IJBPAS, April, 2016, 5(4): 873-886

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYTOCHEMICAL ANALYSIS AND PHARMACOLOGICAL SCREENING OF SOME PLANTS OF RAFHA, NORTHERN BORDER PROVINCE, SAUDI ARABIA

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ABSTRACT

This study was designed with the aim to evaluate the phytoconstituents and pharmacological activities of three plants i.e. *Plantago lanceolata, Portulaca oleracea* and *Rumex vesicarius* which are grown wild in Rafha, Northern Border Province, Saudi Arabia and to give scientific proof for their traditional uses. The plants were extracted using solvents of varying polarities. Preliminary phytochemical screening was performed to identify the various classes of phytoconstituents. Quantification of the total phenolic acid content, flavonoids content and condensed tannins was estimated for the extracts by the Folinciocalteu method, Aluminum chloride colorimetric method and Vanillin hydrochloride colorimetric method respectively.TLC was performed to identify some of the constituents. Wound healing activity was performed by the excision wound model, Anti-inflammatory activity of extracts was carried out by using formalin induced paw edema and analgesic activity was evaluated by Eddy's hot plate method. The amount of phenolic acids, condensed tannins and flavonoids were found to be more in *Portula caoleracea* when compared to *Plantago lanceolate* and *Rumex vesicarius*. The phytoconstituents detected by TLC were rutin, quercitin, gallic acid, ellagic acid, salicylic acid, vanillic acid and β Sitosterol. *Portulacaoleracea* showed the best wound healing activity and analgesic activity when compared to the other two extracts, and this was attributed to the higher amount of phenolic acids and flavonoids. All the three plants exhibited significant wound healing, analgesic and anti-inflammatory activity when compared to the control, thereby giving scientific proof for the use of these plants by the traditional healers for healing of wounds.

Keywords: ***Portulaca oleracea, Plantagolanceolate, Rumex vesicarius*, phytochemical, pharmacological**

INTRODUCTION

Natural products are those chemical compounds derived from living organisms, plants, animals and insects. The knowledge of the drugs has been accumulated over thousands of years as a result of man's inquisitive nature[1]. Despite the advances in modern medicine, new drugs from natural products are still considered important. Since 1980 the World Health Organization has been encouraging countries to identify and exploit traditional medicines and phytotherapy[2]. Herbal medicine is used as the primary source of healthcare for approximately 75-80% of the world population. They are in great demand because they are culturally acceptable, have wide spectrum of biological activities, higher safety margins and lesser costs. Today plant based medicines are only 9.5 % of the estimated 250,000 species worldwide. Therefore the field is wide open. Fortunately, the interest and concern to discover novel compounds from natural origin are ever on the increase.

Traditional medicine occupies a significant part of Saudi Arabia's heritage and is widely used across the country.The knowledge of the traditional healers is not passed from the older generations and Bedouin to younger generations; Moreover, these traditional healers use wild herbs without any scientific knowledge. Hence there is a need to screen the plants for their phytoconstituents and medicinal properties and give scientific proof for their traditional uses.Herbal products are popular as a result of a widespread belief that the preparations are natural and therefore safe [3-5]. The first attempt to cover the flora of Saudi Arabia was in 1974 and it is widely practiced until now [6-7].The flora of Saudi Arabia comprises of several medicinal plants. A total of 2250 species in 142 families are represented in the flora of the Kingdom. Two volumes of Medicinal Plants were published with 300 species from the flora; representing 12% of the total species of the flora. Review of literature also reveals that investigation for medicinal plant diversity in seven families of 254 species, of which 86 are medicinal and were used by local and several tribal people including medicinal healers for the cure of more than 150 ailments. Forty nine species are classified under threatened status in the wild. Drug discovery form traditional medicinal plants continue to provide new and important leads against a number of diseases[8-15].

