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BIOACTIVE COMPOUNDS TO TARGET ANTI APOPTOTIC PROTEINS- Bcl 2 AND Bcl XL AN *IN SILICO* APPROACH

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ABSTRACT: Phytochemicals from several herbaceous and spice plants have been reported to have defense properties against various diseases. Potential of some of such plants like *Allium sativum, Curcuma longa, Boerhaavia diffusa, Pterocarpus marsupium* and *Zingiber officinale* in cancer therapy has already been proved. The state of Kerala in southern India hosts large number of medicinal herbs and is also referred as a hotspot of biodiversity. The current study aimed at computational screening of potent lead molecules from ten selected medicinal plants and five spices widely used in Kerala, which are rich in biological diversity and its traditional values. Even though, the anticancer properties of all these plants and spices were reported, their mode of action and target molecules is not well explained. Hence, the interaction of these compounds with anti-apoptotic proteins Bcl-2 and Bcl-XL are studied and discussed in this paper. The -CDOCKER energy, -CDOCKER interaction energy, Hydrogen bonds, binding energy, and complex energy were predicted through molecular docking studies. Based on binding energy and CDOCKER scores, the active components of *Pterocarpus marsupium, Aegle marmelos, Pseudarthira viscida, Allium sativum, Phyllanthus amarus, Boerhaavia diffusa and Catharanthus roseus* were observed to have strong interaction with both the proteins. Pharmacokinetic analysis such as absorption, solubility, BBB penetration, and toxicity were also performed for these compounds. Comparative docking analysis showed higher interaction than the known inhibitors such as Obatoclax and apogossypolone. Finally, the lead molecules were screened based on their hepatotoxicity. Ten of them passed the analysis and some with high binding energy failed to do so. The lead molecules identified and their probable functional groups could be further reported to transform them as potential anti-apoptotic inhibitors.

INTRODUCTION: Cancer deaths are set to increase at an alarming rate in India and approximately one million new cases of cancer are identified in each year among the population of 1·2 billion [1]. Cervix and breast cancer are the most prevalent cases in women [2] and the top five in men are lip/oral cavity, lung, pharynx, stomach, and colorectal cancer.

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Cytotoxic chemotherapy, radiation, and surgery have been used to treat this killer disease for the past few decades. Even though these methods are still widely used in the treatment, targeted therapies using small inhibitors and monoclonal antibodies are gaining more importance now [3].

In targeted therapy, drugs aim a specific biological target which is either a gene, tissue environment or a protein and arrests the uncontrolled growth of the cell. Using computer aided drug design methods, molecular interaction of targets and drugs can be easily studied. It is one of the most new approaches which span almost all the drug discovery stages. The main goal of the approach is to predict how the small molecule binds with the target and how

strong it is. And the advantage is that it reduces time, cost, and risk factors than the traditional drug development methods.

Several proteins in apoptosis or programmed cell death play a major role in the development and progression of cancer. It triggers through the sequential activation of two distinct pathways [4]. The intrinsic pathway is mediated by the Bcl-2 protein family, which includes the pro apoptotic proteins- BAD, BAX and anti apoptotic proteins- Bcl-2 and Bcl-XL. It regulates apoptosis by controlling the permeability of the mitochondrial membrane and prevents the release of the electron carrier, cytochrome c into the cytosol. Over expression of both Bcl-2 and Bcl-XL leads to cancer [5] and hence they are identified as therapeutic targets for various cancers including breast, leukemia, lung, colorectal etc [6, 7, 8, 9, 10]. Even if Bcl-2 and Bcl-XL are of the same family, it shares only 43 % of sequence identity and differs largely in expression. Bcl-2 resides in mitochondrion, ER and nuclear envelope [11]. Bcl-XL is located in the nuclear envelope, extra-nuclear membranes like mitochondrion and also in cytosol [12]. Therefore, specific targeting is more relevant in the drug design processes.

The Kerala folk medicinal plants have been used for many traditional treatments since ancient times, but to understand the mechanism of their anti cancer pharmacological activity molecular docking analysis was performed. In search for new anticancer inhibitors, we focused on potent lead molecules from selected medicinal plants and spices widely used in Kerala. Our current interest was on ten medicinal plants which include *Catharanthus roseus*, *Aegle marmelos*, *Aloe vera*, *Boerhaavia diffusa*, *Phyllanthus amarus*, *Pseurdarthira viscida*, *Pterocarpus marsupium*, *Sida rhombifolia*, *Trigonella foenum*, *Wrightia tinctoria*, and five spices such as *Allivum sativum*, *Curcuma longa*, *Murraya koenigii*, *Piper nigrum*, and *Zingiber officinale*.

