Quantum Chemical and Experimental Exploration of Biological Activity and Inhibitory Potential of New Acylated Oligosaccharides from *Pistacia integerrima* J. L. Stewart ex Brandis

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ABSTRACT: *The new biologically active integrisides A (**1**) and B (**2**) have been isolated from the methanolic extract of Pistacia integerrima J. L. Stewart ex Brandis. The antibacterial activity of both the integrities was tested against four pathogenic bacterial strains, two Gram-positive (Staphylococcus aureus, Streptococcus pyogenes) and two Gram-negative (Escherichia coli, Pseudomonas aeruginosa) as well as four fungal strains (Microsporum canis, Aspergillus clavatus, Candida albicans, and Candida glabrata). Both the isolated compounds showed significant results analogous with Imipenam and Miconazole standard drugs. Carbonic anhydrase-II inhibition of integriside A **(1)** and B **(2)** with IC_{50} value 1.56 µM and 2.85 µM respectively, as compared to standard drug acetazolamide (1.57 µM). Cholinesterase activity was carried out with acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) IC_{50} values of integriside A **(1)** (8.6, 4.8)*

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*and B **(2)** (0.91, 2.5) were found as compared with standard galanthamine (0.05, 0.92) and Eserine (0.6, 8.7). Here, various molecular descriptors, frontier molecular orbitals (FMO), electron affinity (E.A), ionization potential (IP), molecular electrostatic potential (MEP), and Hirshfeld analysis were carried out to understand the active sites and biological active nature of the integrisides A **(1)** and B (**2**). The energy gap, MEP, Hirshfeld analysis, and reactivity descriptors values demonstrate that the integriside A **(1)** and B (**2**) retain decent reactivity, which is in good agreement with current experimental and quantum chemical studies.*

KEYWORDS: *Pistacia integerrima J. L. Stewart ex Brandis; Integrisides; Cholinesterase activity; Antimicrobial activity; Quantum chemical study.*

INTRODUCTION

Herbal plants have played a vital role to maintain human health and the standard of their living for centuries. According to the report of the WHO (World Health Organization), more than 80% population all over the world depends upon the secondary metabolites of these medicinal plants to cure diseases [1]. Family Anacardiaceae contains 70 genera and over 600 main species found in the Mediterranean, sub-alpine regions, Asia, and America [2]. *Pistacia integerrima* (kakrashinghi; shani; chakra) medium-sized dioecious tree, 17 m tall is found at 400 m altitude [3]. Its rich chemical constituency especially tannin as a major component made it a favorite for medicinal use. The stem, flowers, leaf, seeds, and resin of pistachio attributes to antimicrobial, antioxidant, and anti-inflammatory properties [4, 5]. *Pistacia integerrima* J. L. Stewart ex Brandis (*P. integerrima*) ethnobotanical is used include as a remedy for snakebite, fever, dysentery, hepatitis, diarrha, vomiting, skin diseases, psoriasis, scorpion sting, liver disorder, respiratory tract, and asthma. Hamdard laboratories made several herbal medicines such as *habb-e-suranjan* [6, 7]. The plant bark resin is used as stimulant and diuretic while its roots possess antioxidant properties [8]. *Pistacia integerrima* possesses immunomodulatory activity, hyperuricemic, analgesic, and anti-inflammatory properties. The major isolated phytochemicals included phenolic, fatty esters, steroids, monoterpenes, and flavonoids [5, 9].

Carbonic anhydrase (EC 4.2.1.1., CA) is actually a pH regulatory and metabolic enzyme for all life kingdoms, found in organisms all over the phylogenetic tree. It brings about the catalysis by hydration of carbon dioxide (CO_2) to bicarbonate (HCO_3^-) ion while the dehydration of HCO_3^- in acidic medium leads to regeneration of CO_2 [10]. CA isoforms are also found in a variety of tissues where they are involved in numerous significant biological processes that include acid-base balance, carbon dioxide, respiration as well as ion transport, bone resorption, formation of glucose, formation of fats as well as secretion of electrolyte [11].

The inhibition of CAs possesses pharmacological implementations in many fields, such as inhibition of glaucoma, prevention of convulsant, obesity treatment, as well as in cancer treatments/diagnostic tools. In addition, it is an evolving paradigm for designing anti-infective agents including antifungal, antibacterial, and antiprotozoal. Consequently, designing as a drug of CA inhibitors is actually a significant field, with a variety of novel derivatives mentioned in the scientific literature [12]. These are clinically used as anti-epilepsy, anti-tumor, anti-glaucoma, diuretics, and anti-metastatic agents [13]

