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Pharmacological Screening of *Bassia Latifolia* for Anti-Microbial Potential

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Abstract

This research is crucial in order to validate the traditional medicinal uses of these plants and to identify potential in new antimicrobial compounds. By providing quantitative data on plant oils and extracts, we can meet the growing demand for safe and effective natural products in the market. The presence of active pharmacological elements like tannins, phenolic acids, saponins, flavonoids, and alkaloids was detected through phytochemical screening. As a result, ability to defend the plant against infections and obstruct the development of microorganisms, these substances are known to be biologically active. Phytochemicals typically use different mechanisms than synthetic drugs to carry out their antimicrobial effects. The methanolic extract of *Bassia latifolia* underwent phytochemical analysis, revealing the presence of carbohydrates, steroids, proteins, amino acids, glycosides, flavonoids, saponins, and saponins.

Keywords:

1. INTRODUCTION

1.1 Natural Product

Natural products have wide range of multi-dimensional chemical structures; however, the utility of natural products as biological function modifiers has received considerable attention. They have since been successfully used in the discovery of new drugs and have had a far-reaching impact on chemicobiology. [1-3]

The high structural diversity of natural products has been realized over the past century thanks to the view from physical chemistry. Their three-dimensional chemical and steric properties are intricate and well-organized, and they have a variety of advantages, which contribute to their effectiveness. Regarding the effectiveness and specificity of molecular targets. An effective example of a drug Artemisinin and its analogs were developed from natural products and are currently used extensively for the malaria prevention method. Research on natural products significantly contributed to drug development, with 54% of approved anticancer drugs between 1940 - 2002 being derived from natural products or drugs inspired by their knowledge. [4-5]

Some effective anticancer medications that were first derived from plants include the Vinca alkaloids from Catharanthus roseus and the Terpene paclitaxel from Taxus baccata. Natural products have been successfully used in the creation of new drugs, particularly in the search for novel chemical structures, between 1981 and 2002. Drugs made from natural products have played a significant role in that 22-year period. That is especially true for anti-hypertensives, where about 64% of recently synthesized drugs have their roots in the structures of natural products. Natural products have persisted in playing a crucial role in many drug development and research programs because of their unparalleled chemical diversity and novel mechanisms of action.

The ability of those natural products to interact with a wide variety of biological targets has evolved in intriguing and significant ways, and some of them have even become some of the most crucial medications in the medical system. [6]

For example, plants, microorganisms, and animals manufacture small molecules, which have played a major role in drug discovery. Among the 69 small-molecule new drugs approved from 2005 to 2007 worldwide, 13 were natural products or originated from natural products, which underlines the importance of such products in drug research and development. Over the past 50 years, a great diversity of new drugs has been developed using high-throughput screening methods and combinatorial chemistry; however, natural products and their derived compounds have continued to be highly important components in pharmacopeias. Therefore, there is great potential for future discoveries from plants and other natural products, which, thus, offer huge potential for deriving useful information about novel chemical structures and their new types of action related to new drug.[7, 8]

1.2 Traditional Medicines (TM):

The use of TM in the diagnosis, treatment, and prevention of physical and mental illnesses makes it the oldest system of healthcare in existence. Throughout history, various societies have created numerous practical therapeutic techniques to treat a wide range of serious and life-threatening illnesses. Today, TM is still widely used and has many different names, including complementary and alternative medicine and ethnic medicine. [7] The majority of the medications used in TM come from organic sources. "Clinical trials" have been carried out in TM since the beginning of time. In the case of TCM, a great deal of knowledge and advancements have been gathered and developed over the past thousands of years with regard to preparation techniques, herb selection, material identification, and the ideal time to obtain different plants. To increase drug efficacy and decrease drug toxicity, TCM urgently needs appropriate processing and dose regulation. Clinical studies have yielded a sizable amount of data, and in this way, TM has aided in the creation of contemporary medications. The use of natural products gives TM advantages over conventional medicine in areas like the identification of lead compounds and drug candidates, the investigation of drug-like activity, and the investigation of physicochemical, biochemical, pharmacokinetic, and toxicological properties. If any type of TM is successfully implemented, it might surprisingly aid in the creation of new medicines, which would have a variety of positive effects, including significant cost savings. TCM has now become a crucial component of Chinese public health. Western nations have gradually come to accept TCM as a complementary or alternative medicine in recent years. The most significant element of TCM, Chinese herbal medicine, is currently used in the healthcare of an estimated 1.5 billion people worldwide. It should be noted that TCM uses strict guidelines to combine various herbs and ingredients to create prescriptions known as formulas (fang ji in Chinese). According to their various roles in the formula, a classic formula typically consists of four elements: the "monarch," "minister," "assistant," and "servant," each of which contains one to several drugs. The ideal combination of these medications will have the desired therapeutic effect and minimize side effects.[8]

