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Therapeutic Uses and Pharmacological Properties of *Plantago major* L. and its Active Constituents

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ABSTRACT

In the recent years synthetic drugs have been widely replaced with herbal medicines and plant extracts because of their little undesirable and extensive beneficial effects. *Plantago major* L. (also known as plantain and way bread) is a member of the Plantaginaceae family. Leaves and seeds of the plant have been widely used in folk medicine for various purposes, including treatment of an extensive range of diseases and disorders such as respiratory complications and digestive system affections. It has been also used in wound healing and as an antiinflammatory, antimicrobial and antitumor agent. Moreover, plantain contains ingredients which can neutralize internal and external poisons. Recent studies have also shown its anti-fatigue properties. Phytochemical analysis of *P. major* extract has indicated that this plant contains a wide range of chemicals such as polysaccharides, lipids (saturated and non-saturated), amino acids (essential and non-essential), caffeic acid derivatives, flavonoids, iridoidglycosides and terpenoids, which have the potential to exert different biological effects. Phenols (ferulic acid), flavonoids and tannins have the highest amount in *Plantago* leaves. The present review describes the traditional uses and recent findings (Since 2000 till date) about the pharmacological effects of *Plantago major* L.

KEY WORDS: *Plantago major L.*; Plantain; Ferulic acid; Plantaginaceae; Wound healing.

INTRODUCTION

*Plantago major*L.is a member species of the Plantaginacea family. It is an herbaceous perennial with a rosette of leaves15-30 cmin diameter. Each leaf is oval, 5-20 cm long and 4-9 cm broad, rarely up to 30 cm long and 17 cm broad, with an acute apex and a smooth margin; there are five to nine conspicuous veins. The seeds are quite small with an ovate shape (0.4–0.8_0.8–1.5 mm) and a slightly bitter taste (Samuelsen, 2000). The flowers are small, greenish-brown with purple stamens, produced in a dense spike 5-15 cm long on top of a stem 13-15 cm tall (rarely to 70 cm tall) (Fig. 1). It grows better than the most of other plants in compacted soils, and is abundant beside paths, roadsides, and other areas with frequent soil compaction. It is also common in grasslands and as a weed in crops. It is wind-pollinated, and propagates primarily by seeds, which are held on the long, narrow spikes which rise well above the foliage (Blamey and Grey-Wilson, 1989). The plant is native to the most of Europe and Northern and Central Asia, and is widely naturalized elsewhere in the world, where it is a common weed. *P. major*was spread by man from Europe throughout the world4000 years ago (Jonsson, 1983).The Indiansnamed it 'White man's footprint' because it was found everywhere the Europeans had been. This has been adapted into the genus name *Plantago* that is from Latin *planta*, meaning sole of the foot. Plantain was also used in the time of Shakespeare and was also named in the piece "Romeo and Juliet" Act I, Scene II of the period 1592 to 1609(Samuelsen, 2000).The plant is well known in many countries (especially in Iran) and has been used extensively in folk medicine because of its various beneficial effects (table1).Native Americans carried powdered roots of *P. major* as protection against snake bite or to ward off snake. As traditional Chinese medicine, *P. major* has long been used for treating viral related disease from colds and influenza to viral hepatitis (Chiang *et al*., 2002).Studies carried out on the chemical composition of the plant by various methods (for example: simple, rapid and accurate high-performance liquid chromatography) show extensive chemical components (tables 2). Studies conducted by Jamilah*et al*. (2012) on the chemical composition of various extract (petroleum ether, methanol, ethyl acetate, n-butanol and aqueous) from *P. major* leaves showed that all of them have phenol groups in their extract while having different variation of organic acid groups, flavonoids and terpenoids. *P. major* leaves contain 0.07% of oleanolic acid and 0.22%of ursolic acid which are two major terpenoids of the plant(Tarvainen*et al.*, 2009;Samuelsen, 2000)(Fig 2).These compounds exist in almost all parts of the plant. Bioactivity of*P. major* leaves and other herbal preparations which contain these secondary metabolites is attributed to these chemical constituents (Liu, 1995).It has been shown in one study that ursolicacid from *P. major*is a Selective Inhibitor of Cyclooxygenase-2 catalyzed prostaglandin biosynthesis; hence, anti-inflammatory effect of the plant is possibly via this mechanism. So many other studies are found in the literature on the pharmacological effects of these two important chemicals. Leaves of the plant are rich