Acetylcholinesterase (AChE), as well as butyrylcholinesterase (BChE), bring about the ending of nerve impulse transmission at cholinergic synapses by quick acetylcholine (ACh) hydrolysis. While acetylcholine (ACh), as well as butyrylcholine (BCh) hydrolysis, is one of the main reasons for neurodegenerative ailments, such as Alzheimer's Disease (AD), ataxia, or Parkinson's [14]. AD is a major reason for dementia, accounting for up to 75% of all dementia cases. Pathophysiological mechanisms mentioned for the progression of AD involve neurons and degeneration of synapses, which is influenced by the deficit in ACh [15]. The AChE inhibitors are a major class of drugs presently used for the treatment of AD dementia state,

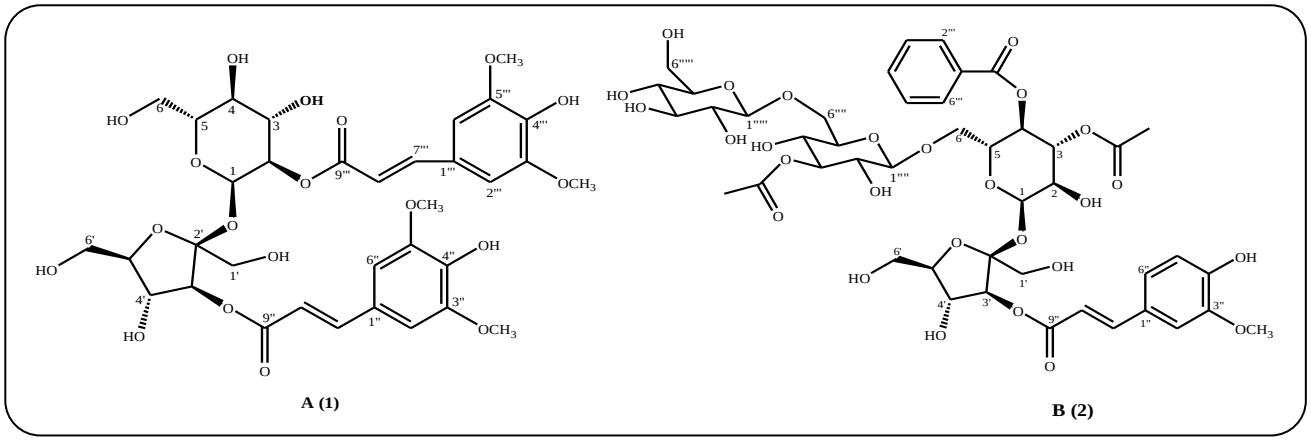

Fig. 1: Structures of the integrisides A (1) and B (2).

for example naturally occurring agent galantamine. Most plant species have different classes of alkaloids, coumarins, terpenes as well as polyphenols which are analyzed for their anti-AChE activity, becoming an exemplary candidate for new AD drugs [16].

The density functional descriptors in the improvement of the Quantitative Structure-Activity Relationship (QSAR) are important to probe the nature of the interaction, active sites, and biological activity of molecules. Here, various molecular descriptors, Frontier Molecular Orbitals (FMOs), electron affinity, ionization potential, molecular electrostatic potential were carried out to understand the active sites and biological active nature of the acylated oligosaccharides from *Pistacia integerrima*, which include integrities A (**1**) and B (**2**) (see Fig. 1). The global reactivity descriptors, *e.g.,* electronegativity (χ), chemical potential (μ), chemical hardness (η), electrophilicity index (ω) and softness (S) were also studied by density functional theory. The main goal of the present work is: (i) to isolate biologically active pure compounds from *Pistacia integerrima*: (ii) to the evaluated *in-vitro* biological activities including antimicrobial, carbonic anhydrase, and cholinesterase (AchE and BchE) enzymatic inhibitory potential: (iii) Quantum mechanical treatment of biologically active constituents.

EXPERIMENTAL SECTION

General

For enzyme inhibition assays, all chemicals and enzymes were obtained from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade. Optical rotations: JASCO P-2000 digital polarimeter, UV/Vis spectrum (MeOH): Hitachi U-3200 spectrophotometer, Infrared spectra (KBr): JASCO 302-A Infrared Spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra in CD_3OD, solvent using TMS as an internal standard source on NMR spectrometers at 500 MHz, respectively (Bruker AMX-500). JEOL JMS-HX-110 MS for exact mass measurement with FAB-MS using the matrix as glycerol. JAILC-908W Japan RP-HPLC with L-80 column used, silica gel, Sephadex LH-20, and Diaion HP-20 ion exchange resin were used as an adsorbent in column chromatography. The pre-coated silica gel (GF_{254} preparative plates) having particle size 20×20, 0.5 mm thick, E. Merck was used by preparative thin-layer chromatography. The samples purity was also monitored on the same plates and detection of the spot was done by spraying ceric sulfate. Sonicator (E-3011 Elmasonic), Shimadzu digital electronic balance: Inolab pH 720, Germany-pH meter, Sanyo Electric Co., Ltd. Incubator, Sanyo Ultra low-temperature freezer of -70 °C.