1.3 Drugs Developed from Natural Products:

In the 1960s, Chinese herb Schisandra chinensis was found to have enzyme-reducing and hepatoprotective effects. Researchers isolated its chemical constituents and discovered that bifendate, an intermediate compound, had stronger pharmacological activity and low preparation costs. They studied the structure and activity relationships of bifendate and its analogs, synthesizing novel derivatives. Bicyclol, a new compound, was designed and synthesized, showing greater in vivo absorption and better bioavailability. Bicyclol showed antifibrotic and hepatoprotective effects against liver injury and liver fibrosis, and also inhibited hepatitis B virus replication in chronic hepatitis B patients. Bicyclol, a new antihepatitis drug, has been approved for treating chronic viral hepatitis in China since 2004. It has significant hepatoprotective effects, antihepatitis virus activity, and fewer adverse reactions. Bicyclol has independent intellectual property rights and belongs to Class 1 of China's New Chemical Drug. It has

been exported to many countries. In 1960, a program screening for cancer drugs from plants began in the United States, leading to the discovery of Taxus brevifolia. Taxol, a new compound, was isolated and developed as a novel anticancer drug. It was approved by the US Food and Drug Administration for treating ovarian cancer in 1992.[9,10]

1.4 Introduction to Anti-microbial activity

Antimicrobial susceptibility testing can be used for epidemiology, therapeutic outcome prediction, and drug discovery. During the "golden era" following the revolution, almost all significant Tetracyclines, cephalosporins, aminoglycosides, and macrolides were among the antibiotics discovered.[11, 12]

Chemotherapy's primary issues were resolved in the 1960s; unfortunately, history is repeating itself today, and the rise in microbial resistance puts these exciting compounds in danger of losing their effectiveness. With treatment failures linked to bacteria that are multi-drug resistant, it has a sizable impact and has elevated to the level of a global public health concern.

Therefore, finding new antibiotics is a particularly important goal. Today, one of the main sources of brand-new drug molecules is still natural products. They come from eukaryotic microorganisms, plants, prokaryotic bacteria, and various animal organisms. The majority of the antimicrobial compounds found so far is made from microbial and plant products. [10]

A wide variety of intricate and structurally diverse compounds can be found in plants and other natural sources. Pure secondary metabolites, essential oils, microbial extracts, and newly synthesized molecules have recently attracted the attention of many researchers who are looking into them as potential antimicrobial agents. The use of various non-standardized approaches in inoculum preparation techniques, inoculum size, growth medium, incubation conditions, and end-point determination made it difficult to compare the results when we reviewed the published articles on the anti-microbial effect of these natural products. The discovery that a plant extract has antimicrobial activity is intriguing, but this initial piece of information needs to be reliable and allow for comparison between studies. This helps prevent work in which antimicrobial activity research is used merely as a supplementary analysis to a phytochemical study. The in vitro antimicrobial activity of an extract or a pure compound can be assessed or screened using a variety of laboratory techniques. The broth or agar dilution and disk-diffusion methods are the most well-known and fundamental techniques. Other techniques, such as the poisoned food technique, are used, particularly for antifungal testing. Time-kill tests and flow cytofluorometric methods are recommended for in-depth investigation of an agent's antimicrobial effect, as they provide information on the nature of the inhibitory effect (bactericidal or bacteriostatic) (time-dependent or concentration-dependent) and the cell damage inflicted on the test microorganism. Because of the renewed interest in the properties of new anti-microbial products, such as combating multidrug-resistant bacteria, it is critical to gain a better understanding of the current methods for screening and/or quantifying the anti-microbial effect of an extract or a pure compound for applications in human health, agriculture, and the environment. As a result, we will discuss two types of antimicrobial activity.[13, 16]

- Agar disk-diffusion method.
- Agar well-diffusion method.

1.4.1 Agar disk-diffusion method

Agar disk-diffusion testing, which was developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. The Clinical and Laboratory Standards Institute (CLSI) now publishes a number of accepted and approved standards for bacteria and yeast testing. Although this method cannot test all fastidious bacteria accurately, it has been standardized to test certain fastidious bacterial pathogens such as streptococci, Haemophilus influenzae, Haemophilus parainfluenzae, Neisseria gonorrhoeae, and Neisseria meningitides using specific culture media, various incubation conditions, and interpretive criteria for inhibition zones. [10,11] Agar plates are inoculated with a standardized inoculum of the test microorganism in this well-known procedure. The agar surface is then covered with filter paper discs (about 6 mm in diameter) containing the test compound at the

desired concentration. The Petri dishes are incubated in the proper conditions. In general, an antimicrobial agent diffuses into the agar and inhibits the test microorganism's germination and growth, after which the diameters of inhibition growth zones are measured. However, because bacterial growth inhibition does not imply bacterial death, this method cannot differentiate between bactericidal and bacteriostatic effects. Furthermore, because it is impossible to quantify the amount of antimicrobial agent diffused into the agar medium, the agar disk-diffusion method is ineffective for determining the minimum inhibitory concentration (MIC). [12, 13]
Nonetheless, the disk-diffusion assay has many advantages over other methods, including simplicity, low cost, the ability to test a large number of microorganisms and antimicrobial agents, and the ease with which the results can be interpreted. [14, 15]

