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NUTRITIONAL ANALYSIS OF SELECTED MEDICINAL PLANTS FROM PUNJAB AND THEIR ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

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Abstract

The medicinal plants can prevent and enhance the potential health of human beings with the nutritional constituents such as fats, proteins and other phytochemical compounds. Since active medicinal constituents are present in plants, 78 percent of drugs have a plant origin. The study aimed to find out the proximate analysis and to ascertain the antibacterial and antifungal properties of five native medicinal plants in Punjab, Pakistan: *Convolvulus arvensis, Sesamum indicum, Capparis spinosa, Annona squamosa, and Anethum graveolens*. The sample plant levels were analyzed in terms of moisture content, ash, protein, fat and carbohydrate. Agar Diffusion was used to ascertain the anti-microbial activities on bacterial and fungal strains and reveal the presence of 5% Protein and 15% fat content in higher in *Convolvulus arvensis* and 83.82% carbohydrate content in *Sesamum indicum*. Moreover, the results of the methanolic extracted plants were constructive against bacterial and fungal strains. *Capparis spinosa* contained 19.73% moisture content amongst all the selected plant species and had high potential against *E. coli* at 10mg/ml minimum inhibitive concentration. *Convolvulus arvensis* and *Annona squamosa* gave an MIC of 1mg/ml which inhibited *Salmonella Typhi*. In the antifungal assay, the plant extracts recorded significant results on the fungal strains except Trichoderma. *Convolvulus arvensis* with MIC of less than 10mg/ml and *Anethum graveolens* with MIC of less than 10mg/ml were used in antifungal research with positive outcome against acremonium and pythium respectively. The paper ends up with the palm of medicinal plants of Punjab in Pakistan that can be used against microbial infections. Based on the findings, the medicinal plants that were selected provided knowledge of value to human health when used in therapeutic treatment and can be used as a substitute to synthetic medicines in the future.

Keywords: Nutritional Analysis, Fat content, Phytocompounds, Antioxidant activity, Antibacterial activity.

INTRODUCTION

Herbal plants have a significant impact on preventing diseases as well as improving the health of mankind. They contain the much-needed fats, proteins, carbohydrates and endow phytochemicals of plant origin that are very important as far as medicine is concerned. The pharmaceuticals are obtained using different medicinal plants, approximately 78 percent of which are (Khan *et al.* 2015). Currently, there is a general trend of exploring the potential of biologically active compounds in plant life, especially the antimicrobial agents, to alleviate the infections by pathogenic bacteria, viruses, and fungi. The use of plant extracts is an important source of therapeutic active (Mohamed *et al.,* 2020), which is effective in microbial growth inhibition and useful in the treatment of diseases (El-Sharkawy *et al.* 2017).

Biogenic compounds have a high concentration in phyto therapeutic agents (Amrati *et al.* 2021), which are also used to formulate medicinal preparations (Bourhia *et al.* 2019).

According to recent studies literature, antioxidant activity is mainly exhibited by medicinal plants. Other familiar antioxidants in vegetation include flavonoids, lignin and tannins besides the vitamin A, C, and E (Suffredini *et al.* 2004).

During the recent years, the treatment of health problems associated with cancer therapy, HIV, along with organ or bone marrow transplant has become prone to opportunistic fungi infection (Wingard *et al*., 1993).

Punjab, Pakistan: several native plants are known to have medicinal use in the state and include *Convolvulus arvensis* (field bindweed), *Sesamum indicum* (sesame), *Capparis spinosa* (caper bush), *Annona squamosa* (Custard Apple) and *Anethum graveolens* (Dill). Pakistan has threatened species of genus *Convolvulus L*. The plant is a perennial weed mostly native to the tropical and temperate areas described by (Iqbal *et al.,* 2011), that is locally referred to as Leli in Pakistan. In the north, it is believed to be the most important medicinal marijuana (Jaradat, 2005) to treat epilepsy (Ashfaq *et al.,* 2019). It can be manufactured with both seeds and the vegetative shoots.

