



## RESEARCH ARTICLE

PHYTOCHEMICAL, FLUORESCENCE AND ANTIMICROBIAL EVALUATION OF  
POLYHERBAL HERBAL EXTRACT

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## ABSTRACT

**Background and Objectives:** Natural products and traditional medicines are of great importance. Such forms of medicine as traditional Chinese medicine, Ayurveda, Kampo, traditional Korean medicine, and Unani have been practiced in some areas of the world and have blossomed into orderly-regulated systems of medicine. *Allium sativum* L., crude drug (Liliaceae) used as herbal medicine to treat hyperglycemia, cancer, cardiovascular disease, hypertension, stress and infections. *Malus domestica* B., fruit (Rosaceae) possess antioxidant, antiproliferative, antimicrobial and anti-inflammatory activity and used for the treatment and prevention of asthma, diabetes and hyperlipidemia. *Capsicum annum* L., crude drug (Solanaceae) is used as anti-diabetic, anti-cancer, anti-inflammatory, analgesic, hypolipidemic, hepatoprotective and antimicrobial agent. *Foeniculum vulgare* M., crude drug (Apiaceae) is used as antioxidant, antimicrobial, bronchodilatory, estrogenic, diuretic, hepatoprotective, gastroprotective and hypotensive agent. *Curcuma longa* L., crude drug (Zingiberaceae) is used various biological actions including anti-inflammatory, antioxidant, analgesic, antifertility, antidiabetic, anticoagulant, antiulcer and antimicrobial. **Methodology:** In the current study these five medicinal plants were purchased, authenticated and extracted by method of maceration. In this research work phytochemical screening, fluorescence analysis, antimicrobial (disc diffusion method) for both antibacterial (gram positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, and *Micrococcus lutes* and gram-negative bacteria such as *Serratia marcescens* and *Escherichia coli*) and antifungal (*Candida albican*, and *Aspergillus flavus*), activity was performed in comparison with ciprofloxacin (standard disc for bacteria) and nystatin (standard disc for fungus). **Results:** Phytochemical evaluation indicates the presence of alkaloids, glycosides, flavonoids, tannins, steroids, phenol, resin and saponins. The poly herbal extract is moderately effective against gram positive bacteria (*Staphylococcus aureus* and *Micrococcus lutes*), active against gram negative bacteria (*Serratia marcescens* and *Escherichia coli*) and is less susceptible against *Bacillus subtilis*. It is active against *Aspergillus flavus* and *Candida albican*. **Conclusion:** The medicinal plants are effective to provide defense against microorganisms.

**Keywords:** Polyherbal, Phytochemical, Antimicrobial

## INTRODUCTION

Nature is a rich source of biological agent that are beneficial to health. These are used for treatment and prevention of many diseases for decades and is main source of medicinal herbs(1). Natural product may define as, anything produced by living organism including plant extract, marine product and microbial composition or their metabolite in the body and inner chemical composition of human and animals(2). Natural products are herb, dietary supplement, or alternative medicine, and are produced by nature or exist in nature. Natural products are products that arise from natural sources such as plants, animals and microbes. It contains some compounds as amino acid, protein, peptide, carbohydrates, lipids, nucleic acid bases, ribonucleic acid and deoxyribonucleic acid(3).

Natural products obtained from medicinal plants are involved in treatment of disease in many countries. Plants have a complex defense system due to presence of complex chemical compounds. The presence of antimicrobial compound in plant tissue was used as botanical pesticide, bactericidal and fungicidal agent against pathogens of human disease(4).

Serious infections in humans are due to microorganisms including bacteria, fungi, virus and nematods. These infections are treated by using antimicrobial drugs but somehow microorganisms developed multiple drug resistance due to undifferentiated consumption of commercial antimicrobial drugs. Due to multi drug resistance, it is necessary to find the alternate way to treat microbial infections. Plants are responsible for food, drugs, shelter and clothing and also studied for drug discovery. Medicinally, plants are used as a source of medicine including antibiotics, antineoplastic, analgesic and cardioprotective(5). Medicinal plants are the major of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs(6). The sources of anti-microbial agents are plants and micro-organism as these are developed due to some disease attack. These antimicrobial agents may possess bacteriostatic or bactericidal activity on other microbes(7).