Folk medicine practiced in southwestern Saudi Arabia has helped people prevent and cure various diseases. In addition, this

ethnobotanical knowledge is not passed on to the younger generations.Moreover; those traditional healers do not make effort to cultivate these medicinal plants threatening to make them extinct. This requires more attention since more than 15,000 plant species may face extinction globally due to over harvesting and habitat loss [16].Hence there is a need to screen the plants for their medicinal properties and give scientific proof for their traditional uses. Review of literature reveals that not much scientific work has been carried out on plants of this region.In this study an attempt was made to screen some of the plants grown in this region for their constituents and activities.

The three plants under investigation i.e. *Plantago lanceolata*, *Portulaca oleracea* and *Rumex vesicarius* are grown wildly in Rafha, a town in the Northern Border Province of Saudi Arabia, close to the border with Iraq. It is located at around 29°38′19″N 43°30′5″E. According to the Köppen-Geiger climate classification system, it is classified as hot desert.

Plantago lanceolate (Arabic name: Azan el-Kabsh) belongs to the family Plantaginaceae. The plant is a rosette-forming perennial herb, with leafless, hairy flower stems. The basal leaves are lanceolate spreading with 3-5 strong parallel veins narrowed to short petiole. The leaf stalk is deeply furrowed, having oblong inflorescence of many small flowers each. It is reported to be used as astringent, demulcent, emollient, expectorant, in cough, in gastrointestinal disorder, in diarrhoea, as diuretic, as anti- inflammatory, anti-spasmodic, wound healing, antibacterial and antiviral [17-18].

Portulaca oleracea (Arabic name: Hamqa) belongs to the familyPortulacaceae. It has smooth, mostly prostrate stems and alternate leaves clustered at stem joints. The flowers are yellow and have five regular parts and are up to 6 mm wide. It has a taproot with fibrous secondary roots .Review reveals that this plant possesses Anti-microbial activity, anti atherogenic, renal protective, immune modulatory, antihyperlipidemic, Antioxidant and nephro protective activity.It also possesses Anti- atherogenic, Reno protective, immune modulatory activity, Anti-haemoerhoidal effect, Anti- arthritic activity and Anti- diabetic activity [19-20].

Rumex vesicarius (Arabic name: Humaidah) which belongs to the family Polygonaceae is an erect plant, usually with long tap roots. The have leathery leaves form a basal rosette at the root. The leaf blade margins are entire or crenate. The flowers are carried above the leaves in clusters. The flowers and seeds grow on long clusters at the top of a stalk

emerging from the basal rosette; the flowers and their stems may be brick-red. Each seed is a 3-sided achene, with a round tubercle on one or all three sides. It is used as a medicinal herb, in treatment of liver diseases, digestive problems, toothache, nausea, pain, anti-inflammatory, antitumor, as anti-schistosomal, and anti-microbial [21-23].

MATERIALS AND METHODS:

Collection of the plant:

Plantago lanceolata, *Portulaca oleracea* and *Rumex vesicarius* which are wildly grown ,were collected from Rafha, Northern Border Province ,Saudi Arabia in the month of September 2015 and were shade dried in the research lab of Faculty of Pharmacy, pulverized and stored until further use. These were identified and authenticated by Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, Northern Border University where the specimen has been deposited for future reference.

Extraction of the plant material:

The three plants were subjected to extraction with solvents of various polarities starting from petroleum ether, followed by chloroform and methanol. The aqueous extract was prepared by macerating the pulverized power in water for 24 hours. The different extracts were collected, evaporated and vacuum dried to get constant weight and percentage yield was calculated.

Phytochemical analysis

Preliminary phyto chemical analysis: The various extracts of the plants were subjected to qualitative analysis for the phytoconstituents like alkaloids, carbohydrates, glycosides, steroids, tannins, proteins, amino acids and flavonoids as per the standard procedures[24].

Total phenolic content was determined by Folin-Ciocalteu (FC) method using gallic acid as standard [25]. The plant extract(1ml)was mixed with 1 ml of FC reagent, and incubated for 5 min,10ml of 7% Na_2CO_3 was added.The absorbance was recorded at 750 nm in the UV spectrometer by plotting absorbance versus the concentration to obtain a standard curve from the various dilution of the standard gallic acid solution.