MATERIALS AND METHODS:

Bcl-2 and Bcl-XL were chosen as the target receptors due to their imperative role in regulating apoptotic pathway and cancer. The three dimensional structures of human Bcl-2 and Bcl-XL with phenylacylsulfonamide inhibitor (4AQ3) and N-(3-(5-(1-(2-(benzo[d]thiazol - 2 - yl) hydrazono) ethyl) furan-2-yl)phenylsulfonyl) – 6 - phenylhexan amide (3ZK6) respectively were retrieved from the Protein Databank (PDB) (http://www.rcsb.org/).

The retrieved protein structures were prepared using Prepare Protein protocol of Discovery studio 4.0. This protocol cleans the experimental target structures by performing tasks such as inserting missing atoms in incomplete residues, modeling missing loop regions, deleting alternate conformations (disorder), removing waters, applying CHARMM forcefield, standardizing atom names, protonating titratable residues using predicted pKs and minimizing the structure.

Binding or active sites of Bcl-2 and Bcl-XL were identified through literature survey and PDB site records.. It is reported that a series of phenylacylsulfonamide have confirmed potent binding affinities for Bcl-2 hydrophobic cleft containing the residues Arg 105, Leu 96, Phe 157, Tyr161, Val 92, Tyr 67, Asp 70, Met 74, Arg 66, Phe 71 and Phe 63 [13]. Another molecular level study on Bcl-XL states that, it inhibits the activation of apoptotic signaling pathways through intermolecular contact with apaf-1 and cytochrome c. The interactional residues of Bcl-XL with cytochrome c were Glu 96, Arg 100, Tyr 101, Asp 132, Asn 136, Trp 137, Gly 138, Thr 190, Phe 191, Leu 194, Tyr 195, and Ser 203 and reveals that these binding sites will be potential druggable binding areas [14]. The binding sites involving these critical residues on both Bcl-2 and Bcl-Xl were defined using "From current selection" protocol in Defined and Edit Binding Site tools of Discovery studio 4.0.

Phytochemicals (ligands) from ten medicinal plants and five spices were identified through literature survey. The identified phytochemicals include,

Allium sativum: 2,3,4-trithiapentane, allixin, kaempferol, allyl methyl trisulphide, 2-methylbenzaldehyde, allyl methyl disulfide, alliin, beta carotene, allyl alcohol, diallyl disulfide, propylene sulfide, allyl sulfide, 2-vinyl-4h-1,3-dithiin allyl propyl disulfide 1,3-dithiane diallyl trisulfide ajoene alpha phellandrene 1-hexanol

piperine, spathulenol, eugenol, germacrene d, n-formylpiperidine, piperitone, terpinenol-4, fenchone, germacrene d, citral, piperonal, terpinolene, gamma elemene, isoborneol, isoborneol, sabinene,trans-calamenene, gamma-cadinene, limonene, 4-cymene, Safrole, trans-piperitol acetate.

***Zingiber officinale*:** cyclosativene, 8-paradol, Zingiberene, Isoborneol, Norcamphor, 8-gingerol, beta-bisabolene, gingerdiol, (8)-shogaol, beta bisabolol, mgingerdione, gingerol, beta-farnesene, gingerol, (10)-shogaol, beta-phellandrene, 6-paradol, alpha-curcumene, beta pinene, 3-(acetylmethyl), shogaol, alpha-farnesene, sesquiphellandrene, camphene, gamma-tocopherol, limonene, 1,8-dimethyl-4-(1-methylethenyl) spiro (4.5)dec-7-ene, Capsaicin, Geraniol, Linalool, Widdrol, Carveol, geranyl acetate, matairesinol, eucalyptol, germacrone, methyl linolenate, citral, (e)-1,7-bis(4-hydroxy-3-methoxyphenyl)hept-4-en-3-one, Naphthalene, farnesene epoxide, guaiol, nerolidyl acetate, gamma-cadinene, lariciresinol, nerolidol.

***Pseudarthria viscida*:** 2'-hydroxygenistein, Diphysolone, Kievitone, Quercetin, 3-o-methylglucose, ferulic acid, leucopelargonidin, rutin, butane, 1,1-diethoxy-3-methyl, gallic acid, n,n-dimethyltryptamine, undecanoic acid, caffeic acid, genistein, methyl-4-tyramine, candicine, palmitic acid, oleic acid, dalbergioidin, hordenine, gangetin, desmodin, lenticin, phenethylamine.

