ORIGINAL ARTICLE

# Potential of Prediction of Activity Spectra of Substances Software to Justify 3Rs Ethics for *In Vivo* Anti-Alzheimer's Studies of Phytochemicals

**Rakesh Kumar[1], Rajan Kumar[1], Abhinav Anand[1], Rishi Mahajan[2], Gopal Lal Khatik[1], Navneet Duggal[1], Meenu Mehta[1], Saurabh Satija[1], Neha Sharma[1], Navneet Khurana[1]**

*[1]Department of Pharmacology, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India, [2]Regeneron Pharmaceuticals, Inc., Tarrytown, New York, USA*

## Abstract

**Context:** Alzheimer's disease (AD) is a progressive neurodegenerative disorder having important concern in today's society. The prediction of activity spectra of substances (PASS) software allows prediction of the pharmacological activity on behalf of canonical simplified molecular-input line-entry system (SMILES) of the substance. PASS predicted the activity of substances in two manners, i.e. probable activity (Pa) and probable inactivity. **Objective:** In this study, we elaborated the benefit of PASS to define 3Rs ethics (replacement, reduction, and refinement) in animal study for anti-Alzheimer's activities of phytochemicals. **Materials and Methods:** Pa values of different phytochemicals important for AD predicted using PASS. For the prediction of activity in the different targets of AD, canonical SMILES of phytochemicals was obtained from PubChem website. **Results and Discussion:** Based on these Pa values predicted for different target sites of AD, screening of pharmacologically important phytochemicals was performed so that those phytochemicals can be further explored for *in vivo* pharmacological activity against AD to justify the 3Rs ethics. **Conclusion:** PASS online software provides an informative data to support the reported activities of the phytochemicals.

**Key words:** 3Rs ethics, Alzheimer's disease, phytochemicals, prediction of activity spectra of substances software

## INTRODUCTION

More than 100 years ago, the Alzheimer's disease (AD) was first identified, but 70 years passed before, it was recognized as the most common cause of dementia and a "major killer." Although research has revealed a great deal about AD, much is yet to be discovered about the precise biologic changes that cause this disease and why it progresses more quickly in some than in others, and how the disease can be prevented, slowed, or stopped. Researchers believe that early detection will be key to prevent, slowing and stopping AD. Under the proposed criteria, the disease begins before symptoms such as memory loss appear, while earlier criteria require memory loss and a decline in thinking abilities for an AD diagnosis to be made. Due to the proposed criteria of the scientific evaluation are ongoing, in this report, AD refers to the disease as defined by the earlier criteria.[1]

AD is a neurodegenerative disorder that generally appears in late adulthood. It is associated with a progressive and rather irreversible decline in memory various other cognitive capabilities. In AD, there is neuronal destruction and deterioration of neural connections in the cerebral cortex region of the brain along with a substantial loss of brain mass. In some rare cases, it appears in people in their 40s and 50s, but otherwise, it is a disease of old age. Based on

**Address for correspondence:**
Navneet Khurana, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab - 144 411, India.
E-mail: navi.pharmacist@gmail.com

**Received:** 21-02-2018
**Revised:** 18-03-2018
**Accepted:** 06-04-2018

clinical, population-based studies, about 200,000 people under 65 years of age are suffering from AD.[2,3]

AD is characterized by the presence of two neuropathological hallmarks, i.e. extracellular amyloid β (Aβ) plaques and intracellular tau neurofibrillary tangles.[4]

The actual etiology behind AD is not yet defined. Even the cause of AD is based on aging, degeneration of anatomical pathways, environmental factors, genetic factors, mitochondrial dysfunction, vascular factors, immune system dysfunction, and infectious agents.[5] However, certain factors such as anomaly in the phosphorylation of tau protein, alterations in calcium metabolism, oxidative stress, neuroinflammation, abnormal energy metabolism, and protein processing, i.e., undesired Aβ formation and aggregation, are considered to be important factors in the pathogenesis of AD.[6]

This period, spanning over a few years, is known as preclinical AD. The progression of AD occurs in three stages, namely mild AD (early stage), moderate AD (middle stage), and severe AD (late stage).[7]

The prediction of activity spectra for substances (PASS) is freely online web resource which is designed for the prediction of phytochemicals or the biological activity spectra of organic compounds. The activities of the compounds are based on its structural formulas for more than 4000 types of biological activity with average accuracy above 95% of about more than 300,000 organic compounds.[8] The structure–activity relationships (SARs) of phytochemicals are used for prediction. The analysis of SAR contains information on the structure and biological activities on different targets of the phytochemicals.[9]