Plant material

Pistacia integerrima was collected from Pakistan (Swat), and identified by Plant Taxonomist Professor Surraiya Khatoon, Karachi university, and deposited specimen in the herbarium.

Extraction, fractionation, and isolation

The *Pistacia integerrima* was divided into small pieces then ground, dried, and extracted with methanol thrice at r.t. The blackish crude residue was obtained after evaporation of solvent methanol. It was dissolved in H_2O and fractionated into *n*-hexane, ethyl acetate (EtOAc) and

n-BuOH portions. The vacuum liquid chromatography was carried out with *n*-butanol portions eluting a combination of solvents in this order *n*-hexane-DCM ≤ DCM and ≤ DCM-methanol to get four major fractions. The DCM-methanol fractions were further subjected to reverse phase CC over Diaion HP-20 and final purification was done over Sephadex LH-20. Finally, semi pure fraction was purified by H_2O-MeOH (5.0:5.0) as eluent with recycling preparative HPLC (L-80 column) to afford integrities A (**1**) and B (**2**) [7].

Antimicrobial assay

Newly isolated integrities A (**1**) and B (**2**) were screened *in-vitro* antimicrobial activity using agar diffusion assay. For antibacterial activity two Gram-positive (*Streptococcus pyogenes* and *Staphylococcus aureus*) and two Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacterial strains were used whereas antifungal bioassay was determined with *Candida albicans*, *Microsporum canis*, *Aspergillus clavatus*, and *Candida glabrata* fungi. The Minimum Inhibitory Concentration (MIC) was compared with imipenem and miconazole for antibacterial and antifungal activity, respectively. The suitable number of perforations was bored using a sterile cork borer and solutions *i.e.*, the testing compound, solvent as well as the reference standard (imipenem) were placed into their specific hole with the aid of a sterilized pipette. The sample was settled at 25 ^{0}C for 120 minutes to let the sample diffusion with incubation at 37 ^{0}C for 24-48 hrs. The diameter of the inhibition zones was determined in mm [17].

Carbonic anhydrase assay

Carbonic Anhydrase (CA) action was measured by spectrophotometric method [18] by making standardization of reaction conditions, which include enzyme concentration, substrate concentration, pH of the buffer, and reaction time. The substrate *p*-nitrophenyl acetate (PNA) was hydrolyzed by CA and transformed to *p*-nitrophenol, which was measured at 348 nm using a spectrophotometer with a 96-well plate reader. The reaction mixture contains 15 mM Tris-sulphate buffer H_2SO_4 with 7.6 pH consisting of 0.1 mM $ZnCl_2$, 5 μL of the tested compound in respective solvent as well as 10 µL of 0.35 U enzyme (CA-bovine erythrocytes, Cat. Not. 147 A2000, Calzyme Lab Inc. the USA) [19]. All the contents were mixed and pre-incubated at 25 ºC for 10 min. The prepared substrate was 3 mM stock using less than 5 % acetonitrile in freshly used buffer and 20 μL was introduced per well to get the concentration of 0.6 mM in each well. The volume of the total reaction was 100 μL after incubation for 30 min at 25ºC. The well-known inhibitor acetazolamide was introduced in the assay as a positive control.

Acetylcholinesterase and butyl cholinesterase assay

The AChE/BChE inhibition effects of integrities A (**1**) and B (**2**) were performed according to previously described Ellman's method [20]. For both cholinergic reactions, acetylthiocholine iodide (AChI) and butyrylthiocholine chloride (BChI) were used as substrates. An aliquot of Na_2HPO_4 buffer (pH 7.7, 100µL) and sample solutions concentration (10 µL, 0.5 mM) were added to 10µL of AChE/BChE enzymes solution (0.005 unit well^{-1}). The solutions were incubated for10 min at 35 °C. The Ellman's reagent DTNB (5,5'-dithiobis-(2-nitrobenzoic acid), 10µL, 0.5 mM well$^{-1,}$ and substrate AChI/BChI were mixed within the incubated mixture and enzymatic reactions were started. The enzymes activities were determined spectro-photometrically with absorbance at 405 nm. Galanthamine and Eserine (0.5 mM/well) were used as a positive control for both enzymes AChE/BChE [21]. The inhibition percentages of enzymes were calculated by the following equation. All experiments were done with their corresponding controls in triplicate.

Inhibition (%) = 100 - (Abs of test sample / Abs of control × 100)

IC_{50} values (concentration of 50% enzyme inhibition) of compounds were calculated by EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

Computational details

Density functional theory is a fascinating method to analyze numerous properties of interest in biological sciences. DFT was systematically used to probe the electronic properties of materials [22-30]. The DFT is a reliable tool for the ground state (S_0) geometries optimization [31-33]. The PBE functional is rational for the S_0 geometries of various biologically active compounds. In the current study, PBE/TZP level of theory was adopted to perform ground state geometries optimizations and electronic properties exploration in Amsterdam Density Functional (ADF) modeling suite.