The flavonoid content ofthe plants was evaluated by the Aluminum chloride colorimetric method [25].The sample of the plant extract was mixed with 1.5 ml of methanol; 0.1 ml of 1M potassium acetate, 0.1 ml of 10 % aluminum chloride and 2.8 ml of distilled water .The absorbance of this mixture was measured at 415 nm.A calibration curve for the standard quercitin was obtained by taking different

concentrations.The total flavonoid contents were calculated as quercitin equivalent from thecalibration curve by plotting the absorbance versus concentration.

Colorimetric estimation of condensed tannins by vanillin assay [26].Aliquots of the standard catechin in the range of 50-350 µg/ml were prepared and transferred to two sets of tubes and the volume in each of the tubes was brought to 1 ml by methanol. The tubes were incubated at 30° C in a water bath. Five ml of working reagent was added at an interval of 1 min to one set of the tubes and 5 ml of 4 % HCl was added to the second set at intervals of 1.0 min (blank).The samples were kept in the water bath for 20 min and the absorbance was recorded at 500 nm. The absorbance of the blank was subtracted from that of the sample containing vanillin reagent. The amount of condensed tannins contents in the extracts were calculated as catechin equivalent from the calibration curve of standard catechin by plotting the absorbance versus concentration

Thin layer chromatographic studies: TLC was performed using precoated silica gel plates 60F254. The extracts were spotted on the TLC plate using capillary. The development chamber was pre-saturated with mobile phase for 20 min before using. Different mobile phases were used. The developed TLC plates were removed, dried and constituentsdetected by observing the TLC plates. The biomarkers used were flavonoids rutin and quercitin, phenolics acids;gallic acid,ellagic acid,salicylic acid, vanillic acid and steroid used was β – sitosterol

Pharmacological activity: The extracts were evaluated for woundhealing, analgesic and anti-inflammatory activities. The guidelines for the animal care were strictlyadhered to during the experimentation.

Wound healing activity[27]:The extracts were formulated as 5% ointments using simple ointment base for performing the wound healing activity.Albino rats of either sex weighing 200-250 g were used. The animals were maintained under standard conditions and were fed with commercial diet and water *ad libitum* during the experiment. The animals were anesthetized using chloroform and an impression was made on the dorsal thoracic region 1 cm away from the vertebral column and 5 cm away from the ear. The skin was excised to the full thickness to obtain a wound area of about 500 mm^2.The animals were divided into six groups group 1 was used as a control, group 2 was treated withFucidin ointment, group 3,4,and 5 were used for ointments prepared using *Plantago lanceolata,*

Portulacaoleracea and *Rumexvesicarius* extracts respectively. The wound area was measured by tracing the wound on a millimeter scale graph paper every alternate day till complete falling of scar. Complete healing i.e. no leaving of the wound was considered as the end point of complete epithelialization and the days required for this was taken as the period of epithelialization.

The analgesic activity of the extract was evaluated using Eddy's hot plate[27].Rats of either sex weighing 250-275 g were used. The animals were maintained understandard conditions and were fed with commercial diet and water ad libitum during the experiment. The animals were divided into 8 groups of six animals each. Group 1 served as the control and was given normal saline, group 2 was administered with standard Tramadol, group 3,4and 5 weretreated with 250 mg/kg body weight (lower dose);while groups 6,7 and 8 were treated with 500 mg/kg body weight (higherdose) of the three extracts. The Eddy's hot plate was maintained around 55-56°C.The animals were placed on the hot plate and the time taken for licking, flicking of the hind paw or jumping from the hot plate was considered as the reaction time of the particular animal which was recorded.

The anti-inflammatory activity of the extract was evaluated by Formalin-Induced Paw Edema.