Pterocarpus marsupium: 4',7-dihydroxyflavone, Dihydrochalcone, beta-d-xylopyranosyl-(1->4)-beta-d-xylopyranosyl-(1->4)-beta-d-xylopyranose, myristic acid, 3,7,4'-trihydroxyflavone, Isoliquiritigenin, 4-hydroxybenzaldehyde, 7-hydroxyflavone, Catechin, 4-[2-hydroxy-3-(4-hydroxyphenyl)propyl]phenol, linoleic acid, liquiritigenin, psi-baptigenin, beta-eudesmol, lupeol, pterostilbene, taraxerone, marsupsin, pterosupin, dibutyl phthalate,midodrine-d6, hydrochloride, pyrocatechin-d4.

***Wrightia tinctoria*:** pyranoid, butane, 2,2-diethoxy-3-methyl, lauric acid, isatin, squalene, 3-o-methylglucose, Campesterol, arachidic acid, lupeol, stigmasterol, linoleic acid, clerosterol, ferulic acid, 4-(but-2-en-1-ylidene)-3,5,5-trimethylcyclohex-2-enone, 1-triacontanol, Amyrin, Warfarin, 3-hydroxyflavone, oleanolic acid, tryptanthrine, alpha cubebene, cycloartenone, palmitic acid, 1,1,3-triethoxypropane, ursolic acid, anthranilic acid, cycloleucalenol, indigo carmine, rutin, amyrin, desmosterol, indirubin, sinapinic acid.

***Trigonella foenum graecum*:** pyrrole-3-carboxylic acid, diosgenin, 3,3-dimethylindolin-2-one, Gitogenin, Allylamine, Harmaline, 3-o-methylglucose, glycolic acid, quararibea lactone, methyl nicotinate, hexopyranose, sarsasapogenin, aziridine, trigonelline hydrochloride.

***Sida rhombifolia*:** 2-deoxyecdysone, Ephedrine, Wogonin, Niacin, Ecdysterone, gamma-sitosterol, beta carotene, stigmasterol, mcryptolepine, cryptolepinone, diethylhexyl phthalate.

Catharanthus roseus: Mitraphylline; Catharanthine, Catharine, Serpentine, Yohimbine, Vindolinine, gamma-sitosterol, quercetin, akuammine, oleanolic acid, camphora, vinblastine, beta carotene, deacetylvinblastine, protocatechuic acid, vinglycinate, malvidin, vindoline, kaempferol, reserpine, ursolic acid, sweroside, cleavamine, isositsirikine, vincristine, raubasine, vinrosidine, raubasine, perivine, vincristine, serpentine, 4'-deoxyvinblastine, Lochnericine, Akuammicine, Furaldehyde

***Aegle marmelos*:** cuminaldehyde, alpha phellandrene, gamma-fagarine, gamma-fagarine, tartaric acid, alpha phellandrene, marmesinin, scopoletin, niacin, 4-cymene, Lupeol, beta carotene, eucalyptol, citronellal, marmesin, marmin, eugenol, imperatorin, luvangetin, anhydromarmeline, ficusin, betulinic acid, limonene, skimmianine, alloimperatorin, citral

***Phyllathus amarus*:** gallic acid, phyllanthin, quercitrin, elaeocarpucin, d phenazine, hypophyllanthin, amariinelaeocarpucin e, catechin, securinine, furosin, elaeocarpucin f, gallocatechol, geraniin, repandusinic acid b, elaeocarpucin g, corilagin, quercetin elaeocarpucin a, elaeocarpucin h, norsecurinine, rutin, elaeocarpucin b, nirtetralin, ellagic acid, elaeocarpucin c.

quercetin allicin brovanexine 3,5-diethyl-1,2,4-trithiolane s-allyl cysteine alliin diallyl tetrasulfide 4-methyl-5-vinylthiazole.