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sources of essential fatty acids (18:2ω6 and 18:3ω3)and also of carotenes (Guil-Guerrero and Rodríguez-García, 1999).Ferulic acid which has high amounts in plantago (especially in seeds)is a hydroxyl cinnamic acid, a type of organic compound(Mohamed *et al.*, 2011). It is an abundant phenolic phytochemical found among plant cell wall components (Fig.2). Ferulic acid, like many natural phenols, is an antioxidant *in vitro.* Recently*in vitro* and animal model studies have suggested that ferulic acid may have direct antitumor activity against breast and liver cancer (Valentão *et al.*, 2001; Pierre *et al.*, 2006).Dosage range is 3-5 g of the powdered herb 1-3 time daily but the most common dose as infusion is 150 ml (one cupful) 3-4 time a day. All in all the plant is safe and there are no limitations and drug contraindications reported so far; however, enough care should be taken regarding usage of the plant in pregnant women(Zagari, 1992).

This article reviews the traditional and newly investigated uses of *P. major* L. along with its pharmacological effects.

Figure 1.*P. major* L. (leaves and seeds).

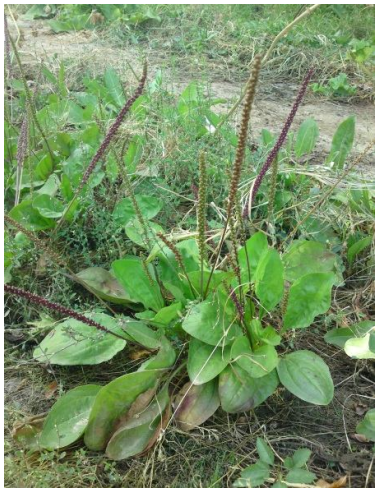


Figure 2. Chemical structures of UA, OA and FA.

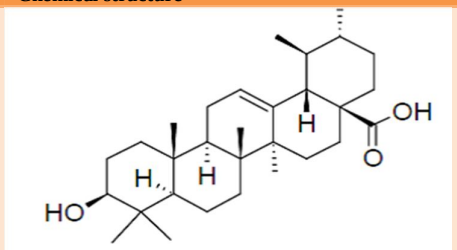
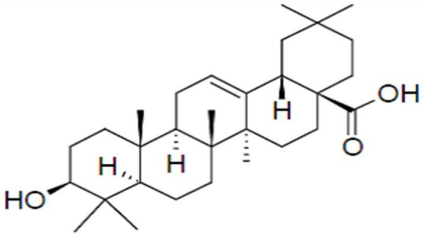
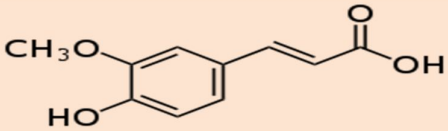
Name	Chemical structure
Ursolic acid	
Oleanolic acid	
Ferulic acid	

Table 1. Traditional uses of *Plantago major* L. in different countries especially Iran.