*Allium sativum* L. belongs to family Liliaceae, consists of allin, ajoene, glutamylcysteines, and hydrogen sulfide and possess, antidiabetic, anti-inflammatory, antioxidant, antithrombotic and cardio-protective effects(8). *Curcuma longa* L., belongs to family Zingiberaceae, consisting of diarylheptanoids (usually called curcuminoids), monoterpenes, diarylpentanoids, sesquiterpenes, triterpenoids, sterols and alkaloids(9). Its extract possesses antioxidant, anti-inflammatory,

hepatoprotective, anticarcinogenic, antidepressant and cardiovascular effects(10). *Malus domestica* B. belongs to family Rosaceae, consists of high level of triterpenoids, flavonoids, hesperetin and phenolic compounds, procyanidins, epicatechin and chlorogenic acid(11). Apples are effective against cancer, cardiovascular diseases, pulmonary dysfunction, Alzheimer's disease (12) and possess antioxidant and anti-inflammatory effect (13). *Capsicum annum* L. belongs to Solanaceae family, consists of steroidal glycosides capsiocside A, D and vitamin B1, B3, C, E, it possess anthelmintic, anti-inflammatory and antioxidant activity and used for treatment of cancer, human nasopharyngeal carcinoma, and human esophageal epidermoid carcinoma (14, 15). *Foeniculum vulgare* M. belongs to family Apiaceae and genus foeniculum, consist of fenchone, trans anethole and small quantities of limonene, camphor, alpha pinene and volatile compound and vomiting. It is beneficial for chronic fever and reduced the obstructions of internal organs especially those in liver, intestine, respiratory and urinary tract(16, 17).

## **MATERIAL AND METHOD:**

### **Identification and authentication of plants:**

The plants *Malus domestica* B. (Rosaceae), *Capsicum annum* L. (Solanaceae), *Curcuma longa* L. (Zingiberaceae), *Allium sativum* L. (Amaryllidaceae) and *Foeniculum vulgare* M. (Apiaceae) were identified and authenticated by Dr. Zaffarullah Zaffar, Associate Professor of Botany, Institute of Pure and Applied Biology Bahauddin Zakariya University Multan.

### **Collection of crude drugs:**

The garlic cloves (5.0 kg), turmeric rhizome (5.0 kg), capsicum (3.0 kg), fennel (2.0 kg), apple (5.0 kg) fruit were purchased from the local market of District Muzaffar Garh, Punjab Pakistan. The garlic cloves, turmeric rhizome, apple and capsicum was washed with water slashed into small pieces. Then these slashed samples and fennel were dried under-shade for complete drying. After drying, dried samples were again weighed. The dried and weighed garlic cloves (3 kg), turmeric rhizome (2.5 kg), apple (3 kg), capsicum (2 kg) and fennel (2 kg) were packed in well closed polystyrene bags for further process (18).

### **Maceration**

500 g of plant powder (capsicum, fennel, apple, garlic cloves and turmeric rhizome) were separately soaked in Methanol (70%) 1500 ml in a well closed glass bottle. Then these bottles

are placed at room temperature for three to seven days with recurrent agitation (19). After that these mixtures were filtered separately and dried by used the rotary evaporator at temperature 64 °C. Then weighed the final extract, labelled and stored in bio-medical freezer at temperature (-20) °C. The marc was re-soaked in Methanol (70%) for re-maceration. This procedure was repeated again with remaining sample to obtained the weight of extract (20).

### **Phytochemical evaluation:**

#### **Test sample**

Properly mixed equal quantity (5.0gm) from each extract (Garlic, fennel, apple, capsicum and turmeric) to made test sample (poly herbal extract).

#### **Preparation of sample:**

Ethanol extract (1.0gm) of test sample was mixed in 100 ml of ethanol. The stock solution is prepared. This stock solution is used for phytochemical evaluation (21).

#### **Screening of phytochemicals:**

Phytochemical is used for the evaluation of the bioactive agents existing in the sample. The specific reagents are added to solution which is being evaluated, this evaluation is performed through visual observation of precipitate formation or color change

#### **Test for Alkaloids**

##### **(a) Hager's test**

In 2.0 ml test sample, few drops of Hager's reagent was mixed. Formation of precipitate in color of bright yellow indicated the presence of alkaloids.

##### **(b) Dragandroff's test**

Dissolved 1.0 ml of test solution in 2.0 ml of Dragandroff's chemical reagent and mixed. Then 2 ml of dilute HCl was added. Orange precipitate confirmed the presence of alkaloids.