Curcuma longa: (1,2,3-trimethyl-cyclopent-2-enyl)-methanol, alpha-atlantone, beta-bisabolene, caryophyllene oxide, 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca - 3, 7, 11, 15 - tetraenyl) cyclohexanol, alpha-bergamotene, beta-curcumene, caryophyllene, 2,3,5-trimethylfuran, Bisabolol, beta-elemene, 2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one, beta-myrcene, farnesol, alpha santalene, beta-phellandrene, 2-carene, alpha-santalol, beta-pinene, 3-carene, alpha selinene, alpha-santalene, 4,5-dimethyl-2,6-octadiene, gamma-terpinene, beta-sesquiphellandrene, 4,8-dimethyl-3,7-nonadien-2-ol, alpha-terpineol, beta-turmerone, dehydrozingerone, alpha-thujene, 2,4-dimethyl-3-nitrobicyclo[3.3.1] nonan – 9 - one, (4s,5s)-(+)-germacrone 4,5-epoxide, zingiberene, bisabolone, nerylacetone, aristolene, isoborneol, 7-epi-sesquithujene, ar-turmerone, bornyl acetate, acoradiene, ascaridole, calebin-a, adoxal, benzene, 1 - methyl – 4 - (1-methylpropyl), camphene, humulene, gamma-sitosterol, camphor, alpha-pinene, (2-methylpropenyl)benzene, d-carvone, alpha-curcumene, carvacrol, (e,e,e)-3,7,11,15-tetramethylhexadeca 1,3,6,10,14-pentaene, Menthofuran, Stigmasterol, chrysanthenyl acetate, procurcumenol, menthol, cyclohexene, eucalyptol, ferulic acid, methyleugenol, teresantalol, (z)-cinerone, (e)-gamma-bisabolene, Citral, terpinenol-4, citronellal, gamma-curcumene, geraniol, terpinolene, citronellyl valerate, gamma elemene, nerolidyl propionate, thymol, corymbolone, gamma-terpinene, geraniol acetate, vanillic acid, 6-cubebene, gamma-terpineol, 1-methyl-2-isopropylbenzene, Vanillin, Curcumin, Citral, oleic acid, xanthorrhizol, curcumenol, decaprenoic acid, palmitic acid, z-, cis - ferulic acid, curcumenone, geraniol, p-cymen-8-ol, zingerone, curcumin, geranyl acetate, 4-cymene, Diferuloylmethane, germacrene d, phytol, demethoxycurcumin, germacrone, d-piperitone, bisdemethoxy curcumin, gitoxigenin, dihydrocarvone, curcuphenol, himachalene, terpinolene, cyclohexyl formate, lupeol p-methylacetophenonek, dehydrocurdione, limonene, procurcumadiol, dehydrozingerone, linalool, procurcumenol, dicumyl peroxide, linoleic acid, pyrazolo[1,5-a]pyridine, 3,3a,4,7-tetrahydro-3,3-dimethyl-, (3as)-, 3,3-dimethyl-4,7-dihydro-3ah-pyrazolo[1,5-a]pyridine, alpha-farnesene, 1-methyl-3-isopropylbenzene, stearic acid.

Murraya koenigii : beta-ocimene, alpha-gurjunene, beta carotene, caryophyllene oxide, isoheraclenin, (z,e)-farnesol, Humulene, Caryophyllene, Cyclomahanimbine, Isopimpinellin, dimethyl allyl xanthyletin, alpha-tocopherol, elemene, dehydroaromadendrene, koenigicine, 7,12-dimethylbenzo[a]anthracene-5-carboxylic acid, benzo(a)pyrene, beta-gurjunene, euchrestine b, koenimbin, linoleic acid, 5-methoxypsoralen, beta-pinene, gamma-terpinene, alpha cadinol, beta-phellandrene, bicyclogermacrene, girinimbine, alpha-pinene, beta-selinene, bornyl acetate, glycozoline, koenoline, mahanine, oxypeucadanin, methoxsalen, limonene, mukonal, sabinene, linalool, mukonidine, scopolin, lutein, murrayanine, terpinenol-4, mahanimbicine, murrayanol, beta-ocimene, mahanimbilol; murrayazolinol, tridecanoic acid, mahanimbine, osthol,7-hydroxycoumarin, Bicyclomahanimbicine, mahanimbidine, murrastifoline, bismurrayafoline, mahanimbinol, murrayacine, bismurrayaquinone, mukeic acid, murrayazoline, isogosferol, mukoenine selinene, koenidine, mukolidine, koenigine, mukoline, koenine, mukonicine.

