JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

IN SILICO ANTI-ANXIETY EFFECT AND PHYTOCHEMICAL EVALUATION OF GALLIC ACID PRESENT IN FLACOURTIA JANGOMAS

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ABSTRACT

According to recent surveys, it is estimated that the global population has been found to be living with anxiety disorders and it is the most prevalent psychiatric disorder. The purpose of this study was to analyze the anti-anxiety action of Gallic acid (GA) by computational docking analysis studies(CDAS) and also to make a survey for bioactive compounds, like, alkaloids, flavonoids, phenols, terpenoids, tannins and saponins, and to determine the total phenol content in the ripe fruits of *F. jangomas*. For this, the natural phenolic compound GA isolated from *Flacourtia jangomas* (FJ) was used as a ligand for molecular interaction and it has found out that *FJ* contains 39.86 µg/mg of GA after following ethanolic extraction of dried ripe fruits *FJ*. The receptors for anxiety were obtained from PDB database (PDB ID: 2Z64, 4KBY, 5I6X). CDAS was performed for these three receptors using Pyrx, Pymol, Openbabel based on scoring functions. The GA showed more binding affinity with receptor 2Z64 and optimum binding affinity with receptors 4KBY, 5I6X. These results indicated that the phenolic compound GA could be one of the potential ligands to manage anxiety.

Keywords: *Flacourtia jangomas,* secondary metabolites, anxiety, docking, gallic acid.

INTRODUCTION

Anxiety is one of the most common mental disorders, affecting more than 10–15% of the population. Anxiety is an adaptive response which prepares the person to face challenges in life. Anxiety is characterized by changes in mood, behavior, somatic function, and cognition. The symptoms of anxiety are commonly associated with depression, panic disorder, agoraphobia and other specific phobias, obsessive-compulsive disorder, eating disorders, and some personality disorder.[1]. According to the report of the WHO, approximately 322 million persons are experiencing melancholy. This report also includes that anxiety disorders affect nearly 3.6% of the

global people (more than 260 million people).[2]

Anxiety affects one-eighth of the total population world-wide and has become an important area of research interest in psychopharmacology during this decade. Benzodiazepines are the major class of compounds used in anxiety and they have remained the most commonly prescribed treatment for anxiety. However, the realization that benzodiazepines present a narrow safety margin between the anxiolytic effect and those causing unwanted side effects has prompted many researchers to evaluate new compounds in the hope that other anxiolytic drugs will have less undesirable effects[3] . Studies reported that between 20–100% of patients experienced withdrawal symptoms, sedation, and dependence when they took benzodiazepines at therapeutic doses for prolonged periods . Therefore, the development of other anxiolytic drugs without the adverse effects of benzodiazepines would significantly improve the treatment of anxiety disorders.

Anxiety disorders, one of the most prevalent mood disorders present themselves in the form of excessive and irrational fear, and uneasiness to a threatening or a potentially threatening situation. Typical symptoms include high blood pressure, tachypnea, chest pain, heart palpitations, increased muscle tension, sweating, and irritability which significantly diminishes a person's quality of life. Anxiety disorders are often found in conjunction with other psychiatric disorders such as depression and substance abuse disorders. They are also found to be comorbid with non-psychiatric disorders such as cardiovascular and respiratory disorders. In 2015, an estimated 3.6% (264 million) of global population were found to be living with anxiety disorders[4] .