Particularly in the small grain fields, the root system of *Convolvulus arvensis* is rhizomatous that develops dense patches that may facilitate the propagation of the weed through cultivation (Sosnoskie *et al.,* 2020). The atmospheric conditions such as the soil contents and the extent of the light impacts on the size of leaves (Kennedy and Crafts, 1931). The presence of convolvulus arvensis signified the presence of large proportion of carbohydrates, amino acids, and fatty acids (Morin *et al*., 1989). Out of the sesame seeds, amino acids, proteins, flavonoid, miners, calcium among others are in plentiful amounts that make them suitable to be consumed by animals and human beings (Pandurang *et al.,* 2022). Its seed contains phytonutrients such as omega-6 fatty acids, flavonoid phenolic anti-oxidants, dietary fibers and so forth that possess a range of health promoting properties. *Sesamum indicum* was discovered in Harapa, Pakistan around 5000 years ago and it is regarded as the ancient oil crop (Bedigian, 2003). It is the oldest oil crop of human belonging to *Pedaliaceae* family (Zech-Matterne *et al.,* 2015) whose size and color of seeds are regulated by the oil content (Eskandari *et al.,* 2015). Sesame seeds also have a high concentration of oil (44-58%), as well as protein (18-25% (Ozcan *et al.,* 2023). This is a diploid, self-pollinating oil crop (21.9 percent protein) that also contains fats (61.7 percent) and has such minerals as FE and CA (Bhattacharjee *et al.,* 2019; Rout *et al.,* 2018).

Capparis spinosa is a flowering plant that blossoms in the summer season and the flowers are pinkish white (Kara *et al.,* 1996). As of (Fici, 2014) described, *C. spinosa* that stretches towards Mediterranean area up to Central Asia. Its sub species are spread in China heterogeneously. The leaf is round, and physiological mechanisms such as stomata opening, cell walls change in response to drought occur in this perennial (Press, 2018; Rhizopoulou and Psaras, 2003). It is capable of locating and storing water due to the existence of big root system (Zuo *et al.,* 2012). This route structure has an optimistic environmental provision during rainfall to the soil and microbes. Since growth rates of many plants in the dry areas are lower, *C. spinosa* can sustain the photosynthetic activity even in arid territories (Levizou *et al.,* 2004). The protein content of its seed is significant (about 27%), as well as its total fats content i.e. 3.3%.

Moreover, the parameters of reducing sugars and crude protein level were determined to

be more in little buds compared to other sizes; however, crude oil, carotenoids, and water were more in massive seeds (El amri *et al.,* 2019). Custard apples are locally referred to as *Annona squamosa*. Its tasty pulp of fruit is white and bitter and their seed-kernel is unhealthy (Nadkarni,1996). It is rampant in tropical and sub-tropical nations such as Malaysia, India, Pennsylvania and Thailand (Nguyen and Nguyen, 2020). *Annona squamosa* is regarded as a fresh plant considering the haematinic properties (Warrier *et al.,* 1994). The *A. squamosa* leaf extract has been identified to be useful in inhibiting the growth of *Fusarium oxysporum* (Safira *et al.,* 2022) specifically *Colletotrichum capsica* (Anil and Talluri, 2021).

Among the vitamins found in *A. squamosa* extract, there is vitamin C (Ronpirin *et al.,* 2012). In their research project, (Shan *et al.,* 2007) reported these 46 extracts which were produced by dietary spices and medicinal herbs exhibiting anti-bacterial effect when subjected to food borne pathogenic bacteria. It is important to note that various extracts of *Pimpinella anisum* L. had high antibacterial activity against *Vibrio cholerae* (Tas *et al.,* 2023). Also, the *Mentha longifolia* L. ssp. longifolia extracted oil showed antimicrobial effects on 23 bacterial species, as well as 15 species and yeasts species of fungi.