The animals were selected as mentioned above and the animals were divided into five groups. Group 1 served as a control, group 2 was given Ibuprofen, group3, 4, and 5 were given 400mg of the three extracts. Paw edema was induced by sub plantar injection of 20 ml of freshly prepared 2% formalin in the right hind paw. The changes in paw size were measured by wrapping a piece of thread round the paw and measuring the circumference of the paw with a meter rule [28]

The percentage of inhibition of edema was calculated by using formula:

% Inhibition of edema = (Vc-Vt/Vc) x100

WhereVt = Paw volume in test group animals.

Vc = Paw volume in control group animals

Statistical analysis: Results of the animal studies were tabulated and the data was expressed as mean ± SEM. The difference between experimental groups and the control were determined using one way analysis of variance (ANOVA) followed by Tukey HSD Test.P-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION:

Preliminary phyto chemical analysis revealed the presence of alkaloids, glycosides, tannins, flavonoids, saponins, carbohydrates and proteins in the various plant extracts. With an aim to identify the sources of potential physiological activities of the evaluated medicinal plants and justify their traditional medicinal uses, we have focused on the methanolic and aqueous extracts, as an attempt to represent the decoctions prepared from various plant organs used as a medicine. It is well known that the medicinal effects of any plant is because of synergistic response of the different constituents, hence there is a need to identify the type and identify as many phytochemicals as possible in order to explain the plant activity. Literature survey has revealed that plant metabolites like phenolic compounds (simple phenols,phenolic acids, flavonoids, tannins etc), and sterols play an important role in many of the activities like wound healing, analgesic, anti-inflammatory and anti-microbial activity [29].Hence the total phenolic acids, flavonoids and condensed tannins were evaluated for the methanolic and aqueous extract and some of the phenolic compounds were identified by TLC.It has been reported that Methanol is the most suitable solvent for the extraction of polyphenolic compounds from plants, as it has ability to inhibit the action of polyphenol oxidase which is responsible for oxidation of polyphenols compared to water, which was true in case of *Plantago lanceolate* and *Rumex vesicarius* ,but the amount of phenolic acids was in a higher concentration in the aqueous extract when compared to the methanolic extract in case of *Portulaca oleracea* (for methanolic extract 400 µgm while 490 µgm in case of aqueous extract). This could be due to the hydrolysis of the glycosidic and ester bonds of the condensed flavonoids and hydrolysable tannins and this is in concurrence with the reported literature [30].The amount of total phenolic acid, flavonoids and condensed tannins is as represented in table no 1

Identification of phytoconstituents by TLC:

TLC of the methanolic extracts of the three plants was performed using a number of solvent and solvent mixtures to identify some of the phyto constituents. The best solvent systems for the different phytoconstituents were as presented in table 2.

Phenolic acids like gallic acid, vanillic acid, and salicylic acid were present it all the three plants, while ellagic acid was detected in *Portulaca oleracea* and *Rumex vesicarius* but was not detected in *Plantago lanceolata*. Quercetin, rutin and β sitosterol was detected

in all the three plants. These constituents could be responsible for the pharmacological activity of the plants.

The literature survey has revealed that plants like *Curcuma longa, Ficusreligiosa, Lantana camara*etc which have been used as wound healing agents also possess significant analgesic and anti-inflammatory activity [31].Hence the extracts were evaluated for analgesic and anti-inflammatory activityalong with wound healing.

The extracts were formulated as 5% ointments using simple ointment base by the fusion method for performing the wound healing activity. The wound healing activity of the methanolic extracts was evaluated using excision wound model. The activity was assessed by the rate of wound contraction (table 3).The days taken for complete epithelization were significantly reduced by all the three extracts when compared to the control.100 % wound closure was obtained by the 10^{th} day by *Portulaca oleracea* extract while *Plantago lanceolata* and *Rumex vesicarius* exhibited 100% wound healing by the 12^{th} day.

The dose of the extracts for analgesic and anti-inflammatory activity were selected based on the reported toxicity studies of *Plantago lanceolata, Portulaca oleracea* and *Rumex vesicarius* [32-34]. The dose selected was i.e. 200 mg / kg (lower dose) and 400 mg / kg (higher dose)body weight.

