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Evaluation of Phytochemicals and Antimicrobial Activity of White and Blue Capitulum and Whole Plant of Silybum Marianum

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Abstract: Phytochemicals are nonessential nutrients derived from plants, most of them are physiologically active. Majority of the phytochemicals have been known to beer therapeutic activities like antibacterial, antioxidant, antifungal, antispasmodic, anticancer, hepatoprotective etc. Chemical compounds with antimicrobial activity isolated from plants have enormous therapeutic potential and are effective in the treatment of infectious diseases while magitating many of the side effects that are often caused by synynthatic antimicrobial agents. The aqueous extracts of blue and white flowering *Silybum marianum* were subjected for qualitative and quantitative phytochemical evaluation and the crud ethanolic extract for antimicrobial activity. Data shows that flavonoids, phenols and tannins are present in both blue and white flowering (whole) plant of *S.marianum* while saponin and alkaloid were not detected. It has been found that, flavonoids are in high quantity (21%) in blue flowering and less (19%) in the white flowering plant. Contents of phenol and tannins are noted very less as compared to flavonoid i.e. 0.430 % phenol is recorded in blue flowering and 0.4321% in the white flowering plant. Contents of tannin is noted high in the white flowering (0.8455%) and less (0.6930%) in the blue flowering (whole) plant of *S. marianum*. Silymarin has been found very active against all gram positive bacteria and demonstrated moderate to significant antibacterial activity against tested pathogens, while inactive against gram negative bacteria and fungi.

Key words: Secondary metabolites · Antibacterial and antifungal activity · White and blue flowering plant

INTRODUCTION

Medicinal plants are abundantly available throughout the world. Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine. These natural compounds formed the foundation of modern prescription drug as we know today [1].

Phytochemicals are naturally occurring compounds of plant kingdom, such as medicinal plants, vegetables, fruits, that work with nutrients and fibbers to act against diseases or more specifically, provides protection against diseases.

Phytochemicals are mainly divided into two groups, which are primary and secondary constituents, according to their activity in plant metabolism. Primary constituents contain common sugars, amino acids, proteins and chlorophyll, while secondary constituents comprise of alkaloids, flavonoids, saponin, tannin, phenolic compounds and many more [2]. The medicinal value of these plants lies in phytochemicals constituents that cause definite pharmacological action on the human body [3].

Infectious diseases are the leading causes of death throughout the world, accounting for nearly one half of all death in the tropical countries, which are also becoming a serious problem in developed countries. It is calculated that, in 8% of the 9 deaths occurring in USA is due to infectious diseases [4]. Nowadays multiple drug resistance has been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this problem, antibiotics are sometime associated with adverse

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effects on the host including hypersensitivity, immuno suppression and allergic reactions. This problem forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, it is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [5]. Antimicrobial of plants origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [6, 7].

Silybum marianum is an annual or biennial tubby, rigid herb that is also wide and jagged. It is commonly known as Milk thistle, Ladys thistle, Holly thistle, Marian thistle, belongs to family asteracaea. *S. marianum* is native of southern Europe, mainly the Mediterranean regions, indigenous to North Africa, Asia Minor and Southern Russian Federations. *S. marianum* is now naturalized throughout Europe, in North and South America, Australia and abundantly available in Khyber Pukhtoon Khwa and Punjab areas of Pakistan [8]. The main active constituents of this plant are Flavonolagnan collectively known as silymarin. It is used for the treatment of many liver disorders characterized by degenerative necrosis and functional impairment [9]. In addition, it is capable to antagonize the toxin of Amanita phalloides [10, 11] and provides hepatoprotection against poisoning by paladin [12], galactosamine [13], thioacetamide[14], halothane[15] and carbon tetrachloride [16]. Keeping in view of the void distribution of s marianum in Pakistan and its medicinal importance, the present study was conducted to evaluate phytochemicals and antimicrobial activity of blue and white flowering of *S. marianum*.

MATERIALS AND METHODS

Phytochemicals

Preparation of Sample: Aqueous extract of each sample was prepared by soaking 10g of powdered samples for 12 hrs in 200mL of distilled water. The extracts were then filtered by means of filter paper or Whatman filter paper [17-19].

Qualitative Analysis

Alkaloid: Dried the extract through evaporation and heated the residue with 2% Hydrochloric acid on a boiling water bath. The mixture was then cooled, filtered and treated with the Mayer's Reagent. The sample was observed for the presence of turbidity or yellow precipitation [17-19].

Flavonoid: To 4 mL of extract solution added 1.5mL of 50% methanol solution. The solution was then warmed and added Metal magnesium. 5-6 drops of concentrated hydrochloric acid was added to that solution and the result was observed for red coloration [17-19].

Tannin: To 0.5mL of extract solution, added 1mL of distilled water and 1-2 drops of ferric chloride solution to it and the result was observed for blue or green black coloration [17-19].

Phenol: To 2mL of the test solution, add alcohol and few drops of ferric chloride solution and the result was observed for coloration [17-19].

Saponin: To 2mL of the test solution was added to 2mL of distilled water and shacked well. The result was for observed frothing [17-19].

