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Lingkan Deka
Department of Pharmacy, NETES Institute of Pharmaceutical Science, NEMCARE Group of Institutions, Assam, India

Bhaswati Kashyap
Department of Pharmacy, NETES Institute of Pharmaceutical Science, NEMCARE Group of Institutions, Assam, India

Rosy Ahmed
Department of Pharmacy, NETES Institute of Pharmaceutical Science, NEMCARE Group of Institutions, Assam, India

Himsikhar Sarma
Department of Pharmacy, NETES Institute of Pharmaceutical Science, NEMCARE Group of Institutions, Assam, India

Riba Doley
Department of Pharmacy, Apex Professional University, NH, Pasighat Smart City, Arunachal Pradesh, India

Nilutpal Sharma Bora
Department of Pharmacy, NETES Institute of Pharmaceutical Science, NEMCARE Group of Institutions, Assam, India

Sameeran Gam
Department of Pharmacy, NETES Institute of Pharmaceutical Science, NEMCARE Group of Institutions, Assam, India

Bitu Gogoi
Department of Pharmacy, NETES Institute of Pharmaceutical Science, NEMCARE Group of Institutions, Assam, India

Rituparna Borah
Department of Pharmacy, NETES Institute of Pharmaceutical Science, NEMCARE Group of Institutions, Assam, India

Koushik Nandan Dutta
Department of Pharmacy, NETES Institute of Pharmaceutical Science, NEMCARE Group of Institutions, Assam, India

Corresponding Author:
Dr. Koushik Nandan Dutta
Department of Pharmacy, NETES Institute of Pharmaceutical Science, NEMCARE Group of Institutions, Assam, India

Exploration of the antioxidant and anti-inflammatory potential of *Vachellia farnesiana* bark by *in vitro* method

Lingkan Deka, Bhaswati Kashyap, Rosy Ahmed, Himsikhar Sarma, Riba Doley, Nilutpal Sharma Bora, Sameeran Gam, Bitu Gogoi, Rituparna Borah, Koushik Nandan Dutta

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Abstract
Ethnomedicine, or the use of plants for medical purposes, has been practiced for thousands of years, and indigenous peoples have been instrumental in the development of this ancient technique. The secondary metabolites found in plants, such as flavonoids and polyphenols, are abundant sources of antioxidants that guard against oxidative damage and provide a number of health benefits. *Vachellia farnesiana,* commonly known as sweet acacia, is a plant with significant pharmacological activities, including antimicrobial, anti-inflammatory, and antimalarial properties. This study focused on extracting bioactive compounds from *Vachellia farnesiana* bark using ethyl acetate, evaluating its phytochemical composition, antioxidant potential, and anti-inflammatory activity. The results showed significant antioxidant and anti-inflammatory properties, indicating the therapeutic potential of *Vachellia farnesiana* as a natural remedy for oxidative stress and inflammation-related disorders.

Keywords: Ethnomedicine, *Vachellia farnesiana,* Anti inflammatory

Introduction
The use of plants for medicinal purposes, known as ethnomedicine, has a long history dating back to at least 60,000 years ago, as evidenced by records in the fossil records from the Middle Paleolithic age. Since that time, the practice of utilizing plants for their healing properties has persisted and been primarily advanced by indigenous peoples [1].

Plants are rich sources of antioxidants that play a crucial role in protecting against diseases linked to free radicals. These antioxidants are primarily in the form of secondary metabolites produced by plants. Phytochemicals, often referred to as 'plant chemicals,' represent the non-nutritive chemical constituents of plants with a wide array of health benefits and disease-prevention properties. These phytochemicals include flavonoids, polyphenols, carotenoids, and other compounds that contribute to the antioxidant capacity of plants. By scavenging free radicals and reducing oxidative stress, phytochemicals help prevent various diseases such as cardiovascular disorders, inflammation, cancer, neurodegenerative conditions, and aging-related ailments. Incorporating phytochemical-rich foods into one's diet, such as fruits, vegetables, herbs, and teas, can contribute significantly to overall health and well-being by harnessing the protective effects of antioxidants against oxidative damage [2].

