

PCOS Modulatory Activity of *Tinospora cordifolia* leaves – An *Insilico* Approach

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The polycystic ovarian syndrome affects women of all age groups is mainly due to hyperinsulinemia and hyperandrogenism. The insulin action in the ovaries is mediated by binding to its receptor namely IRS (Insulin receptor substrate). The present investigation is undertaken to select a suitable antagonistic ligand from the bioactive phyto components of *Tinospora cordifolia* leaves to inhibit IRS1 and IRS 2 receptors to prevent the binding of insulin and subsequent hyperandrogenism. The phyto ligands used for the study were obtained from the previous literature and the IRS1 and IRS 2 receptor protein structure were retrieved from PDB (protein Data bank). Using the Corina 3D converter the ligand 3D structures were obtained. Molecular docking analysis was performed through Patch dock server to select a suitable ligand based on docking score for the IRS1 and IRS 2 receptor protein. Three ligands namely Berberine, Rumphioside I and Syringin showed better docking score among the ligand selected and their inhibitory activity were analysed by intermolecular interactions using "LIGPLOT" analysis. It can be concluded that these three ligands can be used for the successful treatment of PCOS after proper preclinical and clinical studies.

Keywords: IRS, phytochemicals, patch dock.

Ovaries of human are functionally active organs which produce steroids, release eggs, and consume energy cyclically in women of reproductive age ¹. The gonadotropin-driven event is also characterized by sudden enhancement of ovarian glucose uptake during ovulation ². Polycystic ovarian syndrome is characterized by anovulation and ovarian hyperandrogenemia. This occurs not only due the overproduction of insulin but also resistance to the action of

insulin to stimulate glucose uptake in classic target tissues, such as muscle and fat ³. Hoarding evidence suggests that in polycystic ovaries, the androgen-stimulating effect of insulin in thecal cells occurs through the classic insulin receptor ⁴, whereas insulin-mediated glucose uptake in granulosa-lutein cells is substantially impaired compared with that in cells obtained from persons with normal ovulation ⁵. It can be clearly seen that the metabolic and steroidogenic actions of

insulin differ from one another in the polycystic ovary. Insulin has various numerous actions in target tissues, such as stimulation of glucose uptake, steroidogenesis, gene transcription, DNA synthesis, and lipogenesis. The action of insulin is initiated when it binds to its cell surface receptor. The insulin receptor belongs to a family of protein tyrosine kinase receptors that include insulin-like growth factor I (IGF-I), epidermal growth factor, fibroblast growth factor, and cytokine receptors⁶. Signal transduction is initiated by the activated insulin-receptor which phosphorylates tyrosine residues in intracellular substrates. Among these substrates Insulin-receptor substrate (IRS)-1 and IRS-2 were recently characterized^{7,8}. Different Insulin-receptor substrates appear to specify diverse signal cascades and subsequent insulin actions, which makes it reasonable to hypothesize that the altered expression of IRSs serves as the basis of the altered signalling pathways and also responsible for insulin action imbalance and abnormal ovarian functionality in PCOS.

Diverse of phytochemicals accumulated in plants accounts for their constitutive activities. The worldwide trend of using botanical drugs and strategies for developing global drugs has been discussed by Kyungseop Ahn⁹. The drug discovery and development need not always be limited to new molecular entities. It can also be designed rationally by carefully standardizing botanical drug products with robust scientific evidence. Synergistic formulations of traditional herbs can also be the alternatives as suggested by Patwardhan and Mashelkar¹⁰. This study performed the molecular docking for the phytochemicals of the medicinal plant *Tinospora cordifolia* to identify the phyto constituent that fits well with IRS 1 and IRS 2 as potent inhibitor.

MATERIALS AND METHODS

Ligand and Receptor preparation was carried out for Patch Docking.

Receptor protein preparation

In the present study the target protein molecule is the Insulin Receptor Substrate (IRS1 and IRS2). The protein structure were retrieved separately in the PDB format from the Protein Data Bank (<http://www.rcsb.org/>) (PDB ID: P35668, PDB ID: Q9Y4H2) The 3D structures of

the receptors IRS1 and IRS2 were obtained from SWISS-MODEL which was evaluated by the RAMPAGE online server (Lovell *et al.*)¹¹.

Ligand preparation

The Gas chromatography-mass spectrometry (GC-MS) studies conducted by Singh *et al.*,¹² and Sinha *et al.*,¹³ in *Tinospora cordifolia* leaves revealed the presence of alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics compounds From them the phytochemicals belonging to alkaloid and terpenoid category such as Berberine, Palmatine, Magnoflorine, Tembeterine, Ecdysterone, Makisterone A, Tinocordiside, Rumphioside 1, Syringin, and Octacosanol were selected for the docking analysis with the IRS1 and IRS2 receptor using patch dock type of molecular docking. The two-dimensional structures of the above mentioned phyto components from the *Tinospora cordifolia* were obtained from PUB chem site and their 3D structures were drawn using Corina 3D converter.