##### **(c) Wagner's test**

1.0 ml test sample was mixed with 1.0 ml of Wagner's reagent. Red precipitates confirmed the presence of alkaloids.

##### **(d) Mayer's test**

1.0 ml test sample was mixed in few drops of Mayer's reagent. Precipitates were formed which were cream in color and it confirmed the presence of alkaloids.

##### **(e) FeCl<sub>3</sub> test**

Added some drops of  $\text{FeCl}_3$  solution in 1.0 ml of test solution, which result in yellow precipitate confirmed the presence of alkaloids.

### **Test for Glycosides**

#### **(a) Keller Kiliani test**

Dissolved 1ml of test solution in 1.0 ml of glacial acetic acid, cooled. After this 2-3 drops of  $\text{FeCl}_3$  mixed. Carefully added 2.0 ml conc.  $\text{H}_2\text{SO}_4$  along the test tube walls. Reddish-brown ring at the junction of two layers formed which indicate the presence of glycosides.

#### **(b) Conc. Sulphuric acid test**

1ml of test solution was dissolved in 1.0 ml of concentrated sulphuric acid was mixed and placed them for 2.0 minutes. Formation of red precipitate confirmed the presence of glycosides.

#### **(c) Molisch's test**

Took 1ml of test sample and 2-3 drops of molisch reagent was mixed. Then conc.  $\text{H}_2\text{SO}_4$  was added with the test tube walls. Reddish purple ring was formed at the junction of two layers confirmed the presence of glycoside.

### **Tests for Tannins**

#### **(a) $\text{FeCl}_3$ test**

Few drops of  $\text{FeCl}_3$  was mixed with the test sample. Black precipitate indicated the presence of tannins.

#### **(b) Gelatin test**

Few drops of gelatin were added in 1.0 ml of test sample. Formation of white precipitate confirmed the presence of tannins.

#### **(c) Lead acetate test**

In 1.0 ml of test sample lead acetate was added. Bulky red precipitate confirmed the presence of tannins.

#### **(d) Alkaline reagent test**

The test sample was mixed with NaOH solution. Reddish yellow precipitate confirmed the presence of tannins.

### **Test for flavonoids**

#### **(a) Alkaline reagent test**

The test solution was mixed with NaOH solution. Reddish yellow color indicated the flavonoids.

#### **(b) Lead acetate test**

Basic lead acetate was mixed separately to 1.0 ml of test sample. Bulky reddish-brown precipitate confirmed the presence of flavonoids.

**(c) FeCl<sub>3</sub> test**

In test sample few drops of FeCl<sub>3</sub> solution was added. Black precipitate confirmed the presence of flavonoids.

**Test for Steroids:**

**Salkowski test**

1.0 ml of test sample was added in 5.0 ml of chloroform and added few drops of Concentrated sulphuric acid and mixed them. Place the solution for some-time, in the lower layer red precipitates were formed indicated the presence of steroids.

**Test for fixed oil:**

**Stain test**

Small amount of extract was pressed between the two Whatman No. 1 filter papers; if stain is present on filter paper indicated the fixed oil.

**Test for Phenols**

**Phenol test**

2.0 ml test sample dissolved in 1.0 ml of ferric chloride solution. Phenol was indicated by an intense color.

**Test for Resins**

**Copper sulphate:**

1.0 ml test sample was mixed in CuSO<sub>4</sub> 2-3 ml, the mixture was mixed for 2 minutes, and then the solution was separated. Formation of green precipitate indicated resins.

**Test for Saponins**

**Foam test:**

3.0 ml test sample mixed with water of 5 ml and dynamically shaken. Formation of persistent foam indicated the saponins (22).

**Fluorescence analysis:**

0.5 g powdered drug was taken in the dried test tubes, and 5.0 ml of various reagents including distilled water, ethanol, acetone, glacial acetic acid, benzene, chloroform, picric acid, diethyl ether, methanol, sulphuric acid, nitric acid, hydrochloric acid, 1N sodium hydroxide, 5% ferric chloride and 5% iodine were added separately in test tube and test tubes were gently shaken and

place them for 20-25 minutes. The solution was examined under the visible light (day light) and ultraviolet light at 254 nm wavelength (short wavelength) and 365 nm (long wavelength) for specific color change (22).