1.4.2 Agar Well diffusion method

Agar well diffusion is a popular method for determining the antimicrobial activity of plant or microbial extracts. The agar plate surface is inoculated in the same way that the disk-diffusion method is, by spreading a volume of the microbial inoculum over the entire agar surface. The well is then aseptically punched with a sterile cork borer or tip, and a volume (20-100 mL) of the antimicrobial agent or extract solution at the desired concentration is introduced. The agar plates are then incubated under appropriate conditions based on the test microorganism. The antimicrobial agent diffuses through the agar medium and inhibits the growth of the tested microbial strain. [10,13]

2. MATERIAL AND METHOD

Bassia latifolia, a medicinal plant, was collected in Bhopal, Madhya Pradesh. After cleaning, the fruit was dried in the shade at room temperature until completely dry. To avoid contamination and deterioration, dried plant parts were stored in airtight glass containers in a dry and cool environment. A plant taxonomist authenticated the medicinal plant *Bassia latifolia* to confirm its identity and purity.[19, 20]

2.1 Extraction of plant by soxhlet extraction method

Coarsely powered plant parts of *Bassia latifolia*(300 gm) was then extracted by successive extraction using different organic solvents, defatted with petroleum ether and successively extracted with methanol for 36 hrs using soxhlet apparatus. To ensure complete extraction each extract was evaporated to dryness under reduced pressure by rotary evaporator and the resulted dried residue was stored in airtight container for further use Formula;

$$\% \, yield = \frac{\text{Actual yield}}{\text{Theoretical yield x100}}$$

2.2 Phytochemical investigation

An experiment was carried out to determine the presence or absence of various phytoconstituents using detailed qualitative phytochemical analysis. The color intensity or precipitate formations were used as medical responses to tests.[21, 25]

2.2.1 Test for Carbohydrates

- **Molisch'sTest:** The aqueous extract of *Bassia latifolia* was mixed with a few drops of Molish reagent (naphthol) and conc. H_2SO_4 (sulphuric acid) was added dropwise along the test tube wall. When two liquids combine, a purple color ring forms at the junction. It indicates that carbohydrates are present.
- **Fehling's Test:** 1ml of Fehling A and Fehling B solutions were mixed together, and 2ml of *Bassia latifolia* extract aqueous solution was added. Cook for 5-10 minutes in a water bath. The presence of reducing sugar is indicated by the formation of a reddish brown colored precipitate as a result of cuprous oxide formation.[22]
- **Benedict's test:** A test tube containing an equal mixture of Benedict's reagent and *Bassia latifolia*extract was heated in a water bath for 5 to 10 minutes. It appears green, yellow, or red, depending on how much reducing sugar is in the test solution, indicating the presence of reducing sugar.
- **Barfoed's Test:** 1 ml of Benedict solution was added to the aqueous solution of *Bassia latifolia*extract and heated to boiling. Due to the formation of cupric oxide in the presence of monosaccharide, a red color indication was observed.[23,24]

2.2.2 Tests for Alkaloids

- **Dragendorff'sTest**: 1 mL of *Bassia latifolia* extract was taken. With a few drops of acetic acid and Dragendroff's reagent, alcohol was mixed and thoroughly shaken. The presence of alkaloids is indicated by the presence of an orange red precipitate.
- **Wagner's Test:** 1ml of *Bassia latifolia* extract was dissolved in acetic acid. Wagner's reagent was added in small amounts. The reddish-brown precipitate was identified by the presence of alkaloids.
- **Mayer's Test:** 1 mL of extract from *Bassia latifolia* was dissolved in acetic acid with a few drops of Mayer's reagent added. The formation of a dull white precipitate indicated the presence of alkaloids.
- **Hager's Test:** 1-2 mL of *Bassia latifolia* extract was dissolved in acetic acid. 3 mL of Hager's reagent was added to it, and the presence of alkaloids indicated the formation of yellow precipitate.

2.2.3 Test for Saponins

- Froth Test: 1 ml of *Bassia latifolia* extract was added to distilled water and thoroughly shaken. Stable froth formation indicated the presence of saponin.

2.2.4 Test for Triterpenoids and Steroids

- **Libermann-Burchard Test:** Chloroform was used to dissolve the *Bassia latifolia* extract. 1 mL of acetic acid and 1 mL of acetic anhydride were added to it, which was then heated on a water bath and cooled. Then, along the sides of the test tube, a few drops of concentrated sulphuric acid were added. Steroid presence is indicated by the appearance of a bluish green color.
- Salkowski Test: The extract of *Bassia latifolia*was dissolved in chloroform, and an equal volume of concentrated sulfuric acid was added. The formation of bluish red to cherry red color in the chloroform layer and green fluorescence in the acid layer indicated the presence of steroids.

2.2.5 Test for Tannin and Phenolic Compounds

- **Ferric Chloride Test:** In distilled water, a small amount of *Bassia latifolia*extract was dissolved. Add a few drops of dilute ferric chloride solution to it. The presence of tannins was indicated by the formation of a dark blue color.
- **Gelatin Test:** In distilled water, a small amount of *Bassia latifolia*extract was dissolved. The 2ml solution of 1% gelatin containing 10% sodium chloride was added. The formation of white precipitate indicates the presence of phenolic compounds.
- **Lead Acetate Test:** In a test tube, a small amount of *Bassia latifolia* extract was dissolved with distilled water, and a few drops of lead acetate solution were added. The presence of phenolic compounds is indicated by the formation of white precipitate.

