



**Effect Of Combination Administration Of Leaf Extract Star Fruit (*Averrhoa Bilimbi* L.) and Ginger Rhizome Red (*Zingiber Officinale* var. *Rubrum*) Until Against Creatinine In Mice (*Mus Musculus*) CCl<sub>4</sub> Induced and Its Utilization As A Learning Medium**

**Ratna Dwi Rahayu<sup>1\*</sup>, Achmad Ramadhan<sup>1</sup>, Sutrisnawati<sup>1</sup>**

<sup>1</sup>Biology Education Study Program, Faculty of Education and Teacher Training, Universitas Tadulako, Palu, Indonesia

\*Corresponding Author e-mail: [ratnadwirahayu01@gmail.com](mailto:ratnadwirahayu01@gmail.com)

**Article Info**

**Article history:**

Received 29 December, 2025

Revised 25 January, 2026

Accepted 04 May 2026

**Keywords:**

Star Fruit Leaves

Red Ginger Rhizomes

Creatinine

CCl<sub>4</sub>

Learning Media

**ABSTRACT**

The combination of starfruit (*Averrhoa carambola*) and red ginger (*Zingiber officinale* var. *rubrum*) is important to investigate because both plants contain bioactive compounds with antioxidant potential. Starfruit contains vitamin C and phenolic compounds, while red ginger contains gingerol and other antioxidant compounds that may support physiological functions and help prevent disease. Therefore, this combination has potential as a natural antioxidant source. This study aims to understand the combined effects of starfruit leaf extract and red ginger rhizome on creatinine levels in mice that have been given CCl<sub>4</sub> treatment. The method used is a laboratory experiment with a Completely Randomized Design (CRD) design consisting of 24 mice divided into 6 groups and 4 experimental replications. The normal control group (Kn) is a group that is only given food and water, the negative control group (K-) is given CCl<sub>4</sub> induction, groups P1, P2, P3 and P4 are given CCl<sub>4</sub> induction and a combination of starfruit leaf extract and red ginger rhizome with concentrations of 20%, 40%, 60% and 80%. Data were analyzed by ANOVA statistical test via SPSS version-26. The results of the study showed that the combination of starfruit leaf extract and red ginger rhizome significantly reduced creatinine levels in mice induced by CCl<sub>4</sub>, with an effective concentration of 80%. The learning media applied in this study, a flipbook e-module, met the very good category as a learning medium.

This is an open access article under the [CC BY-SA](https://creativecommons.org/licenses/by-sa/4.0/) license



**Corresponding Author:**

Ratna Dwi Rahayu

Biology Education Study Program, Faculty of Education and Teacher Training, Universitas Tadulako, Palu, Indonesia

Email: [ratnadwirahayu01@gmail.com](mailto:ratnadwirahayu01@gmail.com)

**INTRODUCTION**

Herbal plants have long been used as an alternative natural treatment in maintaining and restoring the health of the body. The use of medicinal plants is not only based on local wisdom, but also in line with various scientific studies that prove the composition of the active compounds in them. One of the main benefits of herbal plants is their potential to help maintain the function of body organs, including the kidneys. Some of the plants that are widely used by the community in their daily lives are star fruit leaves



and red ginger, which have been used for generations as an effort to prevent and treat various diseases (Putra et al., 2022).

In Indonesia, star fruit is a plant that is commonly found and cultivated in the environment around the house. Traditionally, star fruit leaves are used to treat various health disorders such as indigestion, joint pain, swelling of the parotid glands (mumps), and antipyretics. In addition, star fruit is also used to treat whooping cough, acne, hypertension, canker sores, and digestive system disorders (Aseptianova & Yuliany, 2020). The content of active compounds in star fruit leaves such as alkaloids, flavonoids, phenols, terpenoids, tannins, and saponins that act as natural antioxidants and potentially as therapeutic agents (Novika et al., 2021).

Red ginger is also an herbal plant that is widely used for traditional medicine, especially to reduce inflammation and help the health of organs in the body. The content of active compounds such as gingerol, shogaol, zingerone, phenolics, and flavonoids plays a role in anti-inflammatory and antioxidant activities that can support organ function including the kidneys (Duppa et al., 2020). Various recent studies prove that red ginger ethanol extract has the potential to be nephroprotective, which is able to indicate a significant decrease in serum creatinine levels in experimental animals, especially at certain optimal doses, as well as show repair of damaged kidney tissue due to toxic induction (Anselia et al., 2020). In addition, other experimental studies have also reported that ginger extract shows therapeutic potential in reducing toxin-induced kidney damage in animal models (Ali et al., 2024).

