International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Evaluation of Phytochemical Constituents in Five Different Types of Green Leaves (Moringa Oleifera, Alternanthera Sessilis, Amaranthus Dubius, Centella Asiatica, and Chenopodium Album)

S. Jeeva[1], A.P.A. Rani[2]

[1,2]Department Of Zoology and Research Centre, Lady Doak College, Narimedu, Madurai.

ABSTRACT

Chemicals found naturally in plants are known as phytochemicals. These phytochemicals are becoming increasingly popular because of their numerous medical applications. Green leaves are commonly consumed as a vegetable, but they also have therapeutic properties, and they contain an abundance of phytochemicals. This research work aimed at the screening of phytochemical constituents in five different types of green leaves namely *Moringa oleifera, Alternanthera sessilis, Amaranthus dubius, Centella asiatica, and Chenopodium album*. Ten different types of phytochemical tests were carried out for selected five different types of green leaves. These green leaves were extracted as a dried powder and were subjected to phytochemical tests. The tests revealed the presence of phlobatannins, saponins, alkaloids, flavonoids, glycosides, steroids, terpenoids, amino acids, carbohydrates, and protein. The result of the present study promises the uses of these phytochemical essences in cosmetics and medicines.

Key words: Phytochemicals, Moringa oleifera, Alternanthera sessilis, Amaranthus dubius, Centella asiatica, and Chenopodium album.

INTRODUCTION

Plant diversity has been utilized for medicinal purposes, herbalism, and in cosmetic products. Approximately 75-80% of the world's population uses partially or exclusively plant-based remedies, according to World Health Organization. Bioactive principles found in plants are potential sources of antimicrobial agents and different types of medicines **(Azubuike *et al.*, 2015)**.

Essential oils, alkaloids, tannins, flavonoids, terpenoids, phenolic compounds, and saponins are bioactive phytochemical elements that may contribute to therapeutic characteristics. **(Ibrahim *et al.*, 2018).**

For example,

- Saponins can help with pro-collagen production.
- Tannins and flavonoids are antiseptic and antibacterial.

Green leaves are commonly consumed as a vegetable but have therapeutic properties. According to **Priyadarsini *et al.*, (2018)**, carotenoids, beta-carotene, and lutein are abundant in green leaves. Green

leaves are high in the bioflavonoid quercetin, which has antioxidant, antiproliferative, anti-inflammatory, antihistaminic, gamma radiation protection, hepatoprotective characteristics, and some other flavonoids. The plant, the sea, and the earth world all provide novel 'bioactive' substances. Vitamins, Chinese herbs, antioxidants, minerals, hormones, enzymes, and a variety of 'naturals' are all popular constituents. Plants have been used since the dawn of time, and in the upcoming years, the market will be flooded with new goods incorporating natural herbs and oils. **(Aburjai *et al.*, 2003).**

FIVE DIFFERENT TYPES OF GREEN LEAVES:

1. Moringa oleifera
2. Alternanthera sessilis
3. Amaranthus dubius
4. Centella asiatica
5. Chenopodium album

PHYTOCHEMICALS OF *Moringa olifera* LEAVES-

Moringa oleifera, commonly known as Moringa.

- Moringa leaves included high quantities of saponins, flavonoids, alkaloids, and tannins according to phytochemical analysis **(Natsir *et al.*, 2019).**
- Alkaloids, phenols, glycosides, tannins, saponins, hydrolyzable tannins, and volatile oils were found in the leaf extract of *Moringa oleifera* (**Dahiru *et al.*, 2006).**
- Both water and methanol extracts of *M. oleifera* contain anthraquinones, alkaloids, tannins, phenol, and flavonoid according to the early phytochemical screening process (**Idris *et al.*, 2016).**

PHYTOCHEMICALS OF *Amaranthus dubius* LEAVES

It is a leafy vegetable in South and Southeast Asia and Africa. *Amaranthus* is commonly known as red spinach, Chinese spinach, and spleen Amaranth.