Anethum graveolens L. is also known as Dill that is also a component of the *Apiaceae family* (Solouki *et al.* 2012). It belongs to Central Asia, however, and was greatly popular in Egypt. It is also of very medicinal value hence the reason it is grown in cultured areas in Europe, United States and Pakistan (Jana and Shekhawat, 2010). The plant is growing to a height of 90cm and the flowers which are yellow, transform into umbels (Warrier *et al.,* 1994). It upholds its nature within the schedule of 5.3 to 7.8. The propagation is brought about by seeds which need hot summers to produce (Pulliah., 2002). Dill is known as an abundant source of such materials (Kaur *et al.,* 2021). Given the nature of top of form the selected plants, this paper aims at examining their nutritional values and comparing their antimicrobial and antifungal activities.

MATERIALS AND METHODS

Collection of Plant

The plants were chosen in various parts of Punjab i.e. Bahawalnagar, Bahawalpur, Lodhran and Rahim Yar Khan. The Institute of Forest Sciences, The Islamia University of Bahawalpur was the place of botanical identification.

Preparation of crude extract

Plant samples were ground and extraction was done using methanol (CH_3OH) at room temperature. The resulting synergistic methanolic extract was filtered and then evaporated under vacuum conditions so as to produce a concentrated mass. These, so obtained, extracts were evaluated under consideration of the antibacterial and antifungal effects they had (Kuete *et al.,* 2007).

Proximate Analysis

Proximate analysis of all samples (carbohydrates, lipids, proteins, moisture, and ash) of plant samples was done through AOAC methodologies. The difference in weight technique identified the moisture content and ash content (Afzal *et al.,* 2026; Afzal *et al.,* 2024; Haro *et al.,* 1968).

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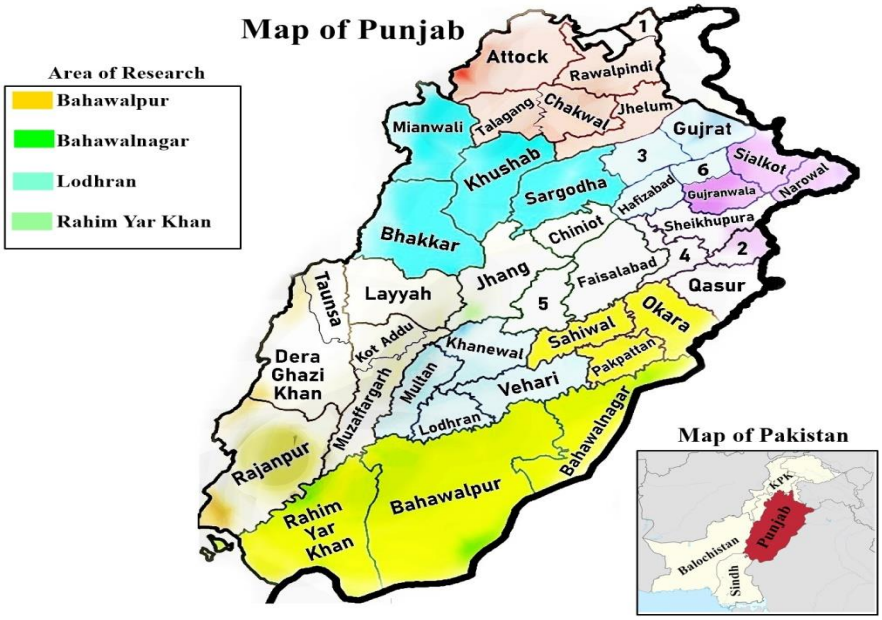

Figure 1: Map of Punjab, Pakistan with highlighted Districts

Determination of moisture

Dried plant material in the quantity of 5g were put in a Petri dish and subjected to a heating temperature of about 105 o C and allowed to burn over a period of six hours. After that, the material was left to cool in a desiccator and moistened. The formula below was used to calculate the content.

$$Moisture\ (\%) = \frac{Weight\ of\ fresh\ sample - weight\ of\ dry\ sample \times 100}{Weight\ of\ sample}$$