Part of plant and preparation	Usage	Country	References
Whole plant decoction	Healing different kinds of wounds such as (snake bite, intestinal worms and infectious wounds), cold treating, Remedy for diabetes	Colombia, Italia	Watkins *et al.*, 2011. Idolo *et al.*, 2010. Jarald *et al.*, 2008.
Fresh leaf of the plant	Internal inflammations such as cystitis, enteritis and swollen abdomen	Mexico	Watkins *et al.*, 2011.
Internal use of leaves(oral)	Respiratory catarrh; astringent effect; bleeding, skin problems; eye inflammations; also fresh leaves applied to treat livestock hematomas and their skin problems; pruritus.	Colombia, Iran,	Rahimi *et al.*, 2010. Mir-heidari, 1994. Zagari, 1992. Neves *et al.*, 2009.
Topical use of leaves (lotion)	Antipyretic, Antitussive, Emollient. Blood rectifier, Kidney pain	Portugal, Italia, Iran	Viegi*et al.*, 2003. Idolo*et al.*, 2010. Zagari, 1992.
Mix of Leaf and Root	Anti-infective	Iran	Mir-heidari, 1994.Zagari, 1992.
Decoction and infusion of fresh leaf	Kidney pain	France	Boulogne *et al.*, 2011.
Decoction of leaves of Plantago major	Remedy for hemorrhagic-diarrheal, Tonic, stimulant	Central America and Mexico	Vera-Kua*et al.*, 2010.
Seeds of plant	mouth inflammation Eye inflammation	Iran, India	Mir-heidari, 1994.
Oral use of the extract	Remedy for tuberculosis Soothing effect	Iran	Mir-heidari, 1994. Zagari, 1992.
Decoction of root Decoction of *P. major*	Anti-hemorrhagic Remedy for pulmonary disease Antipyretic	Iran	Mir-heidari, 1994. Mir-heidari, 1994.
Decoction of leaf with vinegar	Anti-infective	Iran	Mir-heidari, 1994.
Mixture of the sap of leaves and honey	Remedy for Ear pain and Bruises	Iran	Mir-heidari, 1994.
Extract of the root	Urinary tract infection; toothache	Iran	Mir-heidari, 1994.
Decoction of *P. major*, *Euphorbia schlechtendalii* and *Melochianodiflora*	Stomatitis, asthma, bronchitis, ear ache, anti-tussive	Iran, Central America and Mexico	Vera-Kua *et al.*, 2010.
Brewed leaves and root	Remedy for Ear pain	Iran	Mir-heidari, 1994.
Whole plant **Root of plant** **Aqueous extract**	Bruises, Urinary tract, toothache Stomatitis, Asthma, bronchitis, Ear ache	Iran	Zagari, 1992. Zagari, 1992. Zagari, 1992.
Juice of the plant and honey	Anti tussive	Iran	Zagari, 1992.

Table2. Biological active compounds of *P. major* L. leaves and seeds (mg/g on dry weight basis) (Mohamed *et al.*, 2011)

Constituent	*P. major L. leaves*	*P. major L.* seeds
Total phenol (mg gallic/g)	13.05±0.10	7.43±0.07
Total flavonoid (mg Quercetin/g)	6.41±0.04	3.03±.03
Tannins (mg Catechine /g)	5.63±0.06	2.43±0.03

PHARMACOLOGICAL EFFECTS

Immune Enhancing Effects

Endotoxin-free methanol extracts of *P.major*leaves, in the absence of IFN-γ or LPS, increased production of nitric oxide (NO) andTNF-$\alpha$by rat peritoneal macrophages andstimulated lymphocyte proliferation in a dose-dependentfashion.NO and TNF-α production by untreated macrophages was negligible. The regulation of immune parameters by the extract of *P. major*may be helpful in treatment of numerous diseases (Gomez-Flores*et al.*, 2000).For instance, activated macrophages produce mediators of cytotoxicity such as nitric oxide and tumour necrosis factor-alpha (TNF-α), kinds of lymphokines which protect the host against the development of tumors and infections by organisms such as *Cryptococcus*, *Schistosoma*, *Leishmania*, *Francisella*, *Listeria* and *Mycobacteria* (Nathan and Hibbs, 1991; Hibbs*et al.*, 1988.)

Hepatoprotective Effects

Hepatic disorders have grown in recent years and are the cause of billions of deaths all over the world (Williams, 2006). In one study the hepatoprotective activity of *P. major* seed extract in an experimental rat model of carbon tetrachloride (CCl_4) induced hepatotoxicity was evaluated. Control, CCl_4 and reference groups received isotonic saline solution, CCl_4 and silibinin, respectively. *P. major* groups were injected CCl_4 (0.8 ml/kg) and the extract at doses of 10, 20 and 25 mg/kg, respectively for seven days. After sacrificing animals on

the 8th day blood and liver samples were collected, and then changes in plasma marker enzymes of hepatic damage and histopatholgical alterations were recorded. Significant decrement in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels was observed at the dose of (25 mg/kg) in the treated group. Microscopic examination of the livers depicted significant recovery and minimization of necrobiotic changes, in same group (Türel*et al*., 2009). Parallel study was carried out by Atta*et al.* (2006).The results of these studies indicate potential hepatoprotective effect of *P. major* extract. Further studies may suggest replacement of synthetic drug with this natural preparation.

