CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
http://doi.org/10.5281/zenodo.3749294

Available online at: http://www.iajps.com Research Article

EXPLORATION OF THE PHYTOCHEMICALS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *PLANTAGO OVATA* (ISPAGHOL)

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Article Received: February 2020 **Accepted:** March 2020 **Published:** April 2020

Abstract:

Plants are of great importance. They are the major source of secondary metabolites. Antibiotic resistance and side effects of synthetic drugs urge the use of medicinal plants as a therapeutics or curative aid against infectious diseases. Thus, the present study conducted to evaluate the anti-microbial and anti-oxidant potential of Plantago ovata (Isapaghol). Ethanolic and methanolic extracts of plantago ovata were prepared and screened for various phytochemicals such as phenols, tanins, flavonoid, terpenoids etc. Furthermore, these extracts were used to analyze antioxidant activity using DPPH as a scavenging agent. Antimicrobial properties of the extracts were assessed against Staphylococuss aureus IARS-4, Shigella dysenteriae IARS-9, Proteus vulgaris, Escherichia coli IARS-3, Enterobacter cloacae IARS-7, Acineto bacterbaumannii IARS-10 and A. niger. It was observed that majority of the phytochemicals were present in both extracts. The maximum anti-oxidant activity of Plantago ovata was observed for ethanolic husk (31.67%). It has been observed that husk extracts efficiently inhibit the growth of microorganisms as compared to seed extracts except for A. niger. Hence, it is concluded that the P. ovata extracts has a great ability to be used as anti-microbial. The anti-oxidant property of Plantago ovata made it a potential candidate to be utilized in food, nutraceuticals and cosmetic industry.

Keywords: ***Plantago ovata, antibiotic resistance, phytochemical, anti-microbial, anti-oxidant.***

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QR code

Please cite this article in press Muhammad Talha Yasin et al, ***Exploration Of The Phytochemicals, Antimicrobial And Antioxidant Activities Of Plantago Ovata (Ispaghol),*** *Indo Am. J. P. Sci, 2020; 07(04).*

1.INTRODUCTION:

Medicinal plants are vital component of non-timber forest products. These plants considered an important part of health care in rural areas all over the world. Collection of medicinal plants contributes to poor people's livelihood. In (2002) World Bank has surveyed on the use of medicinal plants in which they reported that more than 1.6 billion people around the world are really depending on forests for their lives [1, 2]. WHO have highlighted that majority of rural area in developed countries are still using traditional medicine as first defense of health care i.e. 85% derived from plant sources [3]. Most medicinal plants serve as raw material to produce the traditional and modern medicine, nutraceuticals, food supplement, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. For a long time, plants with their therapeutic properties have an important place in healing wounds and treatment of diseases [4]. From thousands of years plants have been served as medicine in the form of tea, powder, poultices and other herbal formulations.

The world`s biggest problem is Infectious diseases caused by human pathogenic bacteria that result in killing of about 5000 people per day. Sudden increase in usage of antibiotics induce antibiotic resistance that pose an alarming situation for both developing and developed countries. Secondary metabolites produced by medicinal plants act as therapeutic agents that inhibit the growth of pathogens considered as potential antimicrobial compounds [3]. Medicinal plants used as self-medication in skin diseases as well as natural cosmetics in local habitation [5, 6, 7]. Plants are the source of food, feed, energy and pharmacological materials of medical importance [8]. Medicinal plants are of great importance in a sense that these do not have any side effects in comparison with synthetic drugs that have several side effects [9, 10]. Medicinal plants contain anti-microbial, anti-viral, anti-cancer, anti-inflammatory, anti-tumor and mutagenic agents due the presence phytotoxic secondary metabolites such as Alkaloids, Glycosides, Flavonids, Terpenoid, Steroid, Saponin, Tannin, Coumarins and carbohydrates [11, 12, 13, 14]. The usage of medicinal plants decreases due to the availability of several new synthetic drugs [5]. According to the World Health Organization (WHO), Any part of medicinal plants can be used as a precursor for chemo-pharmaceutical and semi-synthetic medicine [15]. Plants along with their products are still used as anti-parasites by farmers and veterinary surgeons in developed countries all around the world [16, 17, 18].