Several other studies have proven the effectiveness of both plants. Andriani et al. (2019) revealed the highest antioxidant in star fruit leaf extract with a radical inhibition percentage of 89.66%. Meanwhile, Misfadhila et al. (2020) also revealed that star fruit leaf extract is able to affect calcium solubility in human kidney stones. The combination of bioactive content from star fruit and red ginger has the potential to provide a synergistic effect in maintaining organ function, especially the kidneys.

The kidneys as the main organ have an important role in stabilizing fluids, electrolytes, and the body's detoxification process. Creatinine levels in the blood are the main benchmark in assessing kidney function. Increased creatinine levels indicate the presence of kidney filtration dysfunction. Thus, controlling creatinine levels is the main indicator in efforts to maintain kidney health, especially in conditions of exposure to toxic substances such as carbon tetrachloride (CCl<sub>4</sub>) which is known to cause damage to kidney tissue.

Based on this description, it is necessary to carry out testing efforts to see the effect of giving a combination of star fruit leaf extract (*Averrhoa bilimbi* L.) and red ginger rhizome (*Zingiber officinale* var. *rubrum*) on creatinine levels in CCl<sub>4</sub>-induced mice (*Mus musculus*). In addition to contributing to the field of health and the development of herbal therapy, this research also has educational value as a learning medium. The use of research results in the form of experiment-based learning media, such as flipbooks, is expected to increase students' understanding of the concept of kidney physiology, scientific methods, and the application of biology in real life, especially in excretory system materials.

## METHOD

The experiment was carried out at the Biology Education Laboratory of Tadulako University, Palu, from March to May 2024. The study was conducted by designing a quantitative approach to post-treatment experiments on 24 mice divided into six treatment groups and consisting of four replicas of each group.

Before treatment, all mice were acclimatized in the laboratory for seven days. In the acclimatization phase, the test animals are fed and drunk ad libitum. Post-adaptation, mice were divided into a control group and a treatment group. The control group was given only feed and drink without treatment, while the treatment group was induced carbon tetrachloride (CCl<sub>4</sub>) for seven days to induce impaired kidney function.

The test materials include star fruit leaf extract and red ginger rhizomes prepared with 96% ethanol maceration. A total of 800 grams of simplicia were each soaked in 3.75 liters of ethanol for three days, then filtered and evaporated with a rotary evaporator at a



temperature of 50°C to obtain a concentrated extract. wuluh starfruit leaf extract and red ginger rhizome are then combined in a 1:1 ratio and made in four concentrations, namely 20%, 40%, 60%, and 80%.

The administration of test materials is carried out orally (gavage). The treatment groups consisted of four groups receiving the combination of wuluh starfruit leaf and red ginger rhizome extracts at concentrations of 20%, 40%, 60%, and 80%. In addition, the study included a normal control group and a negative control group; however, no positive control group was used. The treatment was given for seven days after CCl<sub>4</sub> induction. On the 15th day, the whole mice were sacrificed for blood sampling through the heart vein. The obtained blood is put into a vacutainer tube and centrifuged at 3000 rpm within 15 minutes until serum is obtained. Serum creatinine levels were then analyzed as an indicator of kidney function.

Quantitative data from the measurement of blood creatinine levels in mice were statistically analyzed using the ANOVA test assisted by the SPSS program version 26. If the results of the analysis indicate a significant difference, a follow-up test is carried out with the Smallest Real Difference (BNT) test at a significance value of 0.05 to determine the difference between treatments.