Piper nigrum : caryophellene, ethyl linoleate, ethyl palmitate, globulol, piperamide, piperylline, eucalyptol, alpha-eudesmol, copaene, beta-bisabolene, cadina-1,4-dien-3-ol, sabinene hydrate, 2,4-di-tert-butylphenol, Humulene, alpha cubebene, beta-elemene, camphene, citronellal, 2-nonanone, phellandrene epoxide, elemene, beta-myrcene, camphor, cyclopropanebutanoic acid, 2,2-difluoro-, 2-naphthalenylmethyl ester, 2-methylnaphthalene, alpha phellandrene, guaia-1(5),11-diene, beta-phellandrene, 6,14-endotheno - 7 – alpha - (p-methylfumaroylaminophenylacetylamino)tetrahydroripavine, 3-carene, undecan-2-one, alpha-pinene, gamma-terpinene, beta-pinene, d-carvone, isovalerylaldehyde, alpha-bulnesene, alpha-terpineol, isoborneol, caryophyllene, (e)-calacorene, Humulene, alpha-ylangene, bornyl acetate, caryophyllene oxide, 2,3-dimethoxyphenol, gamma-terpinene, linalool, piperazine, sarmentine, beta-ocimene, citral, methyl geranate, piperettine, sarmentosine, elemol, germacrene b, myristicin,

***Boerhaavia diffusa*:** palmitic acid, Eupalitin, boeravinone d, arachidic acid, boeravinone e, coccineone b, lignoceric acid, boeravinone f, hentriacontane, coccineone e, ursolic acid, boeravinone c, 1-triacontanol, boeravinone a, gamma-sitosterol, boeravinone b.

***Aloe vera*:** Niacin, Aloenin, Alloin, Emodin, Aloenin, (1-6)-alpha-glucomannan, Sorbitol, Campesterol, aloe emodin, gamma-sitosterol, aloe emodin,beta carotene, acemannan, aloesone, aloesin, tocol.

Three dimensional structures of all the compounds were retrieved from PubChem database. Before performing the molecular docking using Discovery studio 4.0, the ligands were prepared using Prepare Ligands protocol of Discovery studio 4.0. This includes removal of duplicates, enumerating tautomers / isomers, and generating three dimensional conformations. Druglikeness of these compounds was determined using Filter by Lipinski's and Veber rule.

Docking procedures were done using CDOCKER protocol of Discovery studio 4.0 to identify the best pose of ligands in the active site region of the targets. It allows flexible docking of ligands and by default; it was set to generate 10 different poses. In addition to molecular docking, pharmacokinetic analysis also was performed to check the bio availability of the identified compounds using ADMET descriptors protocol of Discovery studio 4.0. The results were analyzed and interpreted to highlight the best phytocompounds for drug development.

RESULTS AND DISCUSSION: Inhibiting apoptotic regulator proteins Bcl-2 and Bcl-XL with compounds may potentially overcome the development of cancer. There are drugs developed which explicitly targets these proteins and Oblimersen sodium, Obatoclax, Gossypol, Apogossypol, Apogossypolone are few among them [15, 16]. We here in used molecular docking method to identify the lead compounds from plants which interacts with these proteins. The target proteins from PDB were prepared and the binding sites were defined. Stable form of Bcl-2 protein and Bcl-XL protein was obtained in -3141.7145 and -5986.4987 kcal/mol respectively. A total of 574 phytochemicals were retrieved from selected plants. To find the best fit ligand for the target; the protocol "Prepare Ligands" was applied to generate tautomers and isomers of 2929 ligand structures from 574 phytochemicals. Compounds generated when filtered to determine whether it possess pharmacological properties yielded 868 ligands which satisfied Lipinski's and Veber rule and these compounds were further used for the molecular docking procedures.

Molecular docking studies were performed for both the proteins because even if they belong to same family, they are not identical and studies reported that each protein has different affinities to compounds. Docking calculations were based on – CDOCKER energy, -CDOCKER interaction energy, number of hydrogen bonds involved, binding energy and complex energy. Analysis of docking was performed based on the following criteria's:

CDOCKER Energy:

- CDOCKER score reported as the negative value (i.e., -cdocker_energy) where a higher value indicates a more favorable binding.
- -cdocker_energy is a total of internal ligand strain energy and receptor-ligand interaction energy.

CDOCKER Interaction energy:

- A pose with a negative value of "-cdocker_interaction_energy" score would indicate a very poor receptor-ligand interaction.
- The higher (more positive) the value, it indicates a more favorable binding.

Complex energy, Binding energy and Hydrogen bonds

- We prioritize the complexes of ligand and protein target which has least energy than the protein energy.
- Least negative binding energy which tends to be a favorable stable conformation.

- Hydrogen bond formed to the amino acids involved in the defined residues.

Docking analysis with the target- Bcl 2:

Molecular docking analysis was performed on anti apoptotic protein Bcl 2 and phytocompounds. It predicts the interaction of ligand with receptor protein and amino acid residues involved in this complex. After docking, the ligands were ranked based on their CDOCKER and binding energy. Out of 868 ligands tried 20 were screened out based on the high CDOCKER and binding energy. The docking results of selected ligands were provided in **Table 1**.

