal activity-

The synthesized compounds were screened for antifungal activity by using MIC (minimal inhibitory concentration, broth dilution method). A Sabouraud-dextrose media (double strength test tubes) was prepared. A test tube without inoculum was used as a negative control. Inoculums (three to four drops) are added to reach the requested concentration of microorganism (10^6 cell/test tubes); The test compounds were added ranging from 0.5 to 5 mL except for uninoculated (negative control) and control (positive control) tube. The final volume was adjusted (10 mL) by using sterile water. All test tubes are properly shaken and then incubated at 37 °C for two days.

Results and discussions

The tetrazole derivatives were synthesized using conventional as well as microwave-assisted synthesis methods according to Scheme. Spectral data confirmed the structure of all synthesized derivative.

5-Phenyltetrazole (compound **1)** was prepared by the reaction of benzonitrile with sodium azide in the presence of ammonium chloride and DMF. 5-Phenyltetrazole (**1**) was converted to 5-phenyl-1-acetyltetrazole (**2**) by the reaction with acetic anhydride and sulphuric acid. Compounds **3a-3j** were obtained by treatment of **2** with aromatic aldehydes in the presence of NaOH. Compounds **3a-3j** on treatment with o-phenylenediamine in the presence of ethanol and NaOH yielded a compounds **4a-4j**, respectively.



Reagents and condition: i) DMF/ammonium chloride (conventional method), ii) acetic anhydride/H_2SO_4 (conventional method); iii) ArCHO/NaOH (conventional or microwave method); iv) o-Phenylenediamine (OPD)/NaOH (conventional or microwave method)

The IR spectra of compounds **4a-4j** show absorption bands at 2929 cm^{-1} due to Ar-H and at 1625 cm^{-1} due to C=N ring stretches. Absorption bands occur at 1280 (N-N=N-), 1108 and 1140 cm^{-1} (tetrazole ring).

The ^{1}H-NMR spectra show the chemical shift at 6.9-7.8 due to aromatic protons, 3.2-5.6 due to (5H benzodiazepine part). The results of spectral data are in good agreement with the structure of synthesized compounds.

Table 1. Physicochemical data of compounds **4a-4j** prepared by conventional (CM) and microwave-assisted (MW) methods

	'R' group	Molecular formula	M.wt.	Time		M.P. (^{0}C)		% yield		R_f Value
				CM, h	MW, min	CM	MW	CM	MW	
4a	4-Cl	$C_{22}H_{19}ClN_6$	402	5	3	187	186	65.6	70.6	0.56
4b	4-OH	$C_{22}H_{20}N_6O$	384	5	3	155	155	62.5	68.9	0.63
4c	4-Br	$C_{22}H_{19}BrN_6$	447	5	3	160	161	66.5	74.9	0.69
4d	2,4-$(OMe)_2$	$C_{24}H_{24}N_6O_2$	428	5	3	190	188	56.4	65.8	0.67
4e	4-NO_2	$C_{22}H_{19}N_7O_2$	413	5	3	150	149	74.3	80.9	0.52
4f	2-Cl	$C_{22}H_{19}ClN_6$	402	5	3	181	182	72.5	75.9	0.66
4g	3-NO_2	$C_{22}H_{19}N_7O_2$	413	5	3	154	154	59.8	70.8	0.45
4h	Furoyl	$C_{20}H_{18}N_6O$	358	5	3	145	146	68.3	70.8	0.62
4i	4-NMe_2	$C_{24}H_{25}N_7$	411	5	3	165	164	71.1	68.5	0.55
4j	cinnamoyl	$C_{24}H_{22}N_6$	394	5	3	172	172	63.5	66.8	0.56

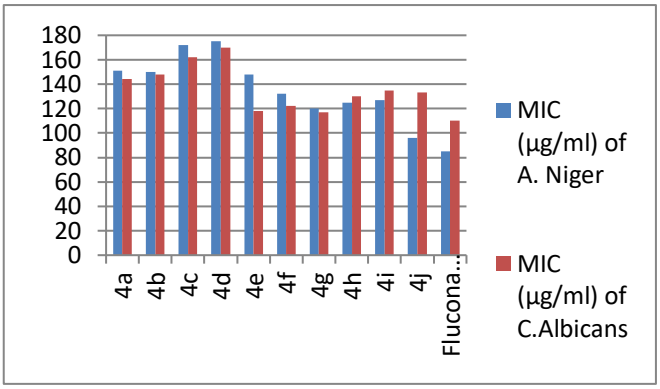


Figure 1. Antifungal activities of synthesized compounds (**4a-4j**)

The results of antifungal activity are depicted in Table 2 and Fig. 1, revealing that all compounds show antifungal activity against *Aspergillus niger* and *Candida albicans.* The activities are comparable with control standard Fluconazole shows potent activity at MIC of 85 and 110 µg mL^{-1}.

Table 2. Anti-fungal activity of synthesized compounds (**4a-4j**)

Compound	MIC, µg mL^{-1}	
	A. niger	***C. Albicans***
4a	148	144
4b	125	148
4c	172	117
4d	175	170
4e	148	118
4f	132	122
4g	120	142
4h	172	161
4i	150	146
4j	172	166
Fluconazole	85	110

Compounds **4b** and **4g** (4-OH, 3-NO_2) have shown good antifungal activity against *A. niger* while compounds, **4c, 4e** and **4f**, (4-Br, 4-NO_2, 2-Cl) have shown good antifungal activity against *C. Albicans.* Compounds **4a** and **4i** (4-Cl, 4-$N(CH_3)_2$) have shown moderate while compounds **4d, 4h** and **4j** showed weak antifungal activity against *A. niger* and *C. albicans.*

Conclusions

Tetrazole derivatives were synthesized from 5-phenyl-tetrazole which was synthesized from benzonitrile and sodium azide in good yields. The compounds **4b** and **4g** possess 4-OH, 3-NO_2 potent anti-inflammatory activity in comparison with control. The compounds **4c, 4e** and **4f** containing 4-Br, 4-NO_2, 2-Cl substitution produce moderate anti-inflammatory activity. The compounds **4d, 4h** and **4j** have shown weak anti-fungal activity against *A. niger* and *C. Albicans.*

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SUPPORTED LIQUID MEMBRANE EXTRACTION OF TERBIUM(III) BY CYTOS IL102/D_2EHPA (TRIHEXYLTETRADECYLPHOSPHONIUM BROMIDE/DI(2-ETHYLHEXYL) PHOSPHATE) EXTRACTANT MIXTURE

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Keywords: Terbium(III); trihexyltetradecylphosphonium di(2-ethylhexyl) phosphate (D_2EHPA); trihexyl (tetradecyl)phosphonium bromide(Cytos IL102); membrane extraction; ionic liquid.

We have developed a membrane impregnated with ionic liquid Cytos IL102/D_2EHPA (trihexyltetradecylphosphonium bromide/di(2-ethylhexyl) phosphate) for the extraction of Tb(III) from aqueous solutions at different pH values. Various parameters such as the mixing effect Cytos IL102/D_2EHPA, the initial terbium concentration, the stirring speed and the extraction time have been studied. The amount of Tb(III) retained per gram of extractant (Cytos IL102/D2EHPA) is 4.37 mg g^{-1} for a concentration of Tb(III) of 10^{-3} M. The optimal yield was obtained at 240 min and stirring speed at 900 rpm.