The software predicts the activity of the compound in two probabilities; one is probable activity (Pa) and another one is probable inactivity (Pi). The value of Pa and Pi varies from 0.000 to 1.000. If Pa > Pi for any activity, only then that activity can be designated to the compound. If Pa value is more than 0.7 for any activity, then probability of that action is high in pharmacological experiment.[10] If Pa value is more than 0.5 but <0.7 for any activity, then probability of that action is less in pharmacological experiment. If Pa value is <0.5, then probability of observing that activity in pharmacological experiment is less.[11]

In biological and biomedical sciences, animals are used for research to enhance the knowledge of human. The experimental research on animal models also enhance concern, awareness of animals used ethically, the use of animals in research and education as per an application of ethics, the role of National Council for the Control of Animal Experimentation.[12]

In 1959, W.M.S Russel and R.L Burch first described 3Rs (replacement, reduction, and refinement) give the principles to the use of animals in testing ethically. The 3Rs have better future scope for alternatives to animal testing. The 3Rs main objective is to ameliorate quality of science and animal welfare to minimum use of animals in research.[13] Replacement is the first R, as the name suggests "replacing" the use of animals using of non-animal methods over animal methods. In absolute replacement, no animals are used, and in relative replacement, the tissue of animals is used.[14]

Reduction is the second R, as the name suggests "reduce," in this method, the number of animals used per study is minimized. The techniques of modern imaging are also reducing the number of animals used in research.[15]

Refinement is the third R, third R itself represent "refine" which is used to minimize pain, suffering of animals in research and to improve animal welfare for animals used in research [Figure 1].[16,17]

## MATERIALS AND METHODS

Phytochemicals were selected on the basis of their already reported pharmacological effect in the *in vivo* and *in vitro* models of AD, and one marketed standard drug compound (galantamine) for AD. The activities of phytochemicals were predicted using PASS and canonical simplified molecular-input line-entry system (SMILES). For the prediction of activity in the different targets of AD, canonical SMILES of phytochemicals was obtained from PubChem website [Table 1]. The SMILES works as a molecular formula of the phytochemical and was directly copied into the PASS software to predict the activities of various reported phytochemicals [Table 2].

## RESULTS AND DISCUSSION

The six activities in AD of selected phytochemicals and marketed compound (galantamine) were predicted using PASS online. These activities are as follows:

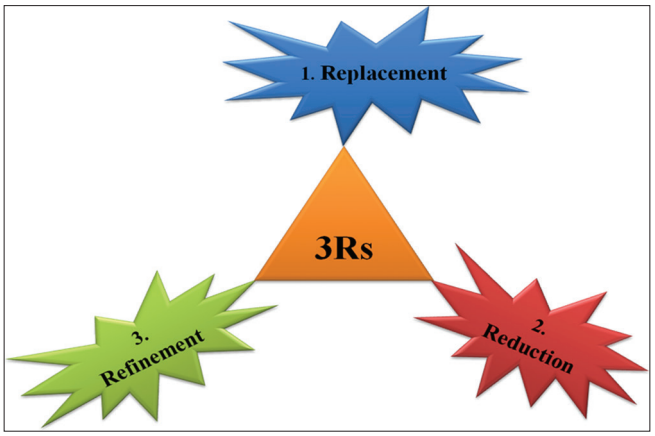


**Figure 1:** Name of 3Rs as per ethical guideline for animal

| Antidementia | Anti -amyloidogenesis | Anti-amyloid β aggregation |
|---|---|---|
| Cholinergic activity | Nootropic activity | Glutamate antagonist |

The PASS online software was used to predict the probable activities which are represented in Table 2 and Figures 2-7.

AD is a progressive neurodegenerative disorder. There is no effective treatment available for AD. Some compounds and nootropic are marketed to treat mild-to-moderate confusion (dementia) that is related to anti-AD, but such compounds and nootropics may improve memory, awareness, and ability to perform daily functions by restoring the balance of certain neurotransmitters in brain. Several phytochemicals are present in plants, but only few phytochemicals are explored for its pharmacological activities and rest are unexplored. Testing every compound by hit and trial method for pharmacological activity is not an easy task as it is tedious and costly. PASS online software is not only providing supportive information for the pre-explored phytochemicals but also gives the information regarding their hidden potential activities which previously was not known.