Docked confirmation revealed that these ligands were interacting to the aminoacid Arg 105 which is an important active site residue. The phytocompounds such as Methyl 4 tyramine, 3,7,4-trihydroxy flavone, Ephedrine, Ellagic acid and Gallocatechol from *Pseudathira viscida, Pterocarpus marsupium, Sida rhombifolia*, and *Phyllanthus amarus* exhibits higher score and strong binding affinity with least complex energy. The study shows that these screened 20 compounds has good inhibitory activity on Bcl2 and can be helpful in cancer treatment.

TABLE 1: PHYTOCHEMICALS SELECTED AGAINST Bcl-2; HAVING HIGH SCORES AND BINDING ENERGY

Sl. No.	Plant name	Compound name	-CDOCKER energy	-CDOCKER interaction energy	H-bond residues	Binding energy	Complex energy
1	*Aegle marmelos*	Aegeline	23.6996	29.7673	Arg 105	-62.0706	-3228.55
2	*Allium sativum*	Alliin	26.1695	22.3103	Glu 95	-103.097	-3337.51
3	*Aloe vera*	Aloe emodin	29.6323	36.8857	Arg 105	-102.754	-3235.55
		Emodin	22.6166	32.6655	Arg 105	-115.593	-3272.18
		Aloesone	31.0281	37.2169	Arg 105	-79.3689	-3227.52
4	*Boerhaavia diffusa*	Boeravinone E	31.3135	41.1647	Arg 105	-99.424	-3290.05
		Boeravinone F	37.7796	37.563	Arg 105	-86.8122	-3296.11
5	*Phyllanthus amarus*	Ellagic Acid	32.2874	36.7473	Arg 105	-122.417	-3354.58
		Gallocatechol	44.4459	40.2894	Arg 105	-120.585	-3261.91
6	*Pseudathira viscida*	Dalbergioidin	35.1914	38.9739	Arg 105	-116.326	-3267.91
		Methyl-4-tyramine	36.2645	34.5365	Arg 105	-182.261	-3374.81
7	*Pterocarpus marsupium*	3,7,4'-trihydroxyflavone	28.643 40.6178	36.4609	Arg 105	-141.041	-3175.93
		2,4',7-dihydroxyflavone	31.6285	37.7676	Arg 105	-125.606	-3310.41
		Isoliquiritigenin		37.9238	Arg 105	-128.523	-3272.6
8	*Sida rhombifolia*	Wogonin	27.5357	36.8209	Arg 105	-103.051	-3219.34
		Ephedrine	19.8881	22.8712	Asp 70	-128.523	-3272.6
9	*Trigonella foenum-graecum*	4-hydroxy isoleucine	29.9118	24.5451	Arg 105	-87.4593	-3328.41
		Trigonelline hydrochloride	22.0834	21.6099	Arg 105	-107.135	-3300.04
10	*Zingiber officinale*	Gingerdione	33.3165	33.0368	Arg105Leu 96	-74.0799	-3223.12
		Capsaicin	20.011	32.8397	Arg105	-48.2607	-3196.91

Docking analysis with the target, Bcl XL:

An *in silico* study about the involvement of active site residues with several chemically synthesized compounds such as ABT-737 and ABT-263 has been reported. It suggests that the drugs show close contact with these residues and are potent inhibitors [7]. In our study, docking procedures of Bcl-XL was performed with the ligands in the same druggable binding areas. Docking results were tabulated and shown as **Table 2.**

Metalline and 5-deoxy kaempherol from *Pterocarpus marsupium* found to be satisfied with the docking scores and binding energy followed by Boeravinone D from *Boerrahavia diffusa*, Vanillic acid from *Curcuma longa* and kaempherol from *Allium sativum*. All 23 compounds showed high scores and binding energy possess complex energy lesser than the protein energy. So we can conclude that all these compounds are stable when they are seen as complexed.