Analgesic activity: Analgesic activity was evaluated using the Eddy's hot plate method. Theextracts showed significant analgesic activity within 15 minutes .The extracts of *Portulaca oleracea* showed maximum activity at 60 mts at both the lower and the higher dose, while the other two extracts showed maximum activity at 30 mts (Table 3). As per the literature this method produces two measureable behavioral components as a response to thermal pain i.e. paw licking and jumping; which are considered to be supraspinally integrated [36]. Thus, the extract has significantly inhibited these behaviors on hot plate method indicating that these extracts may be acting at supraspinal level.

Anti-inflammatory activity: Formalin-Induced Paw Edema model is based upon the ability of the extract to inhibit the edema produced after injection of formalin. The nociceptive effect of formalin is reported to be consisting of two phases, which involves an early neurogenic component which is followed by tissue-mediated response. There is release of histamine, 5-HT, and kinin is in the first phase, followed by the release prostaglandins .The results of our studies have revealed that the % inhibition of edema

is more in the first two hours suggesting that the extracts may have inhibiting activity of the first phase[36].

The various phytoconstituents present in plants have been reported to possess great potential in treatment of various diseases, many of them have been shown to be very useful inwound care i.e. promoting the rate of wound healing, bringing about decrease in pain, discomfort, and scarring. These constituents owe their activity to direct effect on the wound healing processes and sometimes to their various other activities like anti-oxidant,analgesic,anti-inflammatory and anti-microbial properties or it could be a synergistic effect due to the combination of these properties.Wound healing consists of different stages i.e. inflammatory, proliferate, and remodelingphases. During the inflammatory phase, bacteria and debris are phagocytosed and removed, the proliferative phase is characterized by angiogenesis, granulation, collagen deposition, tissue epithelialisation, and wound contraction and the remodeling phase, and collagen is remodeled and realigned along tension lines[37]. The initial phase of wound healing is the inflammatory phase, which is followed by the phase of proliferation and then the phase ofmaturation. Our studies have revealed that the three plants possess significantanalgesic and anti-inflammatory activity whencompared to control. It has been suggested that prostaglandins and bradykinins play amajor role in theanti-inflammatory and analgesic activity which are crucial mediators of inflammation. So it may be predicted thatthese extracts may be acting by inhibiting the synthesis of prostaglandins andbradykinins.The structure and the presence of free hydroxyl groups in the various phenolic compounds make them an important class of compounds which may help in free radical scavenging which is another important factor for wound healing[38].

Table 1: Amount of total phenolic acid, flavonoids and condensed tannins in the methanolic and aqueous extracts of the three plants

Name of the plant	Extract	Total phenolic acid µgm	Total flavonoidµgm	Condensed tanninsµgm
Plantago lanceolata	Methanolic	282	150	170
	Aqueous	122	120	110
Portulacaoleracea	Methanolic	384	400	218
	Aqueous	420	490	192
Rumexvesicarius	Methanolic	263	362	150
	Aqueous	110	254	120

Table 2: Phytoconstituents detected by TLC along with their solvent systems:

Sl no	Biomarker	Solvent system	Plant	Result
1.	Gallic acid	Petroleum ether: Methanol (9:4.5)	*Plantago lanceolata*	+
			Portulacaoleracea	+
			Rumexvesicarius	+
2.	Salicylic acid	Chloroform :Methanol (5:2)	*Plantago lanceolata*	+
			Portulacaoleracea	+
			Rumexvesicarius	+
3.	Ellagic acid	Chloroform: Methanol: Acetic acid 9:1:1	*Plantago lanceolata*	Not detected
			Portulacaoleracea	+
			Rumexvesicarius	+
4.	Vanillic acid	Chloroform: Methanol: Acetic acid 8:1.1:4	*Plantago lanceolate*	+
			Portulacaoleracea	+
			Rumexvesicarius	+
5.	Quercitin	Chloroform :Methanol(3:4)	*Plantago lanceolata*	+
			Portulacaoleracea	+
			Rumexvesicarius	+
6.	Rutin	Chloroform :Methanol (9:5)	*Plantago lanceolata*	+
			Portulacaoleracea	+
			Rumexvesicarius	+
7.	B-sitosterol	Chloroform:ethyl acetate(9:2)	*Plantago lanceolate*	+
			Portulacaoleracea	+
			Rumexvesicarius	+