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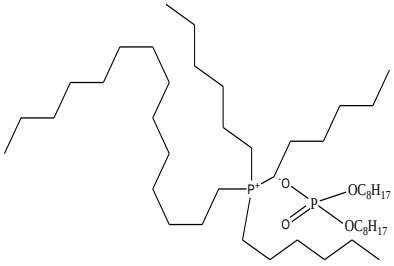
INTRODUCTION

The supported liquid membrane separation (LMS) technique is an advanced solvent extraction technique that provides a simple and effective method for extracting and separating metal ions.[1] The use of membranes is becoming increasingly important in the separation and recovery of toxic and valuable metals as well as in the treatment of effluents containing low concentrations of solutes in large volumes, without generating secondary waste.[2,3] Rare earth removal can be achieved by the supported liquid membrane extraction process.[4-8]

Terbium is used in alloys and in the production of electronic devices and other magneto mechanical devices. Terbium oxide is used in green phosphors in fluorescent in trichromatic lighting technology of lamps and colour TV tubes. In order to meet the fast-growing demand and to ensure sufficient supply of terbium, it is essential to develop an efficient Terbium recovery process from post-consumer terbium containing products.



Scheme 1. Trihexyltetradecylphosphonium di(2-ethylhexyl) phosphate (D2EHPA).

In the present work, the extraction of Tb(III) from a nitrate solution through a supported liquid membrane impregnated with the new ionic liquid D_2EHPA (Scheme 1)/Cytos IL102 (trihexyltetradecylphosphonium bromide) has been studied.

Various parameters have been studied, such as agitation speed, pH of the feed phase and the initial concentration of terbium. The use of the mixture of D_2EHPA and Cytos IL 102 as extractants for the extraction of terbium(III) on a supported liquid membrane has not been reported in the literature.

EXPERIMENTAL

Terbium solution at 10^{-2} M was prepared by dissolving of terbium(III) nitrate (3.025 g) in 1 L of distilled water (purchased from Sigma-Aldrich). The initial pH of the sample solutions were adjusted by using dil. HNO_3 or NaOH (from Sigma-Aldrich). $NaNO_3$ (from Merck) was used to study the salt effect. Arsenazo III 10^{-3} M (from Alfa-Aesar) was prepared by dissolving 0.0820 g in absolute ethanol. Cytos IL102 (trihexyl (tetradecyl)phosphonium bromide) was obtained from Cytec (www.cytec.com).

The membrane support was a microporous polyvinylidene difluoride (PVDF) film, with nominal porosity of 70 %, an average pore size of 0.1 µm and a total thickness of 125 µm (VVHP04700), procured from Millipore, Germany (Figures 1 and 2).

Samples containing Tb(III) were analyzed by spectrophotometer (Analytik Jena Specord 210 Plus), with Arsenazo III as ligand. The morphology of the hydrophobic support membrane at the surface and in the thickness was determined using a scanning electron microscope (SEM) Carl Zeiss EVO®40 EP. pH measurements were taken on a potentiometer Consort C831.

General extraction procedure

The membrane extraction experiments were carried out a one compartment cell with mechanical stirring throughout the experiments, separated by a microporous membrane, one for feed solution and the other for stripping solution. Initial concentration of Tb in the feed phase was 10^{-3} mol L^{-1} in all the SLM studies.

The liquid membrane phase was prepared by dissolving of D_2EHPA and Cytos IL102 (Scheme 1) in diethyl ether. The PVDF support was impregnated with the carrier solution for 24 h, SLMs needed more than 12 h, then removed from the solution and wiped carefully with a tissue paper to remove the excess carrier after with water to remove the excess of the organic solvent from the surface of the membrane.

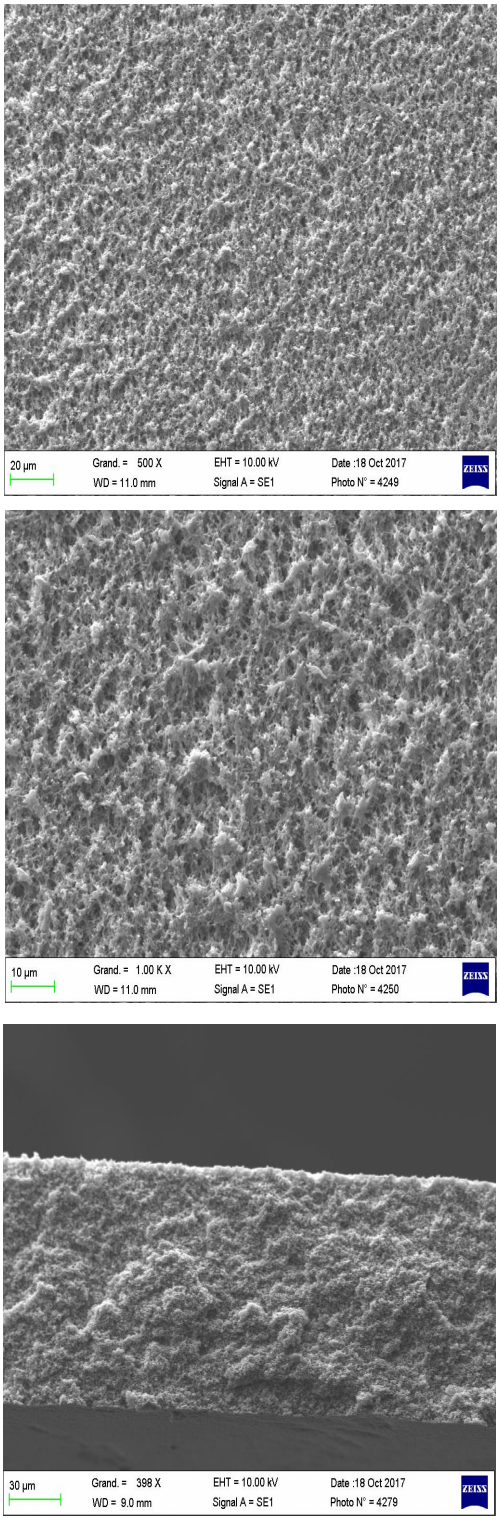


Figure 1. Surface SEM images of a PVDF hydrophobic support membrane and its thickness (Millipore VVHP04700).