**Table 1:** Canonical SMILES and chemical structure of phytochemicals

| Name of compound | Canonical SMILES |
|---|---|
| Quercetin | CC1C(C(C(C(O1)OC2=C(OC3=CC(=CC(=C3C2=O)O)O)C4=CC(=C(C=C4)O)O)O)O)O |
| Rutin | CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O)O |
| Kaempferol | C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O |
| Apigenin | C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O |
| Tangeretin | COC1=CC=C(C=C1)C2=CC(=O)C3=C(O2)C(=C(C(=C3OC)OC)OC)OC |
| Caffeine | CN1C=NC2=C1C(=O)N(C(=O)N2C)C.C(C(=O)O)C(CC(=O)O)(C(=O)O)O |
| Galantamine | CN1CCC23C=CC(CC2OC4=C(C=CC(=C34)C1)OC)O |
| Huperzine A | CC=C1C2CC3=C(C1(CC(=C2)C)N)C=CC(=O)N3 |
| Nobiletin | COC1=C(C=C(C=C1)C2=CC(=O)C3=C(O2)C(=C(C(=C3OC)OC)OC)OC)OC |
| Hesperidin | CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=CC(=C4C(=O)CC(OC4=C3)C5=CC(=C(C=C5)OC)O)O)O)O)O)O)O)O |