Anti-inflammatory drugs play a crucial role in intervening in the pathophysiological process of inflammation, aiming to minimize tissue damage and enhance patient comfort. Various plant materials are valuable in treating inflammation due to the presence of secondary metabolites with anti-inflammatory properties. Therefore, utilizing medicinal plants for resolving inflammation is highly desirable. The treatment of inflammation involves several mechanisms that can serve as therapeutic targets, including inhibiting pro-inflammatory cytokines, reducing the activity of enzymes involved in inflammation like cyclooxygenase and lipoxygenase, modulating immune responses, scavenging free radicals, and regulating inflammatory signaling pathways. By targeting these mechanisms, anti-inflammatory drugs derived from medicinal plants can effectively alleviate inflammation, providing relief to individuals suffering from inflammatory conditions [3].

The plant *Vachellia farnesiana*, also known as sweet acacia, is recognized for its fragrant flowers and is widely distributed in various parts of the world, particularly in tropical and subtropical regions. Due to its appealing fragrance, it has gained popularity and become one of

the most extensively distributed species among all Acacia species [4]. *Vachellia farnesiana* is a compact, prickly, extensively branched, leaf-shedding shrub or small tree characterized by a broad, spreading crown and a height ranging from 2 to 7 meters. *Vachellia farnesiana* exhibits a diverse range of pharmacological activities that make it a valuable plant in traditional medicine. Its pods are utilized for treating sore throat and conjunctivitis, indicating potential antimicrobial and anti-inflammatory properties. The bark is known for its astringent and demulcent effects, suggesting its use in wound healing and soothing mucous membranes. Extracts from the bark and leaves of *Vachellia farnesiana* have been used historically in Colombia to treat malaria. These extracts have demonstrated activity against Plasmodium falciparum in animal models, indicating the plant's potential as an antimalarial. Additionally, crushed and boiled bark and leaf are used for inhaling to treat malaria symptoms. The root of *Vachellia farnesiana* is employed as an antispasmodic, aphrodisiac, astringent, demulcent, febrifuge, and stimulant, demonstrating its versatility in addressing various health concerns like diarrhea, rheumatism, and sore throat pain. Furthermore, active fractions from its extracts exhibit promising anti-diabetic and anti-inflammatory activities, and compounds isolated from its seeds display significant analgesic and anti-inflammatory effects, enhancing its pharmacological profile [5].

Materials & Methods

Plant material

The bark of *Vachellia farnesiana* were collected from the Changsari in the month of April and authenticated at Assam Bio-Resource Centre, Madan Kamdev, Kamrup, Assam under Assam Science Technology and Environment Council, Bigyan Bhavan, Government of Assam, vide accession no ABRC/1025/21.

Preparation of Extraction

The bark of *Vachellia farnesiana* was dried in shade, then made into a coarse powder by using a grinder. The dried powder of the bark was packed in a soxhlet apparatus and continuously extracted with ethyl acetate for two days till the extraction process is complete. After completion of extraction the solvent was removed by distillation process and after that concentrated extract was obtained, dried under reduced pressure using rotary evaporator at a temperature 37 °C.

Phytochemical screening

The phytochemical screening was performed to check the presence of secondary metabolites.

Quantitative standards

The Indian Pharmacopoeia procedure was followed to assess the quantitative standards, such as total ash, acid-insoluble ash, water-soluble ash, extractive values, and loss on drying. This examination was performed thrice, and the averages along with their standard errors were computed [6].

In vitro antioxidant assay

Determination of total phenolic content

The Folin-Ciocalteu assay, employing gallic acid as a reference, was utilized via spectrophotometry to determine the total phenolic concentration in the bark extract. Specifically, 0.5 mL of leaf extract, 3 mL of distilled water, and 0.25 mL of Folin-Ciocalteu reagent were mixed to form the reaction mixture, which was then agitated. Subsequently, 1 mL of 7.5% Na_2CO_3 was added after a 5-minute incubation in darkness, followed by incubation at room temperature in the dark for 90 minutes. Concurrently, a reagent blank was prepared using distilled water. The absorbance at 760 nm was measured using a double-beam UV/Vis spectrophotometer against the prepared reagent blank. The total phenolic compound content in the extract was quantified in milligrams of gallic acid equivalent (mg GAE/100 g) per 100 g of sample. All samples were analyzed in triplicate [7].