Table 1: Antibacterial activity of compounds 1 and 2.

Bacteria	Zone of inhibition of standard (mm)	Zone of inhibition of compounds (mm)		MIC (µg/mL)	
	Imipenem	1	2	1	2
Staphylococcus aureus	39	34	23	95	85
Streptococcus pyogenes	40	40	24	94	91
Escherichia coli	39	36	32	95	95
Pseudomonas aeruginosa	40	35	30	92	90
S.D.	0.58	2.63	4.43	1.42	4.12

Note: S.D. = Standard deviations

RESULTS AND DISCUSSION

The ground, dried whole plant material of *P. integerrima* was extracted with methanol. The crude blackish color residue was obtained after evaporation under reduced pressure in the rotavapor. It was fractionated into *n*-hexane, ETOAc, and *n*-butanol soluble portions. The *n*-butanol soluble portion was subjected to chromatographic separations over silica gel using a solvent gradient of *n*-hexane, dichloromethane, and methanol with increasing polarity. The HPLC and PTLC techniques were also executed in this study. The structures of these newly isolated compounds have been established through advanced spectroscopic techniques and were reported by our group [7].

Antimicrobial activity

Newly isolated compounds **1** and **2** were tested for antibacterial activity using two Gram-positive (*Streptococcus pyogenes* and *Staphylococcus aureus*) and two Gram-negative (*Pseudomonas aeruginosa and Escherichia coli*) bacterial strains. The results were compared with positive control imipenem (10 µg/ml). The present study elaborates the potent antibacterial potential for compounds **1** and **2** as significant antibacterial agents against drug-resistant pathogens. For compound **1** the maximum inhibition was against *Streptococcus pyogenes* (40 mm) while for compound **2**, the maximum inhibition was against *Pseudomonas aeruginosa* (35 mm). The MIC values of each compound against the tested bacterial strains were measured. The lowest MIC values against the tested bacterial strains were observed for A (92 - 95 µg/mL) and B (85 - 95 µg/mL) as shown in Table1 and Fig. 2 using positive control imipenem (10 µg/mL).

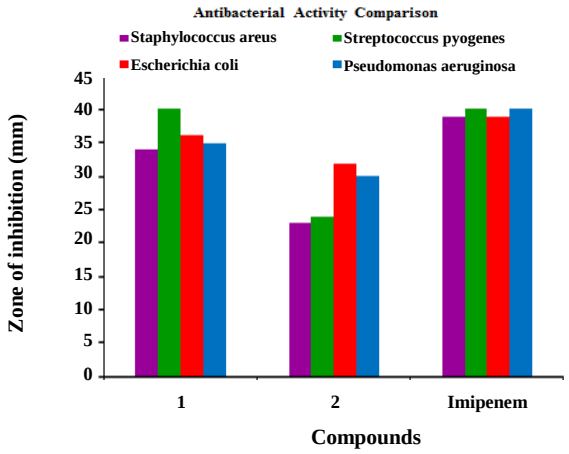

Fig. 2: Antibacterial activity comparison of A (1) and B (2) with imipenem.

The present study displayed compounds **1** and **2** as significant antibacterial agents for resistant drug pathogens. As it has been evident from the literature that the electrochemical binding makes the dispersal of negative as well as positive charges above the surfaces of cell membranes, resulting in weakening as well as the destruction of the membranes that will lead to the release of cell contents. This mechanism was evidenced by electron microscopy that displayed the binding as well as breaking the outermost wall of bacteria [34]. The above discussion provided great support for compounds **1** and **2**, which consist of carboxylate ions at suitable pH, which would enable the variability in charges that induce breakage of the outer wall of bacterial cells. That is the main reason for their antibacterial potential. In the state of antimicrobial mechanism of action, future work should aim to make the molecular aspects of the fundamental mechanisms and their interaction with the antimicrobial action of acylated oligosaccharides

Table 2: Antifungal activity of compounds 1 and 2.

Name of bacteria	Zone of inhibition of standard (mm)	Zone of inhibition of compounds (mm)		MIC (μg/ml)	
	Miconazole	1	2	1	2
Microsporum canis	40	31	30	88	80
Aspergillus clavatus	41	33	38	90	87
Candida albicans	40	30	30	93	89
Candida glabrata	40	28	39	95	92
S.D.	0.50	2.09	4.93	3.11	5.10

Note: SD = Standard deviations

1 and **2**. The development of the biochemical basis of microbial inhibition should minimize microbial side effects as well as maximize useful sensory, nutritional and health effects of treated foods in the diet. This type of effort leads to better as well as safer foods with the enhancement of human health.

