IJBPAS, December, 2022, 11(12): 5986-5998

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF *HEVEA BRASILIENSIS* LEAVE IN DIFFERENT SOLVENT SYSTEM

BAIDYA M[1, 2], MANNA K[3], MAJI HS[1*] AND DEBNATH B[3, 4]

1: Department of Pharmaceutical Technology, JIS University, Kolkata, West Bengal, India-700109

2: Bharat Pharmaceutical Technology, Agartala, West Tripura, 799130

3: Natural Cum Advance Synthetic Lab, Department of Pharmacy, Tripura University (A Central University), Suryamaninagar, India-799022

4: Department of Pharmaceutics, Regional Institute of Pharmaceutical Science and Technology, Agartala, India-799005

***Corresponding Author: Dr. Himangshu Sekhar Maji: E Mail: hsmaji@jisuniversity.ac.in**

Received 26th Dec. 2021; Revised 25th Jan. 2022; Accepted 12th March 2022; Available online 1st Dec. 2022

https://doi.org/10.31032/IJBPAS/2022/11.12.6675

ABSTRACT

Background: *Hevea brasiliensis*, a rubber tree or rubber plant, is a flowering plant of the Euphorbiaceae family Spurge. Milky rubber extracted from the tree is the primary source of natural rubber, so it is the most economically important member of the Hevea species.

Objective: This study aims to identify the phytochemical analysis and antioxidant activity of *Hevea brasiliensis* leaf extract in various solvent systems.

Methods: The leaves of *Hevea brasiliensis* were dried and extracted with five different solvents (pet ether, n-hexane, chloroform, methanol, and ethanol). The qualitative screening of alkaloids, phenols, flavonoids, and tannins was done by Wagner's test, Ferric chloride test, Shibita's reaction test, and Braymer's test, respectively. The quantitative amount of total alkaloids and tannins were determined by titrimetric methods. Total phenols were determined by Folin-Ciocalteu colorimetric method. Total flavonoids were determined by the aluminum chloride (AlCl3) colorimetric method.

2, 2-diphenyl-1-picryl hydroxyl (DPPH) free radical scavenging assay and hydrogen peroxide (H_2O_2) scavenging assay were used for evaluating the antioxidant activity of three different solvent extracts of leaves of *Hevea brasiliensis*.

Results: Preliminary phytochemical screening of five different solvent extracts of leaves of *Hevea brasiliensis* showed that only methanolic and ethanolic extract gives the significant responses against four selected phytochemicals. The ethanol and methanolic extract also gives the significant antioxidant activity against DPPH and H_2O_2 radical scavenging assay compared to standard ascorbic acid.

Conclusions: These findings suggest that the ethanolic and methanolic leave extracts of *Hevea brasiliensis* has potent antioxidant activity which may be responsible for some of its reported pharmacological activities and can be used as an antioxidant supplement.

Keywords: *Hevea brasiliensis,* secondary metabolite, anti-oxidant activity, DPPH assay, H_2O_2 assay, phytochemicals

INTRODUCTION

More than 80% of the world's population relies on herbal or traditional medicine for their simple healthcare needs on a number one basis. The use of herbal medications inside the Asian nations contributes to a primary category inside the records of human interconnections with the surroundings **[1]**. *Hevea brasiliensis (H.Brasiliensis),* the rubber tree, is the number one supplier of natural rubber. It is belonging to the family of Euphorbiaceae **[2]**. Many rubber-producing nations are increasing rubber cultivation to regions the increasing demand for herbal rubber, capture in any other wasteland, enhance rural dwellers, financial activities, and raise export trade to earn foreign exchange. In addition, *Hevea brasiliensis* is cultivated in non-conventional regions as a getaway area for a few devastating sicknesses, inclusive of the South American Leaf Blight in Brazil **[3]**.

The rubber tree is a quick-developing tree that's the maximum economically vital member of the genus Hevea. It is of primary monetary significance because the milky latex extracted from the tree is the number one supply of herbal rubber. As a latex-generating crop, the bark is often tapped. Latex, the supply of Hevea or para rubber, is received with the aid of tapping the trees' trunks. It is cultivated for rubber, food, apiculture, fiber, timber, lipid, gasoline, etc. It is additionally cultivated for soap, insect repellent in Brazil **[4]**.