Determination of total flavonoid content

An aluminum chloride assay was utilized to determine the flavonoid content. In summary, 0.5 mL of the bark extract was mixed with 2 mL of distilled water in a 10 mL test tube. Each test tube contained 0.15 mL of 5% NaNO2, and 0.15 mL of 10% AlCl3 was added after a 5-minute incubation. Subsequently, 1 mL of 1 M NaOH was added after 1 minute, and the volume was adjusted to 5 mL with distilled water. The absorbance of the solution at 510 nm was measured after 10 minutes. The total flavonoid content in each sample was expressed as milligrams of quercetin equivalent per 100 grams of sample (mg QE/100 g sample), with quercetin serving as the standard. All samples were analyzed in triplicate [7].

Determination of hydroxyl radical content

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and MEGL for hydroxyl radical generated by Fe3+-Ascorbate–EDTA– H_2O_2 system (Fenton reaction) according to the method described by Mahendran *et al*. 2021 with trivial modifications in the method. Absorbance was measured spectrophotometrically at 535nm against control [8].

Determination of DPPH radical scavenging activity

The antioxidant potential of any compound can be determined based on its scavenging activity of the stable DPPH (1, 1-diphenyl-2-picrylhydrazyl hydrate) [9]. Radical scavenging activity of the bark extract *Vachellia farnesiana* against stable DPPH was determined spectrophotometrically using the method as described by Baliyan *et al*. 2022 with trivial modifications in the method. The absorption maximum of a stable DPPH radical in methanol was at 517nm. When DPPH reacts with an antioxidant, which can donate hydrogen, it gets reduced [10].

In vitro Anti-inflammatory activity

According to the procedures defined by Anosike *et al*., the *in vitro* anti-inflammatory activity was assessed using the human red blood cells (HRBCs) membrane stabilization method (HRBCs-MSM) [11].

Results

Extraction of *Vachellia farnesiana* bark

The bark of *Vachellia farnesiana* was extracted using the efficient soxhlet extraction method with ethyl acetate as the solvent. The resulting yield was determined to be 17.56% w/w based on the weight of the crude drug used for extraction.

Quantitative standards

Quantitative standards play a crucial role in evaluating crude drugs, whether in powdered form or as a whole. The efficiency and quality of these crude drugs were assessed, and the findings are detailed in Table 1.

Table 1: Quantitative standards of crude drugs

Sl. No.	Parameters	Mean ± S.D.
1.	Total ash	11.53 ± 0.175
2.	Acid insoluble ash	10.5 ± 13.806
3.	Water soluble ash	6.806 ± 0.020
4.	Water soluble extractive	85.55 ± 0.125
5.	Alcohol soluble extractive	33.58 ± 43.12
6.	Loss on drying	90.20 ± 0.06

Phytochemical screening

The bark extract of *Vachellia farnesiana* was individually tested for the presence of different phytoconstituents, including alkaloids, amino acids, carbohydrates, fats and fixed oils, flavonoids, glycosides, saponins, gums, lignins, proteins, steroids, triterpenoids, tannins, and phenolic compounds. The results are expressed in Table 2.

Table 2: phytochemical screening of ethyl acetate extract of *Vachellia farnesiana*

Sl. No.	Plant constituents	Results
1.	Alkaloids	+
2.	Amino acid	_
3.	Carbohydrates	_
4.	Fats & Fixed oils	_
5.	Flavonoids	+
6.	Glycosides	+
7.	Steroids	+
8.	Tannins	+
9.	Phenols	+
10.	Quinones	-

In vitro antioxidant assay

Determination of total phenolic content

The phenolic compound content in the ethyl acetate bark extract of *Vachellia farnesiana* was quantified using the Folin-Ciocalteu reagent, expressed in terms of gallic acid equivalent was found to be 0.15GAE/g. While the Folin-Ciocalteu method is a commonly used and quick assay for assessing total phenolic content, it's important to note that different phenolic compounds can exhibit varying responses within this method.

Table 3: Determination of total phenolic content of the test and the standards

Sample	Concentration (mg ml^{-1})	Absorbance (Mean ± S. E. M)
Standard	20	0.267±0.2902
	40	0.397±0.2052
	60	0.498±0.1675
	80	0.792±0.1451
	100	0.987±0.1297
Test	100	0.097±0.0268

Determination of total flavonoid content

The flavonoid compound content in the bark extract of *Vachellia farnesiana* was determined using the Folin-Ciocalteu reagent and expressed in terms of gallic acid equivalent was found to be 0.21GAE/g. Flavonoids are known to possess significant antioxidant properties and offer substantial benefits to human nutrition and health. Their mechanisms of action are believed to involve scavenging or chelating processes. The bark extract of *Vachellia farnesiana* contains a considerable number of flavonoids, which could play a significant role in the antioxidant activity of the plant.

