International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 3 | JUL-SEPT | 2018 | 438-444
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

PARTIAL PURIFICATION AND CHARACTERIZATION OF A LECTIN FROM THE DIGESTIVE GLAND OF THE STARFISH, *PENTACERASTER MAMMILLATUS*

A.Arokya Jothi Blessy* and M.R. Basil-Rose

Department of Zoology, Holy Cross College (Autonomous), Affiliated to Manonmaniam Sundaranar University, Abishekapatti – 627012, Tirunelveli, Tamilnadu, India.

***Corresponding Author Email: joblessy11anto@gmail.com**

ABSTRACT

An agglutinin/lectin that agglutinated rabbit erythrocytes with high affinity was identified in the extract of the digestive gland of the starfish Pentaceraster mammillatus by hemagglutination assay. The identified digestive gland lectin was isolated and purified by biospecific adsorption using formalinized rabbit erythrocytes. The isolated lectin continued to agglutinate rabbit, human A, B, O, dog, pig, buffalo and cow erythrocytes in the same pattern like the crude extract. The lectin was Ca^{++} dependant. Optimum activity of the lectin was observed between temperature 5 and 40 °C and at pH 8 - 9.5. Agglutinability of the lectin was inhibited by sugars: α - lactose > galactose = D-mannose anhydrosamine > fucose and by glycoproteins: lactoferrin > thyroglobulin > fetuin > PSM > BSM. The lectin appeared as a single band with a molecular mass of 25 kDa on SDS-PAGE.

KEY WORDS

Lectin, Starfish, Agglutination, Erythrocytes, Glycoprotein

INTRODUCTION

Lectins are carbohydrate binding proteins other than immunoglobulins that display no enzymatic activity towards the recognized sugars and are possessing atleast one non-catalytic domain that recognize and bind reversibly to specific carbohydrates inside and outside cell [1, 2]. Echinoderms are primitive deutrostome animals lacking specific immunity. Foreign agents recognition in echinoderms occurs through protein carbohydrate interaction involving lectins, proteins capable of selectively binding carbohydrate components of glycoconjugates [3]. These proteins are neither enzyme nor products of an immune response and are present in many types of organisms from viruses and bacteria to vertebrates [4]. Various lectins have been found in the coelomic fluid and tissue extracts of marine invertebrates [5-7]. Endogenous animal lectins are widely distributed in extra cellular matrix, cell membranes, cytoplasm and nuclei [8, 9]. Recently several lectins have also been isolated from various tissues, such as gonad [10], spermatozoa [11], pedicellariae venom [12], eggs [13] and body wall [14]. Hence an effort is taken in this investigation to search for an agglutinin in the extract of the digestive gland of the starfish *P. mammillatus* and to purify the identified lectin by biospecific adsorption using formalinized erythrocytes.

MATERIALS AND METHODS

Collection and maintenance of animals:

The starfish *P. mammillatus* were collected from the Arabian Sea at Pallam, Kanyakumari District, Tamilnadu, India. They were transported to the laboratory in plastic bucket containing the aerated sea water and fed with small fishes. They were maintained at room temperature (30 ± 2°C).

Preparation of sample:

The starfish *P. mammillatus* was dissected and the digestive glands were removed and thoroughly washed in cold saline (0.7%) to remove the adhering coelomic fluid. After gentle blotting, 100 mg of digestive glands were homogenized in 1 ml of cold TBS (Tris Buffered Saline: Tris - HCl 50 mM, pH 7.5, NaCl 100 mM, $CaCl_2$ 10 mM). The mixtures were filtered and centrifuged at 4000 rpm for 10 min and the supernatant was used for HA assay.

Preparation of erythrocytes:

Mammalian erythrocytes were obtained either by vein puncture of ear (rabbit) or heart puncture (rat, mice and guinea pig) or from the slaughter houses (cow, buffalo, camel, goat and pig) or from Kanya blood Bank, Nagercoil (human A, B and O), Kanyakumari District, Tamilnadu, India. The samples were collected in sterile modified Alsevier's medium and used for HA assay.