The antifungal activity of acetylated oligosaccharides **1** and **2** of *Pistacia integerrima* was analyzed using four fungal strains (*Candida albicans, Microsporum canis, Aspergillus clavatus,* and *Candida glabrata*). These strains were selected based on their clinical and pharmacological significance. Antifungal capacity for compounds **1** and **2** was analyzed in terms of zone inhibition of organism growth. The results of the antifungal activities are shown in Table 2. For compound **1**, the maximum inhibition is against *Aspergillus clavatus* (33 mm) while for compound **2** the maximum inhibition was against *Candida glabrata* (39 mm). The MIC values of each phytochemical against the tested fungal strains were determined. The lowest MIC values were displayed for A (88 - 95 μg/mL) as well as for B (80 - 92 μg/mL) against the tested fungi as shown in Table 2 and Fig. 3. Statistical analysis revealed that the compound 2 zone of inhibition and MIC values are more deviated compared with compound 1.

The electrostatic interactions across the positively charged chitosan (oligosaccharides), as well as the negatively charged cell surface, leads to destabilization of the cell wall or cell membrane, which increases the cell permeability leading to the leakage of cells [35]. That is the reason that acylated oligosaccharides potentially support the above investigation as antifungal. The antimicrobial mechanism of action should aim to make the molecular aspects of the fundamental mechanisms and their interaction to the antifungal action of acylated oligosaccharides. These can be used as future potential

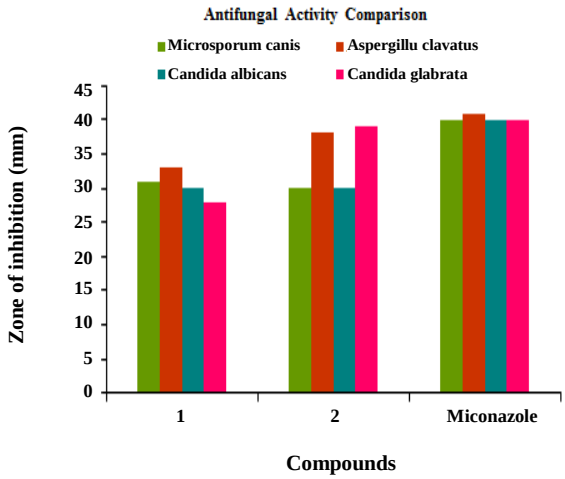

Fig. 3: Antifungal activity comparison of A (1) and B (2) with miconazole.

candidates as antifungal drugs and may be more suitable than already available drugs in terms of consideration of adverse side effects and clinical benefits.

Carbonic anhydrase activity

The phenyl moiety of phenol was present in the hydrophobic part of the active site of CA, while the CO_2 that is a physiologic substrate of the CAs leads to the joining of already catalytic complex, making it easy to understand the behavior of phenol as a distinct CO_2 competitive inhibitor [36]. Joining the phenolic component to other functional groups is a general mechanism to obtain the products with intensified actions. For instance, the "sugar approach" so-called the integration of a hydrophilic sugar, moiety allows the molecule penetration in a distinct manner to the cavity of the enzyme. Moreover, it enhances its specificity to the membrane-bound CA isoforms above the cytosolic ones [37, 38].

Table 3: Carbonic anhydrase-II inhibitions of compounds 1 and 2

Compounds	%inhibition	IC_{50} ± S.E.M[a][μM]
1	81.9	1.56 ± 0.42 µM
2	75.8	2.85 ± 0.09 µM
Acetazolamide	94.0	1.57 ± 0.003

[a] *SE mean of six assays*

Table 4: Inhibition of AchE and BChE by compounds 1 and 2

Compounds	IC_{50} ± S.E.M[a][μM]	
	AChE	BChE
1	8.6 ± 0.15	4.8 ± 0.15
2	0.91 ± 0.015	2.5 ± 0.15
Galanthamine[b]	0.05 ± 0.01	0.92 ± 0.01
Eserine[c]	0.6 ± 0.01	8.7 ± 0.01

[a] *SE mean of six assays*

[b-c] *Positive controls for AChE and BchE, respectively.*

The acylated oligosaccharides having phenolic moiety showed enhanced inhibition potential for carbonic anhydrase enzyme. Sugar moieties attached with both compounds containing hydrophilic parts lead to penetrating with enzyme active sits and showed marvelous potential of inhibition against CA enzyme. Literature observation also gave evidence for its potential inhibition effect. Integriside A **(1)** and Integriside B **(2)** displayed significant inhibition with the IC_{50} value as shown in Table 3. Integriside A **(1)** showed potent inhibitory potential having IC_{50} value very close to standard drug acetazolamide. So compound **1** can be used as a future drug by evaluating *in vivo* animal and human studies that would be our future work.