Ash Determination

The weight of dried plant material which had been taken was 2.5grams, which was then deposited into a pre-weighed crucible where a small amount of incineration was done in presence of a flame. After this, the crucible was moved to a furnace and heated to a temperature of 550^0C over a period of 3 hours until it reached a white-ash state. This content percentage of ash was then calculated.

$$Ash\ (\%) = \frac{weight\ of\ sample\ after\ washing(\%) \times 100}{Weight\ of\ sample}$$

Crude Fat Determination

A Soxhlet extractor was used to remove the fat content of the sample. Four grams of plant material were put in paper thimble and exposed to extraction after six hours using heating mantle. The content of crude fat was calculated in a percentage based on the formula employed in the guidelines of AOCS (2000).

$$Crude\ fat\ (\%) = \frac{Weight\ of\ beaker\ with\ fat - weight\ of\ empty\ beaker\ \times 100}{Weight\ of\ original\ sample}$$

Protein Determination (Digestion, Distillation, Titration)

The plant samples were added to a digestion mixture of 1:15:0.2 (w/w/w) consisting of copper sulphate, potassium sulphate and ferrous sulphate. After that, 25 mL of concentrated sulfuric acid was added to the flask, and the mixture was

heated in a period of 90 minutes. The obtained digest was then moved into the distillation apparatus and 15 mL of 30% sodium hydroxide solution was introduced. Following the distillation, titration of the product was done to remove the ammonium borate produced. The percentage content was obtained as the percentage result of the following formula;

$$Protein\ (\%) = \frac{1.4 \times 6.25 \times 0.1N\ HCl \times Vol\ of\ H2SO4}{Weight\ of\ sample}$$

Carbohydrate Determination

It was calculated by the given formula.

$$carbohydrate(g) = 100 - (crude\ fat\% + crude\ fiber\% + Ash\% + Protein\%)$$

Anti-Bacterial activity

The microbiology lab of The Islamia University of Bahawalpur purchased different strains of *Staphylococcus aureus* (the Gram-positive), *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella flexneri* and *Salmonella typhi*. Pure agar Petri dishes were carefully prepared to determine the effectiveness of plant extracts in killing bacteria. 0.1 mL of diluted bacterial culture was then diligently spread over each plate and a drying period of thirty minutes was then used at 37^0C. Experimentation was carried out using circular discs with diameter being 8 mm. These discs were placed on the agar plates in a strategic manner and incubated over a 24-hour period at a temperature of 37^0C. After incubating, the areas of inhibition were carefully measured. The lowest concentration of the test solution with the ability to inhibit bacterial growth was determined and it is called minimum inhibitory concentration (MIC). The strength of bacterial strains to aqueous plant extracts was analyzed by assessing the agar diffusion assays of the bacterial strains as was defined in the methodology described by (Bauer *et al.,* 1966). Strains of bacteria, indicating a visible inhibition zone of over 12 mm, were regarded as sensitive.

Anti-Fungal activity

Fungal strains that were used in the study included *Alternaria, Acremonium, Verticellium, Pythium* and *Trichoderma*. Same as above procedure and incubation of the discs at room temperature i.e. 27°C was done at a time that is in 24 hours. The Agar well diffusion method is the technique in which the antifungal activities were evaluated (Parekh and Chanda, 2007).

RESULTS AND DISCUSSION

Proximate or Proximate Analysis of plants.