2.2.6 Test for Flavonoids

- **Shinoda's Test:** A few magnesium turnings and a few drops of concentrated hydrochloric acid were added to 1 ml of *Bassia latifolia* extract in alcohol, which was heated on a water bath until the formation of a red to pink color occurred, indicating the presence of flavonoids.

2.2.7 Test for Glycosides

- **Borntragers Test:** Dilute sulfuric acid was added to 3 ml of test solution. It was boiled for 5 minutes before the filtrate was obtained. An equal amount of benzene or chloroform was added to the cold filtrate and shaken thoroughly. The organic solvent layer was separated, and then ammonia was added to it. The presence of anthraquinone glycosides indicated the formation of a pink to red color in the ammonical layer.
- **Keller Killiani Test:** In a test tube, add 2 mL of test solution, 3 mL of glacial acetic acid, and 1 drop of 5% ferric chloride. Add 0.5 mL of concentrated sulphuric acid slowly. The presence of Cardiac glycosides was indicated by the formation of blue color in the acetic acid layer.

2.2.8 Test for fats and oils

Solubility test

- Solubility was observed after adding a few ml of chloroform to 2-3 ml of alcoholic solution of *Bassia latifolia*extract.
- To 2-3 ml of alcoholic *Bassia latifolia*extract solution. Solubility was observed after adding a few mL of 90% ethanol.

2.3 Quantitative Phytochemical Estimation

2.3.1 TPC

The total phenolic content of *Bassia latifolia* extract was determined using the Folin-Ciocalteu Assay. *Bassia latifolia* extracts (0.2 mL from stock solution) were combined with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. Distilled water was used to dilute this mixture up to 7 mL. The solutions were then allowed to stand at room temperature for 2 hours before being spectrophotometrically measured at 760 nm. Calibration curves were created using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Gallic aid was prepared in concentrations of 20, 40, 60, 80, and 100 g/mL. The Folin-ciocalteu reagent is sensitive to reducing compounds such as polyphenols. When they react, they turn blue. This blue color was measured spectrophotometrically.(37)

2.3.2 TFC

Aluminium chloride was used to determine the flavonoid content. 2 mL of distilled water was mixed with 0.5 mL of *Bassia latifolia*extract solution. Then, 0.15 ml of sodium nitrite (5%) was added and thoroughly mixed. Wait 6 minutes before adding 0.15 mL Aluminium chloride (10%) and allowing to stand for 6 minutes. 2 mL of 4% sodium hydroxide was then added. The mixture was thoroughly shaken and mixed. The absorbance of the mixture was measured at 510 nm using a UV spectrophotometer. Calibration curves were created using standard Rutin Equivalent (RE) mg/gm solutions. Rutin concentrations of 20, 40, 60, 80, and 100 g/mL were prepared. The calibration curve was used to calculate total flavonoid content, which was expressed as mg Rutin equivalent.

2.4 Anti-oxidant Activity

2.4.1 DPPH

The antioxidant activity of *Bassia latifolia*extract was determined using the DPPH free radical scavenging assay. A 1 mg/ml methanol solution of extracts/standard was prepared. From a 1mg/mL stock solution and 2mL of 0.1mM DPPH solution, different concentrations of *Bassia latifolia*extracts/standard (20-100g/ml) were prepared. The obtained mixture was vortexed, incubated for 30 minutes at room temperature in a relatively dark place, and then measured using a UV spectrophotometer (Shimadzu 1700) at 517 nm. Take 3 ml of 0.1mM DPPH solution and incubate it for 30 minutes at room temperature in the dark. The absorbance of the control was measured at 517 nm against methanol (as a blank). [26]

Percentage antioxidant activity of sample/standard was calculated by using formula:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100)]

2.4.2 Reducing power assay

Preparation of standard solution

In 3 ml of distilled water/solvent, 3 mg of ascorbic acid was dissolved. This solution was diluted with distilled water to yield concentrations of 20, 40, 60, 80, and 100 g/ml.

Preparation of extracts

Extract stock solutions were made by dissolving 1mg of dried extracts in 1 ml of methanol to achieve a concentration of 1mg/ml. The samples were then prepared at concentrations of 20, 40, 60, 80, and 100 g/ml.

Protocol for reducing power

Aliquots of various concentrations of the standard and extracts (20 to 100g/ml) in 1.0 ml of deionized water were mixed with 2.5 ml of (pH 6.6) phosphate buffer and 2.5 ml of (1%) potassium ferricyanide according to this method. After cooling, the mixture was incubated in a water bath at 50°C for 20 minutes. The mixture was then centrifuged at 3000 rpm for 10 minutes with aliquots of 2.5 ml of (10%) trichloroacetic acid. The upper layer of 2.5 ml solution was mixed with 2.5 ml distilled water and 0.5 ml freshly prepared (0.1%) ferric chloride solution. The absorbance was measured in a UV spectrometer (Systronic double beam UV-2201) at 700 nm. A blank was created without the addition of

extract. Ascorbic acid was used as a standard at various concentrations (20 to 100g/ml). [24]

2.5 Antimicrobial Activity (Well Diffusion Assay)

2.5.1 Anti-Bacterial Activity

Preparation of the Dilutions of Samples

The samples were diluted to concentrations of 100g/ml, 150g/ml, 200g/ml, and 250g/ml, respectively, and then volume makeup was done with distilled water until 1ml.