Cholinesterase activity

The inhibition activity was influenced by the distribution as well as a number of hydroxyl or methoxyl groups substituted at the phenyl ring. Despite that, the research also proved that the methyl or ethyl esters of phenolic acids inhibited cholinesterase (AChE and BChE) more efficiently than the corresponding free acids. The activity of a particular compound against AChE was covered by the presence of the hydroxyl group at ortho position at the phenyl ring as well as the existence of the acidic group [39]. The presence of -OH or -OCH_3 substituted at phenyl ring makes a strong recommendation that the compounds **1** and **2** showed potent inhibition potential against AChE/BChE. Integrisides A and B **(1** and **2)** were screened against enzymes AchE and BChE, which showed moderate activity with IC_{50} values being shown in Table 4. In the light of our study, we can say that compounds **1** and **2** bring about the inhibition of ChE activity.

Most of the studies carried out by authors also supported that diverse plant material as well as substances present in extracts inhibit AChE and/or BChE. This type of inhibitory effect is found to be useful in the treatment of Alzheimer's. Finally, it's important to have more data from clinical trials as well as toxicity tests that would be future considerations of our research.

Electronic properties

The highest occupied / lowest unoccupied molecular orbitals (MOs), *i.e.*, HOMOs / LUMOs of new acylated oligosaccharides from *Pistacia integerrima*, *i.e.*, integrisides A and B as well as some reference compounds (imipenem and miconazole) were probed at PBE/TZP level, as shown in Fig. 4. The intramolecular charge transfer (ICT) from HOMO-1 → LUMO+1 and HOMO → LUMO was found in integrisides A (**1**) and B (**2**). The ICT in reference compounds from occupied to unoccupied MOs was also noticed. The energies of HOMO-1 (E_{HOMO-1}), HOMO (E_{HOMO}), LUMO (E_{LUMO}), LUMO+1 (E_{LUMO+1})

and HOMO-LUMO energy gaps (E_{gap}) are important parameters to explore the electronic properties. E_{HOMO-1}, E_{HOMO}, E_{LUMO}, E_{LUMO+1}, and E_{gap} of reference compounds, as well as integrities A (1) and B (2) at PBE/TZP level at S0, are displayed in Table 5. E_{HOMO-1} at S_0 decrease as: integriside B (-5.41) > integriside A (-5.79) > imipenem (-6.24) > miconazole (-6.52). The tendency in the E_{HOMO} is as: integriside B (**2**) (-5.10) > imipenem (-5.20) > integriside A (**1**) (-5.47) > miconazole (-6.08). The E_{LUMO-1} at S_0 decrease as: integriside B (**2**) (-2.54) > imipenem (-2.61) > miconazole (-2.68) > integriside A (**1**) (-3.34). E_{LUMO} at S_0 decrease as: imipenem (-1.32) > integriside B (**2**) (-2.51) > miconazole (-2.64) > integriside A (**1**) (-2.94). The E_{gap} decrease in the following order: miconazole (3.40) > imipenem (2.59) > integriside B (**2**) (2.56) > integriside A (**1**) (2.13). The energies of frontier molecular orbitals (FMOs) and E_{gap} revealed that integriside B (**2**) would be better biological active compound than integriside A (**1**).

Global chemical reactivity descriptors (GCRD) are important parameters to realize reactivity and structural stability. Here, we have calculated GCRD parameters like chemical hardness (η), chemical potential (μ), electronegativity (χ), softness (S) and electrophilicity index (ω) of integrisides A, B (**2**) and referenced compounds using HOMO and LUMO energy values, see Table 5 (for computational details see supporting information). The η of the compound is interrelated to aromaticity [40, 41]. The μ expresses the electron tendency to rush out from the electronic cloud. The η also symbolizes the extent of the obstruction of the electronic cloud to deformation and ω signifies the stabilization energy. The antioxidant compound delivers an electron to the free radical then produced radical cation should be stable enough for better radical scavenging ability in a one-electron transfer mechanism. In this way, the antioxidant ability can be evaluated by IP and is a physical parameter enlightening the electron transfer range. It is anticipated that radical scavenging nature might be superior for those compounds, which show smaller IP. These results disclosed that Integriside B would have good antioxidant ability than integriside A. The tendency in the E_{HOMO} is as integriside B (**2**) (-5.10) > imipenem (-5.20) > integriside A (**1**) (-5.47) > miconazole (-6.08).