Table 3: Effect of the methanolic extracts on 50% wound contraction and period of epithelialization in excision wound model:

Groups	50 % falling of scar(days)	Wound Contraction(days)
Control	8.0±00	19.9±0.89
Fucidin ointment	6.4±0.89	15.6±0.89*
Plantago lanceolata,	5.6±0.54**	12.8±1.09**
Portulacaoleracea	3.8±0.44**	10.8±1.09**
Rumexvesicarius	6±00	12.8±1.09**

All values are mean ± SEM, n=5, all the data was found significant at P<0.0001.* significant,very significant.**

Table 3:Analgesic activity of the methanolic extracts:

		Reaction time in seconds				
Groups	dose	0 min	15 min	30 min	60 min	120 min
Control		2.82±0.40	2.9±0.69	2.52±0.63	2.31±0.24	2.73±0.47
Tramadol	2mg/ml	3.03±0.18	4.4±0.43	5.26±0.59	7.51±2.16**	7.76±4.06**
Plantago lanceolata	250mg	3.08±0.23	4.61±2.01	4.96±1.44	3.91±1.30	3.05±0.88
	500mg	3.21±0.75	4.57±1.45	5.15±2.23	4.64±1.35	3.19±0.75
Portulacaoleracea	250mg	2.25±0.12	4.39±2.0	6.79±1.72**	7.85±1.56**	6.80±2.61**
	500mg	3.07±0.42	7.45±1.26**	7.82±0.76**	8.54±1.28**	7.78±2.31**
Rumexvesicarius	250mg	2.71±0.21	6.46±0.64**	6.28±1.72**	4.43±1.05	4.22±0.77
	500mg	2.49±0.42	6.74±0.99**	7.46±2.68**	6.83±2.71**	5.57±2.71

All values are mean ± SEM, n=6, all the data was found significant at P<0.01,very significant when compared with control**

Table 4:Anti-inflammatory activity of the methanolic extracts:

Groups	Oedema size(cm) (% inhibition)				
	0 h	1hr	2hr	3hr	4hr
Control	1.85±0.54	2.38±0.25	2.71±0.14	2.73±0.15	2.36±0.15
Standard	1.5±.01	1.46±.01 (38.65)	1.38±.07* (49.07)	1.3±0.08* (52.38)	1.11±0.7* (52.38)
Plantago lanceolata (500mg)	1.56±0.16	1.14±.01 (41.17)	1.21±0.14* (55.35)	1.2±0.06* (56.04)	1.03±0.05* (56.35)
Portulacaoleracea (500mg)	1.58±0.98	1.48±0.09 (37.81)	1.41±.07* (47.97)	1.31±.14* (52.01)	1.1±0.06* (53.85)
Rumexvesicarius(500mg)	1.46±0.18	1.41±0.14 (40.75)	1.3±0.10* (52.02)	1.26±0.1* (53.8)4	1.08±0.07* (54.23)

All values are mean ± SEM, n=6, *P<0.01 is significant when compared with control

CONCLUSIONS

The results of our study give scientific proof for the use of these extracts for different types of diseases by the traditional healers. The activities of these plants were attributed to the presence of different phytoconstituents.Further detailed investigations are needed so as to isolate and identify the active phytoconstituents that may be responsible for pharmacological activities exhibited; and to elucidate their detail mechanism of action,which may lead to the discovery of molecules which may prove to be of importance to mankind. The plant extracts can alsofurther be studied for other unexplored activities.

ACKNOWLEDGEMENT:

The authors are grateful to the Deanship of Scientific Research, Northern Border University, Saudi Arabia for approving,providing necessary facilities and financial support by; grant NO.(3-2-1436-5) in 2015–2016.

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