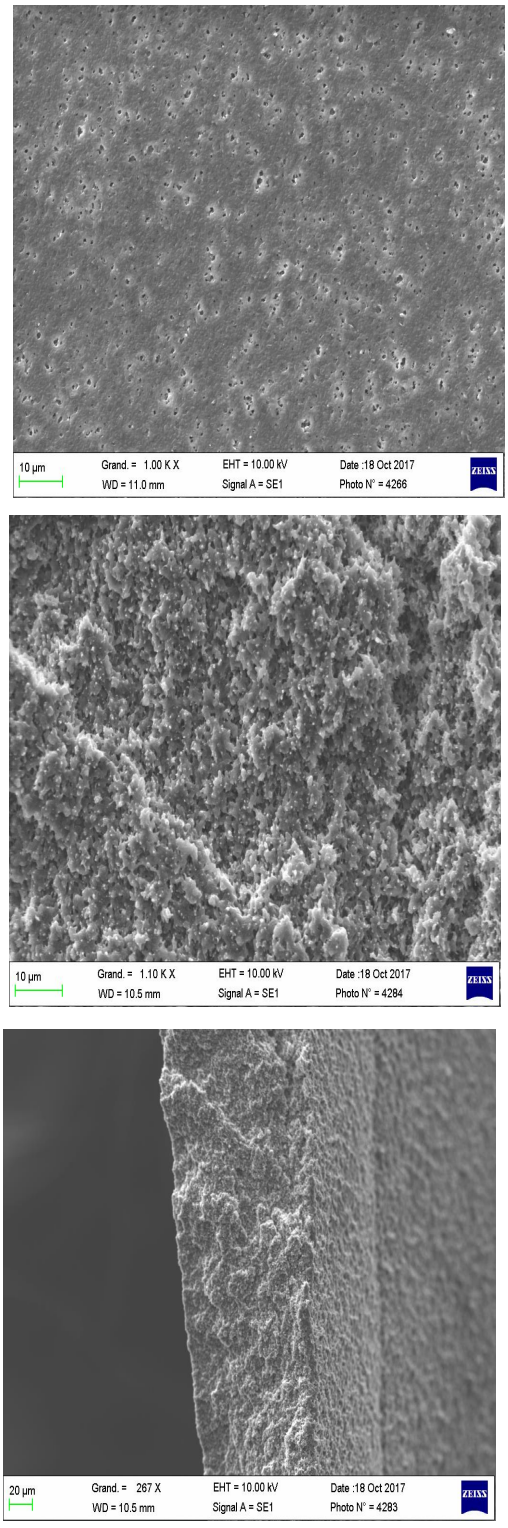
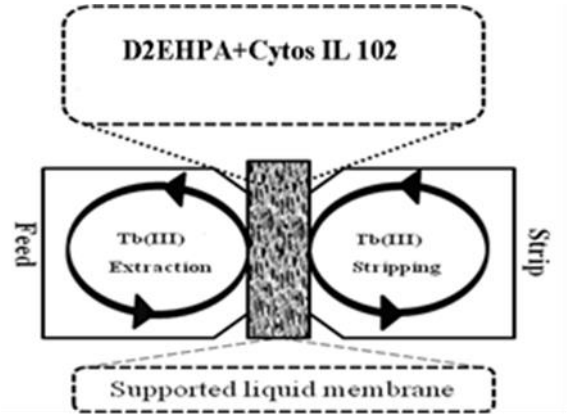


Figure 2. SEM image of the hydrophobic support membrane impregnated with D_2EHPA/Cytos IL102.

After this, each membrane was leaved and dripped for 30 second before being placed in the transport cell, which consists of two identical compartments of 55 mL separated by the impregnated membrane. The effective membrane area was 11.2 cm^2 (see Scheme 2). The extraction of Tb(III) was monitored by taking 100 µL from the compartment at different times for the spectrometer analysis after the addition of a buffer solution (pH = 4.0) and 150 µL 10^{-3} M of Arsenazo III. All experiments were performed at 25°C.[9,10]



Scheme 2. Illustration of transport of terbium ion in SLM.

The reaction of Arzenazo III with Tb(III) is very fast to form a green complex, which absorbs in the visible range (λ_{max} = 654 nm).[11] Three concentrations of Tb(III) variants from 1.10^{-4} M to 10^{-3} M were prepared to plot the calibration. The percentage of Tb(III) that was extracted by MLS was determined using Eqn.(1).[12]

$$Extraction\ yield\,(\%) = \frac{c_i - c_t}{c_i} \times 100 \qquad (1)$$

The uptake rate of Tb, q_t (mg g^{-1}) was determined by Eqn. (2),

$$q(mg/g) = \frac{(C_0 - C_e)VM}{m} \qquad (2)$$

where

C_i, C_t and C_e were the initial, at time, *t*, and equilibrium Tb(III) concentration (mol L^{-1}), respectively;

V (55 mL) was the volume solution;

M molecular weight (g. mol^{-1}), and

m was the mass of extractant used.

The yields are obtained with an error of ± 0.01 %.

RESULTS AND DISCUSSION

In this study, we have used of the mixture of D_2EHPA and Cytos IL102 as extractants for the extraction of terbium on a supported liquid membrane. Cytos IL102 is a commercial phosphorous ionic liquid (trihexyl(tetradecyl)phosphonium bromide). In this study, the hydrophobic membrane support was a microporous polyvinylidene difluoride (PVDF) film with nominal porosity of 70 %, an average pore size of 0.1 µm and a total thickness of 125 µm (VVHP04700), was procured from Millipore (Germany), was used for the extraction of Tb(III) from a solution of Terbium nitrate. A parametric study was conducted to optimize the extraction conditions.

Effect of stirring speed

One of the main resistances in the liquid membrane technique is the extraction of metal ions. To minimize this resistance, the solutions in the two compartments must be kept in agitation. Figure 1 represents the extraction yield of Tb(III) as a function of time by a hydrophobic membrane for two stirring speeds of 180 rpm and 900 rpm. It is observed that the extraction yield of Tb(III) increases with increasing stirring speed. The best yield was obtained for a stirring speed of 900 rpm. Thus, the stirring speed of 900 rpm was used for the other experiments to be carried out (see Figure 3).

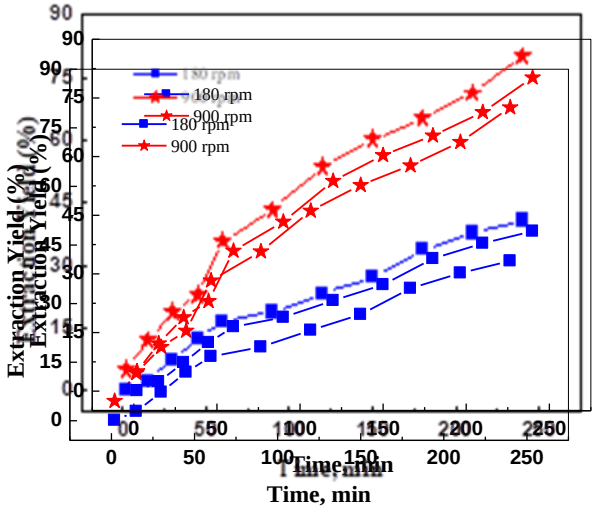


Figure 3. Kinetics of extraction of Tb(III) on MLS hydrophobic at different stirring speeds. [Tb(III)] = 10^{-3} M, molar ratio D_2EHPA/Cytos IL102 (1/1), *T* = 25 °C, pH_i = 5.3, membrane thickness = 125 µm.

Effect of initial pH

The effect of pH in the feeding phase on Tb(III) extraction was studied in a pH range of 2.0 to 5.3 where the predominant species is Tb(III). The solution was adjusted with HNO_3 solutions. The initial concentration of Tb(III) in the feeding phase is 10^{-3} M, the volume of the solution to be extracted in the feed phase is 55 mL and with a molar ratio of D_2EHPA/Cytos IL102 = 1. The results obtained are illustrated in Figure 4.

The curves in Figure 4 show that as the pH decreases from 5.3 to 2.0 in the feed phase, the extraction yield decreases; a maximum yield (80%) was observed at pH 5.3 and for 240 min.

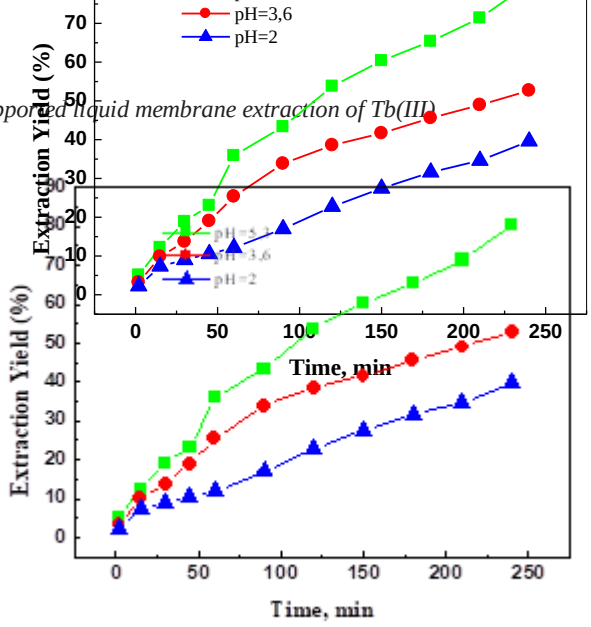


Figure 4. Tb(III) extraction kinetics on MLS hydrophobic at different initial pH. [Tb(III)] = 10^{-3} M, D_2EHPA/Cytos IL102 (1/1), *T*=25°C, pH_i = 5.3, membrane thickness = 125 µm.