#### **Antibacterial activity:**

##### **Preparation of Discs:**

The different dilution of poly herbal extract was prepared by using dimethylsulphoxide (DMSO). Dissolved 50.0 mg, 75.0 mg and 100.0 mg of extract in 1.0 ml of dimethylsulphoxide separately (20). Then the disc of 6 mm diameter prepared by using Whatman No 1 filter paper, these discs were soaked in the prepared dilutions (23).

##### **Culture media:**

The culture media used for antibacterial screening was nutrient agar media. This media was made by dissolving 28.0 g of agar media in 1.0 L of distilled water and mixed until it completely dissolved. Then the prepared media is sterilized in autoclave under pressure of 15 PSI and at temperature of 121°C for 15 minutes (20).

##### **Preparation of bacterial strain:**

Prepared media was poured in petri-dishes and allowed to solidify. Bacterial strains were applied on these petri dishes by streaking and incubate for 24 hours at 37 °C. After 24 hours, this culture was collected from petri-dishes and stored in sterilized vials (20, 24).

##### **Determination of minimum inhibitory concentration (MIC):**

The minimum inhibitory concentration was minimum concentration of sample required for inhibition of the growth of tested microorganism. The already prepared nutrient agar media was poured into petri-dishes and solidified them. Bacterial strains were applied on these petri dishes by streaking. The prepared discs were applied on agar media plates and these plates were incubated for 24 hours under suitable condition and then determined the minimum concentration of the extract that inhibit the growth of microorganisms (25, 26)

##### **Assay procedure:**

The prepared agar media was poured into petri dishes and solidified them. These petri dishes were inoculated with a uniform inoculum of the test microorganism by using spreader. Then, filter paper discs (about 6 mm in diameter), containing extract at 200 mg, 400 mg and 600 mg concentration, are located on the agar surface. In each petri-plates 5 disc are applied 3 discs are from extract concentration (200.0 mg, 400.0 mg and 600.0), positive control disc and negative

control disc. The ciprofloxacin disc was used as positive control and disc infused with dimethyl sulphoxide (DMSO) was used as negative control. These petri dishes were incubated for 18-24 hours at 37 °C. After incubation, zone of inhibition was determined. The assay was performed in triplicate and their zone of inhibition was measured in mean value with standard deviation(23, 24, 27)

#### **Antifungal activity:**

##### **Preparation of Discs:**

The different dilution of poly herbal extract was prepared by using dimethylsulphoxide (DMSO). Dissolved 100.0 mg, 200.0 mg and 300.0 mg of extract in 1.0 ml of dimethylsulphoxide separately (20). Then the discs of 6 mm diameter were prepared by Whatman No. 1 filter paper, these discs were soaked in the prepared dilutions (23).

##### **Culture media:**

SDA (Sabouraud dextrose agar) media was used as a culture media for antifungal screening. This media prepared by dissolving 62.0gm of agar media in 1.0 L of distilled water and mixed until it completely dissolved. Then the prepared media was sterilized in autoclave under pressure of 15 PSI and at temperature of 121 °C for 15 minutes(20)

##### **Preparation of fungal strain:**

Prepared agar media poured in petri dishes and solidified these petri dishes. Fungal strains were applied on media agar plates by streaking through spreader and incubate at 37 °C for 24 hours. After 24 hours, this culture was collected from petri-plates and stored in sterilized vials (20, 24)

##### **Determination of minimum inhibitory concentration (MIC):**

The minimum inhibitory concentration was minimum concentration of the sample required for inhibition of the growth of tested microorganism. The already prepared sabouraud dextrose agar media was poured into petri dishes and solidified them. The fungal strain were applied on these plates by streaking trough spreader. The prepared discs were applied on agar media plates and these plates were incubated for 24 hours under suitable condition and then determined the minimum concentration of the extract which inhibit the growth of microorganism (25, 26).

##### **Assay procedure:**

The prepared agar media was poured into petri dishes and solidified. These agar dishes were inoculated with a uniform inoculum of the test microorganism by using spreader. Then, filter



paper discs (about 6 mm in diameter), containing extract at 300.0 mg/disc, 600.0 mg/disc and 900.0 mg/disc concentration, are located on the agar surface. In each petri-plates 5 disc are applied 3 discs are from extract concentration (300.0 mg, 600.0 mg and 900.0), positive control disc and negative control disc. The nystatin disc was used as positive control and disc infused with DMSO was used as negative control. Incubated these petri dishes for 48-96 hours at 28 °C. After incubation, zone of inhibition was determined. The assay was performed in triplicate and zone of inhibition was expressed in mean value with standard deviation(23, 24, 27).