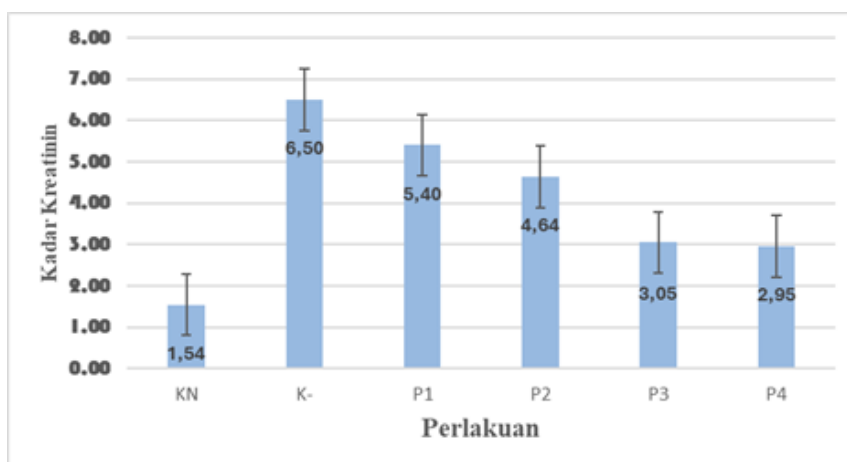
In addition, this research also produces learning media in the form of flipbooks which are used as a supporting media for biology learning. The media feasibility assessment is carried out by calculating the percentage score based on the calculation of the eligibility percentage. The results of the assessment are categorized based on the media eligibility criteria as presented in Table 1:

**Table 1.** Learning outcome

No	Score	Category
1	<21%	Very Unworthy
2	21% - 40%	Not Eligible
3	41% - 60%	Quite Decent
4	61% - 80%	Worthy
5	81% - 100%	Highly Worth it

## RESULTS AND DISCUSSION

Based on the data on the results of the blood creatinine levels of mice presented in Figure 1, there is a difference in creatinine levels for each treatment group. The normal control group (KN) which was carried out ad libitum produced a creatinine level of 0.386 mg/dL, this value still meets the normal criteria of mouse creatinine levels, which is 0.2–0.9 mg/dL where this result is in accordance with the range of the report of (Arifin et al., 2018).



**Figure 1.** Creatinine Level Test Results



In the negative control group (K-) induced by carbon tetrachloride (CCl<sub>4</sub>), there was an increase in creatinine levels of up to 1,625 mg/dL indicating impaired renal function. This condition is strengthened by the results of Ramadhan et al. (2021) who reported that induction of CCl<sub>4</sub> causes a significant increase in serum creatinine levels as an indicator of kidney damage due to oxidative stress. Furthermore, recent research by Noroozi et al. (2025) reinforces these findings by showing that exposure to CCl<sub>4</sub> triggers the formation of free radicals in the form of trichloromethyl (CCl<sub>3</sub>) and trichloromethyl peroxide (CCl<sub>3</sub>O<sub>2</sub>), which cause lipid peroxidation, cell membrane dysfunction, and damage to kidney structures. This process has a direct impact on the reduction of the kidney's filtration ability and increased creatinine levels in the blood, thus reflecting the occurrence of nephrotoxicity due to CCl<sub>4</sub> exposure.

Gliselda (2021) explained that an increase in plasma creatinine levels by up to two times indicates a decrease in glomerular filtration rate (LFG) by 50%, which indicates a significant decrease in kidney function. Therefore, increased creatinine levels in the negative control group indicated impaired renal function due to CCl<sub>4</sub> induction.

In the treatment group with a combination of star fruit leaf extract and red ginger rhizome (P1–P4), there was a gradual decrease in creatinine levels as the concentration of the extract increased. Creatinine levels in the P1 group (20%) were 1,350 mg/dL, P2 (40%) was 1,160 mg/dL, P3 (60%) was 0.762 mg/dL, and P4 (80%) was 0.739 mg/dL. These results revealed that the administration of a combination of extracts was able to reduce creatinine levels, especially at concentrations of 60% and 80% which are close to normal values.

The decrease in creatinine levels in the P3 and P4 groups showed that the combination of star fruit leaf extract and red ginger rhizome had a higher effectiveness at this concentration. This is suspected because at high concentrations, the content of active compounds in the extract is more optimal in providing a protective effect on the kidneys. Bioactive compounds such as flavonoids, saponins, tannins, and polyphenols act as antioxidants that have the potential to neutralize free radicals while inhibiting lipid peroxidation processes.

