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EXPLORING BIOACTIVE PROPERTIES OF METHANOLIC EXTRACTS FROM AYURVEDIC PLANTS: AN IN VITRO ASSESSMENT OF ANTI-MUTAGENIC, ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITIES

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ABSTRACT

Medicinal plants have been used in healthcare since ancient time. Medicinal plants play dynamic roles in disease prevention and fit into all prevailing prevention strategies. They are significant source of molecule with medicinal or healing properties like phytochemicals and other bioactive compounds. These compounds, metabolites have therapeutic potential and give medicinal properties to the plant. The constituent compounds, bio actives and metabolites represent a significant pool for discovery of new drugs. Medicinal plants are precious assets in the fight against serious diseases like malignancies, cardiac diseases, arthritis and other bacterial and pathogenic infections etc. The goal of the current study was to determine the anti-mutagenic potential of Daruharidra (*Berberis Aristata*), *Guduchi* (*Tinospora Cordifolia*), *Saptarangi (Salacia Oblonga), Vekhand (Acorus Calamus*), it was evaluated using the Ames test. Where *Vekhand*, *Guduchi*, and *Daruharidra* exhibited significant anti-mutagenic activity, while *Saptarangi* showed lower efficacy. The well diffusion method was assessed to determine the antimicrobial activity of plant extracts, the Vekhand and Daruharidra have shown notable inhibition against all three bacterial species, *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus)* and *Pseudomonas.* The phytochemical screening revealed the presence of tannins, saponins, terpenoids, steroids, and carbohydrates in varying amounts, while alkaloids, cardiac

glycosides were totally absent. The quantitative determination of vitamin P showed varying optical densities, with *Saptarangi* exhibitng the highest concentration. Total phenolic content was assessed using the *Folin-Ciocalteu reagent*, with *Guduchi* displaying the highest value. The antioxidant activity, measured through the *DPPH* assay, demonstrated substantial free radical scavenging capacity, with *Guduchi* and *Saptarangi* showing the highest activity. The results highlight the potential of these plant extracts as sources of bioactive compounds with promising therapeutic applications in preventing microbial infections, mutagenesis, and oxidative stress.

KEYWORDS: Phytochemicals**,** *Acorus Calamus, Tinospora Cordifolia, Salacia Oblonga, Berberis Aristata,* Anti-mutagenic, Antioxidant, Antimicrobial.

INTRODUCTION

India is known for its traditional medicinal system, *Ayurveda*. Which is considered the oldest health care system in the world.[1],[2] It serves natural resource like plants, based medication, curative therapies. *Ayurveda*, the ancient system of traditional Indian medicine, continues to thrive as one of the oldest living medical traditions. While India has made significant strides in advancing *Ayurvedic* therapies through research and a science-based approach.[2] The importance of medicinal plants and traditional health systems in addressing global health challenges, is gaining increasing recognition. As a result of this growing interest, research on medicinal plants is rapidly expanding worldwide.[3]

Medicinal plants been used as safest curative modality since thousands of years. Plants were being used in different forms like crude extract or pure constituents isolated from them. The phytochemical constituents and metabolites possess therapeutic potential, contributing to the medicinal properties of the plant. The constituent compounds, bio-actives, and metabolites form an important reservoir for the discovery of new drugs. Medicinal plants have been proved to exhibit a spectra of therapeutic properties like, anti-microbial, anti-inflammatory, anti-oxidant, anti-diabetic and antitumor /anticancer etc. The World Health Organization (WHO) has reported that 80% of the global population depends on traditional medicine for their primary healthcare needs, with the majority of these treatments involving plant extracts and their active compounds.[4]

According to WHO (WHO, 2018) Cancer is the second leading cause of death worldwide, responsible for approximately 9.6 million deaths in 2018. Mutation is the root cause of

majority cancers or malignancies and Mutagens are involved in the initiation and promotion of several human diseases including cancer.[5] It is reported that phytochemicals like flavonoids and phenolic compounds are responsible for anti-mutagenic property and exhibiting various other biological activities like antimicrobial, antioxidant (free radical scavenging) and anti-inflammatory.[6] Plant-derived agents are also being utilized in cancer treatment. Several anticancer drugs, including *Vincristine*, *Taxo*l, *Vinblastine*, their derivatives, *Irinotecan*, *Topotecan*, and Etoposide derived from *Epipodophyllotoxin*, are in clinical use globally.[7]