The history of ancient Arab, Chinese, Iranian and African civilizations provided written evidence of using vast varieties of plants used as medication for many diseases. The biggest landmark regarding medicinal plants have been started at that time when French scientists Caventou and Pelletier have discovered Quinine from the bark of Cinchona [19]. It has been estimated by WHO that 60% of anti-infectious and anti-cancerous drugs are synthesizing from cultivated and wild plants [20]. In recent years, the major Global concern in developing countries is antibiotic resistance due to uncontrollable increase in mortality and morbidity rate of infectious diseases. *Plantago ovate* is belonging to family of Plantaginaceae. The seed and husk of *Plantago ovata* is used a popular household folk medicine in Pak-Indo subcontinent. In recent years it has gained popularity as alternative medicine. It is used commonly as anti-daihrria, in intestinal irritation bowel syndrome, weight loss, obesity, high cholesterol, constipation, ulcerative colitis, laxative and antidiabetics [21, 22, 23, 24]. The fibers present in the husks (outer covering of seeds) of *Plantago ovata* reduce the level of glucose which has been reported by American diabetic association [23, 25]. *Plantago ovata* have major medicinal importance and industrial significance. Mucilage present in Seed husk is used as dyeing, Cosmetics and food supplements such as Bread, Cookies, Candies and Ice cream [26]. Hence, the present study is designed to analyze the anti-microbial and anti-oxidant potential of seed and husk extracts of *P. ovata.*

2.MATERIAL AND METHODS:

2.1 Plant extract preparation

The *Plantago ovata* (seeds and husk) were purchased from the local grocer (pansari). The samples were grinded to dust powder using electrical grinder. This dust powder was used to prepare the organic extracts of ethanol and methanol. A known concentration of dust powder added into 100 ml of ethanol and methanol separately and kept in dark for a week. The concentrated methanolic and ethanolic extracts were then filtered using whatmann filter paper. Hot electric fan was used to evaporate ethanol and methanol present in extracts. The dried extracts were stored at 4^0C for further use (Figure 1).

2.2 Phytochemical screening

Different phytochemical screening tests including saponins, tennins, phenol, glycosides, flavonoids, , carbohydrates, terpenoids, steroids and alkaloids were performed to identify secondary metabolites of biological importance. To identify the presence of Saponins, a test was performed by mixing the crude extract with 5ml of distilled water in a test tube and shake for 15 minutes. Stable foam formation indicates the presence of Saponins [27].

The extract of *Plantago ovata* was mixed with 2-3 drops of ferric chloride. The solution conversion into blue-green and blackish color indicates the

presence of Phenol and Tannin. A test was conducted for the presence of Flavonoids. For the purpose, the Crude extract was mixed with 2ml of 2% solution of NaOH. By the addition of few drops of dilute acid, the change in colour from yellow to colorless indicates the presence of flavonoids. Crude extract was dissolved in chloroform and allow to evaporate in the presence of sunlight. Then, concentrated sulfuric acid (H_2SO_4) was added into the mixture and subjected to heat for 2 minutes. Production of Grayish color indicates the presence of Terpenoids. The presence of Steroids was observed by dissolving the extract with 2ml of chloroform. Then 2ml of each of concentrated H_2SO_4 was poured into the mixture side wise. The reddish color in lower chloroform layer indicated the presence of Steroids. Salkowiski's test was conduct for the presence of carbohydrate by adding 2ml of iodine solution into crude extract. A dark blue or purple coloration indicated the presence of the carbohydrate [28].

For the detection of alkaloid, Crude extract was mixed with 1% HCl followed by heating. Addition of Wagner's reagent (Iodine in Potassium Iodide) into mixture result in brown/reddish precipitates which indicate the presence of alkaloids. Fehling's Test was conduct for the presence of Carbohydrate, crude extract was mixed with 5ml distilled water and filtered. The Filtrate was hydrolyzed with dilute HCl and heated with Fehling's A & B reagents. Formation of red precipitate indicates the presence of carbohydrate [29].

2.3 Antioxidant activity

The extracts of different concentrations were used to evaluate the antioxidant activity (Figure 3.4). The DPPH (1, 1-diphenyl- 2 picryl hydrazyl) were used as scavenging agent against all *P. ovata* extracts (Seeds and Husks). The anti-oxidant activity of these extracts was measured with the help of Spectrophotometer. DPPH solution is prepared by dissolving 2.5 mg of DPPH in 100ml of Ethanol. 25 µl of *P. ovata* extract was added to 975 µl DPPH and shake gently. The mixture was subjected to dark place in incubation for 30 min at room temperature. Afterwards, the absorbance at 517nm is measured through spectrophotometer [30]. The ascorbic acid (Vitamin C) was used as standard to compare the anti-oxidant property of *plantago ovata* extract. Calculations of the percentage of scavenging activity were determined by using the following equation.