Preparation of Nutrient Agar Media

In 1 litre of distilled water, 28 g of Nutrient Media was dissolved. Before sterilization, the pH of the media was tested. For 15 minutes, the media was sterilized in an autoclave at 121 degrees Celsius and 15 lbs of pressure. Nutrient media was poured into plates and placed in a laminar airflow to solidify the agar.

Well Diffusion Assay

Bacterial strains (E. coli) were cultured on Nutrient agar media (NAM). The wells were then made for the inoculation of the samples (*Bassia latifolia*) at various concentrations, with volume make-up up to 1 ml. A total of 100 µl of the sample was loaded. The plates were permitted to move. For best results, incubate at 37℃ for 48-72 hours. The bacterial suspension was standardized to a concentration of 10^8 CFU/ml of bacteria added to the shaker. Then, 100l of the broth inoculum (containing 10^8 CFU/ml) was collected using a micropipette and transferred to a fresh, sterile solidified Agar Plate for media.[26, 27]

3. RESULTS

3.1. Percentage Yield

In phytochemical extraction, the percentage yield is critical in determining the standard extraction efficiency for a specific plant, different sections of the same plant, or different solvents used. The table shows the yield of extracts obtained from *Bassia latifolia*.

Table: - Percentage Yield of crude extracts of *Bassia latifolia*extract

S.no	Plantname	Solvent	Theoreticalweight	Yield(gm)	%yield
1	*Bassialatifolia*	Petether	300	1.29	0.43%
2		Methanol	288.07	6.10	2.11%

3.2 Preliminary Phytochemical study

Table: - Phytochemical testing of extract

S. No.	Experiment	Presenceorabsenceofphytochemicaltest	
		Pet.Etherextract	Methanolicextract
1.	**Alkaloids**		
1.1	Dragendroff'stest	Absent	Absent
1.2	Mayer'sreagenttest	Absent	Absent
1.3	Wagner'sreagenttest	Absent	Absent
1.3	Hager'sreagenttest	Absent	Absent
2.	**Glycoside**		
2.1	Borntragertest	Absent	Present
2.2	Legal'stest	Absent	Present
2.3	Killer-Killianitest	Absent	Present
3.	**Carbohydrates**		
3.1	Molish'stest	Absent	Present
3.2	Fehling'stest	Absent	Present
3.3	Benedict'stest	Absent	Present
3.4	Barfoed'stest	Absent	Present
4.	**Proteins andAminoAcids**		
4.1	Biurettest	Absent	Present
5.	**Flavonoids**		
5.1	Alkalinereagenttest	Absent	Present
5.2	LeadAcetatetest	Absent	Present
6.	**TanninandPhenolicCompounds**		
6.1	FerricChloridetest	Absent	Absent
7.	**Saponin**		
7.1	Foamtest	Present	Present

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8.	**TestforTriterpenoids andSteroids**		
8.1	Salkowski'stest	Present	Present
8.2	Libbermann-Burchard'stest	Present	Present

3.3 QuantitativeAnalysis

Preliminaryphytochemicaltestingofcrudeextractsconfirmedthepresenceofphenolicsandflavonoidsin plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC)assays wereperformed.

TotalPhenolic content(TPC)estimation

Table: - Standard tableforGallicacid

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.135
2.	40	0.173
3.	60	0.189
4.	80	0.228
5.	100	0.266

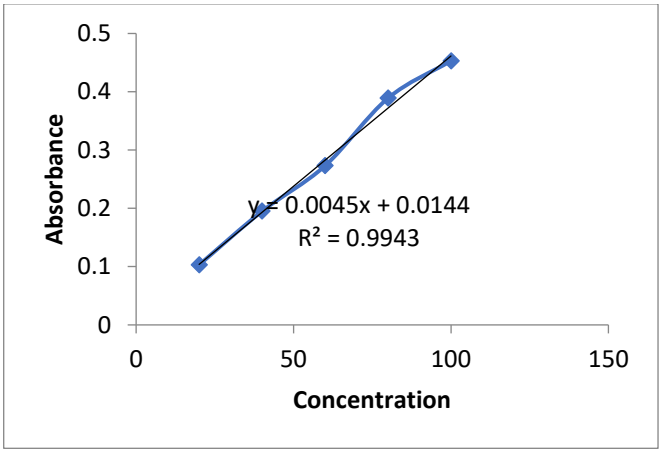

Figure 1: Representation of standard curve of Gallic acid

Total Phenolic Content in extract

Table: - Total Phenolic Content

S.No	Absorbance	TPCinmg/gmequivalentof GallicAcid
1	0.140	
2	0.172	64.66mg/gm
3	0.191	