Plants used in traditional medicine contain various ingredients used to treat acute and chronic diseases. Plants are of medical importance because they incorporate chemicals that have a specific physiological impact on the human body **[5]**.

Alkaloids, flavonoids, tannins, and phenolic compounds are the essential bioactive molecules found in plants. Used of medicinal plants as traditional medicine are well known in rural areas of many developing countries **[6]**. Traditional healers argue that their medicine is more affordable and effective than modern medicine. Low-income individuals, farmers, residents of small isolated villages, and indigenous communities in developing countries use folk remedies to treat common ailments **[7]**. Phytochemistry involves studying plant-based organic components, which are given the pharmacological activity inside the leaving cell. Medicinal plants are used worldwide to treat various diseases such as inflammation, heart disease, and cancer **[8]**. Most of the medicinal plants contain phenolic compounds that exhibit antioxidant properties. Antioxidants from plant substances neutralize the action of free radicals, thus protecting the body from various diseases **[9]**. The literature showed that the leave extract of the plant has not yet been researched for its antioxidant action. The aim of this study was to phytochemicals analysis and evaluates the anti-oxidant properties of *Hevea brasiliensis* leaves extract.

MATERIALS AND METHODS

Plant martial collection and identification

The Fresh leaves of *Hevea brasiliensis* were collected from South Tripura, India, in July 2021. The leaves were identified by Prof. Badal Kumar Datta, a Taxonomist Department of Botany, Tripura Central University.

Chemicals

Concentrated HCl, methyl red indicator, tannic acid, Folin-Ciocalteu reagent, sodium bicarbonate, sodium hydroxide, quercetin, aluminium chloride, KMnO4, picric acid, ferric chloride, n-butanol, metal magnesium, sodium carbonate, sodium potassium tartrate, sodium chloride, pet ether, indigo solution, n-hexane, chloroform, methanol, ethanol, nitric acid, $KMnO_4$, hydrogen peroxide, DPPH, ascorbic acid, had been purchased from Sigma-Aldrich GmbH, Sternheim, Germany.

Processing of the plants materials

The collected leaves of *Hevea brasiliensis* were thoroughly rinsed with distilled water, cleaned and cut into pieces, and air-dried thoroughly. Then the samples were powdered in a grinder and passed through a sieve (mesh no. 40) to obtain the exact size of the powder material. These powders were used for further testing.

Preparation of Plant Extract

Dried leaves powder (30 g) was packaged inside the Soxhlet apparatus. The extracts were prepared by the consecutive extraction method using a series of natural solvents with a polarity sequence according to their dipole moments (petroleum ether, n-hexane, chloroform, methanol, 50% ethanol) at 40° C for 72 hours. The extract became filtered using Whatman No. 1 filter paper, and the filtrate was immersed below reduced pressure at 40^0 C inside the rotary vacuum evaporator (IKA HB 10). Collected extract was dried in a water bath at 40^0 C, measured and stored for testing. Percentages of extract yield (pet ether 6.8%, n-hexane 1.66%, chloroform 15.66%, methanol 16%, 50% ethanol 15.66%) become decided using the subsequent equation.

$$\% \text{ of Yield} = \frac{\text{Weight of the extract}}{\text{Weight of the plant material}}$$

Qualitative Analysis of Plant Secondary Metabolites

Prepared extracts were made to test the quality of four secondary metabolites (alkaloid, phenol, flavonoid, and tannin) were performed.

Alkaloid test

Wagner's test: Test solution (2ml) when treated with iodine in potassium iodide (Wagner's reagent) gives a brown precipitate if alkaloids are present **[10]**.

Phenol test

Ferric chloride test: Test solution (2ml) became handled with one or two drops of 5% (w/v) aqueous ferric chloride solution. The solution will become deep blue or black color which indicated the presence of phenol **[11]**.

Flavonoid test

Shibita's reaction test: 2ml of alcoholic test solution was heated. Then magnesium was added and treated with a few drops of concentrated hydrochloric acid. The formation of a darkish red color indicated the presence of flavonoid **[12]**.