$$\% \text{ scavenging activity} = \frac{(\text{Control} - \text{Sample}) \times 100}{\text{Control}}$$

Control = the absorbance of DPPH

Sample = the absorbance of sample consisting of extract and DPPH

2.4 Antimicrobial activity

Ethanolic and methanolic extracts of *Plantago ovata* seeds and husks have been used to evaluate antimicrobial activity against Gram-positive *Staphylococcus Aureus* IARS-4 and gram negative *Shigella dysenteriae* IARS-9, *Escherichia coli* IARS-3, *Acineto, bacterbaumannii* IARS-10, *Proteus vulgaris*, *Enterobacter cloacae* IARS-7 (obtained from NUST-ASAB) and fungal strain *Aspergillus niger* (obtained from AMB lab IIUI) by using disc diffusion method. Many different concentrations of *Plantago ovata extracts* were used i.e. 5mg, 10mg, 25mg and 50mg. Ciprofloxacin was used as positive control in case of bacteria while clotrimazole was used for fungal strain of *A. niger*. DMSO has been used as negative control.

3.RESULTS AND DISCUSSION:

3.1 Phytochemicals

Medicinal plants play a vital role in the production of natural resources and natural wealth. Most of the medicinal plants have therapeutic properties due the presence of secondary metabolites and other phenolic compounds therefore, these are extensively used in manufacturing of traditional and modern medicine. A wide range of biological properties has been reported for *plantago ovatta* as it contains certain phenolic and secondary metabolites. *P. ovata* is a rich source of phytochemicals that are important for the production of medicine. Methanolic and ethanolic extracts of seed and husk (*P. ovata*) were evaluated for qualitative phytochemicals analysis. The results showed that both ethanolic and methanolic extract of *Plantago ovata* (Seeds and Husks) contained different kind of secondary metabolites such as saponin, tannin, flavonoids, alkaloids, steroids etc that are indicated in table below (Table 1). The present results are in accordance to Zhou et al., and Mahmood et al., [31, 32] who reported the presence of similar phytochemicals i.e. flavonoids, alkaloids, terpenoids, steroids etc in *plantago ovata.* Similar results were reported by seyyednejad and motamdi [14] for presence of phytochemicals in plantago ovata. All the phytochemicals were observed except the carbohydrates (reducing sugar).

Table 1. Phytochemical screening of *plantago ovata*

Phytochemicals	Ethanol Husks	Methanol Husks	Ethanol Seeds	Methanol Seeds
Saponins	+	+	+	+
Tannins	+	+	+	+
Phenols	+	+	+	+
Glycosides	+	+	+	+
Flavonoids	+	+	+	+
Carbohydrates	-	-	-	-
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Alkaloids	+	+	+	+

3.2 Antioxidant activity

The antioxidant activities of the *Plantago ovata* (seeds & husks) were evaluated with help of DPPH as scavenging agent while Ascorbic acid has been used as standard. The absorbance was recorded by using several dilutions that are represented in the form calibration curve. The standard calibration curve for ascorbic acid is represented as R^2= 0.959 (Figure 1). The scavenging activity of methanolic and ethanolic extracts of *P. ovata* was compared with ascorbic acid (standard). Husk extract showed the highest anti-oxidant activity (34.613%) in comparison to seed extract of *P. ovata* (Table 2). The anti-oxidant activity for all extracts followed the trend as Ethanolic husk> methanolic husk> ethanolic seed> methanolic seed. The methanolic extract has lowest scavenging activity. In this study, anti-oxidant activity of ethanolic and methanolic extracts of plantago ovata were comparatively better than those reported by [31, 33] Zhou et al., and Souri et al., in his findings. Oliveira et al., [34] also reported the strong relationship between anti-oxidant activity and phenolic contents of plants. Nofal et al., [35] examined the antioxidant potential of *plantago major* plant by inducing genotoxicity and oxidative stress of CCl^4 in rats. A significant change was observed in variating levels of low-density lipoprotein cholesterol, high density lipoprotein cholesterol, alkaline phosphatase, serum albumin, bilirubin and other components involved in kidney function and concluded that *plantago major* plant can be the better protective and anti-oxidative agent against oxidative stress damage. Karima et al 2015 [36] reported that phenolic, terpenes and flavonoid are important phytochemical components held responsible for anti-oxidant activity of *plantago ovata*. Now-a-days, many commercially marketed cosmetics and nutraceutical products contain active natural ingredients of plants with antioxidant properties. These components have the ability to scavenge free radicals in the body by preventing oxidation reactions and inhibition of oxygen radical`s effect that can damage skin cells by starting chain reaction and ultimately result in initiation of drying, pigmentation and wrinkling on skin. These active natural ingredients incorporate synergistic effect and stability to commercial product, therefore, considered as essential component in the formulations of cosmetic merchandise for effective treatment [3]. Thus, *Plantago ovata* that comprise of antioxidant phytochemicals and owing to cheaper and ease of availability could be potentially utilized for commercial cosmetic, nutraceuticals and food products.