The work functions (W) of Au (Al) are 5.10 (4.08 eV) [42]. Here, we have calculated and compared the hole and electron injection energies (HIE and EIE) of integrisides A (**1**), B (**2**) and referenced compounds to electrodes. In case of Al, EIE for integriside A (**1**), integriside B (**2**), imipenem and miconazole were estimated (1.14 eV = −2.94 − (−4.08)), (1.57 eV = −2.51 − (−4.08)), (2.76 eV = −1.32 − (−4.08)) and (1.44 eV = −2.64 − (−4.08)), respectively. The HIE for integriside A (**1**), integriside B (**2**), imipenem and miconazole were observed (1.39 eV = −4.08 − (−5.47)), (1.02 eV = −4.08 − (−5.10)), (1.12 eV = −4.08 − (−5.20)), and (2.00 eV = −4.08 − (−6.08)), respectively. In case of Au, EIE for integriside A (**1**), integriside B (**2**), imipenem and miconazole were observed (2.16 eV = −2.94 − (−5.10)), (2.59 eV = −2.51 − (−5.10)), (3.78 eV = −1.32 − (−5.10)), and (2.46 eV = −2.64 − (−5.10)) respectively. The HIE for integriside A (**1**), integriside B (**2**), imipenem and miconazole were observed (0.37 eV = −5.10 − (−5.47)), (0 eV = −5.10 − (−5.10)), (0.10 eV = −5.10 − (−5.20)), and (2.00 eV = −4.08 − (−6.08)), respectively. One can see that integriside B (**2**) has no hole injection barrier smaller than integriside A (**1**) revealing that prior compound might be better charge transfer contender than latter one.

Molecular electrostatic potential

The molecular electrostatic potential surfaces views of acylated oligosaccharides integrisides A (**1**), B (**2**), imipenem, and miconazole are illustrated in Fig. 5. The negative electrostatic potential (ESP) was found on the oxygen atoms while the positive one was at hydrogen atoms in integrisides and reference compounds. In integriside B (**2**), sugar moieties contained more negative ESP revealing that in the case of nucleophilic attack substantial repulsion would be at sugar moieties (oxygen atoms) while significant attraction would be toward hydrogen atoms of sugar moieties and the rest of the compound. It is also expected that in case of electrophilic attack noteworthy repulsion would be at hydrogen atoms of sugar whereas the notable attraction would be at oxygen atoms of sugar moieties. The integriside B (**2**) have more prone sites for electrophilic attack than that of integriside A which would lead to the enhanced biological activity of the prior compound.

Table 5: The ground state ground state HOMO energies (E_{HOMO} and E_{HOMO-1}), LUMO energies (E_{LUMO} and E_{LUMO+1}), energy gaps, IP, EA, η, μ, S, χ and ω in eV of integriside A (1), integriside B (2), imipenem and miconazole.

Parameters	1	2	Imipenem	Miconazole
E_{HOMO}	-5.47	-5.10	-5.20	-6.08
E_{HOMO-1}	-5.79	-5.41	-6.24	-6.52
E_{LUMO}	-3.34	-2.54	-2.61	-2.68
E_{LUMO+1}	-2.94	-2.51	-1.32	-2.64
$\Delta E_{HOMO-LUMO}$	2.13	2.56	2.59	3.40
$\Delta E_{HOMO-1-LUMO+1}$	2.85	2.90	4.92	3.88
Hardness *(η)*	1.06	1.28	1.29	1.70
Potential *(μ)*	-4.41	-3.82	-3.91	-4.38
Softness *(S)*	2.57	1.99	2.01	1.79
Electronegativity *(χ)*	4.41	3.82	3.91	4.38
Electrophilic index *(ω)*	9.11	5.70	5.89	5.64
Ionization potential (IP)	5.47	5.10	5.20	6.08
Electron affinity (EA)	3.34	2.54	2.61	2.68