Anxiety, which has neurobiological, cognitive, and behavioral aspects, is one of the leading mental disorders of modern world experienced by children and adolescents. It has been also noted that in certain conditions, stress and anxiety might find helpful as they will motivate individuals, but when it becomes excessive, it leads disturbances in psychological states of individuals. Anxiety is a central nervous system (CNS) disorder with negative emotional state, causing uneasiness, fear, etc. in response to factors perceived from internal or external sources. It has also proved that incidence of morbidity associated with anxiety associated community found to be very high. However, like other CNS disorders, is also linked with CNS neurotransmitter imbalances. There are four neurotransmitters playing key roles in mood regulations, which are norepinephrine, gamma-aminobutyric acid (GABA), serotonin, and dopamine. GABA, glycine, and serotonin are considered as inhibitory neurotransmitters. Gamma-aminobutyric acid (GABA) is the primary inhibitory transmitter in the central nervous system (CNS). It has been suggested that dopaminergic systems have central roles in regulation of anxiety-like behaviors. Gamma-aminobutyric acid is found to be assisting neurons to recover after impulse transmission and thereby reducing the stress and anxiety. GABA also regulates both epinephrine and norepinephrine in order to reduce neuronal excitability during neuronal transmission[5] .

The most important neuronal circuits regulating the stress response system are the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system. Under acute stress, CRF is released from the paraventricular nucleus of the hypothalamus , which in turn stimulates adrenal synthesis and release of cortisol. Cortisol is often referred to as the “stress hormone. The neuropeptide arginine vasopressin, which is co-localized in the PVN, has effects on HPA function similar to those of CRF; the 2 neuropeptides interact in complementary ways, especially

during chronic stress. After activation by stress, HPA axis activity can be inhibited via cortisol via a negative feedback mechanism. Outside the hypothalamus, CRF-containing neurons exist in several brain nuclei and, along with other neurotransmitters, are involved in the ex- pression of fear, mediation of conditioned fear, and stress reactivity. These areas include the amygdala, locus ceruleus, hippocampus, prefrontal cortex, and anterior cingulate gyrus. Pathologic stress, depression, and/or anxietysustains overactivity of the HPA axis that cannot be sufficiently controlled by the fear/stress systems. This may lead to chronic hypercortisolemia, a condition associated with the destruction of hippocampal neurons.

The GABA system plays a role in homeostasis during stress. As with cortisol, GABA opposes the actions of CRF/arginine vasopressin on HPA axis function and inter- acts with numerous other neurotransmitter systems relatedto stress modulation at both the hypothalamic and extra- hypothalamic level.

Currently, the most widely prescribed medications for anxiety disorders are the benzodiazepines. However, the clinical uses of benzodiazepines are limited by their side effects such as psychomotor impairment, potentiating of other central depressant drugs and dependence liability. It has lead to investigate plants, which are commonly employed in traditional and alternate system of medicine for sleep disorders and related diseases. Various plants are being used in complementary and alternative medicines for management of anxiety[6] . Synthetic drugs available for treatment of anxiety have various adverse effects. Drugs obtained from natural sources are known to cause fewer side effects compared to synthetic drugs despite of same ability to cure disease.

Flacourtia jangomas (Lour.) Raeusch., otherwise known as Indian plum tree is a Salicaceae family tree usually found in the wild in Bangladesh. The fruits are edible and is a semi-cultivated fruit plant found frequently in the Brahmaputra valley of Assam and adjoining areas in the northeastern parts of India. The plant has some medicinal as well as economic values. It is mainly cultivated for its edible fruit and hard wood. The fruits are dark red or purple when ripe (Photo-plate 1) and eaten raw or used for making jams and preserves. Different plant parts are also pharmaceutically used for the treatment of asthma, pre- and post-natal blood purification . The fruits are used in bilious conditions and in diarrhea[7]. *Flacourtia jangomas* Lour known as locally Painnagola, Lukluki, Paniamra, Indian plum, coffee pulm that is widely distributed in Chittagong Hill Tracts, Cox's Bazar and Sylhet district of Bangladesh and south east Asia. The plant fruit having a remarkable reputation in the treatment of stomachic and digestive; allay thirst, useful in biliousness, fevers and relieves nausea.

As of today, *in silico* drug design should not be seen as a 'voilà' technique able to suggest directly a small number of compounds with a high affinity and selectivity for the targeted macromolecule, along with favorable pharmacokinetic and pharmacodynamics properties, quantitative structure-activity relatioships, pharmacophores, similarly searching, homology models and other molecular modeling approaches[8].