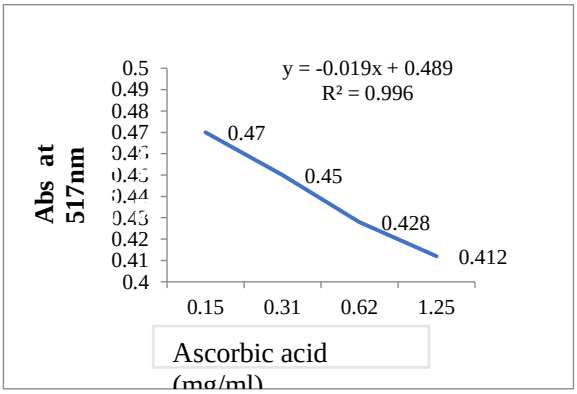

Figure 1. Anti-oxidant activity of standard Ascorbic acid.

Table 2. DPPH scavenging activity of Seed and husk extracts of *Plantago ovatta.*

Extracts	% Scavenging activities
Ethanolic Husks	31.67
Methanolic Husks	25.56
Ethanolic seeds	25.98
Methanolic seeds	24.84

3.3 Antibacterial activity

The medicinal plants came into attention for their potential as anti-microbial agent due to the alarming situation of anti-microbial resistance in the world. The microorganisms genetically becoming resistant against antibiotics. Medicinal plants are the alternative that scientists are trying to explore their potential as new antibiotic source. The antibacterial activity of the *Plantago ovata* seeds and husks extracts of two solvents has been tested against six strains of bacteria. Several concentrations (5, 10, 25 and 50 mg/ml) were used to evaluate the anti-microbial activity of ethanolic and methanolic extracts of *P. ovata* by using DMSO as negative control while ciprofloxacin as positive control for bacteria whereas clotrimazole for fungi. After 24 h of incubation, the values for zone of inhibition were recorded. Zone of inhibitions were observed at 50 mg/ml of ethanolic husk extract against *Staphylococcus aureus* IARS-4 (17.5 mm), *shigella dysenteriae* IARS-9 (15 mm), *Escherichia coli* IARS-3 (9 mm), *Acineto, bacterbaumannii* IARS-10 (9.5 mm), *Proteus vulgaris* (15 mm), *Enterobacter cloacae* IARS-7 (11.5 mm). Minimum inhibitory concentration (MIC) of ethanolic seed extract was 10 mg/ml against *Proteus vulgaris*, *Escherichia coli* IARS-3, and *Enterobacter cloacae* IARS-7 while in case of ethanolic seeds, MIC was observed at 25mg/ml for *Escherichia coli* IARS-3 and 10 mg/ml for *Acineto, bacterbaumannii* IARS-10 and *Enterobacter cloacae* IARS-7. Methanolic husk showed MIC at 25 mg/ml for *Enterobacter cloacae* IARS-7 and 10 mg/ml for *Acineto, bacterbaumannii* IARS-10. The ethanolic and methanolic extract showed satisfied anti-bacterial activity while the fungus was resistant to both extracts of *P. ovata*. Methanolic and ethanolic extracts of husk shown higher anti-microbial activity than seed extracts. In this study, *Staphylococcus aureus* IARS-4 and *shigella dysenteriae* IARS-9 were highly susceptible to husk extracts than any other strains (table 3a and 3b) and *Aspergillus niger* has no susceptibility to any kind of extract.

Table 3. (a): The antimicrobial activity of the ethanolic extract of *Plantago ovata.* (The values are in term of Means ± Standard deviation of triplet analysis. DMSO has no activity).

