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Phytochemical and Biological Evaluation of *R. hastatus* and *R. himalyica* (Polygonaceae) from Galiyat Pakistan

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Authors' contributions

This work was carried out in collaboration between all authors. Author SA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors IA and SSUH managed the analyses of the study. Authors HY and JB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The medicinal plants have been known for their wide variety of applications for centuries. *R. hastatus* and *R. himalyica* are such medicinal plants which have been used in Ayurveda for their excellent antioxidative and scavenging properties. The present study is designed to explore the importance of phytochemical constituents and their biochemical activities due to which they have been known for the treatment of wide variety of diseases. The standardized extraction of the said plant was successfully performed using dichloromethane and methanol as solvents at 25°C for 24 hours. Phytochemical analysis was performed and plant showed good content of flavonoids, anthraquinone glycoside and tannins. Antioxidative activity was evaluated by nitric oxide scavenging activity, ferrous reducing power, and DPPH assay. *In vitro* phytotoxic activity was carried out through brine shrimp assay to calculate the cytotoxicity of the standardized extract of

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R. hastatus and *R. himalyica.* Antifungal activity was also performed. Significant anti oxidative activity was observed, nitric oxide scavenging activity (37.8% at 0.125 mg/ml, p≤0.05), ferrous reducing power (71.48% at 0.125 mg/ml, p≤0.05) and DPPH assay (92.47% at 3.79 µg/ml, p≤0.05). Insignificant antifungal activity was obtained. Cytotoxicity was significant as *R. hastatus* caused 78% inhibition at 1000 µg/ml and *R. himalyica* showed 78% inhibition at 1000 µg/ml against standard drug. The results indicate that both *R. histatus* and *R. himalyica* have significant role in combating oxidative and fungal ailments and can be used for the treatment of diseases.

Keywords: Methanol; dichloromethane; cytotoxicity; antioxidant; phytochemical; phytotoxic antifungal.

1. INTRODUCTION

Nature has been the primary source of cure for diseases and discomforts. It is quite astonishing that a large number of plants have therapeutic properties. Nearly 7000 plant species, from lichens to big trees, have been used from time to time for medicinal purposes [1]. The small herbs, serve as the starting material for the isolation or synthesis of conventional drugs. [2]. In Ayurveda, about 2000 plant species are considered to have medicinal properties [3]. If we focus on the Chinese Pharmacopoeia, there are over 5700 traditional medicines, most of which are obtained from plant origin [4]. It is estimated that about 500 herbs are still employed within conventional medicine, although there are some cases where the whole plant is used for its therapeutic benefit. The traditional medical treatments all over the world were all based on natural products [5].

Rumex belongs to the polygonaceae family and is used as herbal drug for different therapeutic purposes. The Rumex genus includes many edible plants which is the reason for attraction to many researchers. They are used for the treatment of AIDS, herpes, influenza, and sexually transmitted diseases [6]. In addition, genus Rumex is also known for antitumor effect towards different tumor cell lines [7]. *R. japonicas, R. crispus* and *R. nervosus* have potent antibacterial activity against both gram-positive and gram negative bacteria [8]. *R. crispus L.* extracts have antioxidant and antimicrobial activities. The leaves and stem of *R. abyssinicus* is powdered and used to cure diseases like pneumonia and cough. The infusion of the plant as a whole is used orally for the treatment of stomach-ache [9]. Rumex species in Kenya have anthraquinone pigments. *R. usambarensis* leaves are used for curing coughs. Decoction of the leaves are effective in stomach ache. Whole plant is used for the treatment of small pox. *R. tingitanus* leaves extracts are reported to be used for meat preservation. *R. baquaertii* is used for treatment of abscesses; roots are used for abdominal pains [10]. In Ireland, *R. obtusifolius* is the most common Irish wayside weed and commonly known as 'broad-leaf dock'. It is reported as antidote to nettle, purifying/detoxifying agent, astringent, laxative, and in the treatment of painful sores, blisters, skin burns, cancer and tumorous conditions [11]. *R. chalepensis* is commonly known as Hula. Fresh paste of the roots after mixing with common salt is used to treat diarrhea, dysentery and to kill intestinal worms. *R. ecklonianus* is a wild herb of Southern Africa. Its young leaves are edible [12]. It is also used as a mild purgative and in the treatment of chlorosis and anaemia [13]. *R. patientia L.* has anti-inflammatory effects on experimental animals [14]. Methanol and ethanol extracts of *R. alveollatus* leaves are used as natural antibacterial sources for treatment of some diseases, especially local skin diseases [15]. Root of *R. nepalensis* is used in constipation [16]. They are used as antidote in scorpion sting. Crushed leaves are used to cure irritation caused by stinging nettles and to stop bleeding from wounds [17]. Leaves are also cooked and eaten as vegetable. A strong decoction is effective for the treatment of bone dislocation. Paste of the root has the analgesic effect. Plant decoction is used to cure body pain. This genus consists of almost 200 species and belongs to the family Polygonaceae. Many of these were investigated for medicinal values and have been reported positive as well [18]. *R. nervosus* as well as of the root of *R. abyssinicus* has anti-microbial and anti-inflammatory activities [19]. *R. cyprius* showed antimicrobial properties (Mekkawy, 1995). *R. nepalensis* also showed antimicrobial properties. There are many species of *Rumex* that are present in Pakistan and found in temperate regions. These are *R. vesicarius, R. crispus, R. hastatus, R. nepalensis, R. acetosa, R. dentatus, R. angulatus, R. chalepensis, R. arcuatoramosus, R. acetosella, R. paulsenianus, R. punjabensis, R. crispellus,*