Molecular docking is used to recognize and optimize drug candidates by examining and modelling molecular interactions between ligand and target macromolecules. Molecular docking are used to generate multiple ligand conformations and orientations and the most appropriate ones are selected.

Fig 1: ***Flacourtia jangomas***

AIM

The aim of this study was to analyze the anti-anxiety action of Gallic acid (GA) by computational docking analysis studies(CDAS) and also to make a survey for bioactive compounds, like, alkaloids, flavonoids, phenols, terpenoids, tannins and saponins, and to determine the total phenol content in the ripe fruits of *F. jangomas*.

MATERIALS AND METHODS:

Collection and identification of plant materials:

The fruits were collected from certain areas of Thiruvananthapuram in the month of September. After collecting it was authenticated from department of Botany, Christian college, Thiruvananthapuram. Later the samples, thoroughly washed with fresh water and water content is removed by drying. The fruit then cut into small pieces and dried under shade for 10 days. After that it is pulverized into a coarse powder and stored in an airtight container.

Preparation of plant extract:

About 50g of the plant powder mixed with 250ml of ethanol and it is then subjected to extraction by using Soxhlet apparatus for 48h at 50 ^{0}C. The extract then concentrated to dryness under reduced pressure and controlled temperature and stored in air-tight container until further use. The residue was used to perform preliminary qualitative tests for detection of secondary metabolites as well as to conduct quantitative analysis for total phenol contents.

Phytochemical Screening for secondary metabolites

Chemical tests were carried out qualitatively on the extract following standard procedures to identify the phytochemical constituents[9,10]

A. Test for alkaloids

Dragendorff's test:

In a test tube containing 1 ml of extract, few drops of Dragendorff's reagent was added and the colour developed was noticed. Orange colour did not appeared, indicated the absence of alkaloids.

Mayer's test:

To 1 ml of the extract, 2 ml of Mayer's reagent was added, a dull white precipitate did not found, indicated the absence of alkaloids.

B. Test for flavonoids

Alkaline reagent test:

To the test solution, a few drops of sodium hydroxide solution were added. Formation of intense yellow colour which turns to colourless by addition of few drops of dilute acetic acid indicated the presence of flavonoids.

Shinoda test:

To the test solution, a few drops of concentrated HCl and a few pieces of magnesium turning were added. Development of pink or magenta red colour indicated the presence of flavonoids.

C. Test for phenolic compounds

Ferric chloride test:

To the test solution, a few drops of ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

D. Test for tannins

Lead acetate test:

To the test solution, a few drops of 10% lead acetate solution were added. Precipitate formation indicated the presence of tannin.

E. Test for terpenoids

Salkowski's test:

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken well and allowed to stand. Appearance of red colour in the lower layer indicates the presence of steroids. Formation of reddish brown colour of interface after addition of concentrated sulphuric acid to the side carefully (without shaking) indicated the presence of terpenoids.

F. Test for saponins

Foam test:

Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously then some drops of olive oil were added. The formation of stable foam was taken as an indication for the presence of saponins.

Determination of total phenol content

Folin-Ciocalteu method, was used for phenol content determination. One mg/ml of the samples were pipetted out and added 0.5ml Folin-Ciocalteau reagent and 2mL, 20% Na_2CO_3. The tubes were placed in a boiling water bath for exactly one minute. The tubes were cooled and the absorbance was read at 750nm in a

spectrophotometer against a reagent blank. Standard gallic acid solutions (2.5-100µg/ml) were also treated as above [11,12]

DOCKING

The interaction between the ligand and protein was determined by using Auto-dock vina Pyrx virtual screening tool.

AIMS OF MOLECULAR DOCKING

- Docking methodology aims to predict the experimental binding modes and¬ affinities of small molecules within the binding site of particular receptor targets.
- used as a standard computational tool in drug design for lead compound¬ optimisation and in virtual screening studies to find novel biologically active molecules
- basic tools of a docking methodology include a search algorithm and an energy¬ scoring function for generating and evaluating ligand poses.
- They increase the chance of success in many stages of the discovery process.
- They facilitate accessing huge amount of data generated.