Acorus Calamus, Berberis Aristata, Salacia Oblonga and *Tinospora Cordifolia* are commonly known as *Vekhand, Daruharidra, Saptarangi and Guduchi* in India. These plants are widely studied for their therapeutic properties and have a long history of use in traditional medicine systems. The *Acorus Calamus* known to possess therapeutic activities like antimicrobial, anti-inflammatory etc. *Acorus calamus* (*Vekhand)*demonstrated a significant percentage of inhibition, ranging from 60.92% to 83.60%, against mutagenicity induced by *Sodium azide* (NaN3) and *methyl methane sulphonate (MMS)*.[5] *Berberis aristata* (*Daruharidra*) is commonly used in *Ayurveda* to treat infections, digestive disorders, jaundice, and skin diseases. In addition, it possesses antimicrobial and anti-inflammatory, Antioxidant, anti-cancer properties.[8] It has been reported to exhibit strong anti-proliferative activity and is suggested for use in cancer treatment.[9] *Salacia Oblonga (Saptarangi*) primarily used in *Ayurvedic medicine* for controlling blood sugar levels, managing diabetes, and improving overall metabolic function. It is also known for its ability to reduce body fat and support digestive health,[10] also has notable antibacterial activity against pathogenic bacteria.[11] It is reported that plyphenols can be the potent antioxidant and free radical scavenging compounds in *S. oblonga* aerial and root extracts, the presence of phytochemicals which contribute to activities like antimicrobial, antioxidant and anticancer.[12] *Tinospora Cordifolia (Guduchi)* a highly revered herb in *Ayurvedic medicine*, known for its immunomodulatory, anti-inflammatory, anti-oxidative and detoxifying effects. It is proven that *Tinospora Cordifolia* possess antibacterial activity and plays a significant role in combating antimicrobial resistance in clinical isolates,[13] shown significant hepato-protective activities at the concentration (200 mg/kg) against carbon tetrachloride induced damage in rats.[14]

MATERIALS and METHODS

The dried plant powder of *Daruharidra (Berberis Aristata), Guduchi (Tinospora Cordifolia), Saptarangi (Salacia Oblonga), Vekhand (Acorus Calamus)* was collected from local Ayurveda shop.

Bacterial strains

1. *Escherichia coli (E. coli) (ATCC 25922)*
2. *Staphylococcus aureus (S.aureus) (ATCC 29213)*
3. *Pseudomonas aeruginosa (P.aeruginosa) (ATCC 27852)*
4. *Salmonella (ATCC 1028)*

Preparation of plant extract

To prepare 10 ml of methanolic extract, 2 g of dried plant powder was dissolved in 10 ml of methanol (99.5 %) and kept undisturbed for 24 hours at room temperature. Then the solution is centrifuged, the supernatant is collected in labelled falcon tube and the residue discarded.

Qualitative analysis of phytochemicals

1. Test for alkaloids

Wagner's reagent

Procedure

- Take 500 µl of plant extract in test tube using a micro pipette.
- Add 100 µl of Wagner's reagent (2g of iodine, 6g potassium iodide in 100ml of distilled water)

Observation: Reddish orange or brownish red color precipitations taken as indicators for the presence of alkaloids.

2. Tests for tannins

Braymer's test

Procedure

- Take 500 µl of plant extract in test tube using a micro pipette.
- Add few drops of 5 % $Fecl_3$

Observation: The appearance of brownish, green or blue black color indicates the presence of tannin.

3. Tests for saponins

Foam Test

Procedure

- Take 500 µl of plant extract in test tube using a micro pipette.
- Mixed with the same amount of distilled water and kept in a water bath for 10 minutes at 50ºc.
- After that add 6 ml of distilled water and leave for 2 minutes.

Observation: The presence of foam on top indicates presence of saponins.

4. Tests for terpenoids

Procedure

- Take 500 µl of plant extract in test tube using a micro pipette.
- Add 500 µl of Concentrated H_2SO_4 and heat for 2 minutes.

Observation: The greyish color indicates the presence of terpenoids.

5. Tests for steroids

Salkowski method

Procedure

- Take 500 µl of plant extract in test tube using a micro pipette.
- Add 1ml of chloroform and 100 µl of concentrated H2SO4.

Observation: The reddish brown color was taken as positive.

6. Tests for cardiac glycosides

Keller-Killani test

Procedure

- Take 500 µl of plant extract in test tube using a micro pipette
- Add 750 µl of glacial acetic acid and 1 drop of 5 % Ferric chloride.

Observation: The appearance of a blue color in the acetic acid layer indicates the presence of cardiac glycosides.

7. Tests for carbohydrates

Fehling's test

- Preparation of Fehling's reagent A: In 5 ml of distilled water add 0.35 g of $CuSO_4$.