Haemagglutination (HA) Assay:

Haemagglutination assay was carried out as described by Ravindranath and Paulson [15]. The digestive gland extract (25 µl) were serially diluted with 25 µl of TBS (pH 7.5) in U shaped microtiter plates and mixed with 25 µl of 1.5% erthrocyte suspension and incubated for one hour at room temperature (30±2°C). The haemagglutination or HA titer (the unit of agglutinin activity) was considered as the reciprocal of the highest dilution of samples that gave positive agglutination. Agglutinated erythrocytes formed a diffuse mat whereas, non-agglutinated erythrocytes rolled to the bottom of the well forming a button

Purification of lectin by affinity adsorption

Preparation of formalinized erythrocyte:

Rabbit erythrocytes obtained in modified Alsevier's solution, were washed 3 times in 20 volume of phosphate-buffered saline pH 9 (75 mM NaCl, 75 mM Na_2HPO_4/KH_2PO_4) by centrifugation at 1000 × g for 5 min. The packed cells were suspended as 8% solution in phosphate-buffered saline (PBS), pH 9 and mixed with an equal volume of formalin (3% solution in PBS with pH adjusted to 9 with 0.1 M NaOH). The mixture was incubated at 37 °C for 16 h with moderate shaking. The cells were then washed 4 times in 5 volumes of PBS, pH 9 per packed cell volume and stored at 4 °C as 10% suspension in this buffer. This could be used for agglutination assay even for months after preparation. It should be emphasized that relatively fresh erythrocytes must be used for formalization procedure since cells stored for more than 2 weeks crenate during the formalinization procedure and cannot be used for agglutination assay.

Purification of agglutinin:

The stored formalinized rabbit erythrocytes were prepared for use as an affinity reagent by washing 6 times in 10 volumes of TBS (50 mM Tris HCL, 100 mM NaCl), pH 9.5. The packed cells were then incubated with 20 volumes of the clarified digestive gland extract in plastic tubes for 2 h with moderate shaking at 4 °C and then washed 3 times with 20 volumes of TBS pH 7.5 containing 10 mM $CaCl_2$. Elution of the absorbed hemagglutinin was accomplished by incubation of the cells with 10 volumes of 10 mM disodium EDTA in TBS. The elution was continued for 5 h. with moderate shaking at 4°C and the elution mixture was centrifuged for 10 min at 28000 × g to remove particulate material and the resultant supernatant was dialyzed and checked for HA assay.

Polyacrylamide gel electrophoresis:

SDS - Polacrylamide (12.5%) gel electrophoresis was performed. Samples were heated for 3 min at 100 °C in sample buffer (25% 1 M Tris-HCl, pH 6.8; 4% SDS; 2% β-mercaptoethanol and 5% glycerol). Gels were fixed and stained with solution containing 0.05% Coomassie blue R-250, 10% acetic acid, and 25% isopropyl alcohol and destained with a solution containing 5% methnol and 7% acetic acid at room temperature (30-35°C).

Characterization of the purified lectin

Hemagglutination (HA) Assay:

Haemagglutination assay was carried out as described by Ravindranath and Paulson [15]. The digestive gland lectin (25 µl) were serially diluted with 25 µl of TBS (pH 9) in U shaped microtiter plates and mixed with 25 µl of 1.5% erthrocyte suspension and incubated for one hour at room temperature (30± 2 °C). The haemagglutination or HA titer (the unit of agglutinin activity) was considered as the reciprocal of the highest dilution of samples that gave positive agglutination.

Effect of pH and thermal stability:

To assess the effect of pH, 25 µl of the digestive gland lectin was serially diluted with equal volume of TBS at different pH (5-10) in a microtiter plate and was incubated at room temperature (30±2 °C) for 1 h, before adding the erythrocyte suspension. To study the effect of temperature on HA activity, 300 µl of digestive gland lectin in several aliquots were placed in a water bath at a temperature range from 5-100 °C and incubated for 1

h. After incubation the HA activity was determined against rabbit erythrocytes.

Effect of cations, EDTA and trisodium citrate:

Digestive gland lectin (25 µl) was serially diluted with equal volume of TBS (pH 9) with different concentration (0-100 mM) of cations and chelators (EDTA and trisodium citrate) and incubated for 1 h. before adding erythrocytes suspension. After incubation, the HA activity was determined against rabbit erythrocytes.