This result is in line with the dose-response principle of plant extracts, where at low concentrations the biological effects tend to be not optimal because the number of active compounds interacting with the target is still limited, while increasing concentrations increases the availability of bioactive compounds so that the effect is more pronounced. In the DPPH test, this can be seen from the use of the extract concentration series to form the % inhibition curve and calculate the IC<sub>50</sub>. Juniyanti et al. (2024) showed that the determination of antioxidant activity was carried out based on the absorbance of extracts at several concentrations and analyzed as a relationship of % scavenging (% inhibition) to concentration to obtain IC<sub>50</sub>. These findings are consistent with the report of Mutmainnah et al. (2024) The higher the concentration of the extract, the greater the percentage of inhibition produced (decreased absorbance), which indicates an increase in free radicals that have been successfully inhibited.

Star fruit leaves have been shown to contain major secondary metabolites such as flavonoids, tannins, saponins, and alkaloids (Serli et al., 2024). These findings are relevant to the quantitative evidence of the ethanol extraction content of star fruit leaves, namely total phenolics and total flavonoids and shows very strong antioxidant activity in the DPPH test (IC<sub>50</sub> around 21.53 ppm), so it makes sense that these compounds play a role in suppressing oxidative stress (Ningsih et al., 2024). On the other hand, red ginger is also rich in antioxidant bioactive compounds; The oleoresin fraction of red ginger is reported to contain gingerol and shogaol, and the presence of metabolites such as flavonoids/phenols supports red ginger's ability to neutralize free radicals and help repair tissue damage (Sembiring et al., 2024).

The antioxidant activity of these two plants works through proton donation and electron transfer mechanisms that neutralize free radicals, including DPPH radicals. Antioxidant compounds are also able to increase cell defenses against oxidative stress and improve kidney cell function. This is emphasized by Andriani et al. (2019) who reported that



the extraction of star fruit leaves was active as the highest antioxidant using an inhibition percentage of 89.66%.

**Table 2.** ANOVA Test Results

Source of Variation	Number of Squares (SS)	df	Average Squared (MS)	Calculation	Sig.
Intergroup	4,168	5	0,833	8,771	0,000
In a Group	1,710	18	0,095		
Total	5,877	23			

Based on the results of statistical analysis using the ANOVA test (Table 2), the acquisition of the  $F_{cal} > F_{table}$  value was 5% significant, which showed that the extraction of star fruit leaves and red ginger rhizomes had a significant effect on the blood creatinine levels of mice. This result was strengthened by the results of the BNT follow-up test which indicated a real difference between treatment groups.

**Table 3.** Smallest Real Difference Test (BNT) of Mouse Creatinine Levels

Treatment	Average Treatment	Average	BNT 5%
Kn	0,386		0,458
K-	1,625	1,239*	
P1 (20%)	1,350	0,964* 0,275 <sup>tn</sup>	
P2 (40%)	1,160	0,774* 0,465* 0,190 <sup>tn</sup>	
P3 (60%)	0,762	0,376 <sup>tn</sup> 0,863* 0,588* 0,398 <sup>tn</sup>	
P4 (80%)	0,739	0,353 <sup>tn</sup> 0,886* 0,611* 0,421 <sup>tn</sup> 0,023 <sup>tn</sup>	

In the BNT test (Table 3), the P3 (60%) and P4 (80%) groups showed significant differences compared to the negative control group, while the differences observed in P1 (20%) and P2 (40%) were not statistically significant. This condition shows that the optimal concentration of the combination of extracts in lowering creatinine levels is at 60% and 80% concentrations.

Thus, the conclusion of the study showed that the combination of star fruit leaf extract (*Averrhoa bilimbi* L.) and red ginger rhizome (*Zingiber officinale* var. *rubrum*) contained potential nephroprotective agents and was able to reduce blood creatinine levels in mice after induction of  $CCl_4$ , especially at concentrations of 60% and 80%.

**Table 4.** Learning Media Qualification

No	Rating	Assessment Score	Percentage %
1	Content Expert	4,2	84
2	Media members	4,6	92,7
3	Design expert	4,1	82
4	The 20th Student Trials	4,5	89
	Quantity	17,4	347,7
	Average	4,35	86,92

Based on the feasibility test of flipbook e-module learning media (Table 4), the use of this media for the learning process is categorized as very feasible. The feasibility evaluation stage was carried out by three expert validators and the trial was limited to 20 students.

The eligibility percentage of the three experts was 84%, 92.7%, and 82%, while the results of the test for students showed a percentage of 89%. Based on the eligibility criteria for learning media proposed by Arikunto (2009), the percentage range of 81–100% is classified as very feasible. Thus, the flipbook e-module is stated to be very feasible to implement in the learning process.