Docking analysis with known inhibitors:

For comparative analysis, known drugs were developed to inhibit the activity of these proteins. As a comparative study, we performed the docking of known inhibitors Obatoclax and apogossypolone with Bcl-2 and Bcl-XL respectively. The results were tabulated as Table 3. Hydrogen bond formed with the protein and inhibitors were found to be in the defined sites and the interaction was shown as in **Fig. 1 and Fig. 2.**

Pharmacokinetic analysis:

Parameters such as absorption, solubility, BBB penetration, and toxicity for the 43 compounds were predicted using ADMET descriptor protocol of Discovery studio 4.0. Analysis was performed based on the criteria's suggested in the same protocol. Results are provided as **Table 4.**

Properties of compounds against Bcl-2: The 20 compounds that showed good interaction and binding energy were analyzed for their pharmaco kinetic properties. The compounds such as 3,7,4-trihydroxy flavones, Gallocatechol, Ellagic acid failed to pass ADMET due to their hepatotoxic effect. These molecules Alliin, Aegeline, Ephedrine, Capsaicin, Methyl 4-tyramine, Gingerdione from *Allium sativum, Aegle marmelos ,Zingiber officinale, Pseudarthira viscida and Sida rhombifolia* passed ADMET analysis.

Properties of compounds against Bcl-XL: Compounds with high dock score and binding energy such as Metaline, 5-deoxy kaempherol, Boeravinone D, Kaempherol etc. failed to pass the pharmacokinetic analysis based on toxicity. But a total of four compounds such as Curcumin II, Vanillic acid, Alliin and Dodecanoic acid were selected. Curcumin II and vanillic acid from *Curcuma longa* passed all the ADMET analysis and the rest two Alliin and Dodecanoic acid showed solubility level of 5 and BBB level as 1 respectively. Solubility level-5 denotes too soluble compounds and BBB level-1 denotes as high penetration compounds. The compounds which failed the analysis needs several modifications and can be further used as a potent molecule without side effects.

TABLE 2: PHYTOCHEMICALS SELECTED AGAINST BCL XL; HAVING HIGH SCORES AND BINDING ENERGY

Sl. No.	Plant name	Compound name	-CDOCKER energy	-CDOCKER interaction energy	H-bond residues	Binding energy	Complex energy
I.	*Aegle marmelos*	Scopoletin	24.2494	31.5474	Arg 139, Ser106	-130.3147	-6112.8193
I.	*Allium sativum*	Alliin	27.9445	24.3572	Tyr101	-91.4993	-6172.2727
		Kaempherol	46.0383	53.6462	Tyr101	-174.2071	-6139.0809
		Quercetin	45.2479	50.8503	Tyr101	-140.6202	-6032.7647
I.	*Aloe vera*	Glucomannan	35.6986	39.4821	Arg139,Arg139	-125.909	-6088.2595
/.	*Boerrahavia diffusa*	Boeravinone A	30.4985	44.4257	Asn136,Arg139	-139.838	-6111.1159
		Boeravinone D	45.5653	55.6997	Arg139	-178.0244	-6207.091
		Coccinenone B	30.5163	41.3071	Arg139, Asn136	-129.5071	-6112.8548
/.	*Catharanthus*	Kaemphero	47.4289	54.1123	Arg139	-169.1134	-6121.2188

	roseus	Quercetin	50.1269	53.5007	Arg139,Tyr101, Gly138,Asn136	-167.787	-6145.1686
VI.	*Curcuma longa*	Curcumin II	22.6441	40.9754	Tyr101,Arg139, Asn197	-96.1595	-6068.2675
		Vanillic acid	26.7232	34.9707	Asn136	-176.165	-6122.1048
VII.	*Phyllanthus amarus*	Quercetin	45.2479	50.8503	Tyr101	-140.6202	-6032.7647
		Gallocatechin	38.3297	40.1381	Tyr101,Gly138, Ala93	-127.263	-6119.0686
VIII.	*Pseudarthira viscida*	2-hydroxy genestin	21.3924	42.4683	Tyr101	-103.9219	-6110.0605
		Dalbergoidin					
		Quercetin	40.4307	40.9743	Tyr101	-104.6917	-6123.8339
			45.2479	50.8503	Tyr101	-140.6202	-6032.7647
IX.	*Pterocarpus marsupium*	4,7 di hydroxy flavones	38.6013	41.1641	Tyr101	-116.8655	-6128.6138
		5-deoxy kaempherol	37.4195	43.7124	Tyr101	-200.0727	-6210.6715
		L-epicatechin	35.0866	38.4219	Tyr101	-90.2486	
		Metaline	52.6836	55.129	Tyr101,Glu92, Asn197,Tyr195	-254.7978	-6082.7847
		Pseudobapti genin	21.5017	42.6077	Tyr101	-127.1455	-6295.0102
X.	*Wrightia tinctoria*	Dodecanoic acid	40.0254	38.3247	Arg139	-93.7252	-6109.1752 -6088.5522