Hemagglutination inhibition assay:

Known concentration of (glycoprotein: 5 mg/ ml sugar 100 mM) of inhibitor (25 µl) was serially diluted with 25 µl of TBS in microtiter plate. Then 25 µl of digestive gland lectin diluted to subagglutination concentration in TBS pH 9 (to give a HA titer of 2) was added and incubated for 1 h. After incubation, 25 µl of 1.5% rabbit erythrocyte suspension was added, mixed and incubated at room temperature (30±2 °C). After 1h, the hemagglutination inhibition titer was recorded as the reciprocal of the highest dilution of inhibitors giving complete inhibition of agglutination.

RESULT

HA assay of the digestive gland extract:

Agglutinins are found in the extract of the digestive gland of starfish, *P. mammillatus*. Maximum HA titer was observed with rabbit erythrocytes. In addition, the agglutinin also agglutinated a variety of mammalian pig = rat > human O > human B = human A > guinea pig = dog = cow = goat > buffalo (Table 1).

Table 1: HA titer of the extract of the digestive gland of the starfish, *P. mammillatus*

Erythrocytes (n=5)	HA titer
Rabbit	256
Pig, rat	128
Human O	64
Human A & B	32
Guniea pig, dog, cow, goat	8
Buffalo	4

HA assay of the lectin:

The purified lectin from the extract of the digestive gland of the starfish, *P. mammillatus* agglutinated rabbit, human A, B, O, dog, pig, buffalo and cow erythrocytes (Table 2).

Table 2: HA titer of the digestive gland lectin of the starfish, of the *P. mammillatus.*

Erythrocytes (n=10)	HA titer
Rabbit	512
Human A	64
Human B	32
Human O	16
Dog	6
Pig	4
Buffalo	4
Cow	2

pH and thermal stability of the lectin:

HA activity of the purified lectin, increased with increase in pH from 5.5 to 8 and remained unaltered between pH 8-9.5. The HA titer remained high and unaltered between temperature 5 – 40 °C. A decrease in HA titer was observed on further increase in pH and temperature (Fig. 1 and 2).

Effect of cations and ETDA on the HA titer of the lectin:

Maximum HA titer was observed with the addition of 10 mM Ca^{++} ion. Addition of Mg^{++} had no effect on HA activity. The HA titer of the purified lectin got reduced with the addition of increasing concentration of EDTA and trisodium citrate (Table 3 and 4).

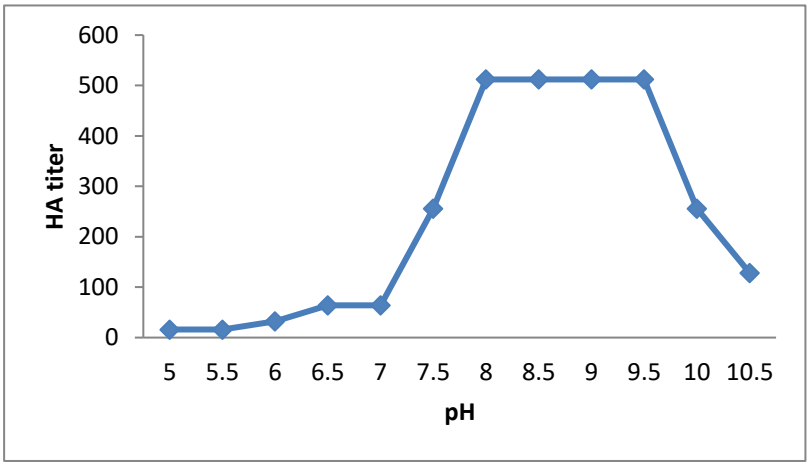

Figure 1: Effect of pH on the HA titer of the digestive gland lectin of the starfish *P. mammillatus*.