The high value of media feasibility shows that the flipbook e-module is able to present material in a structured, attractive, and intuitive manner. This module not only presents text, but also contains visual elements and interactive navigation that have the potential to increase interest and mastery of excretory material. These findings refer to the opinion of Kamelia et al. (2022) who stated that flipbook e-modules can increase student engagement because they present flexible visual, animation, and navigation content.

In addition, the advantage of flipbook e-modules lies in their ability to be accessed flexibly through digital devices, thus supporting independent learning and technology-based learning. This media also allows the integration of research-based materials, such as the results of experiments on the effect of the combination of star fruit leaf extract and red ginger rhizome on creatinine levels, which supports the understanding of biological concepts contextually and applicatively.

The results of this study prove that the existence of flipbook e-module media is not only feasible in appearance and content, but also relevant media to support biology learning, especially in excretory system material. Arikunto (2009) supports the result that if a score is above 81%, it can be said to be very feasible. Thus, the learning products developed in this study can be applied as an innovative alternative as an effort to master biology material for students.

That way, the flipbook e-module developed in this study is classified as very feasible to use with a percentage of 86.92%.

## CONCLUSION

The findings of the study indicated that the combination of star fruit leaf extract and red ginger rhizome had an effect on the creatinine level of CCl<sub>4</sub>-induced mice and the most effective combination of star fruit leaf extract and red ginger rhizome was 80% (P4) with an average creatinine value of 0.739 mg/dl and flipbook was very feasible to be used as a learning medium.

## ACKNOWLEDGEMENTS

Thank you to the Biology Education Study Program of Tadulako University, supervisors and all parties for all contributions during the research process until the compilation of this article.

## REFERENCES

- Ali L. S., Mohamed G., Akeel M., ElBayoumi K. S., El-Beltagy A. E. B. M. & El-Sayeed H. M. (2024). *Zingiber officinale* ethanolic extract improved organs function in lipopolysaccharides-induced organ toxicity by modulating inflammation and oxidative stress in male rats. *Egyptian Journal Of Basic And Applied Sciences*, 11(1), 213-231.
- Andriani, M., Permana I. D. G. M. & Widarta I. W. R. (2019). Pengaruh suhu dan waktu ekstraksi daun belimbing wuluh (*Averrhoa bilimbi* L.) terhadap aktivitas antioksidan dengan metode ultrasonic assisted extraction (UAE). *Jurnal Ilmu dan Teknologi Pangan*, 8(3), 330-340.
- Anselia B., Lubis A. A. & Sembiring N. B. (2025). Efek Nefroprotektif Ekstrak Etanol Jahe Merah (*Zingiber officinale* var. *rubrum*) Terhadap Gambaran Histopatologi Ginjal Tikus (*Rattus norvegicus*) Yang Diinduksi oleh Etilen Glikol. *Journal of Pharmaceutical and Sciences*, 8(3), 1612-1624.
- Arifin, H., Tesa, I. A., Octy, A., & Dian, A. J. (2018). Kajian efek analgetik dan toksisitas subakut dari ekstrak etanol daun kitolod (*Isotoma longiflora* L.) pada mencit putih jantan. *Jurnal Sains Farmasi & Klinis*, 5(2), 112-118.
- Arikunto, S. (2009). *Prosedur penelitian: Suatu pendekatan praktik*. Rineka Cipta.
- Aseptianova, A., & Yuliany, E. H. (2020). Penyuluhan manfaat belimbing wuluh (*Averrhoa bilimbi* Linn.) sebagai tanaman kesehatan di Kelurahan Kebun Bunga, Kecamatan Sukarami, Palembang. *Jurnal Ilmiah Pengabdian pada Masyarakat*, 2(2), 52.