- Preparation of Fehling's reagent B: In 5 ml of distilled water add 1.75 g of potassium sodium tartate and 0.6 g of NaOH.

Procedure

- Take 500 µl of plant extract in test tube using a micro pipette
- Add 250 µl of Fehling's A reagent and then Fehling's B reagent followed by heating for about 10 minutes.

Observation: A brick red precipitate of cuprous oxide.

Quantitative determination of flavonoids in the sample plant extract

Preparation of reagents

1. **Quercetin (1mg/ml)**

- Weigh 50 mg quercetin and dissolve it in 50 ml 80% ethanol.

2. **5 % $NaNO_2$**

- Weigh 2.5 g of sodium nitrite and dissolve it in 50 ml distilled water.

3. **10 % $AlCl_3$**

- Weigh 5 g aluminum chloride and dissolve it n 50 ml distilled water.

4. **1 M NaOH**

- 4g in 100 ml distilled water.

Procedure

Quantitative flavonoids test procedure

- **Blank Preparation**

Combine 3.7 mL distilled water (D.W.), 0.15 mL 5% $NaNO_2$, 0.15 mL 10% $AlCl_3$, and 1 mL 1 M NaOH.

- **Standard Solutions (T1 to T5)**

Prepare standard solutions by mixing varying volumes of D.W. (3.6–3.2 mL) and Quercetin (0.1–0.5 mL) with 0.15 mL each of 5% $NaNO_2$ and 10% $AlCl_3$, followed by 1 mL 1 M NaOH. Incubate all tubes (T1 to T5) for 5 minutes after adding $AlCl_3$ and for 6 minutes after adding NaOH.

- **Samples solutions**

Replace Quercetin with 0.5 mL of plant extracts in the in respective sample tubes and add 0.15 mL each of 5% $NaNO_2$ and 10% $AlCl_3$, followed by 1 mL 1 M NaOH. Incubate all tubes for 5 minutes after adding $AlCl_3$ and for 6 minutes after adding NaOH.

- **Measurement of optical density**

Take the absorbance at 510 nm for all solutions.

Total phenolic content

Principle: The Folin Ciocalteu reagent, is a combination of phospomolybdate and phosphotungstate which is a yellow colored solution, the phenolic compounds causes color change from yellow to blue. The intensity of blue color is directly proportional to concentration of phenolic in sample.

Preparation of reagents

1. **Gallic acid solution (1mg/ml)**

- Weigh 100mg of Gallic acid and dissolve in 100 ml of Distilled water**.**

2. **FC reagent**

- 0.2 N, 1ml in 10 ml

3. **Sodium carbonate**

- 15g of sodium carbonate in 200 ml of distilled water.

Procedure

- **Blank Preparation**

Mix 500 µL distilled water, 500 µL Folin-Ciocalteu (FC) reagent, and 800 µL 7.5% Na_2CO_3 solution.

- **Standard Solutions**

Prepare solutions by varying the volume of gallic acid (20–100 µL) and adjusting distilled water (500–400 µL), keeping 500 µL FC reagent and 800 µL Na_2CO_3 constant. All the tubes are incubated for 10 minutes after adding FC and for 1 hour after Na_2CO_3.

- **Sample Solutions**

Replace gallic acid with 50 µL of four plant extracts and mix with 450 µL distilled water, 500 µL FC reagent, and incubate for 10 minutes then 800 µL Na_2CO_3. Incubate all solutions for 1 hour.

- **Measurement of optical desity**

measure absorbance at 765 nm.

Anti-mutagenic Assay

- **Test bacteria: *Salmonella (ATCC 1028)***

- **Mutagen used: *Sodium azide*** (NaN3) **(1 µg / plate)**

The Ames test by using Salmonella histidine point mutation assay [15] by was used to test the anti-mutagenic activity of the extracts with some modifications. The positive and negative controls were made along with sample plates for comparison. The sample mixture was containing 500 µl of Phosphate buffer, 100 µl of *Salmonella culture* (ATCC 1028), 100 µl *sodium azide* (mutagen) and 100 µl of test extract. The test extract was not added in positive control mixture though all other constituents were similar to sample mixture. The negative control mixture was lacking both test extract and *sodium azide* (mutagen). All the controls and sample mixtures were pre-incubated for 30 minutes at 37 ºc, then the 100 µl of histidine is added in each mixture and immediately pour over minimal agar plate. The plates were incubated for 48 hours at 37 ºc and histidine independent revertant colonies were counted.

