EVALUATION OF PHARMACOLOGICAL EFFECTS AND PHYTOCHEMICAL SCREENING OF RUMEX HASTATUS EXTRACTS

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Background

Rumex hastatus (khatimal) Polygonaceae, 200 species Grow between 700-2500m
Compounds
Chemical constituents
anthraquinones, naphthalenes, flavonoids and phenolic (Zhang *et al*., 2009)
Macromolecules
Moisture, ash, crude fiber (Hameed and Dastagir, 2009)
Leaves
pl eivant acidic taste .Used in chutneys and pickles (Manan *et al*., 2007).
Leaves+young shoot
flavouring agent, carminative, purgative, diuretic (Murad *et al*., 2011).
Astringent, Aperients (Manandhar, 2002).
Whole plant
Laxative, alterative, tonic, refrigerent (Shinwari and Gilani, 2003).
Skin diseases, bilious complaints, piles, bleeding of lungs (Gorsi and Miraj, 2002).
Plant juices for blood pressure and sore throat (tonsillitis) (Manan *et al*., 2007).
To cure sexually transmitted diseases (Vermani and Garg, 2001)).

Objective

To evaluate the antimicrobial, pharmacological and antioxidant potential of R. hastatus
To extracts the phytochemical constituents from the R. hastatus which could be the potential source of therapeutic agents in the field of pharmocology.

MATERIALS AND METHODS

- Collection and identification of plant material
- Extraction
- Bacterial strains
- Agar well diffusion method
- Antibiogram assay
- ABTS Decolorization Assay
- DPPH free radical scavenging assay
- Qualitative phytochemical screening
- Total phenolic contents
- Total flavonoid contents
- Thin layer chromatography
- Antioxidants analysis of TLC-developed plates
- Spot screening of TLC developed plates of *Rumex hastatus*
- Direct Bioautography assay
- Statistical analysis

Result

Table 1: Zone of inhibition recorded against clinical bacterial pathogens by *leaves of Rumex hastatus*.

Conc (mg/ml)	Bacterial pathogens	Zone of inhibition (MSD) in mm against pathogens: Methanol	Ethanol	Acetone	chloroform	Diethyl ether
125.0	E. coli	0.33 ±0.57	2.33±0.57 *	0.33 ±0.57	1.33 ±0.57 *	1.00 ±1.00 *
	S. marcescens	2.00±0.0 *	2.00±0.0 *	2.00±0.0 *	0.0±0.0	0.0±0.0
	K. pneumoniae	1.00±0.0 *	2.00±0.0 *	2.00±0.0 *	0.0±0.0	0.0±0.0
	S. aureus	7.33±0.57	7.66±0.57 **	8.00±0.0 **	0.0±0.0	0.0±0.0
	S. pyogenes	6.00 ±0.0 *	7.00 ±0.0 *	6.00 ±0.0 *	0.0±0.0	0.0±0.0
	P. aeruginosa	7.66±0.57	7.00±0.0 **	0.33 ±0.57	0.0±0.0	0.0±0.0
	S. epidermidis	0.33 ±0.57	6.00±0.0 **	7.00±0.0 **	0.0±0.0	0.0±0.0

Growth inhibition were expressed as (0) for no sensitivity, *(1- 5 mm) for low sensitivity, ** (>5-10 mm) for moderate sensitivity and ***(>10-25 mm) for high sensitivity. (SD±SD) Mean±Standard deviation.

Table 2. Phytochemical screening results of *Rumex hastatus* extracts in different solvents

Phytochemical constituents	Extracts used: Methanol	Ethanol	Acetone	Chloroform	Diethyl ether
Tannins	+	+	+	–	–
Alkaloids	+	+	+	–	–
Saponins	+	+	+	–	–
Terpenoides	+	+	+	–	–
Carbohydrates	+	+	+	–	–
Quinones	+	+	+	–	–
Steroids	+	+	+	–	–
Flavonoids	+	+	+	–	–
Phenols	+	+	+	–	–
Glycosides	–	–	–	–	+
proteins	+	+	+	–	–
Free amino acids	+	+	+	–	–

(+) and (-) indicates presence and absence

The effect of *R. hastatus* aerial parts as antibacterial was analyzed against clinical bacterial pathogens through agar well diffusion method. All the extracts showed some sensitivity against these pathogens. Polar extracts such as methanol, ethanol and acetone showed maximum inhibition. On the other hand *E. coli, S. epidermidis, S. pyogens* and *P. aeruginosa* showed moderate zone of inhibition when polar extracts were applied while *K. pneumoniae* and *S. marcesscens* showed minimum inhibitory zones. The bacterial growth was inhibited by polar extracts as compared to nonpolar **(Table 1)**.