TABLE 3: DOCKING INTERACTION WITH KNOWN INHIBITORS

Sl.No.	Protein name	Compound name	-CDOCKER energy	-CDOCKER interaction energy	H Bond residues	Binding energy	Complex energy
1	Bcl-2	Obatoclax	3.20794	33.1988	ARG105	-31.6005	-3165.39
2	Bcl-XL	Apogossypolone (ApoG2)	8.6031	43.7973	Tyr101, Arg100	-72.0694	-6079.9172

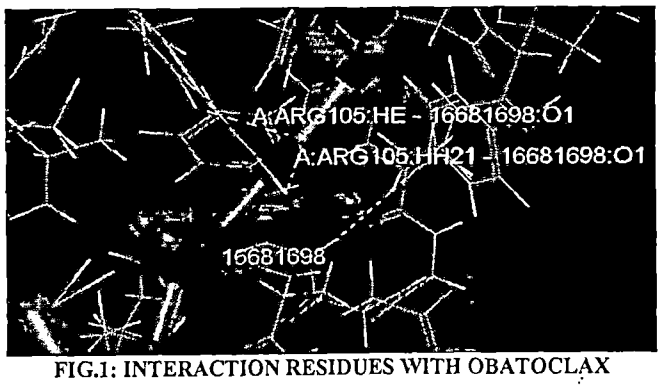

FIG.1: INTERACTION RESIDUES WITH OBATOCLAX

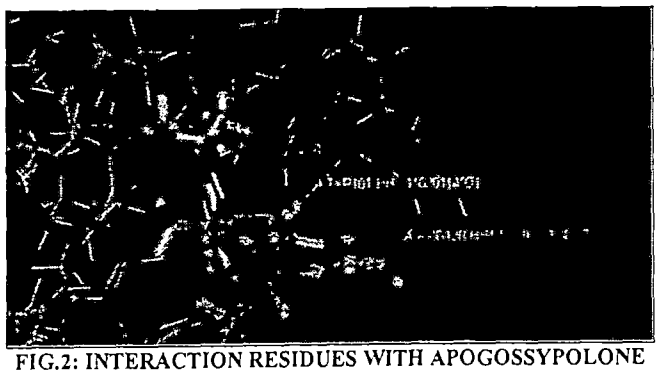

FIG.2: INTERACTION RESIDUES WITH APOGOSSYPOLONE

TABLE 4: PHARMACOKINETIC ANALYSIS OF SELECTED PHYTOCHEMICALS

Sl. No.	Plant name	Compound name	Solubilty level	BBB level	Hepatotoxicity	Absorbtion level	Alogp98
1.	*Curcuma longa*	Curcumin II	3	2	False	0	3.57
		Vanillic acid	4	3	False	0	1.201
2.	*Aegle marmelos*	Aegeline	3	2	False	0	2.562
3.	*Allium sativum*	Alliin	5	3	False	0	-0.912
4.	*Zingiber officinale*	Gingerdione	3	2	False	0	3.739
		Capsaicin	3	1	False	0	3.91
5.	*Pseudarthira viscida*	Methyl 4-tyramine	5	4	False	1	0.216
6.	*Wrightia tinctoria*	Dodecanoic acid	3	1	False	0	4.568
7.	*Sida rhombifolia*	Ephedrine	5	4	False	1	0.003
8.	*Trigonella foenum-graecum*	4-hydroxy isoleucine	5	4	False	3	-3.161

CONCLUSION: Pharmacological validation of large number of promising medicinal plants and spices of Kerala has to be explored more for developing plant based therapeutics. Because, secondary metabolites from the natural resources are potent lead compounds which possess different properties such as anti microbial, anti cancer, anti fungal, anti oxidant and anti diabetic activities. Treatments using small molecules known as targeted therapy are gaining importance and several plant based compounds are already under clinical trials for targeted therapies. Targeted drug design using computational methods, reduces time, cost and risks than the traditional methods.

Herein, we screened a total of 574 phytochemicals identified from fifteen plants so as to help developing novel therapeutic drugs targeting Bcl-2 and Bcl-XL proteins. Phytochemicals with high scores and binding energy were obtained and later performed the pharmacokinetic properties to determine whether these screened compounds can act as lead molecule. Some of the compounds failed to pass the pharmacokinetic analysis even if they possess strong interaction and scores with target protein. These compounds can be further utilized to develop therapeutic drugs or targeted drug after structural modifications which will be a dramatic shift in treating the malignancy and a promising way to develop more personalized cancer therapies.

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