Anti-ulcerogenic Effects

According to some ancient evidence, *major* seeds have been used as a remedy for inflammatory bowel disease (IBD) in Iran, but there are no or not enough studies supporting this use of the plant (Rahimi*et al.*, 2010).In screeninganti-ulcerogenic activity of *P. major*, methanol extract of the plant was assayed using ethanol and aspirin-induced gastric ulcerationsinr at models. Leaf and seed extracts were prepared separately to observe the difference in their pharmacological actions. In alcohol induced ulcer models, the leaf extract significantly decreased the ulcer index with curative ratio of 87.50% but the seed extract had no considerable effect in the same model. Ranitidine (100mg/kg) produced curative ratio of 38.90%.in aspirin-induced ulcer models. Leaf extract at an oral dose of 400 mg/kg, significantly decreased the number of gastric ulcers; however, the seed extract had no effect on the number of ulcers. Both extracts decreased total acidity. It was also observed that *P.major* (leaves and seeds) extract at an oral dose of 400mg/kg did not affect the volume of gastric juice or its protein content. No details have been revealed about the mechanisms of these actions(Atta *et al*., 2005).Also one of the main ulcerogenic agents in digestive system is Helicobacter pylori. According to Cogo*et al*. (2010) studies Plantago major is capable to inhibit H. pylori in vitro.

Antidiarrheal Effects

In a study, the effect of ethanolic extract of *Plantago* Leaves was evaluated on castor oil-induced diarrhea and gastrointestinal movements in rats (charcoal meal) and on the motility of duodenum isolated from freshly slaughtered rabbits. *P. major*ata dose of 200mg/kg (oral) demonstrated significant antidiarrheal effect for at least 4h.This activity was potentiated at a dose of 400mg/kg (oral). In addition, the extract significantly decreased the distance travelled by the charcoal meal when given at both doses (200and 400 mg/kg). The large dose of the plant extract was slightly more effective than the small one. The extract in a concentration of1.6 mg/mL or less produced a transient stimulation the on motility of isolated duodenum. A higher concentration produced rapid relaxation. The initial stimulant effect may be attributed to the presence of irritant substances and may explain the contradiction in the folkloric use of this plant. No clear details were registered about responsible ingredients (Atta and Mouneir, 2005).Further research is, however, needed to determine what compounds are responsible for the Antidiarrheal effect.

Antinociceptive Effects

Methanolic extracts of leaves and seeds separately were studied on acetic acid-induced writhing and tail-flick test in mice, to investigate their anti-nociceptive effects. Oral administration of 400 mg/kg of the seed extract showed significant nociceptive activity against acetic acid-induced writhes with a protection of62.3%. However, at same doses the protection rate of the leaf extract was only 48.8%.These values were compared to 80.5% for the standard dipyrone (50 mg/kg) which is the synthetic drug. The smaller dose (200 mg/kg) of the plant extract did not protect animals from painful acetic acid stimulation. The leaf extract at the dose of 400mg/kg produced significant increase in the latency to the tail response to thermal stimulation. Mild or no effect was observed at the small dose. No detail study has been carried out so far, about the ingredients that can induce such analgesic effect (Atta and Abo EL-Sooud, 2004).

Antioxidant and Free Radical Scavenging Effects

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, and ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen, 1999; Cook and Samman, 1996).Free radical scavengers are any compounds that react with free radicals in a biological system, thus reducing free radical-induced damage and protecting against the indirect effects of free radicals. Ethanolic, hot and cold water extracts of *P. major* leaves and seeds were assayed for determination of free radical-scavenging activity using stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH)*in vitro*. Highest antioxidant activity was observed with the ethanolic leaf extract even at a low concentration of 20 ppm (78% activity). In the same concentration the ethanolic seed extract had really low activity (25%). The antioxidant activity of both mentioned extracts increased in a concentration-dependent fashion, up to 60 ppm. The rate of antioxidant activity for both extracts was so close in a concentration of 100ppm. Hot and cold water extracts of *Plantago* leaves were more effective than the seed extract. It was also observed that, the ethanolic extracts were more active than the hot and cold water extracts of the samples under