Tannin test

Braymer's test: The test solution (2 ml) was treated with one or two drops of 10% alcoholic ferric chloride solution. The appearance of a blackish blue or greenish-black shade indicates the presence of tannins **[13]**.

Quantitative Analysis of Plant Secondary Metabolites

Estimation of Total Alkaloids

The total alkaloid of five different solvent extracts was determined by using the titrimetric technique. 20 mg of extract was taken in 20 ml of volumetric flax, added 10 ml of n-butanol, shaken thoroughly for well mixing, and made the volume was 20ml with n-butanol. 10 ml of this solution have become taken into 50 ml separating funnel, and 10 ml

of 0.1 (N) HCl becomes added then shaken very well for two to three minutes. The upper layer contained n-butanol, and the lower layer contained alkaloid neutralized with 0.1 (N) HCl. 10 ml of 0.1 (N) HCL portion was taken in a beaker, then two to three drops of methyl red were added, and the solution became a slightly reddish color. The beaker contents were titrated with 0.1 (N) NaOH until the shade changed from red to pale yellow. Note down the neutrality point. For blank, add 10 ml of 0.1 (N) HCl to a beaker and add 2-3 drops of methyl red and titrate with 0.1 (N) NaOH, until the color changes from red to pale yellow. The same procedure was done in triplicate **[14]**.

The overall amount of alkaloids was calculated with the aid of considering the following equivalent:

1ml 0.1 N HCL = 0.01629 g of alkaloids

Estimation of Phenolic Content

The total phenolic content of five different solvent extracts was determined using the Folin-Ciocalteu colorimetric method. A stock solution of 1 mg/ml of tannic acid was prepared with ethanol. It was then diluted ten times and used as a standard stock solution. From this standard stock solution, 0.1, 0.2, 0.3, 0.4, 0.5 ml was taken from various test tubes. After that, 0.5 ml of Folin-Ciocalteu reagent and 1 ml of saturated sodium bicarbonate solution were added to each test tube. The volume of each test tube is made up of 5 ml of distilled water. After that, the contents of all test tubes were immersed in a water bath for two minutes. All the test tubes have been cooled at room temperature, and the absorbance was measured at 560 nm by UV-VIS Spectrophotometer (Shimadzu UV-1800) towards the reagent blank **[15]**. For plant extracts, 200µl was taken triplicate, and the color was produced as usual. The overall phenolic content was calculated from the calibration curve.

Estimation of total Flavonoid Content

The flavonoid content was determined using the aluminum chloride (AlCl3) colorimetric method. A standard solution of quercetin (1 mg/ml) was prepared with ethanol. From this standard stock solution, 0.1, 0.2, 0.3, 0.4, and 0.5 ml were taken in 10 ml volumetric flax and made into 1 ml of ethanol. After that, four ml of distilled water was added and incubated for five minutes. After five minutes, 0.3 ml of 10% aluminum chloride was added, then stand for six minutes. After that, 2 ml of 1 mol/lit NaOH solution was added, and finally, the volume was made up to 10 ml, and the absorption was taken at 517 nm. For extract, 200 µl was taken in triplicate, and the color was produced as usual. The overall flavonoid

content was calculated from the calibration curve **[16]**.

Estimation of Tannin Content

The overall tannin content of five different solvent extracts was determined using a titrimetric method. 20 mg of extract was dissolved in 100 ml of a volumetric flask with 50% ethanol. 6.25 ml of infusion was taken in 250 ml of a conical flask and gradually added 6.25 ml of indigo solution and 187.5 ml of 50% ethanol. 0.1 N aqueous solution of KMnO4 was used as a titrant until the blue solution turned green. Then immediately added the few drops of titrant green solution turned to golden yellow, and the borate reading was noted down. For blank, 6.25 ml of indigo solution and 193.75 ml of 50% ethanol were mixed and titrated again with 0.1 N aqueous solution of KMnO4 and noted down the endpoint. The same procedure was carried out for triplicate. The following equation is used to determine the total tannin content **[17]**.

$$\text{The tannin content (T \%)} = \frac{(V-V_0)\ X0.004157X100X100}{g\ X\ 6.25}$$