## RESULTS:

**Table 1:** Phytochemical screening of Poly herbal extract (GFACT)

Sr.No	Phytochemicals Table	Reagents	Observation	Result
1.	Alkaloids	Dragandroff	Orange ppt	Present
		Mayer	Cream ppt	Present
		Wagner	Red ppt	Present
		Hager	Yellow ppt	Present
		Ferric chloride	Yellow ppt	Present
2.	Glycosides	Keller kiliani	Reddish brown ring	Present
		Molisch	No ring	Absent
		Conc. Sulphuric acid	Red ppt	Present
3.	Tannin	Ferric chloride	Yellow ppt	Absent
		Gelatin	White ppt	Present
		Alkaline solution	Red ppt	Present
4.	Flavonoid	Ferric chloride	Yellow ppt	Absent
		Alkaline solution	Red ppt	Present
		Conc. Hydrochloric acid	Yellow ppt	Present
		Conc. Sulphuric acid	Red ppt	Present
5.	Fixed oil	Stain test	Spot on filter paper	Present
6.	Steroids	Salkowski test	Red ppt in lower ring	Present
7.	Phenol	Ferric chloride	Intense color	Present
8.	Resin	Copper sulphate	Green sulphate	Present
9.	Saponin	Water	Froth	Present

**Table 2:** Fluorescence analysis of mixture of crude drugs (GFACT)

Sr. No	Experiments	Visible light	UV fluorescence	
			254 nm	366 nm

1	Powder as such	Brown	Brown	Dark brown
2	Powder +1 N aqueous NaOH	Red	Brown	Orange
3	Powder + dill HCl	Cream	Brown	Green
4	Powder + conc. HCl	Brown	Black	Green
5	Powder + Conc.H <sub>2</sub> SO <sub>4</sub>	Black	Brown	Dark green
6	Powder + 50% H <sub>2</sub> SO <sub>4</sub>	Black	Brown	Green
7	Powder + conc HNO <sub>3</sub>	Yellow	Green	Orange
8	Powder + acetic acid	Bright yellow	Brown	Yellow
9	Powder + ferric chloride	Brown	Black	Black
10	Powder + NH <sub>3</sub>	Red	Orange	Black
11	Powder + Benzene	Yellow	Light brown	Green
12	Powder + chloroform	Orange	Brown	Green
13	Powder + Acetone	Orange	Brown	Gray
14	Powder + Ethyl acetate	Orange	Green	Brown
15	Powder + acetonitrile	Yellow	Brown	Blue
16	Powder + Di ethyl ether	Light brown	Brown	Orange
17	Powder + Picric acid	Orange	Brown	Purple
18	Powder + n-butanol	Yellow	Brown	Green
19	Powder + Methanol	Bright yellow	Brown	Brown
20	Powder +10 % ethanol	Orange	Orange	Green

21	Powder + 99 % ethanol	Bright yellow	Brown	Green
22	Powder + water	Pale yellow	Blue	Orange
23	Powder + Glacial acetic acid	Yellow	Brown	Yellow

**Table 3:** Antibacterial activity of poly herbal extract (GFACT)

Sr. No	Bacterial strain	200mg	400mg	600mg	Ciprofloxacin	MIC
1	<i>Bacillus subtilis</i>	4.3±0.5	5 ± 1	7.6±1.1	16.3±1.5	100
2	<i>Stenotromophonasmaltophilia</i>	10±1	13±1	14.6±0.5	26.6±2.1	100
3	<i>Staphylococcus aureus</i>	3±1.4	5±2.8	7.6±4.2	17.3±1.5	100
4	<i>Micrococcus lutes</i>	3.3±0.5	4.6±0.5	7±1	16.3±1.5	100
5	<i>Serratiamarcescens</i>	10±1	16±3.4	20±0.5	26±2.6	100
6	<i>Escherichia coli</i>	3±2.6	5.3±3.2	7.6±3	16±2.6	100