Procedure

- In laminar air flow, minimal media is prepared and sterilised by an autoclave, then placed into petri plates sterilised with UV light under aseptic conditions.
- Plates were kept undisturbed until the media solidifies.
- The positive, negative controls and sample extract mixtures were made in an Eppendorf tubes and pre-incubated, at the end of incubation time the histidine is added.
- The mixture from each Eppendorf tubes were poured to respective labelled petri plates containing minimal media and spreaded onto it with the help of sterile plate spreader.
- These petri plates were incubated at 37 ºc for 48 hours.
- Plates were observed for revert colonies.

Table 1: Composition of minimal media.

Reagents	Concentration (g/l)
Dextrose	1
Ammonium sulphate	1
Dipotassium phosphate	7
Monopottasium phosphate	2
Sodium citrate	0.5
Magnesium sulphate	0.1
Agar	15

Antimicrobial Assay

- **Test bacteria:** *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Pseudomonas.*

The well diffusion method was used to check the anti-microbial activity of plant extracts. On Muller Hinton Agar (MHA) medium, the bacterial suspension was spread throughout. The sterile cork borer (8mm) was used to form the wells in the medium. 100 µl plant extracts were added in each well. The zones of inhibition around the wells were observed after 24 hours of incubation at 37 ºc.

Procedure

- The MHA media is prepared in laminar air flow and sterilised by an autoclave and petri plates were cleaned and sterilised under UV light.
- The sterilised media was poured in petri plates and kept aside to solidify the media.
- he sterilised plate spreader was used to spread the bacterial suspension onto the media.
- Wells were formed with the help of sterilised cork borer and 100 µl plant extracts were added to the wells.
- The plates were incubated for 24 hours at 37 ºc.
- Plates were observed to check how sample reacted to the bacteria**.**

Table 2: Composition of Muller Hinton Agar media.

Reagents	Concentration (g/l)
Beef	**2**
Peptone	**17.5**
Starch	**1.5**
Agar	**17**

Antioxidant Assay

The DPPH (α, α-diphenyl-β-picrylhydrazyl) is a radical scavenging method offers the first approach for evaluating the antioxidant potential of an extract or compound. The assay is based on the measurement of the scavenging capacity of antioxidants towards it.[16] The DPPH solution is purple in colour, if the sample extract has electron scavenging property that is antioxidant activity the purple colour turns to colourless.

Preparation of reagent

- DPPH stock solution

- Weigh 0.0072 g of DPPH in 30 ml methanol**.**

Procedure

1. Take 5 test tubes labelled as Blank and with respective plant extract.
2. Add 1.5 ml of the DPPH stock solution to each test tube.
3. Then add 50 µl sample extract in respective labelled test tube.
4. Mix well and keep the test tubes in dark for 30 minutes at room temperature.
5. Measure the absorbance of each sample extract at 517 nm using UV spectrometer.
6. Calculate the percentage of DPPH scavenging activity using the following formula :

$$\text{DPPH scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

Table 3: DPPH method.

Tube	DPPH (ml)	plant extract (µl)		
Blank	**1.5**	**50**		
Vekhand	**1.5**	**50**		
Guduchi	**1.5**	**50**	**Incubate for 30 minutes in dark at Room temp.**	**Take O.D at 517 nm.**
Daruharidra	**1.5**	**50**		
Saptarangi	**1.5**			

RESULTS

Qualitative analysis of phytochemicals

The phytochemical screening of plant extracts was out. The test results showed that phytochemicals like Alkaloids, Cardiac glycosides were absent in all plant extracts, while

Tannins were present in all extracts. The other phytochemicals like Saponins, Terpenoids, Steroids and Carbohydrates were present in few any absent in others, the table no.(6) provides the individual results.

Table 4: Phytochemical screening.

Sr. No.	Phytochemicals	Vekhand	Guduchi	Saptarangi	Daruharidra
1.	Alkaloids	-	-	-	-
2.	Tannins	+	+	+	+
3.	Saponins	-	+	+	+
4.	Terpenoids	-	+	-	-
5.	Steroids	+	-	+	-
6.	Cardiac glycosides	-	-	-	-
7.	Carbohydrates	-	+	+	+

Quantitative determination of Flavonoids in the sample plant extract

Table 5: Absorbance of standards of vitamin P assay.