Plantago ovate Seeds & Husks Extracts	Extract Concentration mg/ml	Bacterial strains Zone of inhibition diameter in mm					
		S. aureus IARS-4	*S. dysenteriae* IARS-9	*P. vulgaris*	*E. coli* IARS-3	*A bacterbaumannii* IARS-10	*E. cloacae* IARS-7
Ethanol Seeds	5 10 25 50	7.5± 0.35 10.5± 0.56 11.5± 0.63 12.5± 0.77	9.0±0.48 10.5±0.56 12.5±0.77 14.5±0.84	0 8.5± 0.45 8.5± 0.45 11.0±0.63	0 8.0±0.42 8.5± 0.45 10.0±0.55	8.0± 0.42 10.0±0.55 10.5± 0.56 11± 0.63	0 10.5±0.49 10.5±0.56 10.5±0.56
Ethanol Husks	5 10 25 50	10.0±0.55 15.5±0.9 16.0±0.92 17.5±1.1	11.5±0.63 11.5±0.63 14.5±0.84 15.0±0.88	7.5±0.35 10.0±0.55 12.5±0.77 15.0±0.88	0 0 8.5± 0.45 9.0±0.48	0 7.5±0.35 8.0±0.42 9.5±0.52	0 7.5±0.35 9.5±0.52 11.5±0.63

Table 3 (b): The antimicrobial activity of the methanolic extracts of *Plantago ovata.* (The values are in term of Means ± Standard deviation of triplet analysis. DMSO has no activity)

Plantago ovate Seeds & Husks Extracts	Extract Concentration mg/ml	Bacterial strain Zone of inhibition diameter in mm					
		S. aureus IARS-4	*S. dysenteriae* IARS-9	*P. vulgaris*	*E. coli* IARS-3	*A bacterbaumannii* IARS-10	*E. cloacae* IARS-7
Methanol Seeds	5 10 25 50	7.0±0.3 7.5± 0.35 8.0±0.42 10.0±0.55	6.0±0.25 8.0±0.42 9.5±0.52 9.5± 0.52	2.5± 0.15 6.0±0.25 6.5±0.28 9.5±0.52	4.5± 0.2 4.5± 0.2 6.5± 0.28 8.0±0.42	3.0±0.18 3.0±0.18 3.5±0.18 5.0±0.22	5.0±0.2 2 5.0±0.2 2 8.0±0.4 2 9.5±0.52
Methanol Husks	5 10 25 50	9.0±0.48 9.5±0.52 11.5±0.63 16.0±0.92	9.0±0.48 10.5±0.56 14.5±0.84 18.5±1.25	8.5±0.45 9.0±0.48 10.5±0.56 12.0±0.75	6.5±0.2 8 7.5±0.3 5 9.5±0.5 2 10.5±0.5 6	0 7.0± 0 7.5±0.35 8.0±0.42	0 0 8.5±0.4 5 12.5±0.77

The mechanism behind the action of plant compounds is that they prevent cell growth by disrupting the cell wall cause the cell components to release and expose to the plant compound that ultimately result in plant death [37]. The results of the study are in accordance to Karami et al., [38] who investigated the antimicrobial properties of seed extracts of *plantago ovata* and *Lallemantia iberica* L. by utilizing disc diffusion method. A high and moderate inhibitory effect of *plantago ovata* was reported against *Bacillus saphaericus* and *pseudomonas aeruginosa*. According to sharma et al., [39], the aqueous extracts of plantago ovata had no activity while ethanolic extract showed strong inhibition activity against E. *coli*, K. *pneumoniae*, P. *aeruginosa* and K. *oxytoca*. In a previous study, seyyed nejad and motamedi [14] has reported that microbial strains were susceptible to ethanolic and methanolic extracts at high concentration while the activity decreased with lower concentration. Similar observations have been made in this study for seed extracts of *plantago ovata* against *Staphylococcus aureus* IARS-4 and *shigella dysenteriae* IARS-9 at lower concentration.

4.CONCLUSION:

Most of the medicinal plant extract serve as the therapeutic agent and raw material for the production of traditional and modern medicines. The seed and husk of *Plantago ovata* is used a popular household folk medicine in Pak-Indo subcontinent. The genetic resistance developed by microorganism against antibiotics urge the the importance of medicinal plants and their wide range of biological activities. The present study revealed that ethanolic and methanolic extracts of *P. ovata* has the great medicinal potential to be used as anti-microbial and anti-oxidative agent that might be useful in disease prevention through diet. There may be further studies conducted to identify and purify the specific phytochemical that contributes to anti-microbial and anti-oxidant properties of *P. ovata* for effective treatment. Furthermore, considering the common utilization of *P. ovata* among the general population of indo-pak, lesser or no side effects can be expected from these preparations.

Acknowledgement:
This manuscript is the part of MS thesis of first author Mr. Abdul Jabbar. The authors are very thankful to the ASAB NUST and International Islamic University Islamabad (IIUI) Pakistan for the provision of essential research services to accomplish this research work.

Conflict of interest: There is no conflict of interest among the authors.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical disclosure: N/A

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