Where, V=0.1 N aqueous solution of $KMnO_4$ for the titration of the sample, ml
V_0 = 0.1 N aqueous solution of $KMnO_4$ for the titration of the blank sample, ml
0.004157 = Tannin equivalent in 1ml of 0.1 N aqueous solution of$KMnO_4$
g = Mass of the sample taken for analysis
100 = Volume of the volumetric flax, ml
100 = Percentage (%)

In Vitro Antioxidant Activity

DPPH Free Radical Scavenging Assay

The antioxidant activity of leave extract (chloroform, methanol, ethanol) of *Hevea brasiliensis* was determined by a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay with specific changes of the Sowndhararajan and Kang (2013) approach. A standard solution of ascorbic acid (1 mg/ml) was prepared with methanol. From this stock solution, 10, 20, 40, 80, and 100 µg/ml were taken in different test tubes, and 3.8 ml of 0.1 mM methanolic DPPH solution was added to all test tubes. The reaction was incubated for half an hour at room temperature in the dark. After half an hour, the absorbance was taken at 517 nm, and the percentage inhibition was calculated by the following equation **[18, 19]**. The same process was followed for sample (extract) in triplicate.

$$\text{DPPH scavenging effect} = \frac{\text{Control OD-Sample OD}}{\text{Control OD}} X\ 100$$

Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide scavenging assay of fine different solvent extract of leaves of Hevea brasiliensis was determined by the Debnath and Manna (2019) method with slight modification. Hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). A standard solution of 1 mg/ml ascorbic acid was prepared in phosphate buffer (pH 7.4). From this stock solution, 10, 20, 40, 80, and 100 µg/ml were taken into separate test tubes, and 0.6 ml hydrogen peroxide (40 mM) was added and incubated for 15 minutes at room

temperature in a dark **[20, 21]**. Absorbance was recorded at 230 nm, with the help of the following equation calculated percentage inhibition.

$$H_2O_2 \text{ scavenging effect} = \frac{\text{Control OD-Sample OD}}{\text{Control OD}} \times 100$$

RESULTS

Qualitative Analysis of Plant Secondary Metabolites

The qualitative analysis of plant secondary metabolites of leaves of *Hevea brasiliensis* in five different solvent extract are represented in **Table 1**. After qualitative analysis of secondary plant metabolites, it was found that increase the polarity of solvent extract (pet ether extract> n-hexane extract > chloroform extract > methanol extract> 50% ethanol extract) the colour density of the test gradually concentrates.

Quantitative Analysis of Plant Secondary Metabolites

The content of total phenolic and flavonoids of *Hevea brasiliensis* leave extracts were calculated from the calibration curve (R_2 = 0.98 for phenol and R_2 = 0.97 for flavonoid). A high amount of phenol (93.28±0.753 mg/g tannic acid equivalents) and flavonoid (73.64±1.55 mg/g quercetin equivalents) was found in 50% ethanolic extract of *Hevea brasiliensis.* The titrimetric approach had calculated total tannin and alkaloid contents. To estimate total tannin content, 0.1 (N) aqueous KMnO4 was used as a titrant, and estimated total alkaloid content 0.1 (N) HCl was used as a titrant. A high amount of alkaloid (33.12±0.54 mg/g dry extracts) and tannin (199.016±0.75 mg/g dry extracts) was also found in 50% ethanolic extract. The results of the quantitative analysis of *Hevea brasiliensis* leave extracts are represented in **Table 2**.

Evaluation of Antioxidant Activity

A *Hevea brasiliensis* leave has shown significant scavenging activity compared to standard ascorbic acid against DPPH and the H_2O_2 scavenging assays **(Figure 1 and 2)**. IC_{50} values of three extracts with ascorbic acid against two different scavenging methods are represented in **Table 3**.