investigation (Mohamed *et al.*, 2011). In another study, methanolic extract of the whole plant was studied to measure the antioxidant activity and determine responsible contents. Results showed that the scavenging property against DPPH, is probably due to hydroxyl groups existing in the phenolic compounds of the plant. The extracted concentration of 0.8 mg/g had 89.3±1.5% scavenging activity and with an IC50of 0.32mg/ml. Total phenol content of the plant, responsible for the scavenging activity, was measured as 31±4mg/g (Pourmorad *et al.*, 2006).Another suggested mechanism responsible for the scavenging activity of *P. major* is that highly active flavonoids present in the plant possess a 3',4'-dihydroxy occupied B ring and/or 3-OH group which can embrace oxygen radicals and reduce them into neutralized substances like water (Amic*et al.*, 2003).

Anticancer Effect

In one study, methanolic extracts from seven *Plantago* species used in traditional medicine among them *P. major*, were evaluated for cytotoxicactivity against three human cancer cell lines: the human renal adenocarcinoma(TK-10), the human breast adenocarcinoma (MCF-7) and the human melanoma (UACC-62) cell lines) using the sulphorhodamine B (SRB) assay*in vitro*. *P. major* and the other six *Plantago* species showed cytotoxic activity on the breast adenocarcinoma (MCF-7) and melanoma (UACC-62) tumoral cell lines in a concentration-dependent manner at the recommended NCI (USA) doses. None of the extracts, with the exception of *Plantagobellardii* (GI50 = 86μg/ml), showed cytotoxic activity against renal adenocarcinoma (TK-10) cells. It is thought that the cytotoxic activity depends basically on flavonoids, flavone and luteolin present in the extract (Gálvez*et al.*, 2003).

Another*in vitro* study was carried out on *Plantago*ethanolic, hot and cold water extracts of leaves and seeds separately. A dose dependent inhibition was observed for all tested extracts. The ethanolic extract of *P. major* L. leaves had the greatest effect on tumor cell growth (Dead 74% ±0.35) follow by its hot water extract of the leaves (Dead 54.6% ± 1.21)(Mohamed *et al.*, 2011).Luteolin-7-O- β-glucoside, the major flavonoid found in all species of Plantago, is known to be the responsible agent for the anti-cancer activity of the plant. The precise mechanism responsible for the cytotoxic activity of luteolin-7-O- β-glucosideis not thoroughly understood, however it is thought that topoisomerase-mediated DNA damage is the involved mechanism. Luteolin-7-*O*-β-glucoside acts as a potent DNA topoisomerase I poison as well as its aglyconluteolin. (Gálvez*et al.*, 2003)

Cytotoxic Activity

The cytotoxic activity of *P. major* methanol extract on human transformed cells: HCT-15 (colon carcinoma), SQC-UISO (cervical carcinoma), OVCAR (ovary carcinoma) and KB (nasopharynx carcinoma) cultured in RPMI-1640 medium has been also evaluated, *in vitro*. The extract (1µg/mL) was cytotoxic against the UISO and OVCAR cell lines but stimulated the proliferation of KB cells. (Velasco- Lezama*et al.*, 2006). Ina screening of anticancer effect of forty-five Russian plants, used in folk medicine, a parallel *in vitro* study was carried out using Mouse leukemia cells (L1210). Methanolic extract of *P.major* had 80-100% cytotoxic effect (Goun*et al.*, 2002). Similar work was done in Vietnamese and seventy-seven medicinal plants tested for their antiproliferative activities against human HT-1080 fibro sarcoma cells. *P. major* was not among the most active plants (Ueda, 2002). Studies on the efficacy of hot water extract of *P. major* leaves on Ehrlich ascites tumors in male mice were also undertaken. The extract was most effective at a dose of 25µg/ml against the tumor cells. The results show that *P. major* could be proposed as an effective agent in cancer prevention (Ozaslan *et al.*, 2009).

Hematopoietic Effects

Aqueous and methanolic extracts of the aerial parts of *P. major* were added to bone marrow and spleen cell medium to investigate their hematopoietic potential. The results were as following:

Bone marrow cultures: The aqueous and methanolic extracts stimulated cell proliferation in similar manner using a dose of 0.4and 0.2 gr/mL. Maximum hematopoietic activity was observed at0.1 and 0.05 g/mL doses of the methanolic extract(Velasco-Lezama *et al.*, 2005).