- Plants biological activities, such as antibacterial and anthelmintic properties, are attributed to tannins and flavonoids, which are the main phytoconstituents in these leaves. (**Pulipati *et al.*, 2014)**
- An early phytochemical examination found the presence of amino acids, alkaloids, steroids, cardiac glycosides, tannins, and flavonoids among other phytoconstituents. (**Pulipati *et al.*, 2014)**

PHYTOCHEMICALS OF *Alternanthera sessilis* LEAVES

Alternanthera sessilis, also known as sessile joy weed or dwarf copper leaf.

- All *Alternanthera sessilis* extracts contain flavonoids, alkaloids, anthraquinones, and steroids however tannins are only found in the aqueous extract. **(Monroy and Limsiaco, 2016)**

PHYTOCHEMICALS OF *Centella asiatica* LEAVES-

Centella asiatica, a common perennial herbaceous creeper belonging to the Umbelliferae family, grows abundantly in damp places and is extensively distributed in tropical and subtropical nations **(Dash *et al.*, 2011).**

- *Centella asiatica* contains a variety of chemical constituents such as Asiatic acid, rhamnose, glucose, terpenoids, stigmasterol, sitosterol, and fatty oils containing glycerides of palmitic acid, stearic acid, linoleic acid, linolenic acid, and vitamins such as ascorbic acid. **(Dash *et al.*, 2011)**

PHYTOCHEMICALS OF *Chenopodium album* LEAVES-

- Quercetin, rutin, chenoalbicin, chenoalbuside, calenduloside, cinnamic acid amides, chikusetsusaponin, tannins, saponins, glycosides, steroids, and terpenoids can all be found in *C. album.* (**Said *et al.*, 2021)**

PHYTOCHEMICALS

Plants have a broad and sophisticated arsenal of phytochemicals that not only relax, rejuvenate, and cure the skin, but also withstand the scrutiny of clinical trials and pharmacological testing, according to a scientific study **(Aburjai *et al.*, 2003).**

In the study by **Priyadarsini *et al.*, (2015)**, preliminary phytochemical screening of the plant powder and extracts revealed the presence of saponins, flavonoids, phenols, tannins, sugars, and lipids, steroids, and alkaloids.

In the study by **Priyadarsini *et al.*, (2018)**, they discovered the phytoconstituent type present in each extract and powder of *Spinacia oleracea*, through a qualitative preliminary phytochemical examination using several chemical detecting agents.

Alkaloids, saponins, and tannin kinds of chemicals were found in *Spinacia oleracea* phytochemical investigation **(Islam *et al.*, 2018).**

Tannins, alkaloids, carbohydrates, terpenoids, and flavonoids are bioactive compounds found in medicinal plants that have a distinct physiological effect on the human body **(Sowmya and Lakshimi Devi, 2013)**

Rohini and Padmini (2016) listed the below phytochemicals:

- Plant samples were subjected to phytochemical analysis, which confirmed the existence of elements with medicinal and physiological properties.
- Flavonoids are hydroxylated phenolic compounds that plants produce in response to microbial infection and have been discovered to have antibacterial properties in vitro against a wide range of pathogens. Their capacity to combine with extracellular and soluble proteins, as well as the bacterial cell wall, accounts for their activity.
- Antibacterial properties have been reported for steroids.

MATERIALS AND METHODOLOGY

SAMPLE COLLECTION AND IDENTIFICATION:

Five different types of fresh green leaves; *Moringa oleifera, Alternanthera sessilis, Chenopodium album, Amaranthus dubius, and Centella asiatica* were collected from the Bodi-Meenatchipuram area in Bodinayakanur town, Theni district, Tamil Nādu state, India. Identification of the plant materials was done. The fresh green leaves collected were washed thoroughly with distilled water and allowed to shadow dry for three days at room temperature. The dried leaves were cut into small pieces using sterile scissors and ground into powders. The powder (extracts) was kept in airtight container for future use.

ANALYZATION OF PHYTOCHEMICALS IN THE SELECTED FIVE DIFFERENT GREEN LEAVES EXTRACTED POWDERS

The below ten phytochemical tests were done on all five green leaves extracted powder under laboratory conditions.