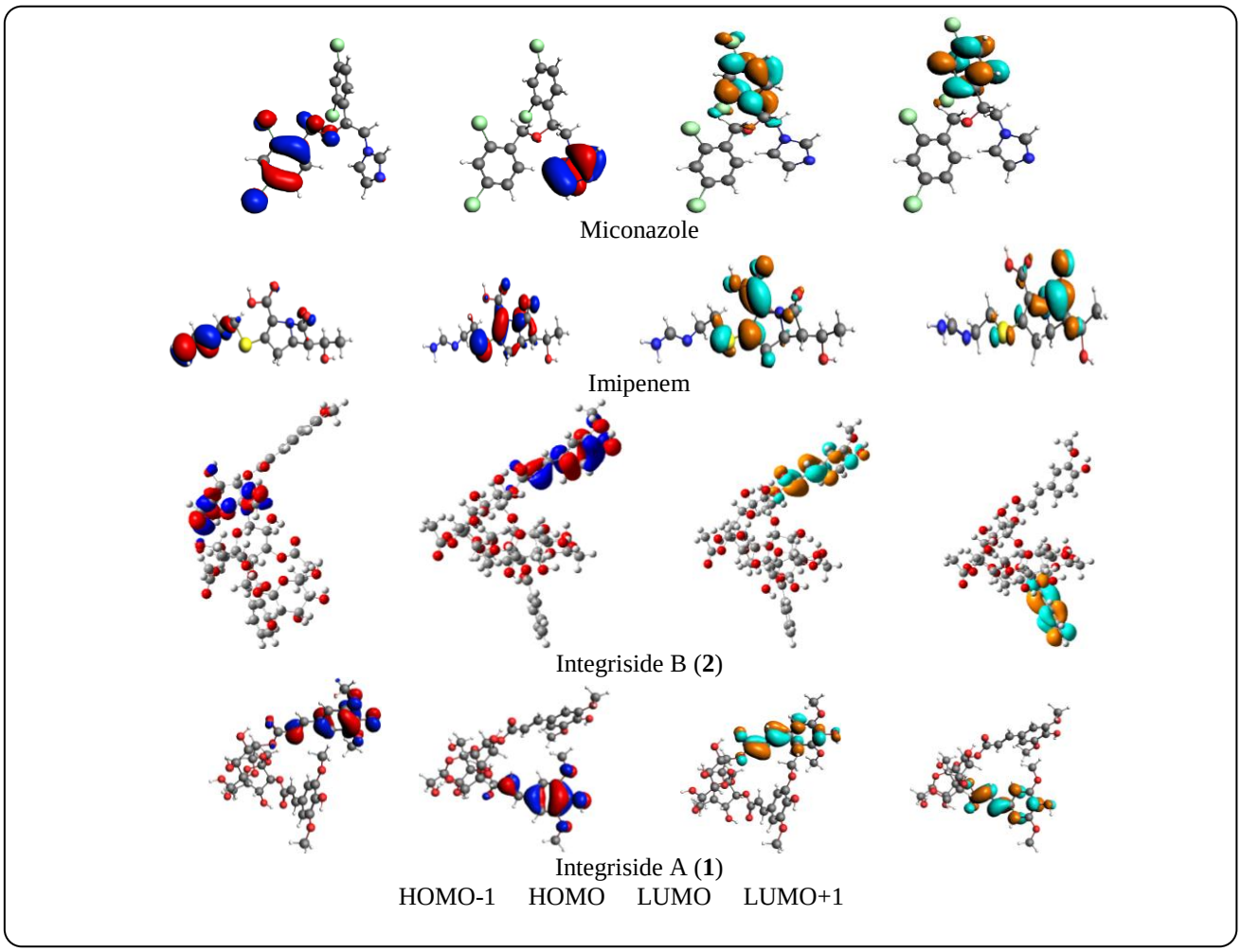

Fig. 4: Ground state charge density of FMOs of integriside A (1), integriside B (2), imipenem, and miconazole (contour value=0.035).

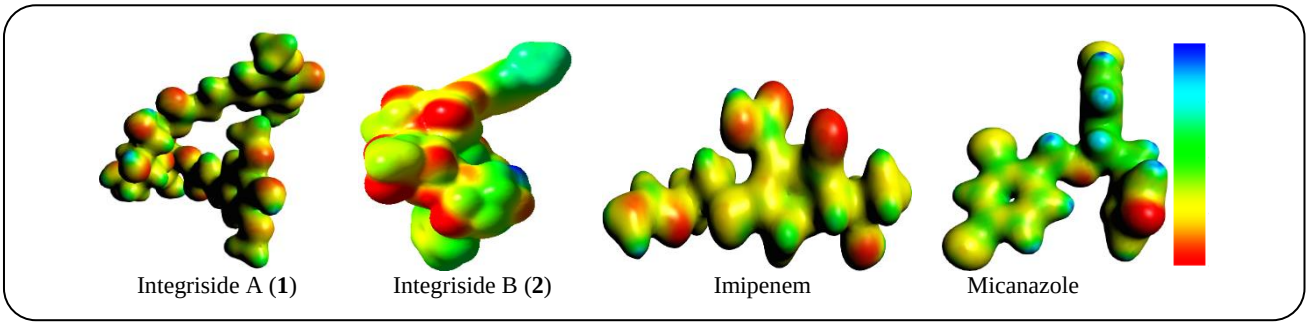

Fig. 5: Molecular electrostatic potential surfaces views of acylated oligosaccharides integrisides A (1), B (2), imipenem and miconazole.

CONCLUSIONS

The bioassay-guided isolation of Integrisides A (**1**) and B (**2**) was performed using reverse-phase chromatographic separation from *n*-butanol fraction of medicinally important plant *Pistacia integerrima*. Compounds **1** and **2** showed significant results against an antibacterial and antifungal activity with positive control drug-resistant pathogens imipenem and miconazole, respectively. Integrisides A (**1**) and B (**2**) were screened against enzymes AchE and BChE which showed moderate activity compared with IC_{50} values of standard galanthamine and eserine. *In-vitro* antimicrobial and enzymatic inhibition showed that compound **2** was more biologically active and fully supported by their first principle investigations. The CA inhibition found that potent inhibitory potential having IC_{50} value was close to standard drug. So these natural products can be used as a future drug by evaluating *in vivo* animal and human studies that would be our future work.

Conflict of interest

The authors declare that they have no conflict of interest.

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