Spleen Cultures: Doses of 0.4 and 0.2 g/mL of the aqueous extract increased the cell population by 3.30- and 4.40-fold, respectively. The same concentrations of the methanolic extract increased the population by 6.25- and 4.28-fold, respectively. The increase was significantly higher in spleen cultures than in bone marrow cultures(Velasco-Lezama *et al.*, 2005).This effect of *P. major* on spleen as a hematopoietic organ is thought to be the second mechanism through which the plant exerts hematopoietic effects.

Wound Healing Effects

Use of *P. major* in wound healing has a very long history. Greek physicians described its wound healing activity in the first century and the leaves were used as a remedy for dog bites (Samuelsen, 2000; Roca-Garcia, 1972).It is also well known for its wound healing property in Scandinavia. The common

Norwegian and Swedish name for *P. major* is *groblad* meaning "healing leaves" (Samuelsen, 2000). Hence, the plant`s Water (fresh and dried leaves) and ethanol-based leaves extracts were studied using scratch assay with the oral epithelial cells *in vitro* to validate it's ancient traditional use. Concentrations of 0.1, 1 and 10mg/ml of the plant extracts added to cell culture media to observe cell proliferation/migration. Apart from the highest concentration of 10mg/mL, ethanol-based extracts had the most beneficial effect, followed by water extracts of fresh leaves, ethanol plus water extracts of dried leaves and, finally, water extracts of dried leaves. Maybe polyphenols are the responsible compounds for wound healing. Phytochemical analysis showed that high levels of plantamajoside and other polyphenols exist in ethanol-based extract compare with other tested extracts. Other involved substances are polysaccharides (Zubair*et al*., 2012).In a whole, a mixture of antioxidants are said to be effective the wound healing process of the plant (Yokozawa*et al*., 1997).

Anti-inflammatory Effects

Inflammation is a complex event linked to tissue damage whether by bacteria, physical trauma, chemical, heat or any other phenomenon and inflammatory response is the critical protective reaction to these kinds of injures remarked by redness, fever, oedema (swelling) and pain of involved tissue (Morais Lima *et al*., 2011; Levine and Reichling, 1999). Methanol extract of *P. major* L. seeds was assayed on carrageenan-induced rat paw oedema to evaluate the anti-inflammatory activity. *P. major* showed anti-inflammatory effect in a dose dependent fashion, but it was not more effective than indomethacin (reference drug). Median effective dose (ED_{50})was determined to be 7.507 mg/kg (Türel *et al*., 2009).It could be thought that inhibition of COX-2-catalyzed prostaglandin biosynthesis may be the involved mechanism for the anti-inflammatory action (Ringbom *et al*., 1998). Furthermore, flavonoid derivatives which are high in *P. major* are other responsible constituents present in the plant (Middleton *et al*., 2000; Havsteen, 2002).

Anti-fatigue Effects

Fatigue is a condition which is marked by the feeling of exhaustion due to heavy physical activity and generally can cause muscular pain. Ethanol extract of *P. major* seeds were studied on forty eight male mice to determine its effect on physical strength. Forced swimming test and biochemical assays of blood were carried out and marker factors were registered. According to the results, the extract increased swimming time by increasing tissue glycogen (as energy source) and decreasing serum urea nitrogen and blood lactate (as fatigue agents). Therefore, it is suggested that the extract possesses anti-fatigue effects and can improve endurance exercise capacity (Mao-yeand and Li-guo, 2011).There is little evidence about this effect of *P. major* and the precise mechanisms responsible, therefore more studies are required to be conducted in this regard.

Pest Organism Managing Properties

An ethno medicinal survey was applied in the city of Pelotas, Brazil, with professionals and patients in the Unified Health System (SUS), showed that the most frequent symptom reported for herbal drug usage was infection (55.3%), and tanc¸ agem (*P. major*) was the plant mentioned to be the most often used to treat this problem (37.3%) (Dias Oliveira *et al*., 2012).