SMILES: Simplified molecular-input line-entry system

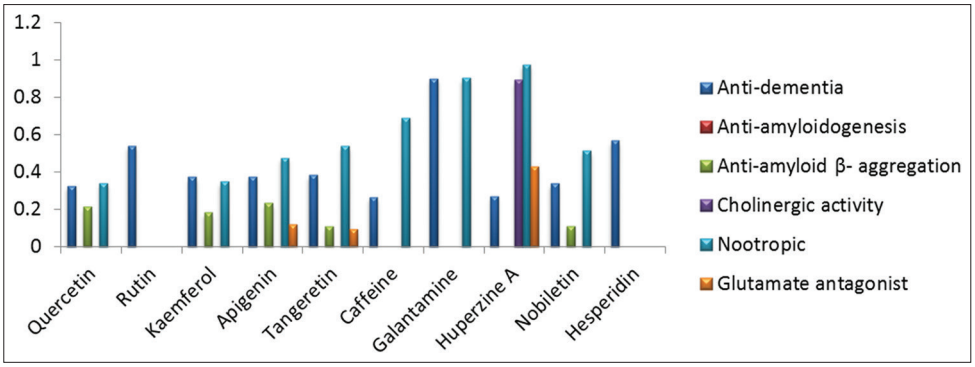


**Figure 2:** Pharmacological activities of all phytochemicals with respect to galantamine

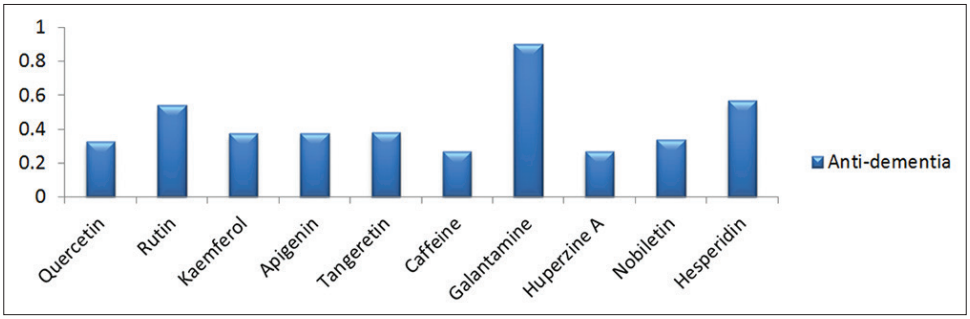


**Figure 3:** Antidementia activity of all phytochemicals with respect to galantamine

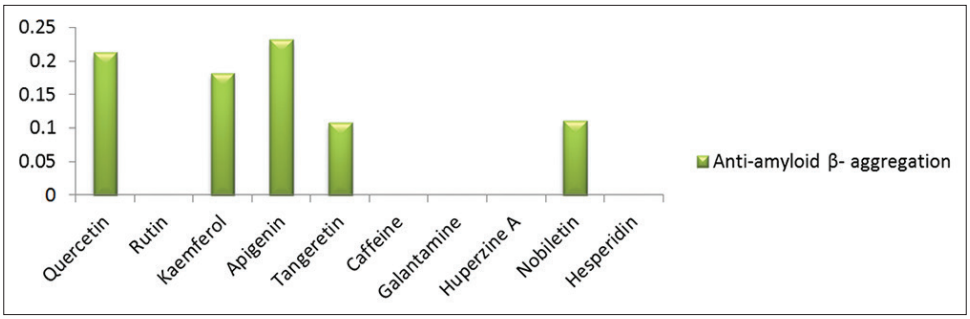


**Figure 4:** Anti-amyloid β-aggregation activity of all phytochemicals with respect to galantamine

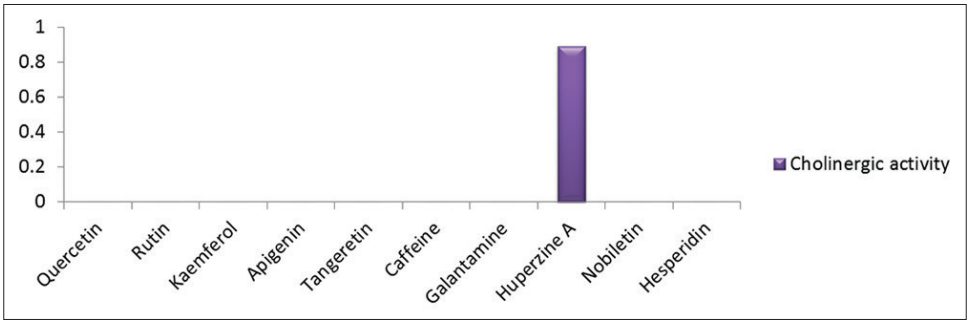


**Figure 5:** Cholinergic activity of all phytochemicals with respect to galantamine

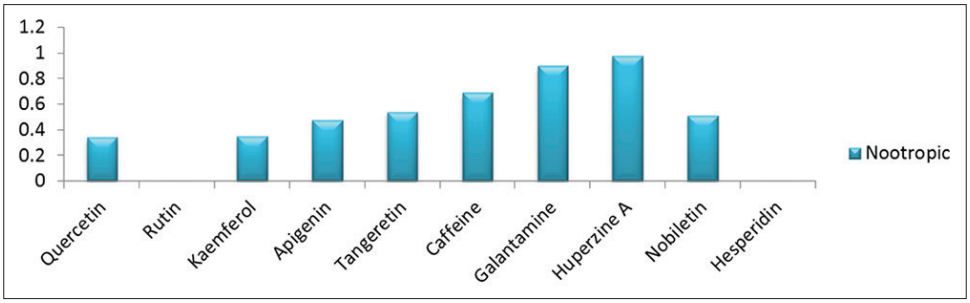


**Figure 6:** Nootropic activity of all phytochemicals with respect to galantamine

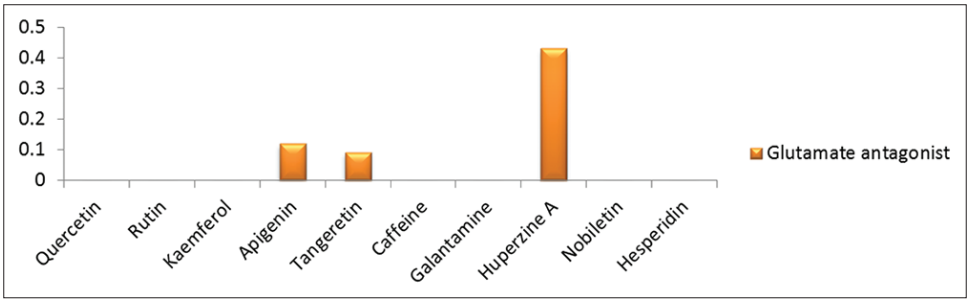


**Figure 7:** Glutamate antagonist activity of all phytochemicals with respect to galantamine

PASS online software can be used by anyone after registration. The PASS online software predicts the biological activity of the chemical compound on the base of SAR of chemical structure and interaction with the different targets. This software gives the information regarding especial compound that can be beneficial in a particular disease. On the basis of predicted Pa, the pharmacological testing can be performed to verify the result from the information provided by the software.

In this study, we selected various phytochemicals which already have been reported to have beneficial effects in the *in vitro* and *in vivo* models of AD. One marketed compound (galantamine) was selected as standard drug for AD to compare with the phytochemicals. Six activities of phytochemicals were tested. In which, we found that galantamine has the highest antidementia activity among the selected phytochemicals. Of these, hesperidin and rutin have the higher antidementia activity and caffeine has the