• Preparation of Ligand

The 3D structure of the compound was obtained from Pubchem, which contains information about the small molecule and their biological activities.

• Preparation of Protein

Protein Proteins are the macromolecule contains one or more amino acid residues. The 3D structure of the protein was obtained from PDB (Protein data bank).

• Conversion of ligand from SDF to PDB format

Openbabel-2.3.2/obgui.exe was used.

• Protein preparation and molecular visualization

pyMOL is software used for the both purposes. pyMOL can produce high quality 3D images of proteins.

RESULT AND DISCUSSION

The present study carried out on the ethanolic extract of fruits revealed the presence of most of the studied secondary metabolites except alkaloids i.e., flavonoids, phenols, tannins, terpenoids and saponins were found to be present. The result is summarized in Table 1. Polyphenols form a large group of chemicals and most commonly occurred polyphenols in food include flavonoids and phenolic acids. Apart from these, various experiments have been demonstrated that phenolic acids are potential antioxidant and antioxidant activity of these compounds is due to their ability to scavenge free radicals. Accumulation of free radicals can cause pathological conditions such as asthma, arthritis, inflammation, neuro-degeneration, heart disease, aging effect, etc. Phenolic compounds, moreover, act as (i) metal chelators, (ii) anti-mutagens and anticarcinogens, (iii) antimicrobial agents

[13]. Tannins and terpenoids are attributed for analgesic and anti-inflammatory activities. Furthermore, tannins contribute property of astringency i.e., faster the healing of wounds and inflamed mucous membrane . Saponin, likewise, has the potential to lower cholesterol levels in humans due to their hypocholesterolemic effect. Saponins form complexes with cholesterol to reduce cholesterol levels . Total amount of phenol contents were calculated from gallic acid ($y = 0.011x + 0.078$, $R^2 = 0.996$) standard curves (Figure 2). The total phenol content in the extract were found 39.86 µg/mg in terms of gallic acid . The phenol content were well confirmed with qualitative investigations. Although, the total amount of phenol content (39.86 µg/mg) comparatively high which is impressive. Therefore the presence of phenolic compound Gallic acid is higher in *Flacourtia jangomas.*

There are many compounds with poor bioavailability shows less effective against disease. To solve this problem, predicting bioavailability properties will be great advantage for drug development. Hence using computer based methods like docking tools were studied. It has found out that *FJ* contains 39.86 µg/mg of GA after following ethanolic extraction of dried ripe fruits FJ. Increased hydrogen bond interaction and binding affinity express the strong binding of Gallic acid with the selected receptors (2Z64, 4KBY, 5I6X). GA shows greater affinity with receptor 2Z64. This docking study shows that GA exhibited anxiolytic like activity. Fig 5,6,7 shows the docking image of GA with receptor 2Z64, 5I6X,4KBY respectively and table 4 shows the hydrogen bond interaction and binding affinity of Gallic acid with receptors.

Secondary metabolites	**Chemical test**	**Indication: + for presence - for absence**
Alkaloids	**Mayer's test**	-
	Dragendorff's test	-
Flavanoids	**Alkaline test**	+
	Shinoda test	+
Phenols	**Ferric chloride test**	+
Tannins	**Lead acetate test**	+
Terpenoids	**Salkowski's test**	+
Saponins	**Foam test**	+

Table 1: Secondary metabolites constituents in the ethanolic extract of fruits of F. jangomas

Standard- Gallic acid (1mg/ml stock)

Standards	**Concentration of gallic acid (µg/ml)**	**OD at 750 nm**
S1	20	0.334
S2	40	0.512
S3	60	0.751
S4	80	0.980
S5	100	1.242

Table 2

Sl. No.	Sample code	OD at 750 nm	Concentration of phenol in Gallic acid equivalent µg/mg of extract
1	DN	0.533	39.86