Effect of initial concentration

The influences of the initial concentration of terbium(III) on the extraction yield were carried out in a concentration range between 10^{-3} and 10^{-4} M. The volume of the solution to be extracted was adjusted to 55 mL and the molar ratio of D_2EHPA/Cytos IL 02 was 1:1. The results obtained are presented in Figure 5.

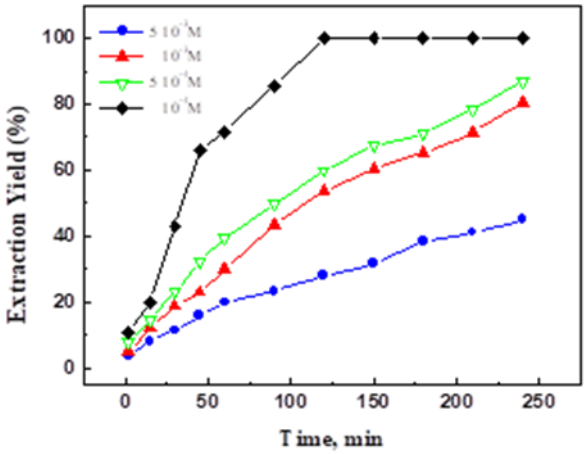


Figure 5. Tb(III) extraction kinetics on MLS hydrophobe at different initial concentrations. D_2EHPA/Cytos IL102 (1/1), *T*=25°C, stirring = 900 rpm, pH_i = 5.3, membrane thickness = 125 µm.

From Figure 5 it is observed that the extraction yield decreases with the increase in the initial concentration of terbium(III) in the feeding phase. In addition, a maximum yield of 100 % is obtained in 120 minutes of stirring, when the initial concentration of terbium(III) is 10^{-4} M.

CONCLUSION

Our work in this study focuses on the extraction of Tb(III) by a membrane impregnated with the ionic liquid D_2EHPA/Cytos IL102. Various parameters, such as the mixing effect D2EHPA/Cytos IL102, the initial terbium concentration, the stirring speed and the extraction time, have been studied. The amount of Tb(III) retained per gram of extractant (D_2EHPA/ Cytos IL102) was 4.37 mg g^{-1} for a concentration 10^{-3} M of Tb(III). The optimal yield was obtained at 240 min and stirring speed at 900 rpm.

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A STUDY OF DISTRIBUTION OF NATURAL RADIONUCLIDES IN SOILS AND ASSESSMENT OF EXPOSURE HAZARDS FROM TERRESTRIAL γ-RADIATION IN THE REGION OF TSALKA (GEORGIA)

K. Kapanadze[a]*, A. Magalashvili[a] and P. Imnadze[b]

Keywords: Natural radionuclides, absorbed dose rate, annual effective dose, radium equivalent activity, Tsalka region (Georgia).

Gamma-spectroscopy method has been used to determine the activity concentrations (in Bq kg^{-1}) of natural radionuclides such as ^{238}U, ^{232}Th, and ^{40}K in soil samples collected from Tsalka region of south Georgia. Based on which contents of radionuclides in soil (in g kg^{-1} and ppm) were calculated. In addition, concentrations of artificial radionuclide of ^{137}Cs were determined, which has shown contamination character of study area. Based on the results of the analysis, some crucial physical values have been calculated, which are necessary for assessment of radiation exposure hazards for the population. Relevant conclusions have been drawn by comparing the results with previous work and recommendations of the international organizations.

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Introduction

It is known that natural radioactive substances in the soil are constant sources of radiation (terrestrial radiation). According to periodic reports published by The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), the average radiation from natural sources equals to 2.4 mSv y^{-1}, whereas the share of radiation from artificial sources is 0.8 mSv y^{-1}.[1,2] Thus, 75 % of total radiation affecting human health is due to natural radiation sources. Consequently, the great importance of studying the existing natural radiation of radioactive sources and assessment of radiation hazards is quite apparent. The major part of the soil radiation comes from the upper layer of the soil,[2,3] in which the sources of radioactivity are ^{238}U, ^{232}Th, their decay products, and radionuclide ^{40}K. Radiological impact of natural radionuclides on humans is mainly expressed by gamma radiation affecting the body, as well as by Radon and the processes caused by inhalation of its decay products.[3]

Aim of this research is to study spatial distribution of natural nuclides in the soils, based on the local geological characteristics of area under research, as well as determination of the contamination characteristics of the area due to artificial radionuclide ^{137}Cs.

Main tasks of the research are to determine concentrations of radionuclides in the soils, to calculate some crucial parameters assessing radiation exposure hazards for the population, namely absorbed dose rate in air, annual effective dose, radium equivalent activity and external hazard index and to compare obtained results with the relevant international monitoring data.

Experimental

Area under research

Natural radioactivity of the soil and ionizing gamma radiation coming from soil depends on the concentration of natural radionuclides it contains, while the latter depends on soil forming parent rock and other forming factors.[1,2,4,] In general, relatively increased radioactivity is associated with igneous rocks and the decreased one with sedimentary rocks. However, there are some exceptions, for instance, some shales and phosphates show relatively high content of radionuclides. Igneous rocks, namely, sialic rocks (especially granitoids) contain a relatively higher concentration of natural radionuclides than ultramafic and mafic rocks.[1,2]

In Georgia, granitoids are occurred in axial region of Caucasus Main Ridge, as well as in the crystal massifs of Dzirula, Khrami, and Loki. Presently, Khrami massif (Tsalka region) as a study area was selected for our research. During the selection, some other important factors, apart from the spread of granitoids, were considered, such as the existence of populated localities, agricultural and mining (of natural industrial materials) activities, etc.

The territory selected for this research covers approximately 20 km^2 of Tsalka municipality in Lower Kartli region (Figure 1). According to existing geological data[5] the most widely spread rocks here are late variscan granitoids building Khrami crystal massif, granodiorites, gneisses, adjacent and partly overlapping continental basaltic lava of neogene-quaternary of calc-alkaline series, continental and shallow marine volcanoclastic rocks, and other (Figure 2). As for soils, the most widely spread ones on the territory under research is black soil.[6]

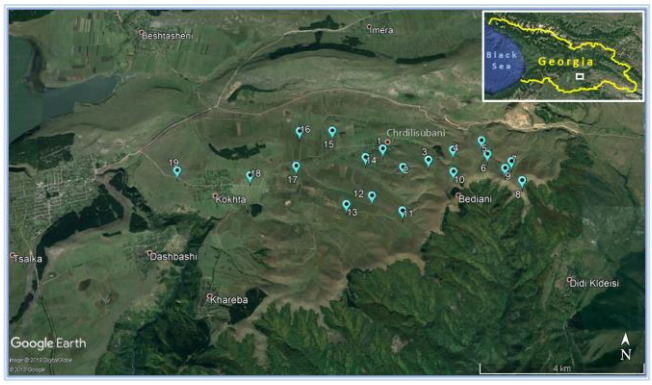

Figure 1. Area under study and sampling sites.