Table 4: Determination of total flavonoid content of the test and the standards

Sample	Concentration (mg ml^{-1})	Absorbance (Mean ± S. E. M)
Standard	20	0.292±0.0011
	40	0.336±0.0020
	60	0.642±0.0012
	80	0.852±0.0131
	100	0.995±0.0011
Test	100	0.084±0.0012

Determination of hydroxyl radical content

The findings regarding hydroxyl radical scavenging activity are depicted in Table.5 and Figure4. The scavenging activity against hydroxyl radicals became evident as the concentration of the ethyl acetate extract from *Vachellia farnesiana* bark and the reference standard ascorbic acid increased, leading to a gradual rise in percentage scavenging activity. The IC50 values were determined to be 156.44 µg/ml for the extract and 153µg/ml for ascorbic acid, respectively.

Table 5: Determination of hydroxyl radical content the test and the standards

Sample	Concentration	% inhibition
Test	20	14.13±0.0014
	40	18.22±0.0016
	60	26.24±0.0017
	80	29.15±0.0011
	100	35.12±0.0022
Standard	20	15.22±0.0017
	40	20.78±0.0014
	60	27.16±0.0015
	80	30.86±0.0012
	100	36.00±0.0011

Determination of DPPH radical scavenging activity

The DPPH reduction capability was assessed by measuring the decrease in its absorbance at 517 nm, which is caused by antioxidants. The extract displayed its highest ability to donate hydrogen in the presence of DPPH free radicals at higher concentrations. It demonstrated a robust capacity to donate hydrogen and effectively scavenge DPPH radicals, showcasing antioxidant activity with an IC50 value of 145µg/ml. In comparison, the well-known antioxidant ascorbic acid showed an IC50 value of 139µg/ml against DPPH radicals. *Vachellia farnesiana* exhibited significant scavenging effects that increased with concentration compared to ascorbic acid.

Table 6: Determination of DPPH radical scavenging activity of the test and the standards

Sample	Concentration	% inhibition (Mean ± S.D.)
Test	20	15.59± 0.020167
	40	17.98 ±0.020275
	60	27.08 ±0.016275
	80	29.11±0.016321
	100	38.52± 0.016275
Standard	20	16.92±0.3259
	40	18.01±0.4684
	60	28.46±0.2556
	80	30.54±0.2848
	100	39.92±0.0254

In vitro Anti-inflammatory activity

The ethyl acetate extract of *Vachellia farnesiana* at varying concentrations (50, 100, 150 mg/mL) demonstrated considerable stabilization effects on HRBC membranes. The level of protection, measured as a percentage, was notably

higher at a concentration of 150 mg/mL compared to lower concentrations. However, protection decreased at higher concentrations. The findings have been organized and presented in a tabular format in Table 7.

Table 7: *In vitro* Anti-inflammatory activity of the test and the standards

Sample	Concentration	% inhibition	IC_{50} value
Test	50	47.89	130.04
	100	42.45	
	150	54.07	
Standard	50	44.31	115.05
	100	47.45	
	150	54.08	
Control	0.092		

Discussion and Conclusion

The study focused on exploring the medicinal potential of *Vachellia farnesiana* bark extract, traditionally used in ethnomedicine for various ailments. The extraction process yielded a significant amount of extract, indicating the efficient extraction method employed. Quantitative standards assessment revealed acceptable levels of total ash, acid-insoluble ash, water-soluble ash, extractive values, and loss on drying, ensuring the quality and efficacy of the crude drug. Phytochemical screening confirmed the presence of valuable phytoconstituents like alkaloids, flavonoids, glycosides, steroids, tannins, and phenols, which contribute to the plant's therapeutic properties. The *in vitro* antioxidant assays demonstrated the extract's potent antioxidant activity, as evidenced by high total phenolic and flavonoid contents and effective scavenging of hydroxyl and DPPH radicals, highlighting its potential in combating oxidative stress-related diseases. Additionally, the extract exhibited significant anti-inflammatory activity, as indicated by its ability to stabilize human red blood cell membranes, suggesting its potential in mitigating inflammatory conditions. These findings collectively support the traditional use of *Vachellia farnesiana* and underscore its promising pharmacological profile as a valuable source of natural medicine for various health applications. Further studies, including *in vivo* evaluations and clinical trials, are warranted to fully elucidate its therapeutic mechanisms and validate its efficacy and safety for medicinal use.