- Duppa, T., Yulia, D. Y., & Mufidah, M. (2020). Uji aktivitas ekstrak etanol jahe merah (*Zingiber officinale* Rosc. var. *rubrum*) dalam memproteksi dan memperbaiki gangguan fungsi hati dan ginjal tikus akibat induksi parasetamol. *Majalah Farmasi dan Farmakologi*, 24(2), 33–36.
- Gliselda V. K. (2021). Diagnosis dan manajemen penyakit ginjal kronis (PGK). *Jurnal Medika Hutama*, 2(4), 1135–1140.
- Hermawan, S. D., Yul, M., & Tahono. (2009). Efek ekstrak buah mengkudu (*Morinda citrifolia*) terhadap kadar enzim SGOT dan SGPT pada mencit dengan induksi karbon tetraklorida. *Biofarmasi*, 7(2), 89.
- Herni, S., Puspita, R. A., Adinugraha, A., Faticha, H., & Sukma, A. N. (2024). Uji aktivitas fagositosis dan antioksidan ekstrak rimpang jahe merah (*Zingiber officinale* var. *rubrum*). *Jurnal Ilmu Kesehatan*, 7(2), 120–129.
- Juniyanti F. Y., Oktapiani H. & Ridwan A. (2024). Antidiabetic activity of *Averrhoa bilimbi* fruit methanol extract through hancement of GLUT4 protein expression in diabetes-induced mice. *Indonesian Journal Of Biotechnology*. 29(4) : 177-189.
- Kamelia N., Yeni L. F. & Candramila W. (2022). Pengembangan flash flipbook sebagai media pembelajaran pada submateri peranan jamur di kelas X SMA. *Jurnal Ilmiah Biologi*, 10(1), 252–265.
- Misfadhila, S., Chandra, B., & Wahyuni, Y. (2020). Pengaruh fraksi air, etil asetat, dan n-heksan dari ekstrak etanol daun belimbing wuluh (*Averrhoa bilimbi* L.) terhadap kelarutan kalsium batu ginjal secara in vitro. *Jurnal Farmasi Higea*, 12(2).
- Mutmainnah P. A., Ariansyah, Ruslan, Agustina S. & Annafi N. (2024). Uji Aktivitas Antioksidan Ekstrak Etanol Rumput Laut *Sargasum* Sp. Menggunakan Metode DPPH. *ORYZA: Jurnal Pendidikan Biologi*, 13(2), 297-303.
- Ningsih E. R., Fitriyati L., Rahayu T. P. & Kiromah N. Y. W. (2024). Uji Aktivitas Antioksidan Metode DPPH dan Nilai SPF Ekstrak Etanol Daun Belimbing Wuluh (*Averrhoa bilimbi* l.). *Jurnal Farmasi Klinik dan Sains*, 4(2), 48-58.
- Noroozi F., Asle-Rousta M., Amini R. & Sahraeian Z. (2024). Alpha-pinene ameliorates liver fibrosis by suppressing oxidative stress, inflammation, and the TGF- $\beta$ /Smad3 signaling pathway. *Iranian Journal of Basic Medical Sciences*, 28(4), 451-460.
- Novika D. S., Ahsanunnisa R. & Yani S. F. (2021). Uji aktivitas antiinflamasi ekstrak etanol daun belimbing wuluh (*Averrhoa bilimbi* L.) terhadap penghambatan denaturasi protein. *Jurnal Sains dan Terapan Kimia*, 3(1), 16–22.
- Putra E. D. L., Wahyuni H. S. & Prasetyo, B. E. (2022). Edukasi pemanfaatan tanaman herbal bagi kesehatan di Desa Liang Pematang. *Jurnal Pengabdian Masyarakat*, 5(4), 1323.
- Ramadhan S., Ramadhan A., Laenggeng A. H. & Kundera I. N. (2021). Pengaruh kombinasi ekstrak daun pare (*Momordica charantia* L.) dan kunyit (*Curcuma longa*) terhadap kadar kreatinin pada tikus (*Rattus norvegicus*) yang diinduksi CCl<sub>4</sub>. *Journal of Biology Science and Education*, 9(2), 780–785.
- Sembiring N. B. & Merry J. (2024). Perbandingan Aktivitas Antioksidan Ekstrak Etanol Jahe Merah (*Zingiber officinale* var. *rubrum*) Metode Maserasi dan Maserasi Digesti. *Jurnal Farmasi dan Herbal*, 6(2), 78-84.
- Serli S. P., Riga R. & Nasra E. (2024). Kajian Fitokimia Ekstrak Metanol Daun Belimbing Wuluh (*Averrhoa Bilimbi* L.) Asal Kecamatan Kota Tangah. *Jurnal Pendidikan dan Sains*, 4(5) , 1004-1018.