Table8- TotalPhenolicContentofextract*Bassialatifolia*

Extracts	TotalPhenoliccontent(mg/gmequivalentof Gallicacid)
Methanol	64.66

Total Flavonoids content (TFC) estimation

Table: - Standard table for Rutin

S. No.	Concentration(µg/ml)	Absorbance
1.	20	0.170
2.	40	0.196
3.	60	0.262
4.	80	0.304
5.	100	0.323

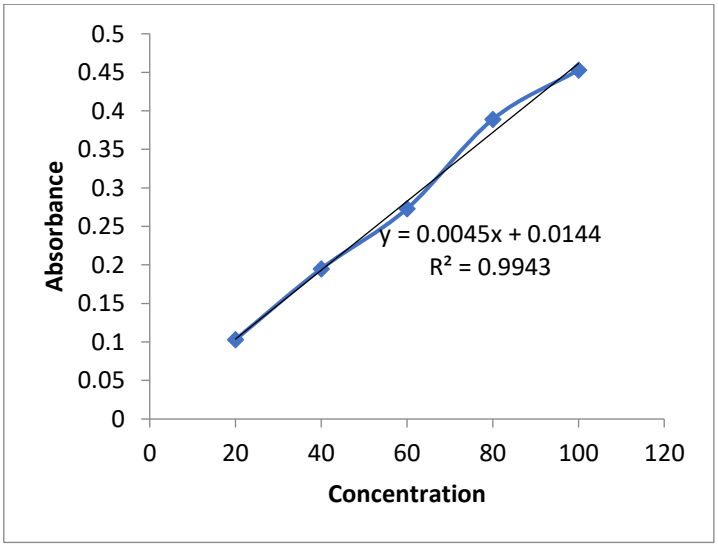

Figure 2: represent standard curve of Rutin

Total Flavonoid Content in extract

Table: Total Flavonoid Content

S.No	Absorbance	TFCinmg/gmequivalentofRutin
1	0.136	
2	0.158	16.33mg/gm

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3	0.182

Table:**TotalFlavonoidContentofextract*Bassialatifolia***

Extracts	**TotalFlavonoidcontent(mg/gmequivalentofrutin)**
Methanol	16.33

3.4. *Invitro*AntioxidantAssays

Inthepresentinvestigation,theinvitroanti-oxidantactivityofextractsof*Bassialatifoliawas*evaluatedbyDPPH radicalscavengingactivity. Theresults aresummarizedin Tables

DPPH1,1-Diphenyl-2-picrylhydrazylAssay

Table:DPPHradicalscavengingactivityofStd.Ascorbicacid

Concentration(µg/ml)	**Absorbance**	**%Inhibition**
20	0.471	52.18274
40	0.420	57.36041
60	0.333	66.19289
80	0.276	71.9797
100	0.135	86.29442
Control	0.985	
IC50		

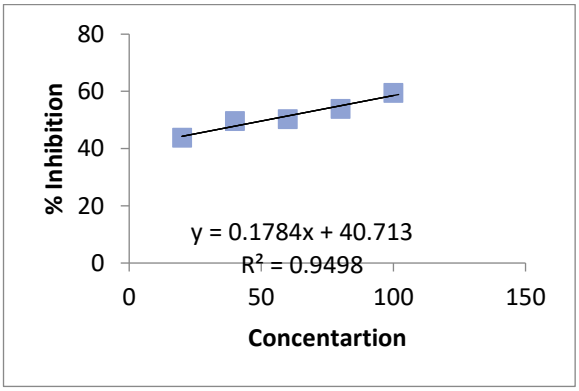

Figure 3: DPPH radical scavenging activity of Std. Ascorbic acid

Table: DPPH radical scavenging activity of methanol extract of *Bassia latifolia*

Concentration(µg/ml)	**Absorbance**	**%Inhibition**
20	0.512	43.79802
40	0.459	49.61581
60	0.453	50.27442
80	0.420	53.89682
100	0.369	59.49506
Control	0.911	
IC50		**52.19**

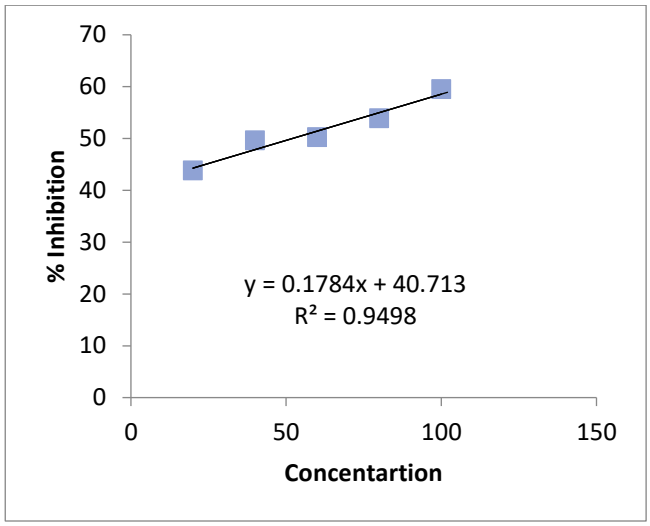

Figure 4: represents the Percentage Inhibition Vs Concentration of extract of *Bassia latifolia*