Tube	Concentration (mg/ml)	Optical Density at 765 nm
1.	0.1	0.09
2.	0.2	0.23
3.	0.3	0.35
4.	0.4	0.455
5.	0.5	0.566

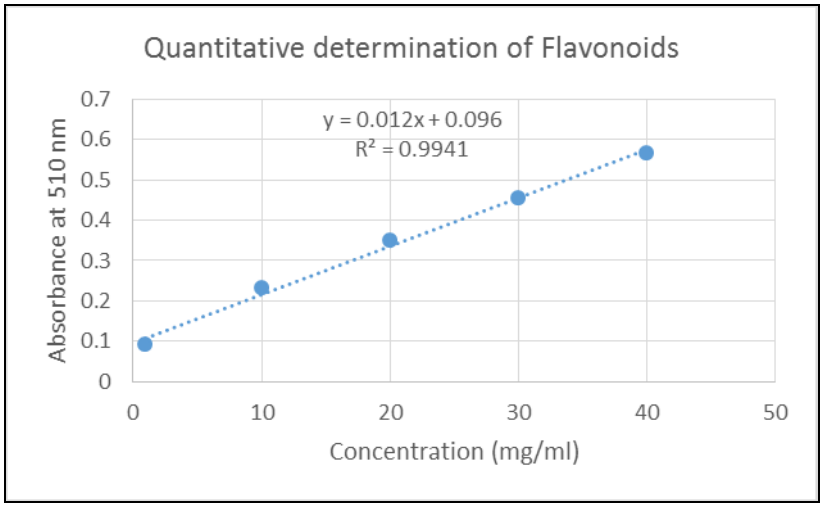


Fig.no. 1: Standard graph of flavonoids.

Table 6: Unknown concentrations of flavonoids in plant extracts.

Plant extract	Vekhand	Guduchi	Daruharidra	Saptarangi
Absorbance at 510 nm	0.337	0.225	0.442	1.384
Unknown concentration(mg/ml)	**20.083**	**10.75**	**28.833**	**107.33**

Total phenolic content

Table 7: Absorbance of standards of total phenolic assay.

Tube	Concentration (mg/ml)	Optical Density at 765 nm
1.	20	0.088
2.	40	0.455
3.	60	0.745
4.	80	1.050
5.	100	1.411

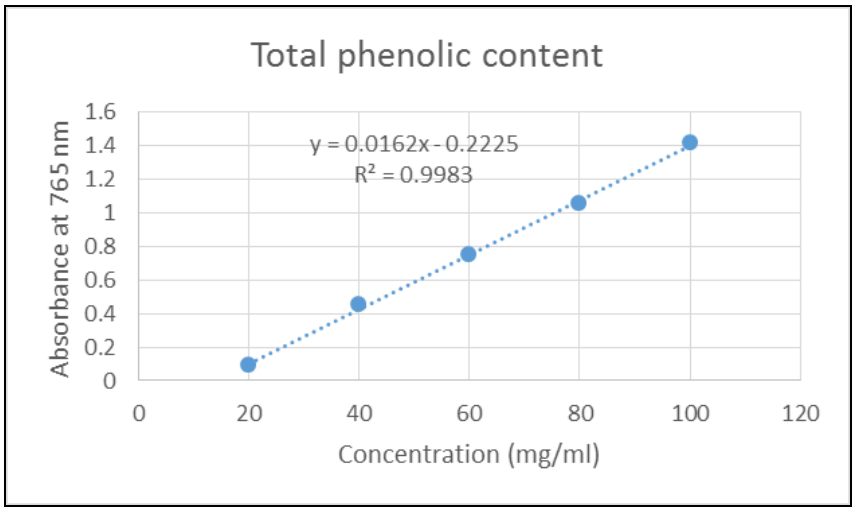


Fig.no. 2: Standard graph of total phenolic content.

Table 8: Unknown concentrations of total phenolic in plant extracts.

Plant extract	Vekhand	Guduchi	Daruharidra	Saptarangi
Absorbance at 765 nm	1.567	1.643	0.868	1.225
Unknown concentration(mg/ml)	**110.462**	**115.154**	**67.314**	**89.351**