Nutritional value of the plant is based on the content of protein, carbohydrates, and fats which is critical in the nourishment of human beings as well as animals (Waziri and Saleh, 2015). The medicinal herbs play a significant role in treatment of microbial diseases because synthetic drugs have severe adverse consequences and are overindulged. The therapeutic compounds available as plants may be effective, safe, and resistant to microbial diseases. Although positively-abiotic properties of some plants have been largely documented, there remains a significant number yet to be discovered. T

he quality and quantity of proteins in plant extracts are considered as the basic criterion used to select plants on the basis of their nutritional value, systematic classification, and plant enhancement

programs (Nisar *et al.,* 2009). *Convolvulus arvensis* had the greatest protein content at 5 percent whereas *Capparis spinosa* had low protein content only at 0.59 percent. *Convolvulus arvensis* was found to have the greatest ash content of 25.31 percent as demonstrated in Table 1 and in figure 2. Other studies have reported a decreasing trend in the contents of the ash as the plants age (Hussain *et al.,* 2009). Carbohydrates have very essential functions in biological systems and are immediate energy sources *Convolvulus arvensis* when oxidized, energy storage, and carbon reservoirs in different biological substances (coenzymes and nucleic acids).

Carbohydrates had the highest carbohydrate content (83.82), whereas *Capparis spinosa* had the highest moisture content (19.73). The amount of moisture in various species depends on certain physiological and environmental actions such as humidity, temperature, time of harvest and climatic conditions as well as storage conditions. Our results support the observation of high moisture level in *Capparis spinosa* at 19.73. In the current experiment, *Convolvulus arvensis* had the highest fat content of 15.1 per cent whereas *Capparis spinosa* plant had the lowest.

Table 1: Proximate Analysis of Medicinal Plants

S. No.	Plant name	Ash%	Moisture%	Protein%	Carbohydrate%	Fats%
1	*Convolvulus arvensis*	25.31	18.33	5	63.72	15.1
2	*Sesamum indicum*	18.09	13.61	4.7	83.82	7.0
3	*Capparis spinosa*	12	19.73	0.59	52.62	6.2
4	*Annona squamosa*	9.16	10.48	3.2	29.34	9.2
5	*Anethum graveolens*	21	11.52	4.4	31.66	7.1