Reducingpowerscavengingactivity

Table: ReducingpowerscavengingactivityofAscorbicacid

Concentration(µg/ml)	**Absorbance**
20	0.103
40	0.195
60	0.273
80	0.389
100	0.453

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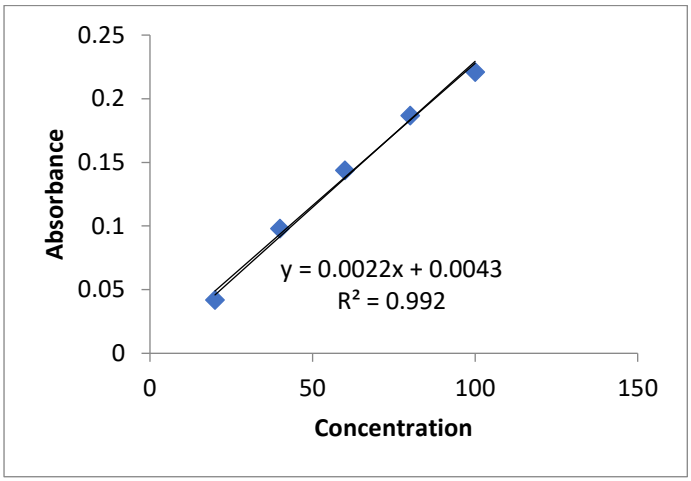

Figure 5: Graph represents the Absorbance Vs Concentration of Ascorbic acid

Table: Reducing power scavenging activity of Bassia latifolia

Concentration(µg/ml)	Absorbance
20	0.042
40	0.098
60	0.144
80	0.187
100	0.221

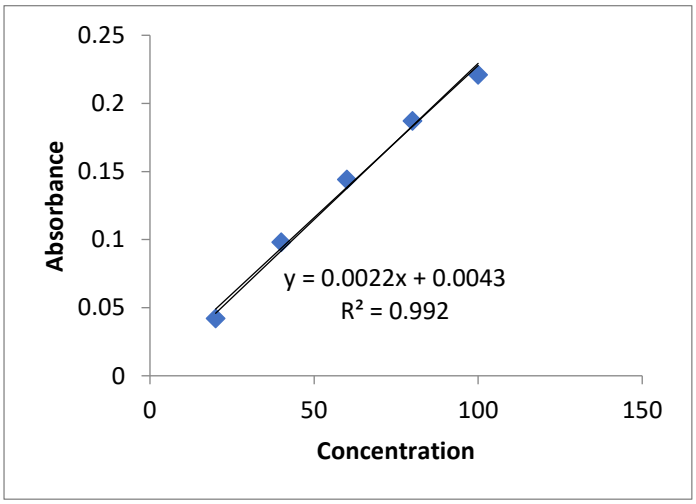

Figure6:GraphrepresentstheAbsorbance *Vs*Concentrationof*Bassialatifolia*

3.5 *In-vitro*antimicrobialactivity

Table16:*In-vitro*antimicrobialactivityof *Bassialatifolia*extractagainstgramnegativebacteria(*Escherichiacoli*)and grampositivebacteria(*Staphylococcus aureus*)

Bacterialstrain	Differentconcentrationsof*Bassialatifolia*extract			
	25µg/ml	50µg/ml	75µg/ml	100µg/ml
Escherichiacoli (Gramnegative)	0mm	7mm	8mm	12mm
Staphylococcus aureus (grampositive)	7mm	9mm	10mm	12mm

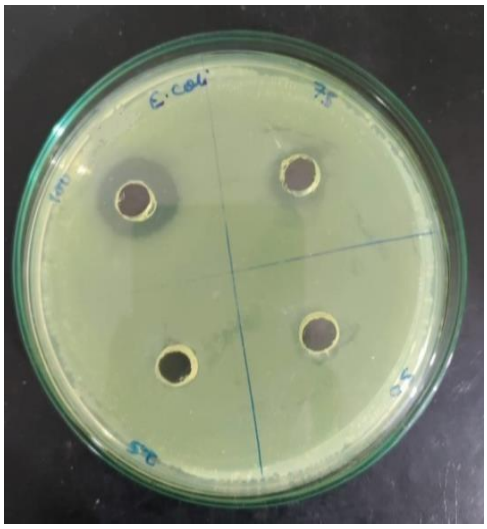

Figure7:Zoneofinhibitionof *Bassialatifolia*extractagainstgramnegative bacteria

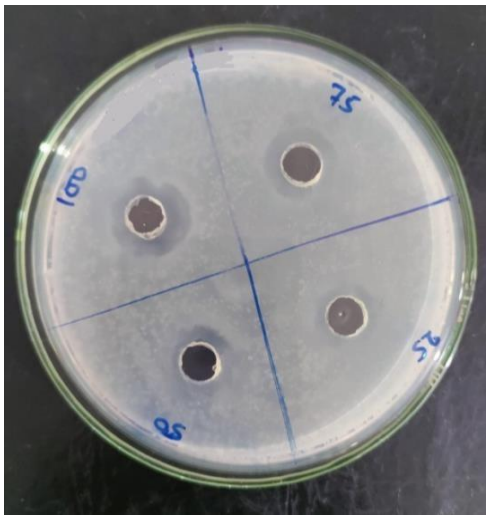

Figure 8: Zone of inhibition of *Bassia latifolia* extract against gram positive bacteria