Table 3

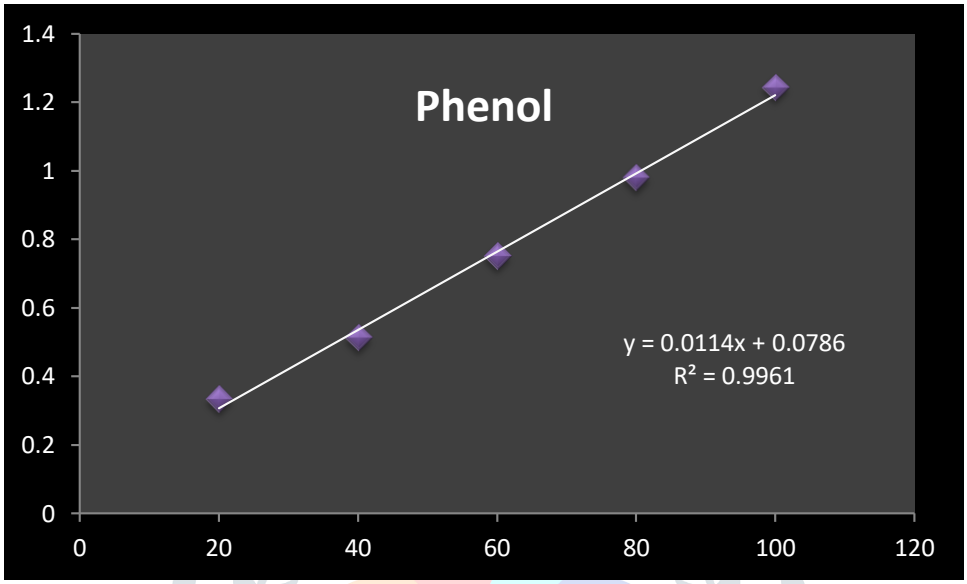

Fig 2: Standard calibration curve of Gallic acid for the determination of total phenol content.