1. **TEST FOR PHLOBATANNINS (Unegbu *et al.*, 2020)**

   About 0.2g of each extracted powder was boiled with an equal volume of 1% HCl. The deposition of

a red precipitate indicated the presence of phlobatannins.

2. **TEST FOR SAPONINS (Unegbu *et al*., 2020)**
   About 0.1g of each extracted powder was dissolved in 5ml of distilled water and shaken vigorously. The formation of fronting bubbles that lasted for 10 minutes indicated the presence of saponin.
3. **TEST FOR ALKALOIDS (Idris and Abubakar 2016)**
   Ten milligrams of the extracted powder were dissolved in concentrated HCl and filtered. Two to three drops of the solution were poured into the center of the watch glass. Mayer reagent was added to the watch glass's sides with a glass rod's help. The formation of a gelatinous white precipitate at the junction of the two liquids showed the presence of alkaloids.
4. **TEST FOR FLAVONOIDS (Unegbu *et al*., 2020)**
   About 0.2g of each extracted powder was dissolved in 2ml of sodium hydroxide solution. The occurrence of a yellow solution that disappears with the addition of HCl acid indicates the presence of flavonoids.
5. **TEST FOR GLYCOSIDE (Unegbu *et al*., 2020)**
   0.5 g of each extracted powder was dissolved in 3ml of Fehling solution. A brick-red precipitate indicated the presence of glycosides.
6. **TEST FOR STEROIDS (Unegbu *et al*., 2020)**
   Five (5) drops of concentrated H_2S0_4 were added to 0.1g of each extracted powder in a test tube. A reddish-brown coloration indicated the presence of steroids.
7. **TEST FOR TERPENOIDS (Unegbu *et al*., 2020)**
   Four milligrams (4mg) of the extracted powder were treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated sulphuric acid solution was added slowly and red-violet color was observed for terpenoid.
8. **TEST FOR AMINOACID (NINHYDRIN) (Banu and Catherine 2015)**
   The extracted powder (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatman No. 1 filter paper and the filtrate is subjected to a test for Amino acids.
9. **Ninhydrin test-**
   Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) are added to 2 ml of aqueous filtrate. The appearance of purple color indicated the presence of amino acids.
10. **TEST FOR CARBOHYDRATES (BENEDICT) (Banu and Catherine 2015)**
    To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated in a boiling water bath for 2 to 5 minutes. A characteristic-colored precipitate indicated the presence of sugar.
11. **TEST FOR PROTEIN (BIURET) (Banu and Catherine 2015)**
    2 ml of filtrate is treated with 1 drop of 2% copper sulfate solution. To this 1 ml of ethanol (95%) is added, followed by an excess potassium hydroxide pellet. The pink color ethanolic layer indicated the presence of protein.

RESULTS

The phytochemical constituents of green leaves; *Moringa oleifera, Alternanthera sessilis, Amaranthus dubius, Centella asiatica, Chenopodium album* are presented in table 1.

In this study, phytochemical screenings were performed on five different green leaves extracted powder. Figure 1 shows the result for the test phlobatannins: the phlobatannins are present in the extracts of *A. dubius* (S-3), *C. asiatica* (S-5), *and C. album* (S-,4) and absent in the extracts of *M. olifera* (S-1) *and A.*