Anti-mutagenic Assay

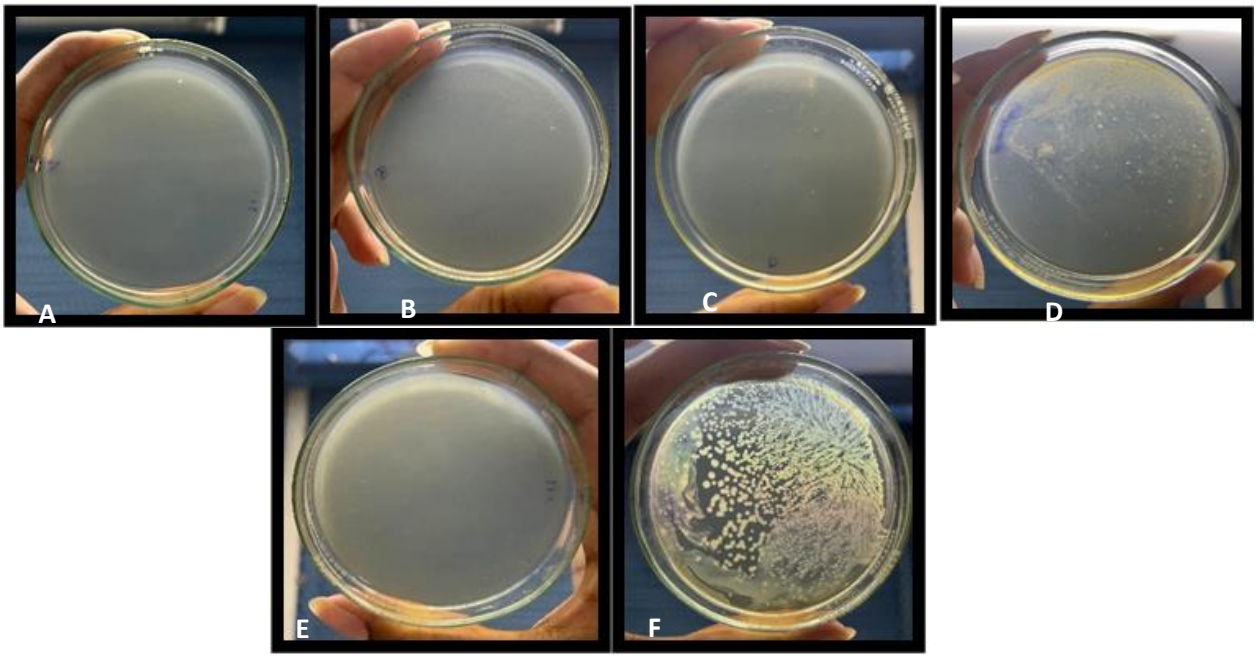
To check the anti-mutagenic activity of plant extracts against the mutagen added the Ames test was utilised. If the growth i.e. revert colonies were observed in the petri plates after the 48 hours' incubation, the plant extract was considering as less effective against mutagen, and if no growth is observed then the plant extract added exerts anti-mutagenic activity against mutagen.

The positive control plate showed no growth while negative control plate showed growth. However, there was absence of colonies (no growth) in the plates of *vekhand*, *guduchi and daruharidra*, few colonies were observed in the plate of *saptarangi.*

This suggests that the plant extract of *vekhand, guduchi and daruharidra* have anti-mutagenic quality, while the *saptarangi* plant extract is less active against the mutagen compare to other plant extract as it was unable to stop the growth completely.

Table 9: Results of Ames test after incubation.

Plates	Observation after 48 hours of incubation	Inferences
Positive control	No growth	-
Negative control	>100 colonies	-
Vekhand	No growth	Anti-mutagenic activity present
Guduchi	No growth	Anti-mutagenic activity present
Saptarangi	14 colonies	Low anti-mutagenic activity
Daruharidra	No growth	Anti-mutagenic activity present



Ames test petri plates after 48 hours of incubation: A: *Vekhand* (*Acorus Calamus*), B: *Guduchi* (*Tinospora Cordifolia*), C: *Daruharidra*(*Berberis Aristata*), D: *Saptarangi* (*Salacia Oblonga*), E : Positive control, F : Negative control.

Antimicrobial Assay

- *Vekhand*

Table 10: Growth inhibition of plant extracts against each pathogens.

Microorganism	Zone of inhibition (mm)
Escherichia coli (ATCC 25922)	15
Staphylococcus aureus (ATCC 29213)	25
Pseudomonas aeruginosa (ATCC 27852)	24

- *Guduchi*

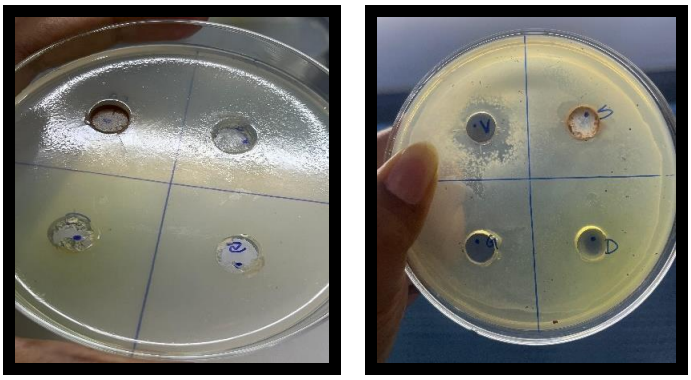
Microorganism	Zone of inhibition (mm)
Escherichia coli (ATCC 25922)	No inhibition
Staphylococcus aureus (ATCC 29213)	18
Pseudomonas aeruginosa (ATCC 27852)	19