R. chinesis, R. conglomerates, R. maritimus, R. scutatus, R. patientia and *R austral* [20].

R. hastatus is commonly known as Churki, churca, khati buti. *R. hastatus* plant is very versatile in the form of juice that is used as an astringent. It is also known for its effectiveness against dysentery. Rhizome is used to treat throat pains when fresh. Root is used in constipation for its laxative effects and some skin disorders [21]. Leaves and shoots possess diuretic property while the seeds are reported to have cooling properties. *R. hastatus* is commonly known as khatimal. It is used for breathing disorder, back pain, rheumatism and weakness in some animals. Its dose is twice daily for 4-5 days. Commercial *R. hastatus* (Tarukay) roots contain 25 - 30% tannin. It is used to enhance digestion [22]. *R. hamalayica* is commonly known as shalkhay in Pakistan. Ethnopharmacologically it is used as digestive agent [16]. Its leaves are used as pot herb.

Depending upon the ethnopharmacological uses of *R. hastatus and R. hamalyica*, research was performed to evaluate the possible beneficial effects of the plant. The standardized extract of *R. dentatus* depicts the antiproliferative activity against the liver injury induced by the standard drug paracetamol in albino mice (*Mus musculus*) [23].

2. MATERIALS AND METHODS

2.1 Plant Material

R. hastatus and. *R. himalayica* was harvested from Galliyat situated in South East of Kheber Pakhtoon Khua province of Pakistan. The study area includes Donga Galli, Bara Galli, Nathia Galli and Changla. Identification was confirmed by Dr. Hassan Sher, Associate Professor (Botanist), Department of Biodiversity, University of Swat KPK. The harvested plant material was kept in open air until all moisture contents were removed. Aerial and root parts were crushed separately. The powdered plant material was then used for further experiment to evaluate various biological activities.

2.2 Extraction of Plant

The aerial parts and roots of the selected plants are shade dried. After drying, extraction was performed with methanol and dichloromethane at room temperature of between 21-24°C. Occasional shaking was done for 24 hours. Extracts obtained were concentrated with Rotavapor-R20.

2.3 Chromatographic Studies

2.3.1 Analytical

Silica gel 60 F_{254} was coated TLC aluminium sheets. Analysis of different components present in extracts of dichloromethane and methanol was performed.

2.3.2 Visualization of components on TLC plates

TLC plates were observed under UV (254nm and 365 nm). Godine reagent was sprayed followed by 10% H_2SO_4. The plates were heated in oven (110°C) for 5 min. Colors were developed and identified.

2.3.3 Solvents and chemicals

All solvents were imported from Merck KgaA Darmstadt Germany.

2.3.4 Preparation of reagents

Pharmaceutical Codex (11^{th} edition) and British Pharmacopoeia specification were consulted for the preparation of reagents e.g; Mayer's reagent, Wagner's reagent, Dragendorff's and Godine reagent.