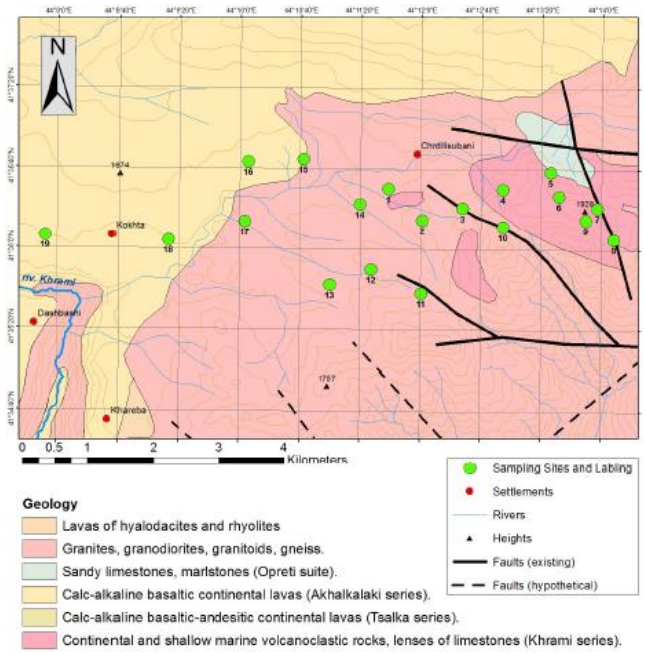

Figure 2. Sampling sites on the geological map.

Sampling and processing

The sampling scheme was selected according to spread of rocks, allowing the determination of the correlations between research parameters and geological and geographical features of the area.

In total, 19 samples were collected from the territory. All samples were taken in the distance from populated localities and buildings or other infrastructural constructions, in order to exclude the occurrence of ecdemic soil or any other materials in the samples to the greatest possible extent.

To get a generalized picture of radionuclide distribution and formation of background radiation by means of existing sampling methodology on the research territory, the so called "envelope" method (Figure 3) was selected,[7] according to which, five samples (30-40 m away from each other) in each sampling site were taken and averaged by means of mixing (i.e. in total 95 samples were taken).

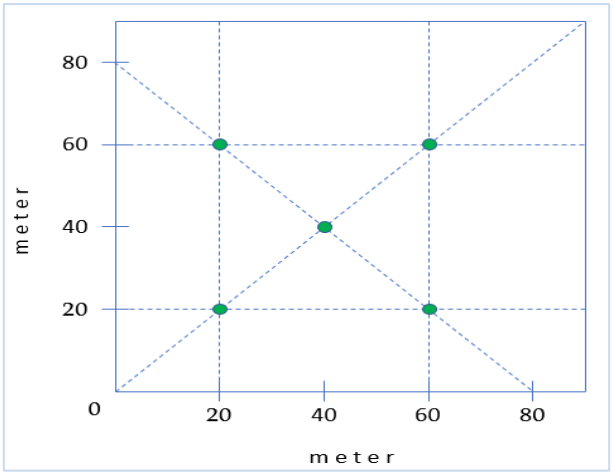

Figure 3. "Envelope" sampling method.

The distance between sampling sites was 600-800 m on an average. Sampling took place at the depth of 15-20 cm under the surface of the soil. The primary processing of samples took place on site (removing stones and roots from the soil samples) and 200-250 g of soil fractions were collected. Table 1 show geographical coordinates recorded on the central point of sampling site and shows agricultural purpose of the soils and parent material of the soil.

For laboratory measurements the samples were further prepared with well established methods.[2-4,8] At first, obtained samples were air dried at room temperature. After this, samples were sifted, first in a sift with 1.5 mm cells and then with 1mm cells and finally, samples were placed in hermetically sealed double polyethylene containers and stored for two months to attain radioactive equilibrium in decay series.

Laboratory research

A well-established gamma-spectroscopy method was used to determine activity concentration of natural radionuclide in soil samples, measurements were made with the use of semiconductor (detector), based on high-purity germanium (HPGe) crystal (manufacturer CANBERRA) and software packages Genie-2000 and ISOCS/LABOCS at Laboratory of Radiological Studies of the Applied Research Centre at the E. Andronikashvili Institute of Physics of the I. Javakhishvili Tbilisi State University.

To measure the activity concentrations of radionuclide i in Bq kg^{-1}, for the peak energy E, eqn. (1) was used,[2-4]

$$A_{\mathrm{Ei}} = \frac{C_{Ei}}{C_{\mathrm{eff}} \gamma m t} \qquad (1)$$

where C_{Ei} is the total count of a peak at energy E, C_{eff} is the detection efficiency at energy E, γ is the percentage of gamma emission probability of the radionuclide i for a transition at energy E, m is the mass in kg of the measured sample, and t is the counting time.

Table 1. Characteristics of sampling sites.

Site	Coordinates	Altitude (m)	Agricultural purpose	Geology
1	41°36.503'N 44°11.643'E	1637	Pasture	Granite
2	41°36.244'N 44°12.014'E	1681	Pasture	Granite
3	41°36.344'N 44°12.465'E	1799	Pasture	Granite
4	41°36.500'N 44°12.914'E	1779	Pasture	Granite
5	41°36.645'N 44°13.437'E	1771	Pasture	Volcanoclastic
6	41°36.447'N44°13.531'E	1839	Pasture	Volcanoclastic
7	41°36.350'N 44°13.953'E	1870	Pasture	Volcanoclastic
8	41°36.090'N 44°14.136'E	1873	Pasture	Granite
9	41°36.248'N44°13.828'E	1912	Pasture	Volcanoclastic
10	41°36.187'N 44°12.914'E	1813	Pasture	Granite
11	41°35.636'N 44°12.011'E	1687	Treated	Granite
12	41°35.833'N 44°11.458'E	1655	Treated	Granite
13	41°35.703'N 44°11.000'E	1624	Treated	Granite
14	41°36.375'N 44°11.329'E	1614	Old treated	Granite
15	41°36.746'N 44°10.701'E	1594	Old treated	Basalt/Granite
16	41°36.724'N 44°10.091'E	1597	Old treated	Basalt
17	41°36.225'N 44°10.056'E	1579	Old treated	Granite
18	41°36.070'N 44°09.216'E	1568	Old treated	Basalt
19	41°36.106'N 44°07.870'E	1573	Pasture	Basalt

Results

Concentrations of radionuclides

As a result of gamma spectrometry analysis for 19 samples activity concentrations of ^{238}U, ^{232}Th, and ^{40}K in Bq kg^{-1} was determined and their contents in g kg^{-1} and in ppm were calculated. The results are provided in Table 2, where apart from natural sources it shows the concentration of ^{137}Cs, which is one of the most important radioactive artificial soil pollutants.

As it can be seen from Table 2, in our case the mean values of activity concentrations are 38.57, 53.18, and 879.76 Bq kg^{-1}, for ^{238}U, ^{232}Th, and ^{40}K, respectively, which exceeds the world mean values (also provided in Table 2) for ^{238}U by 3.57, for ^{232}Th by 18.18, and for ^{40}K by 479.76 Bq kg^{-1}.[1,3]

As for ^{137}Cs, as it can be seen from Table 2, activity concentration of ^{137}Cs fluctuates between 3.75 and 33.00 Bq kg^{-1} with the mean value of 10.53 Bq kg^{-1}.