**Table 2:** Pa of AD in various phytochemicals predicted by PASS software

| Name of compounds | Reported activities | Pa predicted by PASS software | | | | | |
|---|---|---|---|---|---|---|---|
| | | Antidementia | Anti -amyloidogenesis | Anti-amyloid β aggregation | Cholinergic activity | Nootropic | Glutamate antagonist |
| Quercetin | Decreased ROS production and increased MnSOD activity[18] | 0.327 | 0 | 0.213 | 0 | 0.341 | 0 |
| Rutin | Decreased level of reduced GSH and activities enzyme, GPx, and GR[19] | 0.541 | 0 | 0 | 0 | 0 | 0 |
| Kaempferol | Antioxidants[20] | 0.374 | 0 | 0.182 | 0 | 0.348 | 0 |
| Apigenin | Anti-inflammatory activity and anti-AD[21] | 0.377 | 0 | 0.232 | 0 | 0.475 | 0.12 |
| Tangeretin | Neuroprotective[22] | 0.383 | 0 | 0.108 | 0 | 0.539 | 0.093 |
| Caffeine | Neuroprotective, decrease the level of ROS, and increase the level of SOD and GHS[23] | 0.267 | 0 | 0 | 0 | 0.69 | 0 |
| Galantamine | Neuroprotective[24] | 0.902 | 0 | 0 | 0 | 0.904 | 0 |
| Huperzine A | AChE activity and destructed β AP aggregation[25] | 0.271 | 0 | 0 | 0.895 | 0.977 | 0.432 |
| Nobiletin | Anti-inflammation and antioxidant effects[26] | 0.339 | 0 | 0.111 | 0 | 0.513 | 0 |
| Hesperidin | Improve oxidative stress and cognitive dysfunction[27] | 0.57 | 0 | 0 | 0 | 0 | 0 |

Pa: Probable activities, AD: Alzheimer's disease, PASS: Prediction of activity spectra of substances. ROS: Reactive oxygen species, MnSOD: Mitochondrial superoxide dismutase, GSH: Glutathione, GPx: Glutathione peroxidase, GR: Glutathione reductase, SOD: Superoxide dismutase

lowest one. The phytochemicals showed antidementia activity that is as follows the pattern; galantamine>hesperidin >rutin>tangeretin>apigenin>kaempferol>nobiletin>caffeine.

Anti-amyloidogenesis activity of the reported phytochemicals was not predicted.

Anti-amyloid β-aggregation activity of phytochemicals was predicted that is as follows in pattern; apigenin >quercetin >kaempferol >nobiletin >tangeretin >rutin, caffeine, galantamine, huperzine A, and hesperidin.

Among all predicted phytochemicals, huperzine A showed the highest cholinergic activity and rest of the phytochemicals showed no cholinergic activity.

In case of nootropic activity, huperzine A has the higher value than the galantamine, and according to value list is like; huperzine A>galantamine>caffeine >tangeretin >nobiletin>apigenin>kaempferol >quercetin >rutin and hesperidin.

In glutamate antagonist activity, huperzine A has the highest value than another phytochemical and more than galantamine; huperzine A >apigenin >tangeretin >kaempferol, quercetin, galantamine, rutin, nobiletin, and hesperidin.

PASS online software predicts the pharmacological activities of phytochemicals. It justified the 3Rs (replacement, reduction, and refinement) ethics for animal used in research.

In case of "replacement," PASS will help in initial screening of the phytochemicals. Hence, it replaces the unnecessary animal studies or avoids animal study.

In case of "reduction," PASS will assist to reduce the number of animal used in research. For example, we want to check the activity of phytochemicals on six targets in six different animal groups which require large number of animals. However, with the help of PASS, we come to know that selected phytochemicals having activity only on two target site. Hence, on behalf of this information, we will work only on two groups of animals which require less number of animals. That is why, it was concluded that PASS will reduce the number of animals used in research.

In case of "refinement," PASS will help to refine/improve the animal study protocol. As from PASS, we can get information that which type of biochemical parameters can be performed or which can be skipped. For example, initially decided to evaluate glutamate antagonist parameter of the phytochemicals, but from PASS, we come to know that phytochemical not have glutamate antagonist activity so that parameter can be skipped, which refine the research protocol and refine unnecessary pain, distress, or suffering to animals.

## CONCLUSION

From the above study, it can be concluded that PASS online software provides an informative data to support the reported activities of the phytochemicals. It can also help the researcher to decide and to prepare a protocol for testing a new phytochemical for a particular pharmacological action as well as to test a pre-reported phytochemical for a new activity. PASS online software justifies 3Rs ethics for animal usage in research by justifying replacing, reducing number and scarification of animals, saving time and money and at the same time, giving relevant information to enhance human knowledge, and identify potential novel phytochemical for debilitating diseases like AD, in future.

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**Source of Support:** Nil. **Conflict of Interest:** None declared.