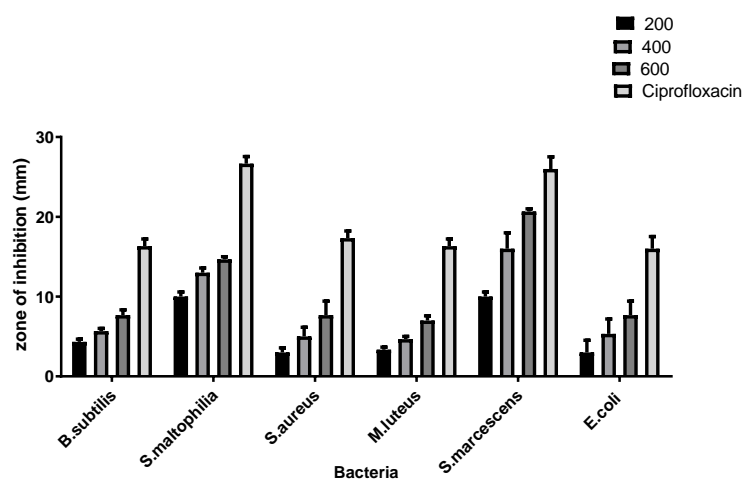


Figure 1: Antibacterial activity of poly-herbal extract at different concentration

**Table 4:** Antifungal activity of poly herbal extract (GFACT)

Sr. No	Fungal strain	300mg	600mg	900mg	Nystatin	MIC
1	<i>Candida albican</i>	3.6±0.57	8.3±1.5	14±0.00	17.6±0.57	300
2	<i>Aspergillusflavus</i>	6.3±1.5	7.6±0.57	7.6±3.2	21±3	300

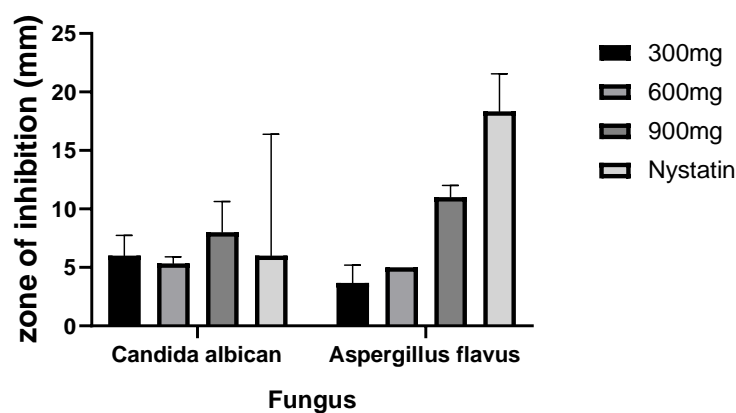


Figure 2: Antifungal activity of poly herbal extract at different concentration

### Discussion:

Plants synthesize an extensive variety of organic compounds which are not involve in primary metabolism, but these compounds take part in various type of biological activities. These compounds are known as secondary metabolites, which include alkaloids, glycosides, flavonoids, tannins, saponins and phenolic compounds and used as dyes, glues, oils, fibers, waxes, flavoring agents, drugs and perfumes. All these compounds are also used as a potential source of new natural drugs, antibiotics, herbicides and insecticides. These are biologically active compounds and have variety of functions. Alkaloids are used in the development of analgesic medicine. Flavonoids show various biological activities like antimicrobial, anti-inflammatory, analgesic and antioxidant effect. Herbs that contain tannin are astringent in nature. Tannins have antioxidant effect and anticancer effect. Steroidal compounds are involved in anabolic hormones such as sex hormones. These compounds show antibacterial and antiviral effects. Phenolic compounds show anticarcinogenic effect and antioxidant effect. These compounds are also involved in protection of blood vessels and also lower the LDL cholesterol in the blood. Saponin have property to reduce the congestive heart failure(28). In the present study, phytochemical evaluation of poly herbal extract shows the presence of saponins, steroids, tannins, glycosides,

alkaloids, flavonoids and phenolic compounds. All these phytochemicals that are present in poly herbal extract show the therapeutic effect of these plant in folk medicine. Results are shown in table 1.