- *Daruharidra*

Microorganism	Zone of inhibition (mm)
Escherichia coli (ATCC 25922)	19
Staphylococcus aureus (ATCC 29213)	25
Pseudomonas aeruginosa (ATCC 27852)	21

- *Saptarangi*

Microorganism	Zone of inhibition (mm)
Escherichia coli (ATCC 25922)	No inhibition
Staphylococcus aureus (ATCC 29213)	20
Pseudomonas aeruginosa (ATCC 27852)	18



Zone of inhibition shown by each plant extract against *S.aureus and Pseudomonas.*

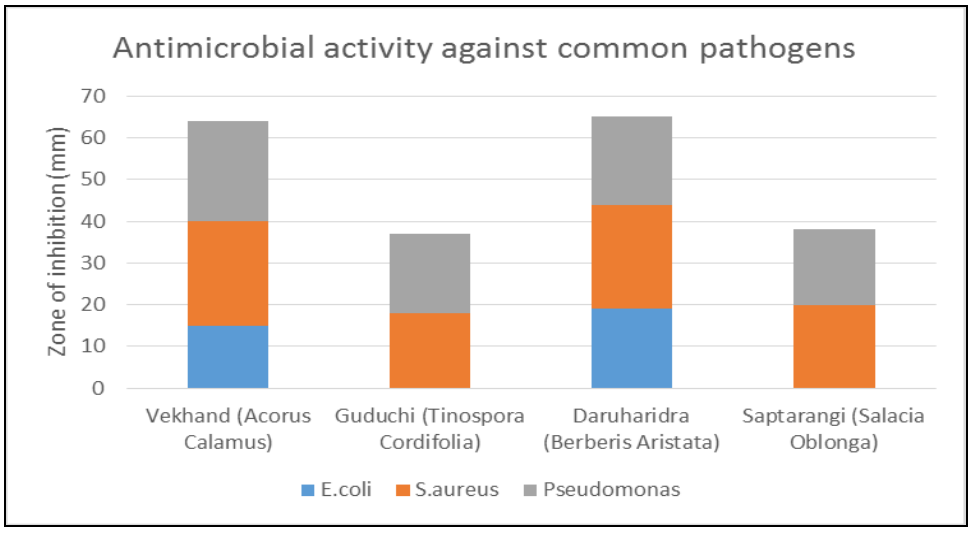


Fig. no. 4: Comparison of growth inhibition shown by each plant extracts against pathogens.

Antioxidant Assay

Table 11: DPPH scavenging activity of each plant extract.

Tube	Optical density at 517 nm	DPPH Scavenging activity(%)
Blank	0.786	-
Vekhand	0.350	**55.47 %**
Guduchi	0.175	**77.73 %**
Daruharidra	0.222	**72.01%**
Saptarangi	0.178	**77.35%**

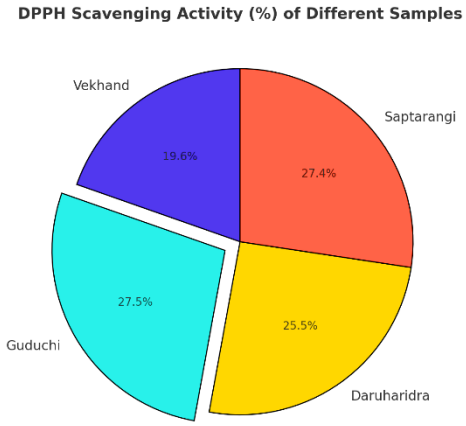


Fig. no. 5: Comparison of DPPH scavenging activity of four plant extracts.

DISCUSSION

Plant-based medicines have historically played a pivotal role in advancing human health, not only providing effective treatments but also serving as a foundation for the development of modern pharmaceuticals. The present study aimed to investigate the antimicrobial, antioxidant, and anti-mutagenic potential of four medicinal plant extracts: *Acorus calamus* (*Vekhand*), *Berberis aristata* (*Daruharidra*), *Salacia oblonga (Saptarangi*), and *Tinospora cord folia* (*Guduchi*). The phytochemical screening of these plants revealed the presence of several bioactive compounds, including saponins, tannins, carbohydrates, and terpenoids, which are directly associated with their therapeutic properties.