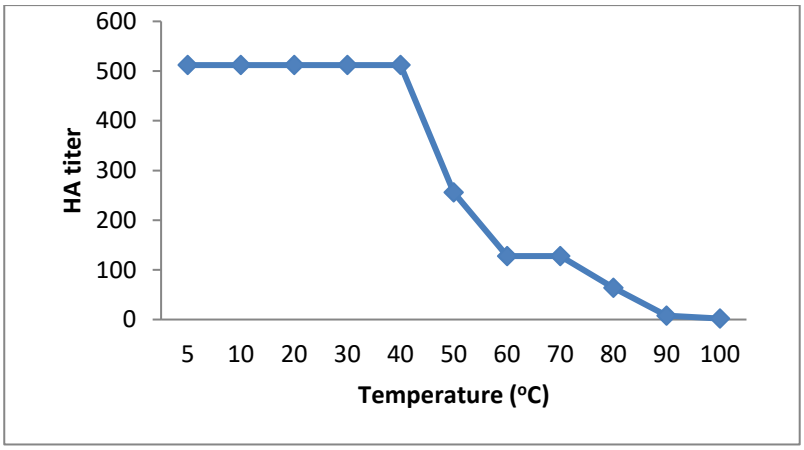

Figure 2: Effect of temperature on the HA titer of the digestive gland lectin ofthe starfish, *P.mammillatus*

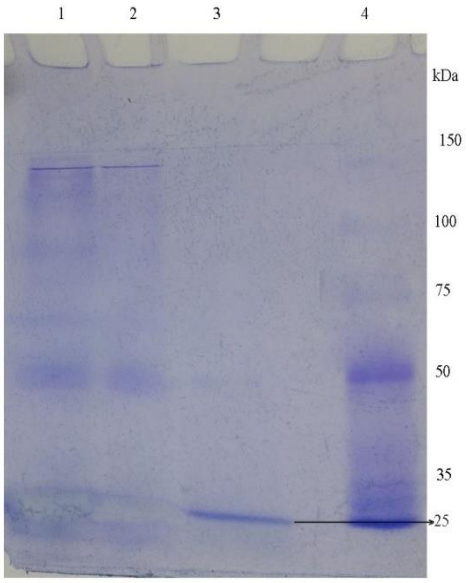

Figure 3: SDS-PAGE of the digestive gland lectin of the starfish, *P. mammillatus*. lane 1- crude, lane 2- clarified, lane 3- purified and lane 4- marker.

Table3: Effect of EDTA on the HA titer of the digestive gland lectin of the starfish *P. mammillatus*

Concentration (mM) (n=10)	HA titer Di sodium EDTA	Tri sodium citrate	Tetra sodium EDTA
0	512	128	128
0.01	512	512	512
0.1	512	512	512
1	512	256	512
5	256	128	256
10	64	128	16
20	4	128	4
30	4	64	4
40	2	64	4
50	2	64	4
100	2	32	2

Table 4: Effect of cations on the HA titer of the digestive gland lectin of the starfish, *P. mammillatus*

Concentration (mM) (n=10)	HA titer	
	$Ca^{+=}$	Mg^{++}
0	128	128
0.01	32	32
0.1	64	32
1	64	32
5	64	32
10	512	32
20	256	32
30	256	32
40	64	32
50	4	32
100	4	32

HAI assay of the lectin:

HAI assay of the purified lectin of the extract of the digestive gland of the starfish *P. mammillatus* with rabbit erythrocytes was inhibited by glycoprotein: lactoferrin > thyroglobulin > fetuin> PSM > BSM and sugars: α-lactose > galactose > D- mannose anhydrosamine > fucose (Table 5).

Table 5: HAI titer of the digestive gland lectin of the starfish *Pentaceraster mammillatus*

Sugars (N=5)	HAI titer
α-lactose	2048
Galactose	1024
D-mannose anhydrosamine	1024
D-fucose	16
Glycoproteins	HAI titer
Lactoferrin	2048
Thyroglobulin	512
Fetuin	32
PSM	16
BSM	4

Molecular weight determination: The molecular weight of the purified lectin was estimated to be 25 kDa on SDS PAGE (Fig. 3).

DISCUSSION

A lectin capable of agglutinating rabbit erythrocytes with great avidity was purified from the extract of the digestive gland of the starfish, *P. mammillatus* by biospecific adsorption using formalinized rabbit erythrocyte. The purified lectin gave a single homogenous band with molecular weight 25 kDa on SDS- PAGE. Presence of single band with molecular weight 200 kDa was reported in the seminal plasma of the sea urchin *Paracentrotus lividus* [16], 355 kDa in the internal organs of the sea cucumber, *Holothuria atra* [14]. The purified lectin agglutinated rabbit erythrocyte with great potency than human A, B, O, dog, pig, buffalo and cow erythrocytes. The purified lectin seems to have tremendous affinity for the sugar residue of the rabbit erythrocyte than the sugar receptors of the surface of the human A, B, O, buffalo, cow and dog erythrocytes. This may be due to the quantitative difference in the distribution of sugar residues on the erythrocytes membranes.