Fluorescence characteristics is used for identification and differentiation of plant material from their adulterants. Fluorescence analysis is used to confirm the uncertain sample. It is important parameter of pharmacognostic evaluation which shown by the various chemical constituents present in plant extract. Some chemical constituent exhibit fluorescence in day light in visible range. Many constituents show fluorescence in ultraviolet light which do not fluorescence in day light. When constituents itself are not fluorescent then these constituents are reacted with different chemical reagents in order to convert these constituents into fluorescent derivatives(29). In the current study the five plants are studied individually and in the form of poly herbal powder. These powders are interacted with various reagents such distilled water, acetone, ethanol, benzene, chloroform, diethyl ether, methanol, glacial acetic acid, sulphuric acid, nitric acid, hydrochloric acid, picric acid, 5% ferric choride, 5% iodine and 1N sodium hydroxide. These samples are observed in visible day light and UV light of both short (254 nm) and long (365nm) wavelength. Sometime these sample show color in visible day light with some certain reagents while some show fluorescence in UV light. Results are shown in table 2.

Antibacterial activity from herbal extract mainly depend on the parts of plants and solvent used for extraction. Antibacterial activity is because of the presence of bioactive compounds including phenol, pyrocatechol, essential oil and polygalacturonases inhibitors proteins. Disc diffusion method is of the method that used to determine the susceptibility, in which disc infused with extract at various concentration were placed on the nutrient agar media and incubated for 18-24 hours at temperature 37°C. Antibacterial agent present in extract diffuse into the agar and inhibit the growth of microorganism that was evaluated by measuring zone of inhibition. If zone of inhibition is  $\geq 10$  mm then extract is considered active. The positive control (standard) was ciprofloxacin disc. Ciprofloxacin belongs to fluoroquinolone class and is effective against both gram positive and gram-negative bacteria by inhibiting the cell division through inhibition of DNA gyrase, type II topoisomerase and topoisomerase IV. Dimethyl sulfoxide is an organosulfur compound with the formula  $(\text{CH}_3)_2 \text{SO}$ . This colorless liquid dissolve both polar and nonpolar compounds and is miscible in a wide range of organic solvents as well as water. It has a relatively high boiling point(6, 30-32). In current study the extract was evaluated against six

bacterial strain including both gram positive bacteria and gram-negative bacteria. In gram positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, and *Micrococcus lutes* are studied, while *Stenotrophomonas maltophilia*, *Serratiamarcescens* and *Escherichia coli* are gram negative bacteria. The poly-herbal extract has maximum zone of inhibition against *Serratiamarcescens* that was  $21 \pm 0.5$  mm in diameter as compare to positive control disc which has zone of inhibition of  $26 \pm 2.6$  mm. However bacterial strain *Bacillus subtilis* and *Stenotrophomonas maltophilia* are less susceptible with poly herbal extract because their zone of inhibition is less than 10 mm. On the other hand, *Staphylococcus aureus*, *Micrococcus lutes* and *Escherichia coli* are active as their zone of inhibition is greater than 10 mm. The negative control disc, dimethyl sulphoxide (DMSO) show no zone of inhibition which indicate the non-toxic effect of DMSO. Results are shown in table 3 and figure 1.

Antifungal agents are used to inhibit the growth of fungus and provide protection from fungal infections. Disc diffusion method is one the method that used to determine the susceptibility, in which disc infused with poly-herbal extract at various concentration were placed on Sabouraud dextrose agar and incubated for 48-96 hours at temperature 28 °C. Nystatin is used as positive control disc. Nystatin is ionophore. It acts by binding with main component of cell membrane which is ergosterol resulting in membrane leakage which is responsible for fungus death(32, 33). In the current study antifungal activity was evaluated against two fungal strain including *Candida albicans* and *Aspergillus flavus*. Poly-herbal extract is more effective against *candida albicans* which show  $14 \pm 1.5$  mm zone of inhibition. The *Aspergillus flavus* is considered active because its zone of inhibition is greater than 10 mm diameter. Results are shown in table 4 and figure 2.

**CONCLUSION** The study shows that poly herbal extract contains secondary metabolites such as alkaloids, glycosides, tannins, flavonoids and resins, due to the presence of these secondary metabolites poly herbal extract possess, antimicrobial (antibacterial and antifungal) activities.

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#### **Conflict of interest:**

The authors declare that there are no conflicts of interest including financial interest.

#### **Authors' contributions**

AT contributed to study concept; RM, UM, TS, AW, AU, FAS and TS contributed to study design, data collection, data analysis and interpretation, literature review, write and critically review the manuscript. All the authors read and approved the final manuscript.

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### **Declarations**

#### **Ethics approval and consent to participate**

Not applicable.

#### **Competing interests**

The authors declare that they have no competing interests.

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