If the activity concentrations of radionuclides in soil are known assuming that radionuclides are uniformly distributed in the soil, then exposure dose rate in air causing these radionuclides can be found.[1-3] The absorbed dose rate in air is calculated by eqn. (2),[1]

$$D = 0.4620A_U + 0.6040\,A_{Th} + 0.0417\,A_K \quad (2)$$

where D denotes the dose rate in the air at 1 m above the ground surface.

A_U, A_{Th}, and A_K are the activity concentrations of ^{238}U, ^{232}Th, and ^{40}K, respectively, in the soil sample. 0.4620, 0.6040, and 0.0417 are dose conversion factors for ^{238}U, ^{232}Th, and ^{40}K, respectively.

The results calculated for absorbed dose rate in the air are presented in Table 3. The mean value of our results is equal to 86.63 nGy h^{-1}. That considerably exceeds the world mean value. which is 57 nGy h^{-1}.[2,8]

Annual effective dose rate (*E*)

When calculating the annual effective dose rate exposure to population, the following factors should be taken into account,[1-3] (a) coefficient of transferring from absorbed dose to effective dose (0.7 Sv Gy^{-1}) and (b) so called "occupation factor", i.e. how long a human stays outdoor and indoor.

These factors are reported[1] to be 0.2 and 0.8 (a person spends 20 % of time outdoors and 80 % indoors). A summarized effective dose rate is calculated by means of the Eqn. (3),[2,4]

$$E = TQD \times 10^{-6} \quad (3)$$

where D is the absorbed dose rate in the air, Q is the conversion factor of 0.7 Sv Gy^{-1}, which converts the absorbed dose rate in the air to human effective dose received, and T is the time during a year, i.e. 8760 h. According to the results given in Table 3, in our case, the mean annual effective dose rate is 0.55 mSv Gy^{-1}, which is a little higher than world mean value[1,3] i.e., 0.48 mSv Gy^{-1}.

Table 2. Concentrations of radionuclides in soil samples.

Site #	Bq kg^{-1}				g kg^{-1}				ppm			
	^{238}U	^{232}Th	^{40}K	^{137}Cs	^{238}U	^{232}Th	^{40}K	^{137}Cs	^{238}U	^{232}Th	^{40}K	^{137}Cs
1	42.50	44.40	690.60	10.60	0.00345	0.01100	0.00267	3.30 x 10^{-12}	3.45	11.0	2.67	3.30 x 10^{-9}
2	39.40	53.80	745.80	9.60	0.00320	0.01330	0.00289	3.00 x 10^{-12}	3.20	13.3	2.89	3.00 x 10^{-9}
3	38.70	50.70	936.00	4.50	0.00314	0.01250	0.00362	1.40 x 10^{-12}	3.14	12.5	3.62	1.40 x 10^{-9}
4	38.30	51.40	933.00	11.50	0.00311	0.01266	0.00361	3.59 x 10^{-12}	3.11	12.66	3.61	3.59 x 10^{-9}
5	39.60	50.00	867.30	5.50	0.00321	0.01232	0.00336	1.72 x 10^{-12}	3.21	12.32	3.36	1.72 x 10^{-9}
6	40.67	50.50	933.00	3.75	0.00330	0.01240	0.00361	1.17 x 10^{-12}	3.30	12.4	3.61	1.17 x 10^{-9}
7	43.44	56.50	1008.00	12.26	0.00352	0.01392	0.00390	3.83 x 10^{-12}	3.52	13.92	3.90	3.83 x 10^{-9}
8	40.45	54.40	944.00	11.30	0.00328	0.01340	0.00365	3.53 x 10^{-12}	3.28	13.40	3.65	3.53 x 10^{-9}
9	38.00	60.20	1004.80	33.00	0.00308	0.01483	0.00389	1.00 x 10^{-11}	3.08	14.83	3.89	1.00 x 10^{-8}
10	33.00	48.90	956.00	8.50	0.00268	0.01205	0.00370	2.70 x 10^{-12}	2.68	12.05	3.70	2.70 x 10^{-9}
11	41.20	59.90	768.50	10.00	0.00334	0.01475	0.00297	3.20 x 10^{-12}	3.34	14.75	2.97	3.20 x 10^{-9}
12	35.70	52.00	784.20	10.20	0.00290	0.01280	0.00303	3.20 x 10^{-12}	2.90	12.80	3.03	3.20 x 10^{-9}
13	29.30	50.70	778.60	13.00	0.00238	0.01250	0.00301	4.10 x 10^{-12}	2.38	12.50	3.01	4.10 x 10^{-9}
14	36.00	54.50	957.50	10.00	0.00292	0.01340	0.00371	3.20 x 10^{-12}	2.92	13.40	3.71	3.20 x 10^{-9}
15	48.80	63.20	954.50	8.50	0.00396	0.01560	0.00369	2.70 x 10^{-12}	3.96	15.60	3.69	2.70 x 10^{-9}
16	44.30	53.90	837.50	10.70	0.00360	0.01330	0.00324	3.40 x 10^{-12}	3.60	13.30	3.24	3.40 x 10^{-9}
17	42.80	64.90	975.00	8.30	0.00343	0.01600	0.00377	2.60 x 10^{-12}	3.43	16.0	3.77	2.60 x 10^{-9}
18	34.90	51.00	918.40	13.30	0.00283	0.01260	0.00355	4.20 x 10^{-12}	2.83	12.60	3.55	4.20 x 10^{-9}
19	25.80	39.60	722.80	7.90	0.00210	0.00980	0.00280	2.50 x 10^{-12}	2.10	9.80	2.80	2.50 x 10^{-9}
Min.	25.80	39.60	690.60	3.75	0.00210	0.0098	0.00267	1.17 x 10^{-12}	2.10	9.8	2.67	1.17 x 10^{-9}
Max.	48.80	64.90	1008.00	33.00	0.00396	0.01600	0.00390	1.00 x 10^{-11}	3.96	16	3.9	1.00 x 10^{-8}
Mean	38.57	53.18	879.76	10.65	0.00313	0.01310	0.00340	3.33 x 10^{-12}	3.12	13.11	3.40	3.33 x 10^{-9}
World's Average[1]	35	30	400	–	0.00284	0.00739	0.00155	–	2.83	7.39	1.54	–

Radium equivalent activity (Ra$_{eq}$)

Radium equivalent activity is calculated by considering the hazards that are connected with the use of building and other types of industrial materials containing ^{238}U, ^{232}Th, and ^{40}K. Assuming that 10 Bq kg^{-1} of ^{238}U, 7 Bq kg^{-1} of ^{232}Th, and 130 Bq kg^{-1} of ^{40}K generate approximately the equal amount of gamma-radiation, the total activity concentration of ^{238}U, ^{232}Th, and ^{40}K is to be calculated. For calculations we use eqn. (4),[3]

$$Ra_{eq} = A_U + 1\,.\,430\,A_{Th} + 0.077\,A_K \qquad (4)$$

where A_U, A_{Th}, and A_K denote activity concentrations for ^{238}U, ^{232}Th, and ^{40}K, respectively. To avoid the expected risks of exposure, the material which contains more than 370 Bq kg^{-1} radium-equivalent activity should not be used for industrial purposes.[2,4] From Table 3, it can be observed that the mean value of radium-equivalent activity according to our results equals to 182.37 Bq kg^{-1}, which is considerably less than above mentioned recommended maximum value.