Docking Analysis

In the field of drug discovery in recent years computer aided drug design minimises the complexity and time. The receptor interaction with the ligand was obtained as docking score using the patch dock server. The docking score of ligand and receptor interaction were calculated based on their binding energies in kJ mol⁻¹. The ligands which show the low binding energy with that of the receptor were selected as the best ligands. The antagonistic nature of the ligand towards the particular receptor was studied in terms of the intermolecular interactions with the active site amino acid residues present in the receptor through the visualization by LIGPLOT analysis.

RESULTS AND DISCUSSION

The selective insulin resistance and hyperandrogenism in PCOS pathology triggers the mitogen-activated protein kinase (MAPK) cascade in the cells of the ovary in response to insulin where as the metabolic actions of insulin will be impaired¹⁴. The insulin action in the theca cells of ovary is initiated through its binding to the insulin receptor substrate (IRS) IRS1 and IRS2. This leads to the tyrosine kinase phosphorylation and production of the secondary messenger phosphatidyl inositol-3,4,5-triphosphate (PIP3)

from phosphatidyl inositol-4,5-diphosphate (PIP2) which in turn activates the mitogenic pathway favouring the uncontrolled folliculogenesis as

Table 1. Molecular docking of IRS1 and IRS2 for phytochemicals of *Tinosporacordifolia*

S. No	Compound name	Binding energy expressed in (kJ mol ⁻¹)	
		IRS 1	IRS 2
1	Berberine	-0.17	-0.29
2	Palmitate	-0.22	-1.35
3	Magnoflorine	-0.70	-0.67
4	Tembeterine	-0.33	-0.57
5	Ecdysterone	-0.29	-0.71
6	Makisterone A	-1.09	-1.56
7	Tinocordiside	-0.39	-0.95
8	Rumphioside I	-0.09	-0.02
9	Syringin	-0.11	-0.14
10	Octacosanol	-0.59	-0.93

observed in PCOS ovary. Arresting the mitogenic pathway through the binding of insulin to IRS receptor will be a therapeutic intervention in correcting PCOS condition. So to select a best antagonistic ligand for the receptor IRS1 and IRS 2 from the phyto molecules of *Tinospora cordifolia* *Insilico* molecular docking analysis was performed using “Patch dock” server In the present investigation based on the docking score and glide energy (Table 1) out of the 10 ligands, 3 ligands namely Rumphioside 1, Syringin and Berberine were selected as they showed lowest binding energy.

These three ligands namely Berberine, Rumphioside I and Syringin are further investigated for their antagonistic potential in terms of inhibiting the IRS1 and IRS 2 receptor through their binding to specific amino acid residues present in the active site. The intermolecular interaction between the IRS1 and IRS2 receptor and the phyto ligands