In order to evaluate efficacy of mayan (a large family of American Indian) traditional potions in treating of infectious bowel disease *P. major* L. and thirty-eight other plants were studied *in vitro* using bacteria including *Escherichia coli*, *Klebsiella pneumonia*, *Shigella flexneri*, *Salmonella typhi* and protozoa such as *Entamoeba histolytica* and *Giardia lamblia*. Mixture of *Melochia nodiflora*, *Euphorbia schlechtendali* and *P. major* was active against *Giardia lamblia* (IC_{50} = 21.78 μg/ml). The efficacy of this formulation for both protozoa was comparable with positive control (metronidazole). Moreover, another formula composed of four herbs, *Trema micrantha*, *Euphorbia schlechtendali*, *Diphysa carthagenensis* and *P. major* showed good activity against *Giardia lamblia* with an of IC_{50} = 12.71 μg/ml. Both formulae of *P. major* were so close to the efficacy index of metronidazole against *Giardia lamblia* and *Entamoeba histolytica*. These two formulae showed no considerable effect against bacteria (Vera-Ku *et al*., 2010). In another study, extracts of 13 Brazilian medicinal plants were screened for their antimicrobial activity against bacteria and yeasts. Leaves extract of *P. major* presented some degrees of antibacterial activity and was not among the most active plants (Holetz *et al*., 2002).

Similar works were carried out by Saltan Çitoğlu and Altanlar (2003), but the results were adverse. According to this study, when compared with the standard antibiotics, *P. major* extract was found to have good activities against *E. coli* and *S. aureus*. However, *P. major* did not show any activity against *B. subtilis*. Further details are available in table 3.

Table3. The inhibition zones diameters of free and aqueous ethanolic extracts of *P. major* (mm) (Çitoglu and Altanlar, 2003).

Diameters of the inhibition zones (mm)							
	E.coli	**P.aeruginosa**	**B. subtilis**	**S. aureus**	**C.albicans**	**C.galabrata**	**C. krusei**
Plantago major	11	10	-	13	7	12	12
Ampicillin (25 fig)	12	N.T	13	15	N.T	N.T	N.T
Fluconazole(25 ng)	N.T	N.T	N.T	N.T	18	20	20

N.T.: not tested. (-): no inhibition zone, Ampicillin and Fluconazole are positive control.

Antibacterial effects of acetone and ethyl alcohol extracts of *P. major* L. leaves were studied, using macro dilution liquid (tube) method. Both extracts were tested for nine bacteria species (*Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, and Salmonella enteritidis*). The ethyl alcohol extract was only effective against *E. coli* and *B. cereus*, but acetone extract was effective on all selected bacteria species at different concentrations (Metiner *et al.*, 2012).

Previously, parallel studies were carried out by Sharifa *et al.* (2009). The whole plant methanolic, ethanolic and aqueous extracts of *P. major* were tested on S*taphylococcus aureus, Bacillus subtilis, Escherichia coli, Candida albicans* and *Candida tropicalis*. The methanolic and ethanolic extracts at concentrations of 100-200 mg/ml showed bactericidal activity against both Gram positive and Gram negative bacteria tested. Electron microscopic observation demonstrated collapse in Gram positive bacteria cell wall and blebs formations on Gram negative bacteria. No activity was observed against yeast by any of the extracts.

In one study, the aqueous, methanol, chloroform and hexane extracts of the aerial parts (leaves and seeds) of *P. major* were added to *Escherichia coli, Bacillus subtilis and Candida albicans* cultures and antibacterial activities were observed in different ranges (Velasco-Lezama *et al*., 2005).

The antibacterial effect of a soluble pectin polysaccharide (PMII), isolated from the leaves of *P. major* was examined against systemic *Streptococcus pneumoniae* serotype 6B using animal model of mice. It was observed that PMII can have prophylactically protective effects (Hetland *et al.*, 2000)