The quantitative assays of Vitamin P (bioflavonoids) and phenolic content further supported the pharmacological potential of these plant extracts. The presence of these compounds suggests that the plant extracts have the ability to reduce oxidative stress and neutralize free radicals, thus contributing to cardiovascular health and the prevention of chronic diseases such as cancer. The analysis of flavonoids levels revealed the following trend in concentration: *Saptarangi* > *Daruharidra* > *Vekhand* > *Guduchi*. For phenolic content, the trend was as follows: *Guduchi* > *Vekhand* > *Saptarangi* > *Daruharidra*. These findings

indicate that phenolic compounds and flavonoids (vitamin P), which are known for their antioxidant activity, are present in considerable amounts in the studied extracts.

The anti-mutagenic activity of the plant extracts was assessed through the Ames test, which measures the ability of a substance to induce mutations in genetic material. The results showed that *Vekhand*, *Guduchi*, and *Daruharidra* completely inhibited revert colony growth, indicating strong anti-mutagenic activity, while *Saptarangi* exhibited only a low level of efficacy. This potent anti-mutagenic activity can be attributed to the presence of phenolic compounds, flavonoids, and tannins, which are known to possess protective effects against genetic mutations and oxidative damage.[17]

The antimicrobial activity of the four plant extracts was evaluated against common pathogenic bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The results demonstrated that *Vekhand* exhibited the most robust antimicrobial activity, followed by *Daruharidra*, *Saptarangi*, and *Guduchi*. Notably, *Saptarangi* and *Guduchi* showed no inhibition against *E. coli*, highlighting their relatively lower antimicrobial potency against this bacterium. The overall antimicrobial trend was as follows: *Vekhand* > *Daruharidra* > *Saptarangi* > *Guduchi*.

Finally, the antioxidant potential of the plant extracts was assessed by measuring their DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity, a common method for evaluating antioxidant capacity. The results revealed that *Guduchi* exhibited the highest antioxidant activity, followed by *Saptarangi*, *Daruharidra*, and *Vekhand*. The antioxidant potential of these plants suggests their potential for therapeutic use in conditions where oxidative stress plays a role, such as cancer and cardiovascular diseases, Arthritis etc.

In conclusion, the findings from this study highlight the significant pharmacological potential of the four plant extracts in the prevention and treatment of chronic diseases, including cardiovascular diseases, cancer, and arthritis. However, it is important to note that medicinal plant-based treatments may also pose potential health risks. Therefore, comprehensive investigations into the side effects and toxicological profiles of these plant extracts are crucial before their widespread clinical application.

CONCLUSION

This study explored the pharmacological properties of four medicinal plant extracts—*Acorus calamus* (Vekhand), *Berberis aristata* (*Daruharidra*), *Salacia oblonga* (*Saptarangi*), and *Tinospora cordifolia* (*Guduchi*)—focusing on their antimicrobial, antioxidant, and anti-mutagenic activities. The phytochemical analysis revealed the presence of bioactive compounds such as saponins, tannins, carbohydrates, and terpenoids, which are linked to various therapeutic effects. Additionally, the quantitative assays for flavonoids and phenolic content indicated the potential of these plant extracts as strong antioxidants, which may contribute to the prevention of oxidative stress-related diseases, including cancer and cardiovascular disorders.

The Ames test demonstrated that *Vekhand*, *Guduchi*, and *Daruharidra* exhibited significant anti-mutagenic activity, effectively inhibiting mutagen-induced revert colony growth. In contrast, *Saptarangi* displayed only moderate anti-mutagenic potential. Antimicrobial assays highlighted *Vekhand* as the most effective against common pathogens, including *Staphylococcus areus* and *Pseudomonas aeruginosa*, while *Saptarangi* and *Guduchi* showed limited or no activity against *Escherichia coli*. Regarding antioxidant activity, *Guduchi* exhibited the highest DPPH scavenging activity, followed closely by *Saptarangi*, *Daruharidra*, and *Vekhand*.

These results underscore the considerable pharmacological potential of the studied plant extracts, suggesting their therapeutic value in combating oxidative stress, microbial infections, and genetic mutations. However, further research is essential to evaluate the safety profiles, including potential toxicity and side effects, of these plant extracts. Overall, this study lays a solid foundation for future investigations into the therapeutic use of these plants in managing chronic diseases, while also highlighting the need for comprehensive clinical evaluation before their widespread application in healthcare.

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