Qualitative phytochemical screening gave the positive indication for the presence of flavonoids, phenols, terpenoids, tannins, protein, carbohydrate, amino acids, phytosteriods, quinones and saponins.**(Table 2)**

Summary

Phytochemical screening of various fractions of leaves and stem of *Rumex hastatus* showed the presence of active secondary metabolites including, alkaloids, flavonoids, saponins, tannins, terpenoids, phytosteriods, phenols and terpenoids that was attributed with strong pharmacological behaviour. Considering the results of biological assays it can be concluded that most of the tested fractions appeared as an important source for the discovery of new antimicrobial drugs for the therapeutic use of animals and humans infectious diseases.

In chromatographic evaluation, data obtained by analyzing sample of *Rumex hastatus* inform the presence of flavonoids as well as phenolic compounds. In fact, these fractions may be a good source of flavonoid for preparation of drugs. The results obtained by *in vitro* studies are remarkable with regard to the antioxidant activities of tested plant samples particularly methanolic, ethanolic and acetonic fractions attributed due to the phenolic and flavonoid contents. It can be concluded that such notorious potential and natural modules could be exploited as efficient food/feed additives for the health of mankind.

Figure 1: Bioautography of TLC developed plates of *Rumex hastatus* after TBTB spray against bacterial pathogens. Para = *P. aeruginosa*, E. coli = *E. coli*, St. A = *S. aureus*.

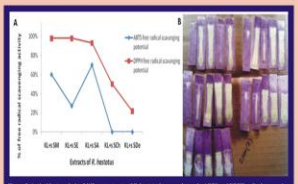

Figure 2: Antioxidant analysis of different extracts of *R. hastatus* leaves and stem by ABTS and DPPH radical scavenging activity. Each value represented as M ± SD. R1 =5 SM = Methanol leaves and stem in solvent methanol, R2 =5 SE = Methanol leaves and stem in solvent ethanol, R3 =5 SA = Methanol leaves and stem in solvent acetone, R4 =5 SCh = Methanol leaves and stem in solvent chloroform, R5 =5 SDe = Methanol leaves and stem in solvent diethyl ether. M = mean and SD = standard deviation.

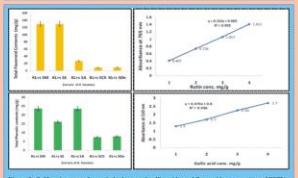

Figure 3: Calibration curve for rutin hydrate and gallic acid, total flavonoid contents (mg of RHE/g of extracts) of *R. hastatus* and total phenolic contents (mg of GAE/g of extracts) of *R. hastatus*.

Conclusions

To evaluate the antimicrobial, pharmacological and antioxidant potential of R. Hastatus
To extracts the phytochemical constituents from the R. hastatus which could be the potential source of therapeutic agents in the field of pharmocology.

TLC-Bioautography was performed against *S. aureus, P. aeruginosa* and *E. coli* which showed good sensitivity to *R. hastatus* extracts **(Figure 1)**. TLC-Bioautography revealed clear zones of growth inhibition around each plate after treatment with TBTB with purple background indicating one or more bioactive antimicrobial compounds in *R. hastatus* extracts **(Figure 1)**. Spot screening indicated the moderate zones of inhibition around each well indicating the presence of active compounds.

ABTS free radical scavenging potential was obtained as acetone>methanol>ethanol > chloroform > diethyl ether (Figure 2.A). The results revealed that tested sample inhibited or scavenge the free radicals. The DPPH scavenging potential of methanol and ethanol was calculated as 98%, acetone (93%), chloroform (50%) and diethyl ether (27%), respectively **(Figure 2.A)**. The conformation of the presence of antioxidant constituents in extracts was done by spraying DPPH solution on TLC plates of various solvent systems. The results evaluated the clear white bands on TLC plates indicating the presence of antioxidants **(Figure 2.B)**.

The total contents of flavonoids were expressed in rutin hydrate equivalents (RHE), varied from 8.5 to 129.4 mg rutin hydrate equivalent/g dry fraction **(Figure 3)**. Methanolic and ethanolic extracts showed maximum value as 129.4 and 129.7 mg rutin hydrate equivalent/g dry fraction while acetone extract showed the moderate amount of flavonoid content as 26.7 mg rutin hydrate equivalent/g dry fraction. On the other hand nonpolar extracts showed lowest account as 8.5 mg rutin hydrate equivalent/g dry fraction. TLC-developed plates also indicated the presence of phytochemicals. The yield percent of total phenolics were expressed as gallic acid equivalents (GAE) shown in **Figure 3.** The extraction yield of these sample varied from 7.4 mg/g to 23.5 mg/g with a descending order of methanol> acetone > ethanol > diethyl ether > chloroform.

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