Antiviral activity of aqueous extract and pure compounds of *P. major* was assayed using *herpes viruses* (HSV-1, HSV-2) and adenovirus species (ADV-3, ADV-8, ADV-11).The aqueous extract possessed only a slight anti-herpes activity. In contrast, the pure compounds were active against selected viruses. Among them caffeic acid exhibited the strongest activity against HSV-1 (EC_{50}=15.3 g/ml, SI=671), HSV-2 (EC_{50}=87.3 g/ml, SI=118) and ADV-3 (EC_{50}=14.2 g/ml, SI=727), whereas chlorogenic acid possessed the strongest anti-ADV-11 (EC_{50}=13.3 g/ml, SI=301) activity. The potency relates to the presence of hydroxyl group in the chemical structure. Compounds that contained hydroxyl groups at the R_1 and R_2 positions (caffeic acid, chlorogenic acid) were more potent than the compounds containing one hydroxyl group at the R_1 position (ferulicacid, p-coumaric acid). Results suggest the use of these compounds as a remedy for infections caused by these two viruses (Chiang *et al.*, 2002). Results are in contrast with those reported by McCutcheon *et al.* (1995).

According to a pilot study by Ali *et al.* (2004), interview with 492 informants from 13 villages indicated that macerated, dried leaves of *P. major* can be used as a remedy for malaria. Screening study must be carried out to determine antimalarial activity of *P. major* and also to isolate and identify the active compounds, which may be regarded as future promising phytotherapeutics in the treatment of malaria.

Antigenotoxic Effects

Anti genotoxic property of the sap from greater plantain (*P. major* L.) was investigated using two bacterial test systems (SOS chromo test and Rec assay). *P. major* showed sizable anti genotoxic effect in none of the test systems. This means that *P. major* extract cannot be used as a dis mutagen (prevent DNA damage) or bio anti mutagen (repair of damaged DNA) agent (Karamova *et al*., 2010).

External Poison Detoxification

Heavy metals such as lead are toxic for humans and animals and can cause various diseases. *P. major* L. was grown hydroponically in a water medium supplemented with concentrations of lead ion under different duration times and temperature regimes to evaluate the efficacy of lead detoxification by different parts of the plant (roots, stems, leaves and whole plant). Roots of the plant showed the highest removal rate of lead than other parts (Akram et al., 2007). As a concern in public health, the use of agents which can purify water and environment from heavy metals is necessary. Therefore, *P. major* can act as a bio filter for the removal of Pbcations from water.

Toxicity Evaluation

In recent years synthetic drugs have been widely replaced with herbal medicines in both developed and non-developed countries (Verma and Singh, 2010). Some possible reasons are: the development of new diseases with severe complications for which there is still no appropriate treatment and the belief that herbal medicine have less or no side effects. In addition to the belief that herbal medicine is naturally superior to synthetic drugs, economically plants are cheaper sources of remedies (Capasso *et al.*, 2000). Apart from advantages of herbal drugs, their safety has not been confirmed scientifically and there are still some risks in their usage. Presence of toxic constituents (pyrrolizidine alkaloids, saponins, cyanogenetic glycosides, etc.) is the main risk in this regard. Evaluation of toxicity of chemical and natural products isolated from 20 plants was carried out using *Artemia salina* L. (Artemiidae) as *in vitro* test and Swiss albino mice as *in vivo* test. LC_{50} and LD_{50} for *P. major* were determined as 4.74 (µg/mL) and 182.54 (mg/kg), respectively. *P. major* was not among the most toxic plants (Parra *et al.*, 2001). Analysis of the anti-nutritional and toxic components showed low content of oxalic acid (6736 mg) and erucic (3.45%) in *P. major* extract (Guil *et al*., 1997). Laboratory studies have reported uterine stimulatory activity of *P. major*; therefore it should be only used under medical supervision during pregnancy (Shipochliev, 1981). In conclusion, *P. major* l is a safe plant, with low content of toxic factors, however some adverse reactions such as: nausea, vomiting, diarrhea, anorexia, bloating, hyper-sensitivity and dermatitis may arise after treatment with the plant. Life threatening anaphylaxis may occur in more serious cases, which is observed in high dose usage.

Conclusion and Further Scope

This review presents up to date findings about *P. major*, based on the most recent pharmacological studies that support its traditional uses. The leaf extract is reliably nontoxic with strong hepato-protective and wound healing activities, however data about the responsible constituents is little and further research is required. Anti-fatigue effect of the plant is also one of the newly investigated effects of *P. major* that needs to be further investigated.

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