Quantitative Analysis

Flavonoid: Ten gram of the plants sample was frequently extracted with 100mL of 80% aqueous methanol at room temperature. The whole solution was filtered through filter paper and transferred the filtrate in to a water bath. The solution was then evaporated into dryness and weighed until a constant weight [17-19].

Phenol: Boiled the plants sample with 50mL of $(CH_3CH_2)_2O$ for 15 minutes. 5mL of the sample was pipetted into 50mL flask and added 10mL of distilled water. Then added 2mL of NH_4OH solution and 5mL of concentrated CH_3 $(CH_2)_3CH_2OH$ to the mixture. The sample was made up to the mark and left to react for 30minutes for colour development and measured at 505nm wave length using a spectrophotometer [17-19].

Tannin: Weighed 0.5g of plant sample and transferred to 50mL flask. 50mL 0f distilled water was added and stirred for 1hrs. The sample was then filtered into a 50mL volumetric flask and made up volume to the mark.5mL of the filtered sample was then pipette out into test tube and mixed with 2mL of 0.1M Ferric Chloride. The absorbance was measured with a spectrophotometer at 395nm wavelength within 10 minutes [17-19].

Antimicrobial Activity

Preparation of Crude Extract: Hundred gram of each of the coarsely powdered plant material was extracted with ethanol. The ethanolic extract was filtered, added sodium chloride solution to the filtered extract to form precipitates. The precipitates were then separated through filter paper; air dried and transfers to air tight amber glass container. The crude extract was dissolved in absolute ethanol to make the final concentration, which kept in refrigerator till used [20].

Preparation of Standard Bacterial Suspension: The average number of viable, *Bacillus subtilis* (NCTC8236), *Escherichia coli* (ATCC25922), *Proteus vulgaris* (ATCC6380) and *Pseudomonas aeruginosa* (ATCC27853) and *Salmonella typhi* (ATCC0650), *Staphylococcus aureus* (NCTC25953) organism per mL of the stock suspension was determined by means of the surface viable counting technique. About (10^8-10^9) colony forming units per mL was used. A fresh stock suspension was prepared each time [21, 22].

Test for Antibacterial Activity: The well agar diffusion method was used to determine the antimicrobial activity of the prepared extracts. Mixed 0.6mL of the standardized bacterial stock suspension (108-109) colony forming units per mL with 60mL of sterile nutrient agar thoroughly. Poured 20 mL inoculated nutrient agar into sterile Petri dishes. Left the agar to set and four well 10mm in diameter was made in each of these plates using sterile cork borer No 8 and then removed agar discs. Filled the entire well with 0.1mL of each extracts using microtiter-pepette and allowed to diffuse at room temperature for two hours. The plats were then incubated at 37°C for 24 hours. Three replicates were also performed for each extract against each of the test organism. Simultaneously addition of the respective solvent instead of extract was carried out as controls.

After incubation the diameter of the results and growth inhibition zones were measured, averaged and mean values were calculated [21, 22].

Preparation of Standard Fungal Suspension: The fungal cultures, *Aspergillus niger* (ATCC 9763), *Candida albicans* (ATCC7596) were maintained on saboraud dextrose agar. It should be incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline and the suspension was stored in refrigerator till used [21, 22].

Test for Anti Fungal Activity: Same, well diffusion method was used to assess the anti fungal activity using saboraud dextrose agar media.

RESULTS AND DISCUSSION

Phytochemicals: Phytochemicals are playing vital role for the treatment of different types of diseases and still are use in, both traditional and modern system of medication. Table 1, data shows that flavonoids, phenols and tannins are present in both blue and white flowering (whole) plant of *S. marianum,* while saponin and alkaloid are not detected. As can be seen from Table 1, high yield of flavonoid (21%) in blue flowering and less (19%) in the white flowering plant has been found. Contents of phenol and saponin are noted very less as compared to flavonoid i.e. 0.430 % phenol is recorded in blue flowering and 0.4321% in the white flowering plant Contents of tannins is noted high in the white flowering (0.8455%) and less (0.6930%) in the blue flowering (whole) plant of *S. marianum*.

Flavonoids, also referred to as bioflavonoids, are polyphenol antioxidants found naturally in plants. Flavonoids are plant nutrients that when consumed in the form of fruits and vegetables are non-toxic

Table 1: Qualitative analysis of Phytochemicals

S.No	Plant Name	Alkaloid	Flavonoid	Phenol	Saponin	Tannin
1	Blue flowering *S.marianum*	-	+	+	-	+
2	White flowering *S.marianum*	-	+	+	-	+

Table 2: Quantitative analysis of Phytochemicals

S.No	Plant Name	Flavonoid (%)	Phenol (%)	Tannin (%)
1	Blue flowering *S.marianum*	21	0.4130	0.6930
2	White flowering *S.marianum*	19	0.4321	0.8455

Table 3: Antimicrobial activity of the crude Silymarin on different bacterial strains