External hazard index (H_{ex})

One of the characteristics of irradiation risk for the population is considered the so called external hazard index, which is calculated by eqn. (5),[3]

$$H_{ex} = \frac{A_U}{370} + \frac{A_{Th}}{259} + \frac{A_K}{4810} \leq 1 \qquad (5)$$

where A_U, A_{Th}, and A_K are activity concentrations of ^{238}U, ^{232}Th, and ^{40}K, respectively. To avoid the expected risks the external hazard index should be less than 1, which corresponds to maximally admissible radium-equivalent activity 370 Bq kg^{-1}.[3,8] In our case the mean value of external hazard index is 0.49 (Table 3), which is less than the recommended limit.

Correlations

Table 4 shows correlations of radionuclide concentrations (in ppm) ^{232}Th/^{238}U, ^{232}Th/^{40}K and ^{238}U/^{40}K, while the Figures 4-6 present them graphically.

Table 3. Absorbed dose rate, annual effective dose rate, radium equivalent activity, and external hazard index.

Site #	Absorbed γ-dose rate in air (nGy h^{-1})	Annual effective dose rate (mSv y^{-1})	Radium equivalent activity (Bq kg^{-1})	External hazard index
1	77.24	0.47	159.17	0.43
2	84.51	0.52	173.76	0.47
3	90.34	0.55	183.27	0.49
4	90.50	0.55	183.64	0.50
5	87.30	0.54	177.88	0.48
6	90.92	0.56	184.73	0.5
7	99.30	0.61	201.85	0.55
8	93.88	0.58	190.93	0.52
9	99.28	0.61	201.46	0.54
10	87.57	0.54	176.54	0.48
11	90.29	0.55	186.03	0.5
12	83.39	0.51	170.44	0.46
13	79.55	0.49	161.75	0.44
14	92.62	0.57	187.66	0.51
15	103.72	0.64	212.67	0.57
16	90.61	0.56	185.86	0.50
17	103.16	0.63	210.68	0.57
18	88.16	0.54	178.55	0.48
19	68.31	0.42	138.08	0.37
Min.	68.31	0.42	138.08	0.37
Max.	103.72	0.64	212.67	0.57
Mean	89.51	0.55	182.37	0.49

Table 4. Correlations between natural radionuclides (ppm ratio).

Site #	$^{232}Th/^{238}U$	$^{232}Th/^{40}K$	$^{238}U/^{40}K$
1	3.19	4.12	1.29
2	4.16	4.60	1.11
3	3.98	3.45	0.87
4	4.07	3.51	0.86
5	3.84	3.67	0.96
6	3.76	3.43	0.91
7	3.95	3.57	0.90
8	4.09	3.67	0.90
9	4.89	3.81	0.79
10	4.50	3.26	0.72
11	4.42	4.97	1.12
12	4.41	4.22	0.96
13	5.25	4.15	0.79
14	4.59	3.61	0.79
15	3.94	4.23	1.07
16	3.69	4.1	1.11
17	4.67	4.24	0.91
18	4.45	3.55	0.80
19	4.67	3.50	0.75
Min.	3.19	3.26	0.72
Max.	5.25	4.97	1.29
Mean	4.23	3.88	0.93

Figure 7 shows the correlation of annual effective dose rates with parent rocks according to sampling sites. In the results presented it can be observed increased concentrations. For instance, an increased concentration of ^{238}U isotope is at point 15, which is one of the main water catchment areas.

Figure 8 shows the correlation of ^{238}U, ^{232}Th, and ^{40}K natural radionuclide concentrations with absorbed dose rates in the air according to sampling sites.

With the aim of taking into consideration geochemical factor during the process of soil formation, Digital Elevation Model (DEM) of the relief in a geo-informational system ArcGIS–10.4.1 has been developed, water flow has been modelled and a combined scheme of natural radionuclide distribution in the soil and geological structure have been created (Figure 9).

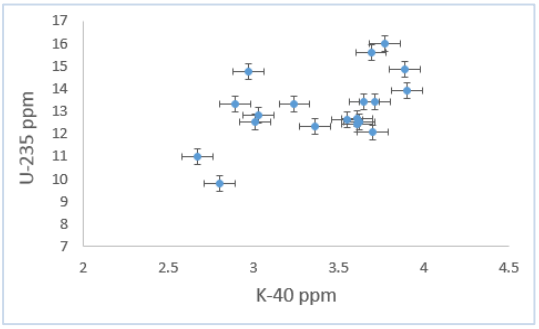

Figure 4. Correlation $^{238}U/^{40}K$.

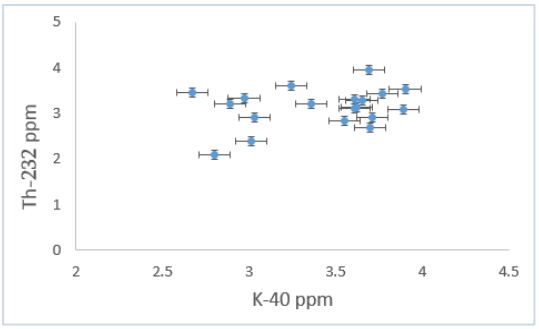

Figure 5. Correlation $^{232}Th/^{40}K$.

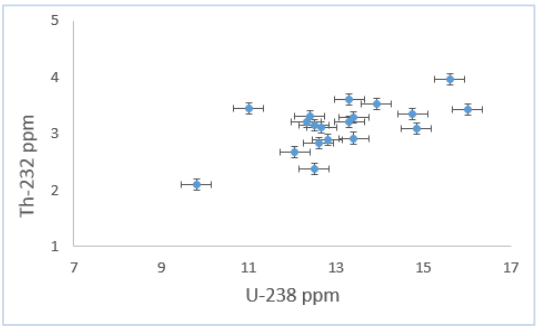

Figure 6. Correlation $^{232}Th/^{238}U$.

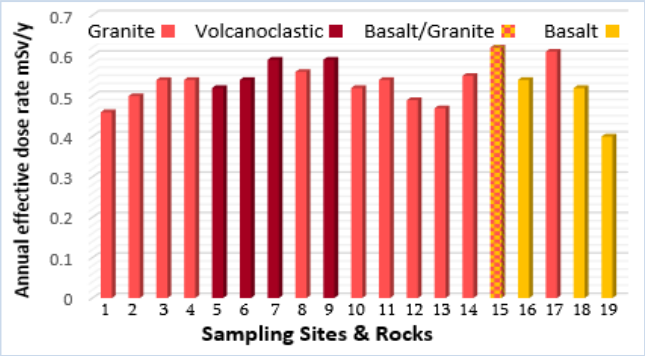

Figure 7. Correlation between annual effective dose and geology of area.

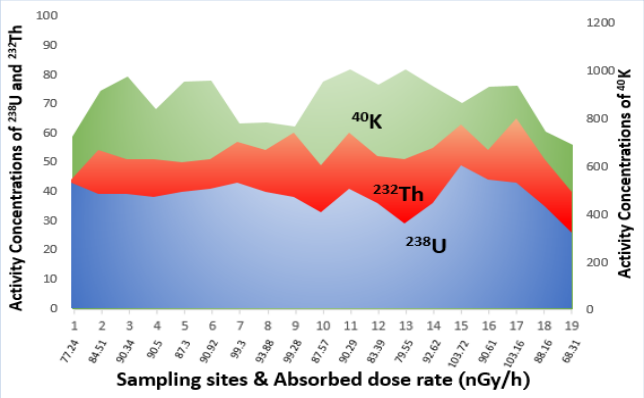

Figure 8. Correlation between activity concentrations and absorbed dose rate.