Table 1: Qualitative response of plant secondary metabolites of *Hevea brasiliensis* leave extracts

Name of the extracts of *Hevea brasiliensis*	Alkaloid	Phenol	Flavonoid	Tannin
Pet Ether Extract (Polarity Index: 0.1)	+	-	-	-
n-hexane Extract (Polarity Index: 0.1)	-	-	-	-
Chloroform Extract (Polarity Index: 4.1)	++	++	++	++
Methanol Extract (Polarity Index: 5.1)	++	+++	++	+++
50% Ethanol Extract (Polarity Index: 5.2 Water: 10.2)	+++	+++	+++	+++

*** + represent low concentration, ++ represent moderate concentration, +++ represent high concentration, - represent absent**

Table 2: Quantitative analysis of plant metabolites of *Hevea brasiliensis* leave extracts

Name of the extracts of *Hevea brasiliensis*	Alkaloid (mg/g dry extracts) (Mean±SEM)	Phenol (mg/g dry extracts) (Mean±SEM)	Flavonoid (mg/g dry extracts) (Mean±SEM)	Tannin (mg/g dry extracts) (Mean±SEM)
Pet Ether Extract (Polarity Index: 0.1)	7.596667±0.543333	-	-	-
n-hexane Extract (Polarity Index: 0.1)	-	-	-	-
Chloroform Extract (Polarity Index: 4.1)	20.08333±1.086667	77.57667±0.668639	62.85667±1.091701	65.42667±1.085333
Methanol Extract (Polarity Index: 5.1)	15.74667±0.543333	86.15333±0.994256	67.82333±1.021312	132.272±0.752
50% Ethanol Extract (Polarity Index: 5.2 Water: 10.2)	33.12±0.54	93.28333±0.753444	73.64±1.555324	199.016±0.752