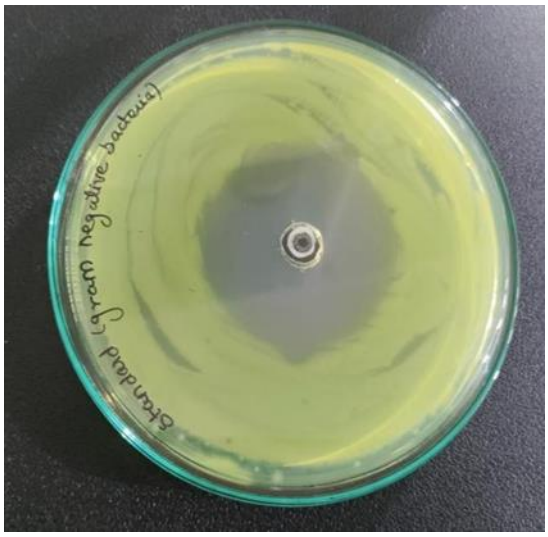

Figure 9: Zone of inhibition of standard (Gentamycin) against gram negative bacteria

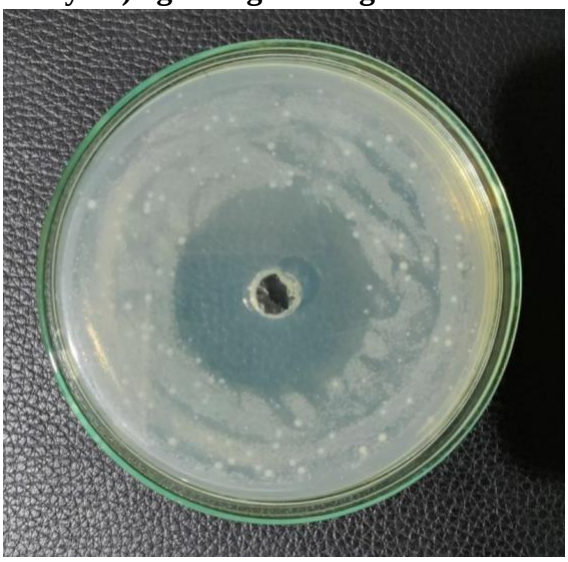

Figure10:Zoneofinhibition ofstandard(Ofloxacin)againstgrampositivebacteria

SUMMARY AND CONCLUSION

The traditional use of plants as medicine provides the foundation for determining which essential oils and plant extracts may be useful for specific medical conditions. Many plant oils and extracts, such as tea tree, myrrh, and clove, have historically been used as topical antiseptics or have been reported to have antimicrobial properties (Hoffman 1987; Lawless 1995). It is critical to conduct scientific research on plants that have been used in traditional medicine as potential sources of novel antimicrobial compounds. Furthermore, the resurgence of interest in natural therapies and rising consumer demand for effective, safe, natural products necessitates quantitative data on plantoils and extracts. The presence of active pharmacological components such as tannins, phenolic acids, saponins, flavonoids, and alkaloids was discovered through phytochemical screening. These components are known to be biologically active because they protect the plant from infections and inhibit microorganism growth. Phytochemicals generally exert antimicrobial activity via mechanisms distinct from those used by synthetic drugs. The presence of carbohydrate, saponins, glycoside, flavonids, saponin, steroids, protein, and amino acid was discovered in a phytochemical analysis of a methanolic extract of *Bassia latifolia.* Total phenolic content (TPC) and total flavonoid content (TFC) were calculated in a quantitative phytochemical assay. TPC was calculated in relation to Gallic acid (as a standard), and TFC was calculated in relation to rutin as a standard. The percent inhibition of DPPH radical scavenging activity of *Bassia latifolia* extract was 59.49%, and the IC 50 value was 52.19g/ml. Ascorbic acid was used as a control compound, with an inhibition percentage of 86.29% and an IC 50 value of 19.46g/ml. A compound's reducing capacity can be a good indicator of its potential antioxidant activity. For comparison, a dietary antioxidant such as ascorbic acid was used. Compounds with reducing power are electron donors that can reduce the oxidized intermediates of lipid peroxidation processes, allowing them to act as primary and secondary antioxidants.

The antimicrobial activity of *Bassia latifolia* extract was determined to inhibit the growth of microbes against both gram positive (Staphylococcus aureus) and gram negative (Escherichia coli) strains. A significant zone of inhibition was observed against two pathogenic bacterial strains. When comparing data from different studies, most publications make broad statements about whether a plant oil or extract has activity against Gram-positive and Gram-negative bacteria and fungi. However, not all provide information about the scope or scope of this activity. Some publications also demonstrate the relative activity of plant oils and extracts by comparing the results of different oils tested against the same organisms.

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