Fig 1: Image of *Vachellia farnesiana*

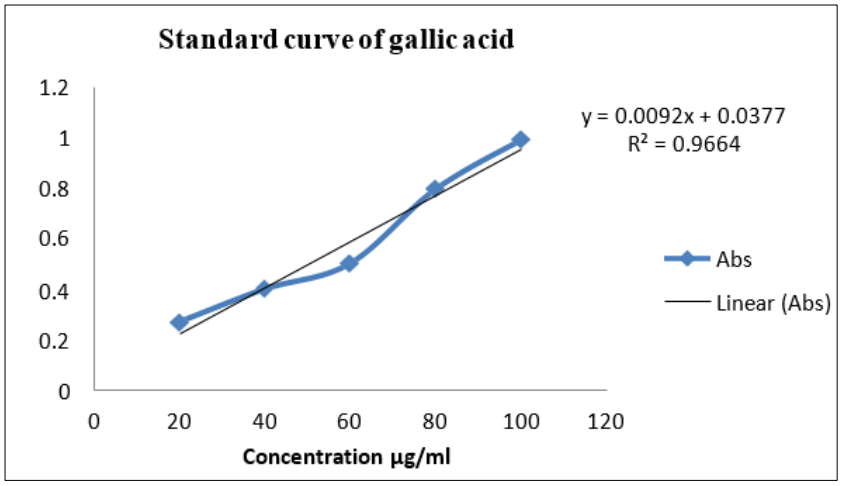

Fig 2: Standard curve of gallic acid

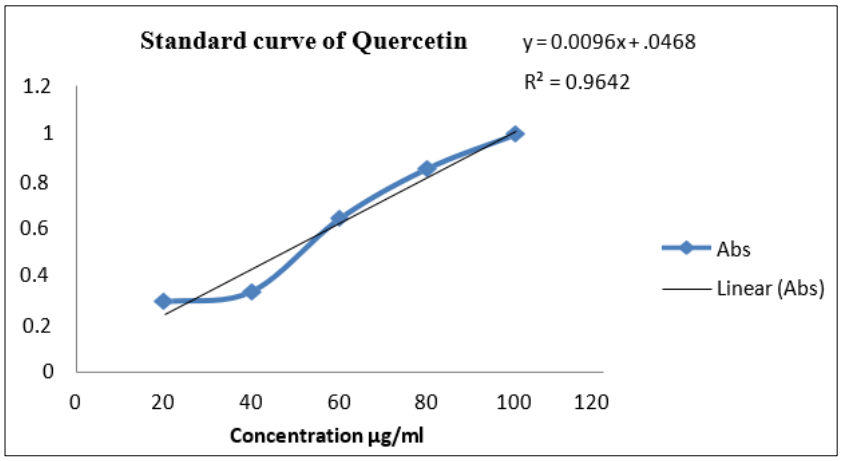

Fig 3: Standard curve of quercetin

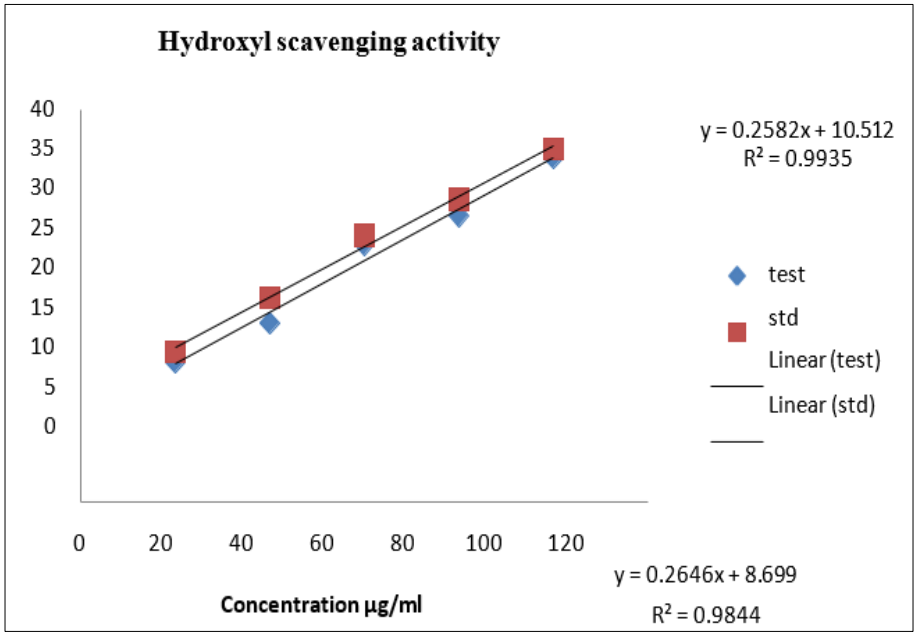

Fig 4: Graph of hydroxyl scavenging activity