sessilis (S-2). The deposition of a red precipitate indicates the presence of phlobatannins.
Figure 2 shows the result for the test of saponins; saponins are present in the extracts of *M. olifera* (S-1), *A. dubius* (S-3), *and C. asiatica* (S-5), *and C, album* (S-4) and absent in the extracts of *A. sessilis* (S-2). The formation of frothing bubbles indicates the presence of saponins.
The result of the test of alkaloids is presented in Figure 3. The alkaloids are present in the extracts of *M. olifera* (S-1), *A. sessilis* (S-2), *A. dubius* (S-3), *C. asiatica* (S-5), *and C. album* (The formation of a gelatinous white precipitate at the junction ensures the presence of alkaloids).
Figure 4 shows the results of flavonoids. The extract of *M. olifera* (S-1) exhibits the presence of flavonoids by the occurrence of a yellow solution which disappears with the addition of HCl acid.
The results of glycoside Figure 5 show that the extracts of *M. olifera* (S-1), *A. sessilis* (S-2), and *C. asiatica* (S- 5) contained glycoside which is evident from the appearance of the brick-red precipitate.
The extracts of *M. olifera* (S-1), *C. album* (S-4), and *C. Asiatic* (S-5) contained the presence of steroids which appears reddish-brown Figure 6.
The results of the terpenoid testing are presented in Figure 7 and the extract of *C. asiatica* (S-5) alone showed the presence of terpenoids (appearance of violet color).
Figure 8 shows the result of amino acids in the extracts. In control C, no color change is seen. But the extract of *M. olifera* (red indicates the presence of asparagine). The extract *A. sessilis* (S-2), *A. dubius* (S-3,) and *C. album* (S-4) color changed to yellow that indicating the presence of proline and hydroxyproline. The extract of *C. asiatica* (S-5) color changed to brown which indicates the presence of asparagine. This result shows the presence of different amino acids in all the green leaves.
Figure 9 shows the result of carbohydrates in the extracts, carbohydrates are present in the extracts of *M. olifera* (S-1) good green colour, *A. sessilis* (S-2) crystal green colour, *A. dubius* (S-3) crystal green colour, *C. asiatica* (S-5) dark good green colour *and C. album* (S-4) crystal green colour and the control showed no colour change. This indicates that the extracts contain the carbohydrate but with a different amount.
Figure 10 depicts the result for protein presence. There was no pink colour ethanolic layer formation which indicated the absence of protein in all the extracts.

DISCUSSION

Phytochemicals such as phlobatannins, saponins, alkaloids, flavonoids, glycosides, steroids, carbohydrates, and terpenoids were found in the plant extracts. They possess biological properties such as anti-aging, anti-inflammation, and cell proliferation activities **(Rohini and Padmini 2016).** Flavonoids are hydroxylated phenolic compounds that plants produce in response to microbial infection and have been demonstrated to be antibacterial agents *in vitro* against a wide range of pathogens. Their ability to interact with extracellular and soluble proteins and the bacterial cell wall is most likely the reason for their activity. Antioxidant properties are also present. Antibacterial properties of steroids have been reported **(Rohini and Padmini 2016).**

CONCLUSION

Different phytochemicals presence proves the effect of various natural properties present in the extracted green leaves.

REFERENCES

1. Aburjai, T., & Natsheh, F. M. (2003). Plants used in cosmetics. *Phytotherapy Research: An Internati-*

onal Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, *17*(9), 987-1000.