*** Each value represents the mean ± SEM calculated as dry weight basis in triplicate**

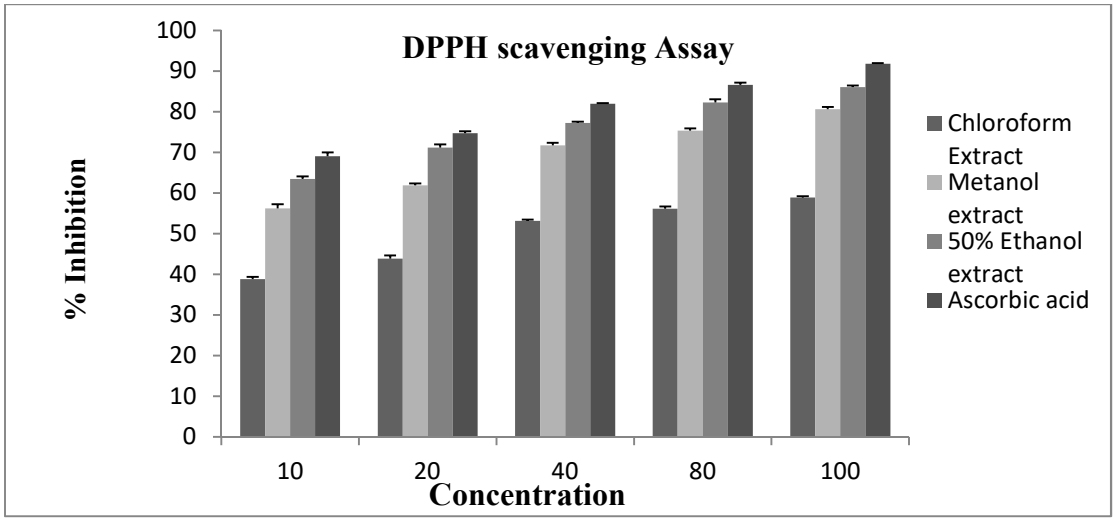

Figure 1: DPPH scavenging assay

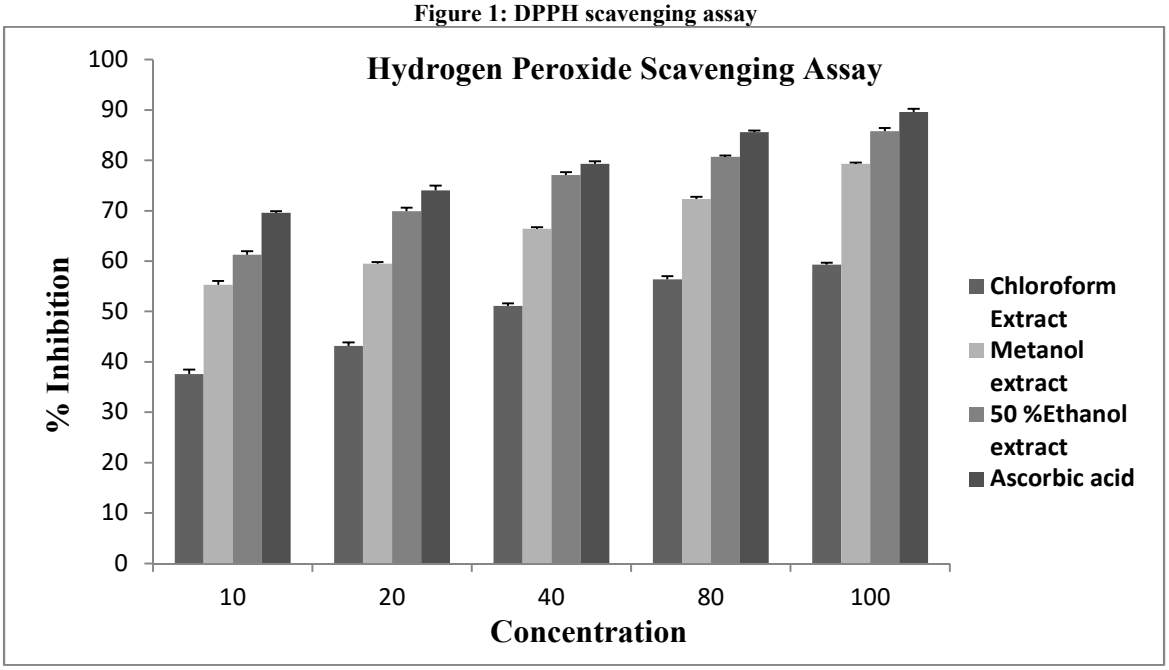

Figure 2: Hydrogen peroxide scavenging assay

Table 3: IC50 values of three extracts of *Hevea brasiliensis* with ascorbic acid against DPPH and H_2O_2 scavenging assay

Name of the *Hevea brasiliensis* leave extracts	IC_{50} Value (µg/ml) of DPPH Scavenging Activity (Mean ± SEM)	IC_{50} Value (µg/ml) of H_2O_2 Scavenging Activity (Mean ± SEM)
Chloroform extract	67.15 ±0.52	67.39 ±0.63
Methanol extract	49.28 ±0.59	51.06 ±0.57
Ethanol extract	45.47 ±0.43	45.94 ±0.52
Ascorbic acid	42.86 ±0.41	43.72 ±0.47

***Each value represents the mean ±SEM calculated as dry weight (DW) basis in triplicate**

DISCUSSION

Antioxidants are chemical compounds that engage with and neutralize unfastened radicals, for that reason stopping those from inflicting cell harm within side the organic system **[22]**. The body makes a lot of antioxidants that uses to reduce free radicals. These antioxidants are known as endogenous antioxidants. However, the body also relies on exogenous (external) sources, especially food; to get the remainder of the antioxidants it desires **[23]**. These exogenous antioxidants are normally referred to as nutritional antioxidants. Fruits, vegetables, and grains are wealthy reassets of nutritional antioxidants **[24]**. In the present experiment, it was observed that *Hevea brasiliensis* leave extracts (pet ether, n-hexane, chloroform, methanol, ethanol) gave a good amount of extracts yield (chloroform extract 15.66%, methanol extract 16%, and ethanol extract 15.66%) and less amount of extracts yield (pet ether 6.8% & n-hexane 1.66%).

Alkaloid and specifically plant alkaloid in a small amount in any fit for human consumption plant elements helps several pharmacological sports in the human and animal body **[25]**. Prolonged use of alkaloids reasons dangerous results like nausea, liver damage, headache, pores, and skin infections **[26]**. Tannin, phenol, and flavonoid found in any plant elements verify that it additionally offers numerous pharmacological activities **[27]**. In the existing experiment, it discovered that *Hevea brasiliensis* leave extracts (chloroform, methanol, and 50% ethanol) contained significant amount of alkaloid, phenol, flavonoid, and tannin. These extracts might also additionally provide dynamic pharmacological activities.