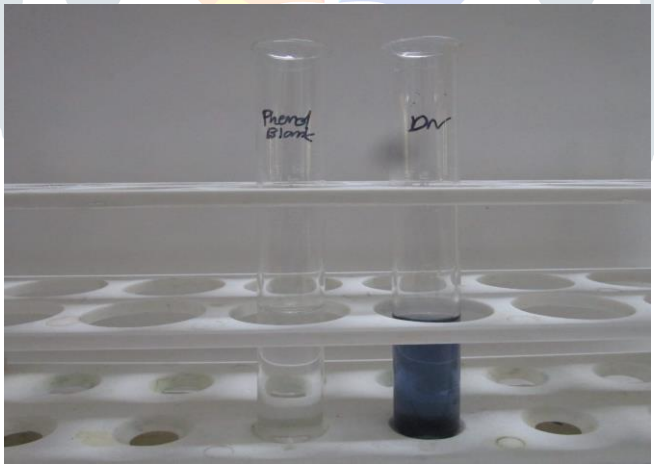

Fig 3

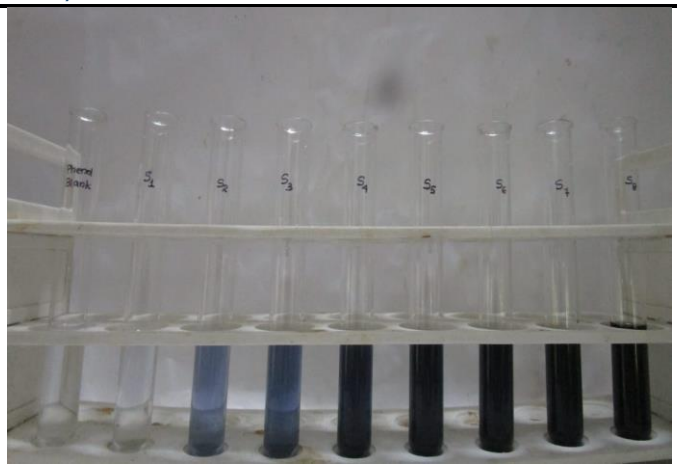

Fig 4

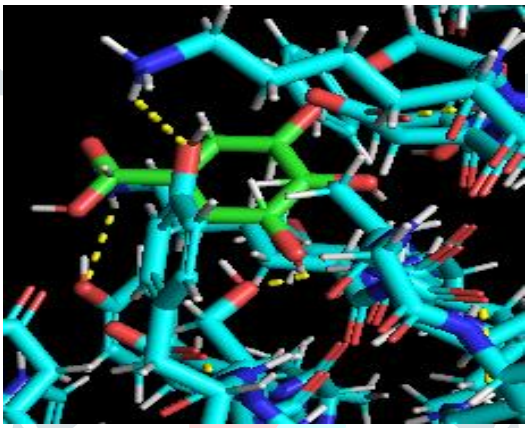

Figure 5:Docking image of Gallic acid with receptor 2Z64

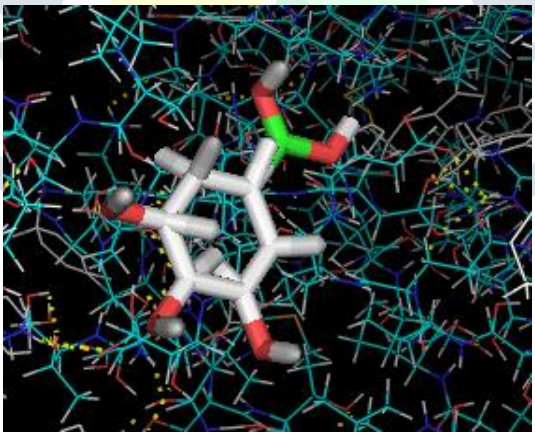

Figure 6:Docking image of Gallic acid with receptor 5I6X

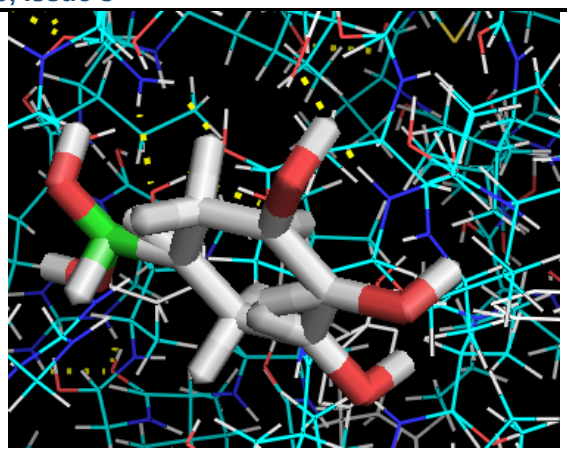

Figure 7:Docking image of Gallic acid with receptor 4KBY

RECEPTOR	NO.OF HYDROGRN BOND INTERACTIONS	BINDING AFFINITY(KCAL/MOL)
2Z64	3	-5.6
5I6X	2	-5.4
4KBY	1	-5.4

Table 4: Hydrogen bond interaction and Binding affinity of Gallic acid with receptors

CONCLUSION

In conclusion, the investigation has revealed that the fruits of *F. jangomas* contains mainly the bioactive phenolic compound such as Gallic acid which are vital for its anti-anxiety activity. Findings of the study have provided evidences that crude extract of the fruits contain medicinally important bioactive compounds and justices the uses of the plant in the indigenous medicine for the treatment of different mental disorders.

Computational tools may be helpful in predicting the binding affinity and also finding geometry of ligand when it binds to the receptor sites. Anxiety is becoming more widespread in today's scenario and their diversity is increasing at high pace thus an effective and efficient treatment is an urgent need of present times. The study shows that Gallic acid is having best binding capacity with the 2Z64 receptor. The binding affinity for Gallic acid with 4KBY and 5I6X receptor is also greater. Thus we can conclude that Gallic acid can be used for the treatment of Anxiety. Further *invivo* and *invitro* studies are required to prove the anti-anxiety activity.

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