2. Azubuike, C. P., Ejimba, S., Idowu, A. O., & Adeleke, I. (2015). Formulation and evaluation of antimicrobial activities of herbal cream containing ethanolic extracts of Azadirachta indica leaves and Aloe vera gel.
3. Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International Journal of Advanced Research in Chemical Science*, *2*(4), 25-32.
4. Dahiru, D., Onubiyi, J. A., & Umaru, H. A. (2006). Phytochemical screening and antiulcerogenic effect of Moringa oleifera aqueous leaf extract. *African Journal of Traditional, Complementary and Alternative Medicines*, *3*(3), 70-75.
5. Dash, B. K., Faruquee, H. M., Biswas, S. K., Alam, M. K., Sisir, S. M., & Prodhan, U. K. (2011). Antibacterial and antifungal activities of several extracts of Centella asiatica L. against some human pathogenic microbes. *Life Sciences and Medicine Research*, *2011*, 1-5.
6. Ibrahim, N. I., Wong, S. K., Mohamed, I. N., Mohamed, N., Chin, K. Y., Ima-Nirwana, S., & Shuid, A. N. (2018). Wound healing properties of selected natural products. *International journal of environmental research and public health*, *15*(11), 2360.
7. Idris, A., & Abubakar, U. (2016). Phytochemical and antibacterial investigations of moringa (Moringa oleifera) leaf extract on selected bacterial pathogens. *Journal of Microbiology and Antimicrobials*, *8*(5), 28-33.
8. Islam, O. (2017). Phytochemical profiling and evaluation of antioxidant and antidiabetic activity of methanol extract of spinach (Spinacia oleracea L.) leaves. *Int J Pharm Sci Scient Res*, *3*, 8-24.
9. Monroy, A. E. M., & Limsiaco, C. L. (2016). Phytochemical and antimicrobial analysis of "Lupo"(Alternanthera sessilis LR BR.). *West Visayas State. Univ. Res. J*, *5*(2), 21-34.
10. Natsir, H., Wahab, A. W., Budi, P., Arif, A. R., Arfah, R. A., Djakad, S. R., & Fajriani, N. (2019, October). Phytochemical and antioxidant analysis of methanol extract of moringa and celery leaves. In *Journal of Physics: Conference Series* (Vol. 1341, No. 3, p. 032023). IOP Publishing.
11. Priyadarsini, S. S. (2018). Formulation and evaluation of a herbal antibacterial cream from ethyl acetate extract of leaves of Spinacia oleracea Linn. against Aeromonas skin and soft tissue infections. *International Journal of Green Pharmacy (IJGP)*, *12*(03).
12. Priyadarsini, S.S., Kumar, P. R., Thirumal, M., Joseph Blessing, A., Prabhu, P., & Vyshnavi, P. (2015). Antibacterial Effect of Successive Extracts of Leaves of Spinacia oleracea Linn. American Journal of PharmTech Research.
13. Pulipati, S., Babu, P. S., & Narasu, M. L. (2014). Quantitative determination of tannin content and evaluation of antibacterial activity of Amaranthus tricolor (L). *Int J Biol Pharm Res*, *5*, 623-6.
14. Rohini, M. V., & Padmini, E. (2016). Preliminary phytochemical screening of selected medicinal plants of polyherbal formulation. *Journal of Pharmacognosy and Phytochemistry*, *5*(5), 277.
15. Said, A., Naeem, N., Siraj, S., Khan, T., Javed, A., Rasheed, H. M., & Wahid, F. (2020). Mechanisms underlying the wound healing and tissue regeneration properties of Chenopodium album. *3 Biotech*, *10*(10), 1-10.
16. Sowmya, S., & Lakshmidevi, N. (2013). Qualitative phytochemical analysis of nonpolar to polar solvent extracts of selected medicinal plants. *International Journal of Current Research*, *5*(12), 3618-3621.

17. Unegbu, V., Nkwoemeka, N., Okey-Ndeche, F., & Obum-Nnadi, C. (2020). Phytochemical and Antibacterial Properties of Moringa oleifera leaf extracts on Escherichia coli and Staphylococcus aureus. *Nigerian Journal of Microbiology*, *34*(1), 5145-5152.

TABLE

Table 1: Phytochemical constituents of green leaves extracted powder

TESTS	EXPECTED RESULTS	*M. olifera* (S-1)	*A. sessilis* (S-2)	*A. dubius* (S-3)	*C. album* (S-4)	*C. asiatica* (S-5)
Phlobatannins	Deposition of red precipitate.	-	-	+	+	+
Saponins	Fronthing bubbles (10 mins)	+	-	+	+	+
Alkaloids (Mayer's)	A gelatinous white precipitate at the junction of the two liquids.	+	+	+	+	+
Flavonoids	Occurrence of a yellow solution which disappears on addition of Hcl acid.	+	-	-	-	-
Glycoside	Brick red precipitate	+	+	-	-	+
Steroids	Reddish brown colouration	+	-	-	+	+
Terpenoids	Red violet colour	-	-	-	-	+
Amino acid (Ninhydrin)	Appearance of purple colour	+ Presence of Asparagine,	+ Presence of Proline and Hydroxyproline	+ Presence of Proline and Hydroxyproline	+ Presence of Proline and Hydroxyproline	+ Presence of Asparagine,