S.No	Active constituent	Zones of inhibitions in millimetre (mm)							
		1	2	3	4	5	6	7	8
1	Silymarin extract of blue flowering *S. marianum*	17	15	21	-	-	-	-	-
2	Silymarin extract of White flowering *S. marianum*	22	13	19	-	-	-	-	-

Gram positive bacteria- 1-*Bacillus subtillis*, 2-*Proteus vulgaris*, 3-*Staphylococcus aureus*

Gram negative bacteria- 4-*Escherichia coli*, 5-*Pseudomonas aeruginosa*, 6-*Salmonella typhi*

Fungi- 7-*Aspergillus niger*, 8-*Candida albicans*

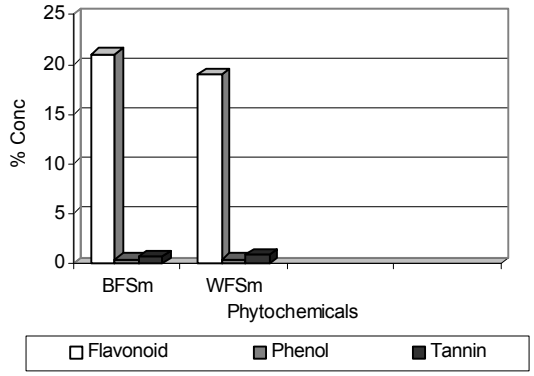

Fig. 1: Graphic representation for quantitative analysis of Phytochemicals
BFSm: Blue flowering *S.marianum* WFSm: White flowering *S.marianum*

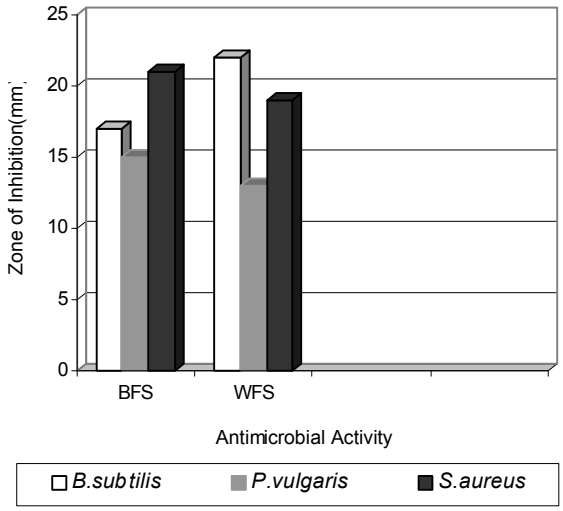

Fig. 2: Graphic representation for antimicrobial activity of the crude silymarin
BFS: Blue flowering silymarin WFS: White flowering silymarin

as well as potentially beneficial to the human. Up to now more than 300 different flavonoids has been isolated. The flavonoids are a common group of contentions in all plants and it plays an important role in the metabolism [23].

Phenols are very wide spread in nature and are probably the largest group of secondary plant metabolites. They range from simple structures having a simple aromatic ring to highly complex polymeric structures and often exist in glycosidic forms [24]. Phenols may be divided into several classes. Those of pharmaceutical importance are the simple phenolic compounds. Simple phenolic compounds consist of a single phenolic ring and often possess alcoholic,

aldehydic and carboxylic acid groups. Examples include vanillin, a phenolic aldehyde and salicylic acid, a phenolic acid. Vanillin is found in the unripe fruits of various species of Vanilla. Capsaicin is found in the dried ripe fruit of different species of Capsicum. It has been used internally for dyspepsia and flatulence. Externally, it is frequently used as counterirritant [25].

Tannins are used in tanning process of animal hides to convert them to leather. They are used as healing agents in inflammation, leucorrhoea, gonorrhea, burn, piles, diarrhoea and as antidote in the treatment of alkaloidal poisoning [26].

Antimicrobial Activity: Microbes are comparatively more prone to mutation, which is the major cause of drug resistance. It is because of these reasons that the search for plant products having antimicrobial properties has intensified in recent years.

Results obtained from Table 3, shows that silymarin both from blue and white capitulum's seeds of *S.marianum* has not shown zone of inhibition against fungus which confirm the results of Ammara Hassan *et al.* (r) and Hannak *et al*. [27], which means that fungus is resistant to silymarin. All the gram-negative bacteria also show resistance to silymarin which confirm the results of Lee, D. *et al*. [28]. Silymarin has been found very active against all gram positive bacteria tested which confirm the results of Ammara, H. *et al*. As can be seen from the Table 3, 17 mm zone of inhibition is detected against *Bacillus subtillis* (silymarin from blue capitulum's seeds) and 22mm zone of inhibition is recorded against the same bacteria (silymarin from white capitulum's seeds). Silymarin from blue capitulum's seeds has given 15mm and from white capitulum's seeds 13mm zones of inhibitions against *Proteus vagaries*, while the zones of inhibition is formed by silymarin from both blue and white capitulum's seeds against *Staphylococcus aureus* is 21mm and 19mm respectively.

CONCLUSSION

S. marianum of blue and white flowering data showed nearly the same values of phytochemicals and antimicrobials activities, therefore if it is cultivated commercially or used for basic manufacturing, both the flowering plants can be utilised.

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