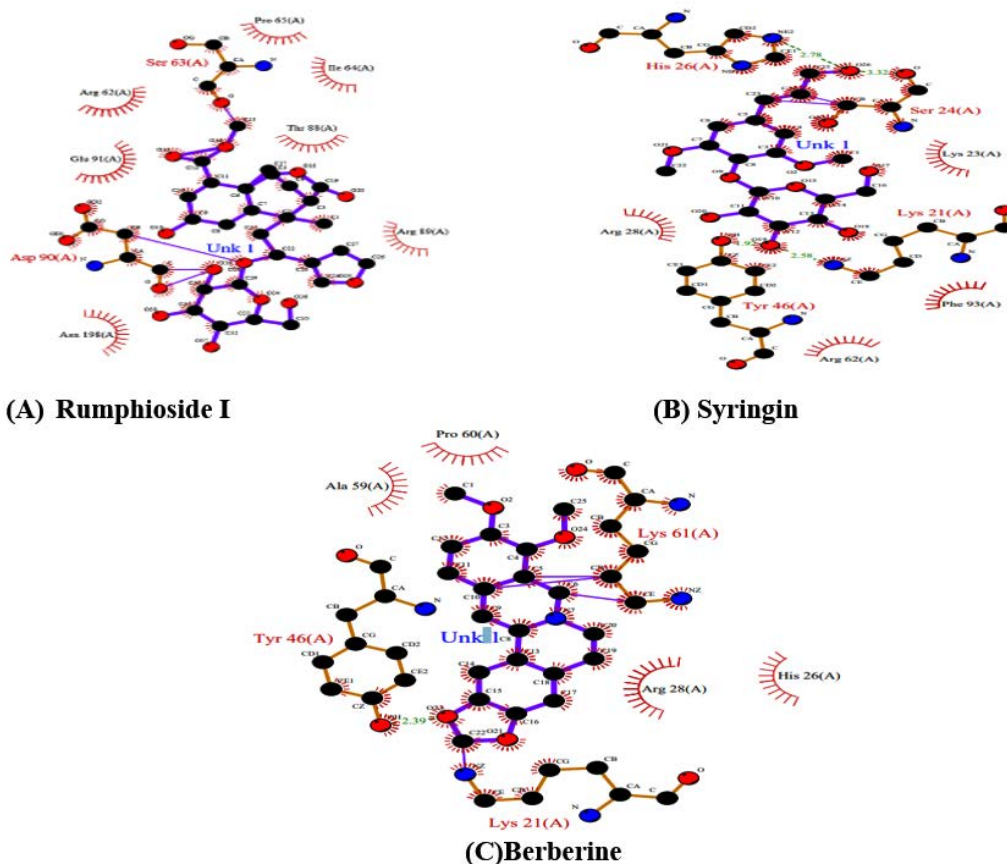


Fig. 1. Amino acid interactions of IRS 1 receptor with specific Ligands

Table 2. Intraction of IRS 1 and IRS 2 receptor with the phyto chemicals of *Tinospora cordifolia*

S. No	Compound	Insulin Receptor Substrate 1 (IRS1) receptor		Insulin Receptor Substrate 2 (IRS2) receptor	
		H bonding sites	amino acid binding site Hydrophobic contact sites	H bonding sites	amino acid binding site Hydrophobic contact sites
1	Rumphiosidel	-	88 Arg 62 (A), Ser 63 (A), Ile 64 (A), Pro 65 (A), Thr 88 (A), Arg 89 (A), Asp 90 (A), Glu 91 (A), Asn 198 (A)	2 Arg 261 (A)	90 Met 243 (A), Asn 244 (A), Ile 245 (A), Arg 246 (A), Gly 260 (A), Arg 261 (A)
2	Syringin	6 Lys 21 (A), Ser 24 (A), His 26 (A), Tyr 46 (A), Tyr 46 (A)	63 Lys 21 (A), Lys 23(A), Ser 24 (A), His 26 (A), Arg 28 (A), Tyr 46 (A), Arg 62 (A), Phe 93	1 Arg 195 (A)	39 Arg 195 (A), Glu 289 (A), Lys 292 (A), Glu 296 (A), Leu 297
3	Berberine	1 Tyr 46 (A)	90 Lys 21 (A), Ser 24 (A), His 26 (A), Arg 28 (A), Tyr 46 (A), Ala 59 (A), Pro 60 (A), Lys 61(A)	- -	74 Lys 292 (A), Lys 295 (A), Glu 296 (A)

were explained in the Table 2 and Figures 1 and Figure 2.

Based on the docking studies Rumphioside I, Syringin and Berberine showed better inhibitory actions against IRS 1 and IRS 2. Berberine is anisoquinoline plant alkaloid which has multiple pharmacological activities including hyperglycaemic, dyslipidemic, anti-microbial, anti-tumoral and immune modulating activities¹⁵. X.Wu¹⁶ and L.Zhao, *et al.*,¹⁷ in their *In vitro* studies have shown that berberine can increase glucose uptake and thereby reduce insulin resistance which accounts for its hyperglycemic activity. The insulin resistance lowering activity of berberine can be considered as a key property for the potential treatment of PCOS, since hyperinsulinemia and insulin resistance plays a prime role in the pathology of PCOS. A significant number of clinical studies^{18,19} has called on for attention of berberine in the role for treating PCOS.

The peptide hormone insulin binds to its receptor and executes its multiple functions by

amplifying the different signal cascade reactions. Since insulin activates the mitogen activated protein kinase pathway specific to ovarian theca cells as stated by Saltiel²⁰ in PCOS, hyperinsulinemia could be responsible for the altered signalling pathways leading to abnormal ovarian functionality. Burghen *et al.*,²¹ also suggested the involvement of insulin in the ovarian function and confirmed that hyperinsulinemia is associated with hyperandrogenaemia in PCOS women. Poretsky *et al.*,²² in his studies using cultured polycystic ovary theca cells indicated that insulin increased production of androstenedione and progesterone. Hyperinsulinemia and insulin resistance are the key components in the pathophysiology of PCOS. So, a potential drug is expected either to sensitize the insulin thereby reducing the hyperinsulinemia or directly act as an antagonistic ligand for the IRS1 and IRS 2 receptors and prevent their binding of insulin to control the signalling cascade in the ovarian theca cells. In the present investigation we have shown through the inhibition of amino