		(Appearance of brown colour)	(Appearance of yellow colour)	(Appearance of yellow colour)	(Appearance of yellow colour)	(Appearance of brown colour)
Carbohydrates (Benedict's)	Characteristic Colours	+ Presence (Good green)	+ Presence (Crystal Green)	+ Presence (Crystal Green)	+ Presence (Crystal Green)	+ Presence (Dark good green)
Protein (Biuret)	Pink colour ethanolic layer	-	-	-	-	-

+ = Presence of phytochemical, - = Absence of phytochemical

FIGURES

Figure 1: Test for Phlobatannins

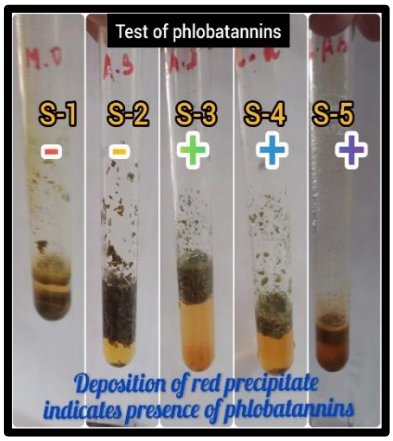

Figure 2: Test for Saponins

Figure 3: Test for Alkaloids

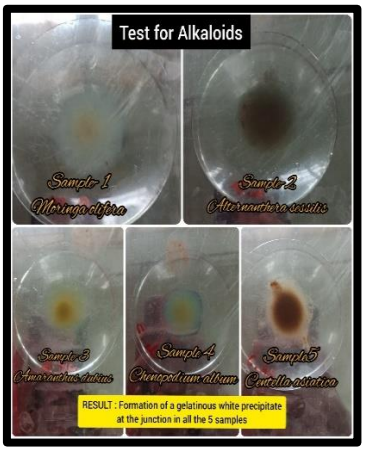

Figure 4: Test for Flavonoids

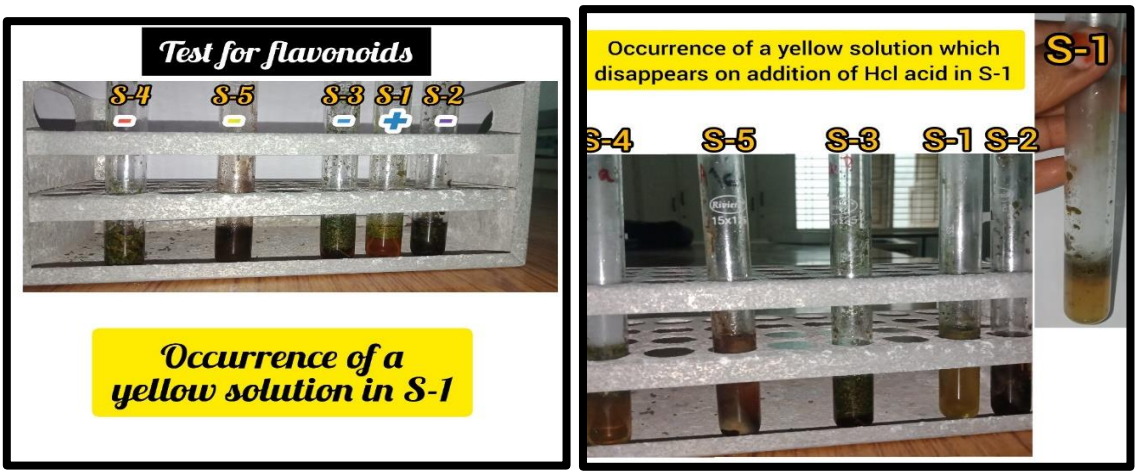

Figure 5: Test for Glycoside

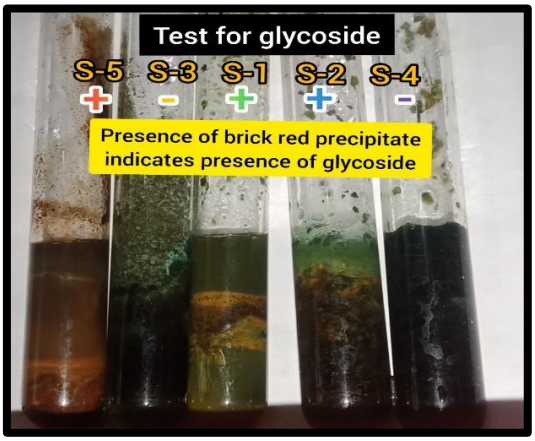

Figure 6: Test for Steroids

Figure 7: Test for Terpenoids

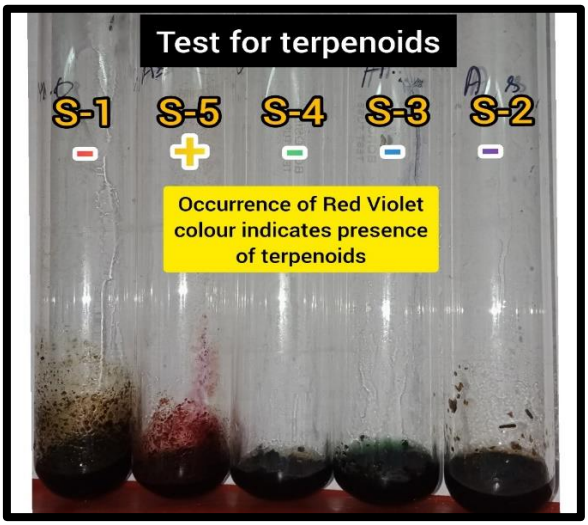

Figure 8: Test for Amino acid

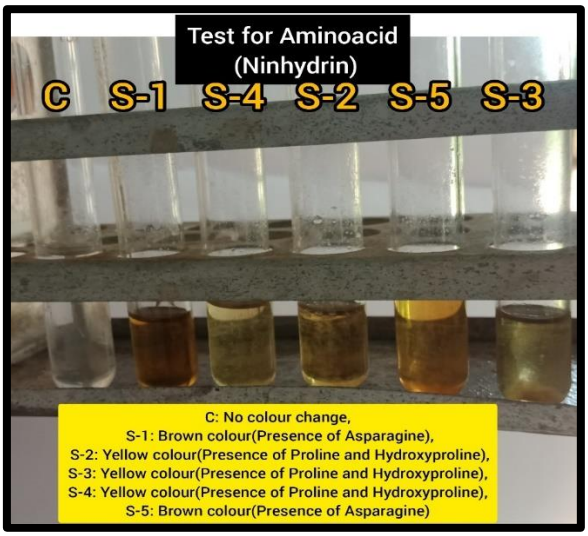

Figure 9: Test for Carbohydrates

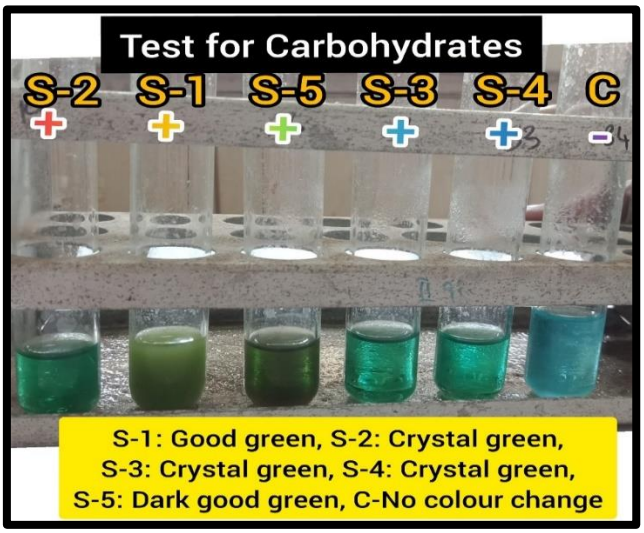

Figure 10: Test for Protein