The lectin activity remained high and stable from 5 – 40 ^{0}C and pH 8 - 9.5 when tested with rabbit erythrocyte. The decrease in HA titer at temperature above 40ºC could be due to the denaturation of lectin at higher temperature. The results on the effect of pH reveal that the lectin is active above the neutral pH. The same result was also reported in the holothuria species [17]. Hemagglitination of the purified lectin was inhibited by glycoproteins lactoferrin, thyroglobulin, fetuin, PSM and BSM. The carbohydrate recognition domain of the lectin can bind to α-lactose, galactose and fucose that in turn, are able to inhibit hemagglutination. The hemagglutinin is generally inhibited by mono and disaccaride, which presumably are part of or closely related to saccaride receptor sites on the erythrocyte surface [18]. Magnesium had no effect on the HA activity, even at concentrations as high as 10 mM. Same result was also reported Paracentrotus *lividus* [16], *Hemicentrotus pulcherrimus* [19]. The HA activity of the purified lectin was enhanced by calcium supplementation and not by magnesium revealing its specific affinity to calcium. Addition of increasing concentration of calcium show an increase in HA titer upto 10 mM. Further increase in the Ca^{++} concentration resulted in a decrease in agglutinating activity. The C-type lectin require Ca^{++} to express their activity (Drickamer, 1988). Most of the marine lectins are from C-type lectin family [20-22]. C-type lectins were also reported in *Asterina pectinifera* [23], *Stichopus japonicas* [24], *Cucumaria echinata* [25]. C- type lectin domains have a diverse range of functions including cell-cell adhesion, immune response to pathogens and apoptosis [26, 27]. So, the result, suggests that a C-type lectin occur in the extract of the digestive gland of the starfish, *P. mammillatus*.

CONCLUSION

A lectin was isolated from the extract of the digestive gland of the starfish, *P. mammillatus* by biospecific adsorption using formalinized rabbit erythrocyte. The isolated lectin agglutinated rabbit erythrocyte with great avidity. Addition of calcium ion increase the HA activity of the lectin. Optimum HA activity of the lectin was achieved between 10 – 40 ^{o}C and pH 8 - 9.5. SDS-PAGE revealed the presence of a single lectin with molecular mass of 25 kDa.

REFERENCES

1. Drickamer K. Two distinct classes of carbohydrate recognition domains in animal lectins. Journal of Biological Chemistry, 263; 9557-9560, (1988).
2. Kilpatrick DC. Animal lectins: a historical introduction and overview. Biochim Biophys Acta, 157; 2187-2197, (2002).
3. Bernondes S.H. Bio functional properties of lectins: lectin redefined. Trends in Biochemical Science, 13(12); 480- 482 (1988).
4. Sharon N., Lis H. History of lectin: from hemagglutinin to biological recognition molecule. Glycobiology, 14; 53-62 (2004).
5. Brown R., Almodovan L.R., Bhtia H.M., Boyd WC. Blood group specific agglutinins in invertebrates. Journal of Immunology, 100; 214-216 (1968).
6. Hatakeyama T., Kohzaki H., Nagatomo H., Yamasaki N. Purification and Characterization of Four Ca2+-Dependent Lectins from the Marine Invertebrate, *Cucumaria echinata*. Journal of Biochemistry, 116; 209-214 (1994).
7. Matsui T., Ozeki Y., Suzuki M., Hino., Titani K. Purification and characterization of two Ca^{++} dependant lectins from coelomic plasma of sea cucumber, *Stichopus japonicas.* Journal of Biochemistry, 116; 1127-2233 (1994).
8. Barondes S.H. Soluble lectin: a new class of extracellular protein. Science, 223; 1259-1264 (1984).
9. Hubert J., Séve A.P., Facy P., Monsigny M. A nuclear lectins and nuclear glycoproteins involved in the modulation of nuclear functions. Cell Differentiation and Developement. 27; 69–81 (1989).
10. Kamiya H., Muramoto K., Goto R., Sakai M. Lectins in the hemolymph of a starfish, *Asterina pectinifera*:

purification and characterization. Developmental and Comparative Immunology, 16; 243-250 (1992).
11. Vacquier V.V., Moy G.W. Isolation of bindin: the protein responsible for adhesion of sperm to sea urchin eggs. Proceeding of the National Academy of Science of the United States of America, 174; 2456-2460 (1977).
12. Edo K., Sakai H., Nakagawa H., Hashimoto T., Shinohara M., Ohura K. Immunomodulatory activity of a pedicellarial venom lectin from the toxopneustid sea urchin, *Toxopneustas pileolus*. Toxins Review, 31; 54-60 (2012).
13. Sasaki H., Aketa K. Purification and distripution of a lectin in a sea urchin (*Anthocidaris crassipina*) egg before and after fertilization. Experimental cell Research, 135(10); 15-9 (1981).
14. Mojica E.R.E., Merca F.E. Lectin from the body walls of black sea cucumber (*Holothuria atra* Jäger). Philippine Journal of Science, 133; 77–85 (2004).
15. Ravindranath M.H., Paulson J.C. O-acetyl sialic acid specific lectin from the crab *Cancer antennarius*. Methods in Enzymology, 138; 520–527 (1987).
16. Stabili L., Pagliara P., Canicatti C. Characterization and isolation of an agglutinin from seminal plasma of the sea urchin. Canadian Journal of Zoology, 71(10); 2042-2046 (1993).
17. Gana, A.E., Merca F.E. Isolation and purification of a cytotoxic lectin from brown sea cucumber by affinity chromatography. Philippine Scientist, 85; 236-247 (2004).
18. Giga Y., Ikai A., Takahashki K. The complete amino- acid sequence of echinoidin, a lectin from the coelomic fluid of the sea urchin *Anthocidaris crassispina.* Journal of Biological Chemistry, 262; 197 – 6203 (1987).
19. Watanabe H., Seike Y., Miyata T., Suyemitsu T. N- Acetyl-D-galactosamine-specific hemagglutinin purified from seminal plasma of the sea urchin, Hemicentrotus pulcherrimus. Zoological Science, 8; 693 -2 25 (1991).
20. Moura R.M., Queiroz A.F.S., Monteiro N.K.V., Moura G.E.D.D., Macedo L.L.P., Santos E.A., Sales M.P. CvL. a lectin from the marine sponge *Cliona vaeians*: Isolation, characterization and its effects on pathogenic bacteria and *Leishmania promastigotas.* Camparative Biochemistry and Physiology Part A. 145; 517-523 (2006).
21. Suzuki T., Takagi T., Furukohri T., Kawamura K., Nakauchi M. A calcium-dependent galactos-binding lectin from the tunicate *Polyandrocarpa misakiensis.* Journal of Biological chemistry, 265(3); 1274-1281 (1990).
22. Bulgakov A.A., Park K., Choi K., Lim H., Cho M. Purification and characterization of a lectin isolated from the Manila clam *Ruditapes philippinarum*in Korea. Fish and Shellfish Immunology, 16; 487-499 (2006).
23. Kakiuchi M., Okino N., Sueyoshi N. Purification and characterization, and cDNA cloning of α-N-acethylgalactosamine specific lectin from sterfish *Patiria pectinifera*. Journal of Glocobiology, 12(2); 85-94 (2008).
24. Hatakeyama T., Himeshima T., Komatsu A., Yamasaki N. Purification and characterization of two lectins from the sea cucumber *Stichopus japonicas.* Journal of Bioscience Biotechnology and Biochemistry, 1736-1739 (1993).
25. Hatakeyama T., Kohzaki H., Nagatomo H., Yamasaki N. Purification and chracterization of four Ca^{++} - dependent lectins from the marine invertebrates *Cucumaria echinata*. Journal of Biochemistry, 116; 209-214 (1994).
26. Drickamer K. C-type lectin-like domains. Current Opinion Structural Biology, (5): 585–90 (1999).
27. Cambi A, Figdor C. Necrosis: C-type lectins sense cell death. Current Biology, 2009; 19 (9), R375–8 (2009).

Received:04.05.18, Accepted: 07.06.18, Published:01.07.2018

***Corresponding Author:**
A.Arokya Jothi Blessy*
Email: joblessy11anto@gmail.com