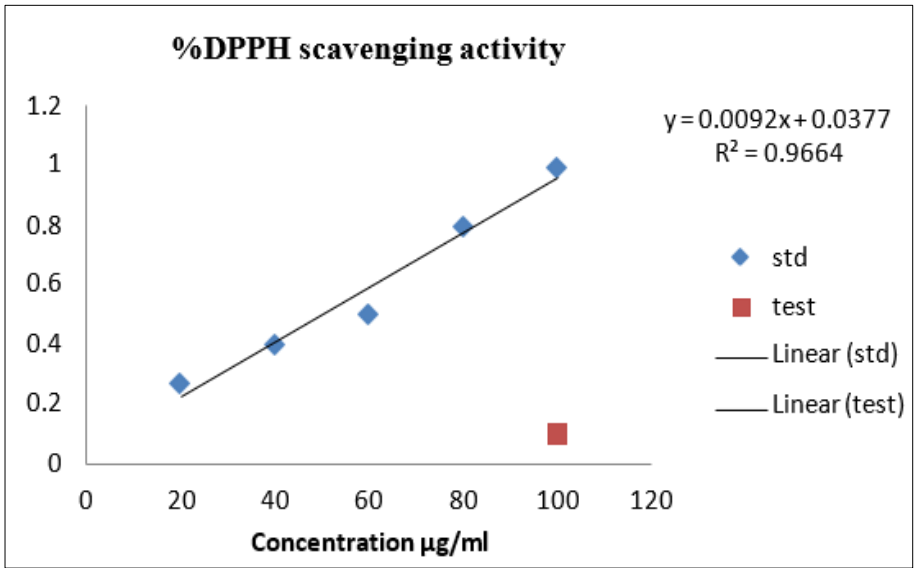

Fig 5: Graph for DPPH scavenging activity

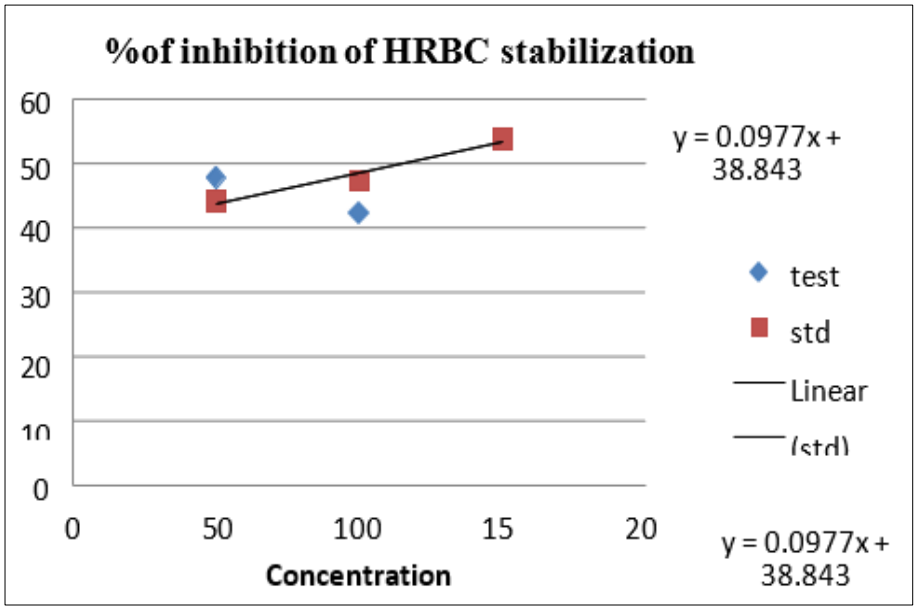

Fig 6: Comparative Graph of human red blood cell membrane stabilization of test

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