Discussions

As it can be seen from the Figure 9, the increased concentrations of natural radionuclides are in a certain correlation with the direction of water flows. Increased concentrations can be observed at their gathering points. Besides, as the combined scheme shows, the distributions of natural radionuclides are obviously related to the type of parent rock. Namely, the soils emerged at the expense of late variscan granitoids of Khrami massif, reveal higher natural radioactivity compared to neogene-quaternary lavas.

Mean value of absorbed dose rate in the air calculated according to natural radionuclide concentrations in the soil, in our case equals to 89.51 nGy h^{-1}. The obtained result is considerably higher (by 32.5 nGy h^{-1}) than the world mean value (Figure 10), which is 57 nGy h^{-1}.[1,2] But as it was mentioned above, our research covers Khrami massif, and where due to the spread of granitoids natural radioactive factors must have been increased.

Mean value of annual effective dose rate of 0.55 mSv h^{-1} is slightly higher than the world mean value (Figure 11), which is 0.48 mSv h^{-1}.[1,3] But the obtained value is less than the recommended limit established by ICRP, which is 1 mSv h^{-1}.[2,3] However, as it is known during the formation of the total radiation hazard, to gamma radiation portion generated by natural radionuclides is added some other significant components such as the portion caused by the spread of artificial pollutants, cosmic radiation, radon inhalation, spread of natural and artificial pollutants and their concentration in drinking water and food, as well as professional activities, radiation impact in medical sphere etc.[1]

Mean value for radium-equivalent activity according to our results is 182.37 370 Bq kg^{-1}, which is less than maximally admissible limit set by UNSCEAR, which is 370 370 Bq kg^{-1}.[2,3] This indicates that the territory under this research is free from the threats caused by radium and its decay product radon, especially that there are no regional deep faults on the territory.[5]

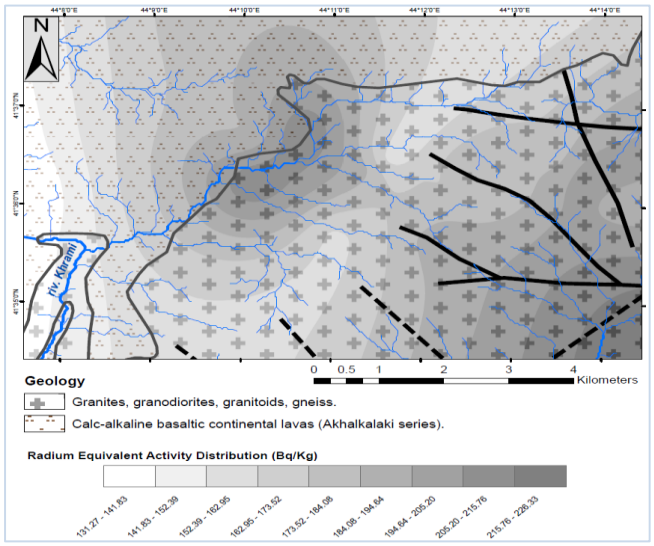

Figure 9. Interconnection of radium-equivalent activities, soil parent materials, and water flows.

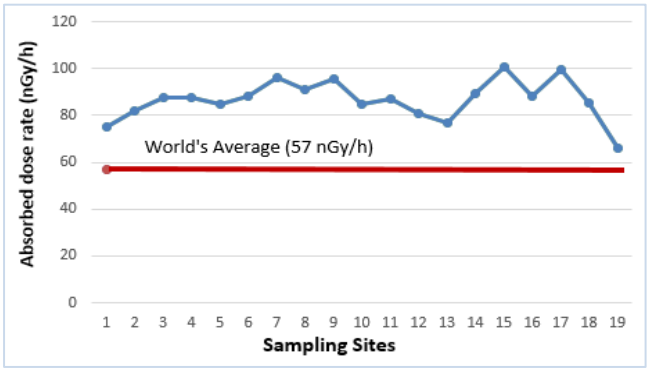

Figure 10. Comparison of obtained values of absorbed dose rate with world mean values.

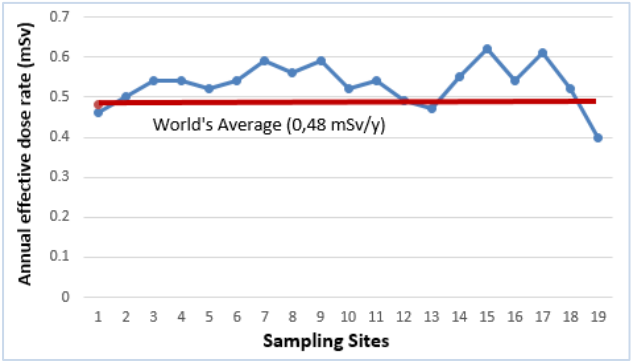

Figure 11. Comparison of obtained values of annual effective dose rate with world mean values.

Figure 12. The 9th site of sampling.

Table 5. Comparison of current results for concentrations of ^{137}Cs with data available in literature.

No.	Country	Bq kg^{-1}
1	Ordu, Turkey	171.35
2	Venezuela	5.00
3	Bangladesh	6.50
4	Majorca, Spain	35.00
5	Inshass, Cairo, Egypt	10.35
6	Algeria	25.00
7	Louisiana, USA)	31.50
8	Montenegrin Coast, Montenegro	14.95
9	Sudan	9.25
10	North-Western Libya	1.30
11	Riyadh, Saudi Arabia)	1.00
12	Northern Taiwan	14.24
13	Punjab – 1, Pakistan	2.80
14	Pakka Anna, Pakistan	3.60
15	Southern Punjab, Pakistan	1.60
16	Mid-Rechna, Pakistan	3.50
17	Punjab – 2, Pakistan	2.18
18	Charsaddah, Pakistan	7.10
19	Mirpur ,Azad Kashmir, Pakistan	1.39
20	Khrami Array, Georgia – present study	10.65

For external radiation index all mean values are below 1, which means that the populated localities on the territory are not exposed to radiation hazard that exceeds the limit.

The maximum concentration of ^{137}Cs (33 370 Bq kg^{-1}) was found at the 9th point (Table 2, Figure 12), which was sampled between the points located at a maximum altitude ASL (Table 1). A comparison of the results for ^{137}Cs obtained in studies conducted in various countries are given in Table 5.[5] As seen in **Table 5** in a number of cases ^{137}Cs concentrations is relatively high, which in our opinion indicates the trace left after the Chernobyl accident in 1986 and nuclear tests during the "Cold War" period. In general, the spread and sedimentation of artificial pollutants (radioisotopes) during the Chernobyl accident fallout depended on the strength of atmospheric motions and their directions. However, due to relatively high intensity of precipitation, pollution in mountainous regions was higher than in the plain, which is proved by corresponding studies carried out for instance, in France and Poland.[10,11]

Conclusion

Results of our research have shown that concentrations of natural radionuclide in the soils of the area under study considerably differ. In our opinion this must be conditioned by specific character of soils and their formation in which the forming parent rock plays a significant role and the factor of geochemical migration of substances is less important. Research results have indirectly revealed that sialic igneous rocks of Khrami massif, namely the soils that have emerged as a result of weathering of granitoids are indeed characterized by relatively high concentrations of natural radionuclides.

In this investigation, radiation character of a specific region of Georgia has been studied and explained according the geological features and correlation factors of the different characteristics have been observed. Although, the research has shown relatively high radioactivity level of the soils in the study area, but all parameters of assessing radiation exposure hazards are below the international limits.

We expect that this investigation and methodology used will stimulate similar study of other regions of Georgia, as well as of whole of south Caucasus. This may lead to a creation of useful generalized analytical information of terrestrial natural and artificial radioactivity of the region.

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