Free radicals are concerned with many degenerative sicknesses along with pain, inflammation, cancer, diabetes, hepatic damage, etc. Antioxidants combat unfastened radicals and shield from exceptional kinds of degenerative diseases. DPPH is a strong nitrogen-targeted unfastened radical and may without difficulty summarize an electron or hydrogen radical from appropriate reducing agents to grow a strong molecule. Hence, the unpaired electron of DPPH receives paired off to make the corresponding hydrazine **[28]**. In

the existing analysis, three extracts (chloroform, methanol, and ethanol) inhibited the DPPH radical in distinctive manners. This result proved that the three extracts of Hevea brasiliensis could donate an electron or hydrogen that could react with DPPH radical.

Hydrogen peroxide (H_2O_2) is debile active non-radical reactive oxygen species (ROS) that may impact many cellular processes via crossing the cell membrane. Low ranges of H_2O_2 entered within the cells in all probability react with Fe2+ and Cu2+ ions to make hydroxyl radicals, and this could be the origin of a lot of its poisonous effects **[29, 30]**. Thus removal of H2O2 is therefore essential to protect the living system. In the existing experiment, three extracts of Hevea brasiliensis (chloroform, methanol, and 50% ethanol) showed hydrogen peroxide scavenging activity in a dose-dependent manner.

CONCLUSION

In the existing study, the antioxidant capacities of the plant extracts had been analyzed the usage of free radical scavenging activity (DPPH), hydrogen peroxide scavenging assay. The extracts of *Hevea brasiliensis* having ability to scavenge the free radical and useful to cure some disease. All the extracts of *Hevea brasiliensis* (chloroform, methanol, ethanol) having antioxidant activity.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

NA

HUMAN AND ANIMAL RIGHTS

No animals/Humans were used for studies that are base of this research.

FUNDING

NA

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGE

We acknowledge the Department of Pharmacy, Tripura University for providing all the facilities to carry out this research work.

REFERENCES

[1] Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotech. 4: 685-688. DOI: 10.5897/AJB2005.000-3127.

[2] Seeam SM, Islam AS. Golam SR, Istiaq A, Rana S, Babu MH, Rahman MDM, Islam MDJ,Hakim MDL, Das P (2018). Biomedical and Phytochemical Study of Methanol Extract of Hevea Brasiliensis Roots in Swiss Albino Mice. Discovery Phytomedicine 5(4): 48-51. DOI:10.15562/phytomedicine.2018.70

[3] Feng An , James R,Guishui, David MC, Xiuqing C, Zhi Z, Lingxue K

(2016). Ethephon increases rubber tree latex yield by regulating aquaporins and alleviating the tapping-induced local increase in latex total solid content. J. Plant Growth Regul 35:701-709. DOI: 10.1007/s00344-016-9573-6.

[4] Singh SK, Kumar SS (2015). Phytochemical and antibacterial efficacy of Hevea brasiliensis. Journal of Chemical and Pharmaceutical Research 7(12):777-783.

[5] Stahl-Biskup E, Venskutonis RP (2004). Thyme. In: Peters, K. V. (Ed.) *Handbook of herbs and spices.* Boca Raton:CRC Press LLC, Vol. 2, ISBN 0-8493-2535.

[6] Yada D, Sivakkumar T, Srinivas N (2021). Aqueous extract of whole plant of hyptis suaveolens (l.) Poit: An antiulcer agent. Jour. of Med. P'ceutical & Allied Sci 10:1068 ,4187 – 3190, DOI: 10.22270/jmpas.V10I4.1068.

[7] Baidya M, Anbu J, Akhtar MS, Sarkar S, Mandal SK (2020). Antimicrobial Evaluation of Ethanolic Extract of Selected Seed Shells. Int J Curr Pharma Res. 12:74-6. DOI: 10.22159/ijcpr.2020v12i6.40286.

[8] Verma SK, Kumar A (Therapeutic uses of withania somnifera (Ashwagandha) with a note on withanolides and its pharmacological actions (2011). Asian Journal of Pharmaceutical and Clinical Research 4:1-4.

[9] Narayanaswamy N, Balakrishnan KP (2011) Evaluation of some Medicinal Plants for their Antioxidant Properties. International Journal of Pharm Tech Research 3(1):381-385.