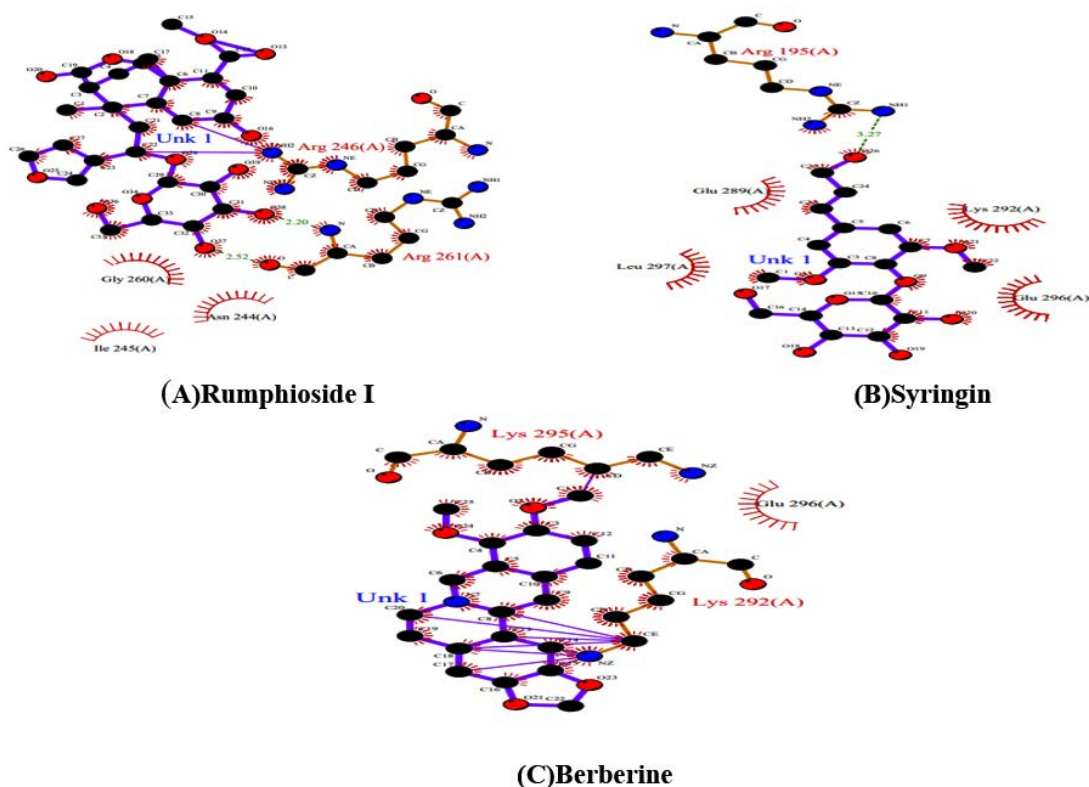


Fig. 2. Amino acid interactions of IRS 2 receptor with specific

acid interactions berberine can be considered as an antagonistic ligand for the IRS1 and IRS 2 receptors to halt the down streaming reactions of insulin by binding through these receptors.

In the present investigation based on the docking score Syringin, also showed the inhibitory activity of IRS1 and IRS 2 receptors in terms of the amino acid interaction. Syringin is a eleutheroside derivative belongs phenyl propanoid glycoside group of secondary metabolite which is reported by Mahadeva Rao *et al.*,²³ to possesses several pharmacological properties including anti-diabetic, antiproliferative, anti-nociceptive and anti-allergic effect. The anti-diabetic activity reported by him may be due to either decrease in insulin resistance or increase in glucose uptake by cells. These two possibilities are further confirmed by Bobae Kim *et al.*,²⁴ who studied the insulin resistance lowering activity of Syringin. Niu *et al.*,²⁵ with his research stated the ability of syringin to enhance glucose utilization and lower plasma glucose level in rats suffering from insulin deficiency. Added to these findings Ko Yu Liu *et al.*,²⁶ also proved that syringin can augment the insulin release which result in plasma glucose lowering action. The same mechanism can be expected in the ovarian theca cells in the ovaries to reduce the androgen production in response to insulin so that the complications of PCOS can be minimized.

Among the three ligands selected for docking with IRS1 and IRS 2 receptors RumphiosideI also showed a best antagonistic potential in inhibiting these receptors. According to Martin *et al.*,²⁷ RumphiosideI is a new furanoid diterpenoid predominantly present in the *Tinospora* species. Waqas Ahmad²⁸ in his studies using *T. crispa* have shown that diterpenoid glycosides are potential anti diabetic agents. Being a new compound the *invivo* or *invitro* hyperglycaemic activity has to be studied to confirm the mechanism of action of this ligand.

CONCLUSION

In our present investigation considerable inhibition of IRS1 and IRS 2 receptors by the antagonistic ligand RumphiosideI, Syringin and Berberine was observed through the *Insilico* studies. It can be concluded that these compounds

can be used for the treatment of PCOS condition after proper preclinical and clinical evaluation.

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Conflict of Interest

All the authors enlisted in the manuscript have no conflict of interest.

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