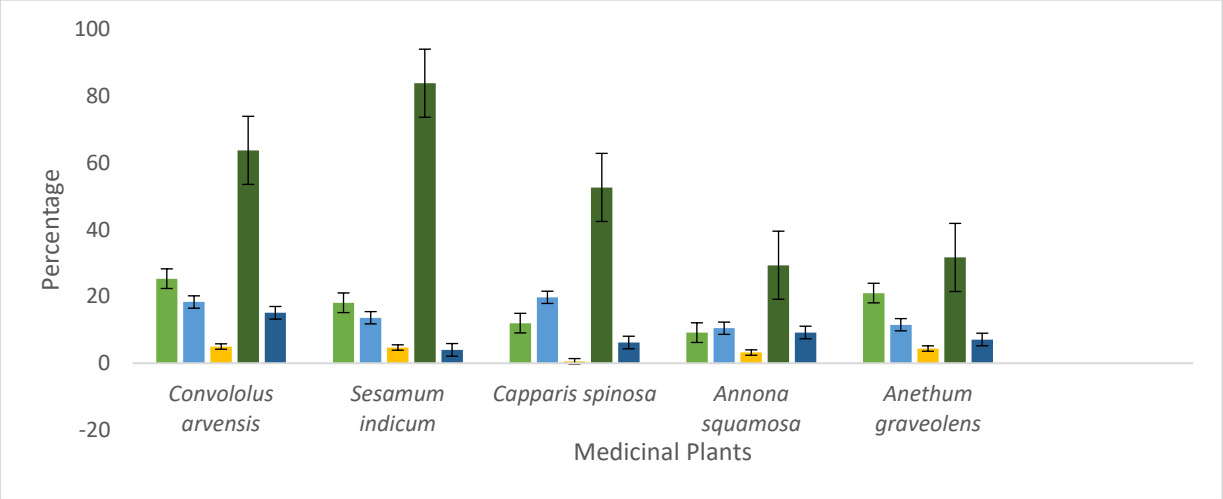

Figure 1: Comparative Proximate Analysis of Medicinal Plants by AOAC Method

Anti-Bacterial Analysis

This paper explored the antibacterial properties of five methanolic plant extracts against a collection of bacterial ciphers, which include *E.coli*, *Staphylococcus aureus*, *Salmonella Typhi, Shigella flexneri* and *Pseudomonas aeruginosa*. Antibacterial activity was also evaluated quantitatively through the use of agar diffusion assay in which the wells or disks that contained the extracts were measured in terms of inhibition zone. Plant extracts with an inhibition zone larger than 12 mm in diameter were then further tested by viable cell count method to find the minimum concentration of the extracts inhibiting the suitable bacterial species. The results as analyzed and reported in Table 2 showed that of all the plant extracts tested, *Capparis spinosa* had the highest effective action against *E.coli* as indicated by an MIC of 10 mg/ml.

On the other hand, *Convolvulum arvensis* and *Annona squamosa* demonstrated efficient inhibition of *Salmonella* Typhi development and the MIC of 1mg/ ml. Nonetheless, no *Annona squamosa* extracts exhibited any visible activities against *S. aureus*. Markedly, *Capparis spinosa* had a higher value of MIC of 10 mg/ mL than the other plant extracts against *S. aureus*.

Anti-Fungal Analysis

The experiment included antifungal properties of five Methanolic plant extracts on five different fungal isolates that had different clinical sources. According to all the plant extracts investigated, there was a remarkable antifungal action of the extract against the fungus, excluding *Trichoderma*. When it comes to inhibitory effectiveness across the spectrum, the calculated range of inhibition of 8 mm, 9-10 mm, and over 10 mm was categorized as minimal activity, moderate activity and strong activity respectively. The most active botanical agent in Acremonium was found in the case of *Convolvulus arvensis* at 10 mm zone of inhibition whereas the strongest 10 mm zone of inhibition was seen in Pythium in the case of *Anethum graveolens* as shown in table 3.

The rest of plant extracts showed an inconsistent moderate-low activity and all the fungal scale strains. The results provided above pronounce unequivocally the nutritional structure of established medicinal plants and their possible resistance to microbial events that can be described as a promising source of therapeutic agents to plants and animals.

CONCLUSION

The findings reached the conclusion that the use of medicinal plants selected possesses nutritional and anti-microbial properties. The proximate analysis showed that the different specimens that were studied had different compositions of ash, moisture, protein, carbohydrate and fat, with the results indicating their different nutritional characteristics. We have come up with the conclusion that *Convolvulus arvensis* recorded good outcomes against *Acremonium* that can be of use in therapeutics. Also, *Capparis spinosa* showed strong inhibition *E. coli*. The paper is used to provide researchers with background information to conduct new studies that would enable tapping the potential of this important plant species that is underutilized.

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AUTHOR'S CONTRIBUTION

All authors have contributed equally.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DECLARATION OF USE OF GENERATIVE AI

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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This research received no external funding.

Table 2: Anti-Bacterial Activity of Medicinal Plants

S.No.	Plants	Bacterial Strains (Minimum Inhibitory Conc. MIC) in mg/mL)				
		E.coli	*S. aureus*	*Salmonella typhi, Shigella flexneri*	*P. aeuroginosa*	
1	*Convolvulus arvensis*	<1	<2	<1	<5	<5
2	*Sesamum indicum*	-	<1	-	<10	<1
3	*Capparis spinosa*	<10	<5	<5	-	<1
4	*Anethum graveolens*	<1	-	<2	<10	<2
5	*Annona squamosa*	-	-	<1	<2	-

Table 3: Anti-Fungal Activity of Medicinal Plants

S.No.	Plants	Fungal strains (Zone of inhibition in mm)				
		Alternaria	*Acremonium*	*Verticellum*	*Pythium*	*Trichoderma*
1	*Convolvulus arvensis*	<10mm	<60mm	-	<5mm	-
2	*Sesamum indicum*	-	<15mm	<20mm	<10mm	-
3	*Capparis spinosa*	<30mm	-	<50mm	-	-
4	*Anethum graveolens*	<35mm	-	<20mm	<10mm	-
5	*Annona squamosa*	<25mm	-	<25mm	-	-

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