[10] Wagner HXS, Bladt Z, Gain EM (1996). Plant drug analysis. Springer Veralag, Berlin, Germany: 360.

[11] Ugochukwu SC, Uche A, Ifeanyi O (2013). Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Baker. Asian J Plant Sci Res 3(3):10-3.

[12] Shah RK, Yadav RN (2015). Qualitative phytochemical analysis and estimation of total phenols and flavonoids in leaf extract of *sarcochlamys Pulcherrima* Wedd. Global Journal of Bioscience and Bio-Technology 4:81-4.

[13] ThilagavathiT, Arvindganth R, Vidhya D, Dhivya R (2015). Preliminary phytochemical screening

of different solvent mediated medicinal plant extracts evaluated. Int. Res. J. Pharm 6(4):246-248 http://dx.doi.org/10.7897/2230-8407.06455.

[14] Auwal MS, Saka S, Mairiga IA, Sanda KA, Shuaibu A, Ibrahim A (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). Vet Res Forum 5(2): 95-100.

[15] Debnath B, Manna K. Phytochemicals and Nutrient Profiles, Anti-oxidant Activity Study of Three Edible Parts (Flower, Fruit and Stem) of Musa paradisiacal (2019). Current Biotechnology 8(1):1-10.

[16] Siddiqui N, Rauf A, Latif A, Mahmood Z (2017). Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (Nepeta bracteata Benth). J Taibah Univ Sci 12(4): 360-3.

[17] Silva L, Pezzini BR, Soares L (2015). Spectrophotometric determination of the total flavonoid content in *Ocimum basilicum* L.(Lamiaceae) leaves. Pharmacogn Mag 11(41): 96-101.

[18] Atanassova M, Christova-Bagdassarian V (2009). Determination of tannins content by titrimetric method for comparison of different plant species. J Univ Chem Technol Metallurgy 44(4): 413-5.

[19] Sowndhararajan K, Kang SC (2013). Free radical scavenging activity from different extracts of leaves of *Bauhinia vahlii* Wight & Arn. Saudi J Biol Sci 20(4): 319-25.

[20] Ruch RJ, Cheng SJ, Klaunig JE (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 10(6): 1003-8.

[21] Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M, Stahl W, Viña-Ribes J (1998). Functional food science and defence against reactive oxidative species. Br J Nutr. 80(1):77-112. DOI: 10.1079/bjn19980106.

[22] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007). Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39(1):44-84. DOI: 10.1016/j.biocel.2006.07.001.

[23] Bouayed J, Bohn T (2010). Exogenous antioxidants--Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. Oxid Med Cell Longev 3(4):228-237. DOI:10.4161/oxim.3.4.12858.

[24] Rahman MS, Islam MB, Rouf MA, Jalil MA, Haque MZ (2011). Extraction of alkaloids and oil from Karanja (*Pongamia pinnata)* Seed. J Sci Res 3(3): 669-75.

[25] Soni P, Siddiqui AA, Dwivedi J, Soni V (2012). Pharmacological properties of *Datura stramonium* L. as a potential medicinal tree: an overview. Asian Pac J of Trop Biomed 2(12): 1002-8.

[26] Aruoma OI (2003). Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutat Res. 523: 9-20. DOI: 10.1016/S0027-5107(02)00317-2.

[27] Droge W (2002). Free radicals in the physiological control of cell function. Physiol Rev 82(1): 47-95. DOI: 10.1152/physrev.00018.2001.

[28] Kumaran A, Karunakaran RJ (2007). *In vitro* antioxidant activities of methanolic extract of Phyllanthus species from India. LWT, Food Sci Technol 40(2): 322-52.

[29] Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohoni DP, Biyani MK, Mohan H (2003).Comparative antioxidant activity of individual herbal components used in ayurvedic medicine. Phytochemistry 63(1):97–104.DOI: 10.1016/S0031-9422(02)00754-9.

[30] Baidya M, Jayaraman A, Maji HS, Ramya Krishna P S, Das D, 2022. Evaluation of acute and sub-acute toxicity of sivanar amirtham in albino mice and wistar albino rats. J. Med. P'ceutical Allied Sci. V 11 - I 1, Pages - 4196 - 4204. doi: 10.22270/jmpas. V11I1.2217.