2.3.5 Preparation of solutions

1. dilute HCl
2. dilute ammonia solution,
3. 70 % alcohol
4. lead subacetate solution
5. 10 M NaOH
6. 10 % Ferric chloride solution
7. 3.5 % Ferric chloride in glacial acetic acid
8. 1 % gelatin solution in 10 % Sodium chloride
9. 10 % Sulfuric acid.

2.3.6 Phytochemical screening for secondary metabolites

Tests for Cardiac Glycosides, Alkaloids, Anthraquinones, Saponin Glycosides, Tannins, Favonoids are performed.

2.3.7 Biological activities

Antibacterial assay by agar well diffusion method (Yasmin et al. 2010), Antifungal assay, Brine Shrimp Bioassay, Phytotoxic Assay, Antioxidant Activity was performed on the samples.

3. RESULTS

There were no alkaloids found in *R. astatus* and *R. himalyica*. Saponins were absent in both plants. Tannins were found in *R. hastatus* and aerial and root part of *R. himalayica*. They are included in the group of polyphenols. They are usually present in the bark, leaves, stem and fruit. Flavonoids were present in large concentration in aerial extract than the root extract of *R. hastatus*. But *R. himalayica* root extract was found to have more concentration of flavonoids as compared to aerial extract.

Pharmacological studies of R. *hastatus* and *R. himalayica*

Antibacterial Activity by Agar Tube Diffusion Method (Carron *et al*. 1987)

Plants show no significant antibacterial activity.

Antifungal activity (Agar Tube Dilution Protocol)

Antifungal activity was conceded out against *Candida albicans, Candida glabarata*, *Aspergillus flavus, Mycosporum canis, Fusarium solani.*

Crude extract (methanolic and dichloromethane) of aerial parts and roots of *R. hastatus* and *R. himalayica* exhibited non-significant antifungal activity against *Fusarium solani, Aspergillus flavus* and *Microsporum canis*.

Table 1. Results of the extraction of plants *Rumex hastatus* and *Rumex himalayica*

Sample	Part	Solvent Used	Extract (g)	Codes
Rumex hastatus	Aerial parts (700g)	Dichloromethane	19	RHSAD
		Methanol	8.9	RHSAM
Rumex hastatus	Roots (700g)	Dichloromethane	3.0	RHSRD
		Methanol	21	RHSRM
Rumex himalayica	Aerial parts (800g)	Dichloromethane	15	RHYAD
		Methanol	30	RHYAM
Rumex himalayica	Root parts (800g)	Dichloromethane	4.5	RHYRD
		Methanol	45	RHYRM

Table 2. Qualitative phytochemical analysis of *Rumex hastatus* and Rumex *himalayica*

Phytochemical Tests	Results			
	R. hastatus **Aerial parts**	***R. hastatus*** **Root parts**	***R. himalayica*** **Aerial parts**	***R.himalayica*** **Root parts**
Cardiac glycosides	_	_	+	+
Anthraquinone glycosides	_	+	_	_
Alkaloids	_	_	_	_
Flavonoids	++	+	++	+++
Saponins	_	_	_	_
Tannins	+	+	+	+

Table 3. Anti fungal activity

Sample	*C. albicans*	*A. flavus*	*M. canis*	*F. solani*	*C. glabrata*
RHSAM					
Sample	100	85	75	80	100
Control	100	100	100	100	100
% inhibition	0	15	25	20	0
RHSRD					
Sample	100	85	75	80	100
Control	100	100	100	100	100
% inhibition	0	15	25	20	0

Sample	*C. albicans*	*A. flavus*	*M. canis*	*F. solani*	*C. glabrata*
RHSRM					
Sample	100	85	75	80	100
Control	100	100	100	100	100
% inhibition	0	15	25	20	0
RHSAD					
Sample	100	85	75	80	100
Control	100	100	100	100	100
% inhibition	0	15	25	20	0
RHYRM					
Sample	100	85	75	80	100
Control	100	100	100	100	100
% inhibition	0	15	25	20	0
RHYR D					
Sample	100	85	75	80	100
Control	100	100	100	100	100
% inhibition	0	15	25	20	0
RHYA D					
Sample	100	85	75	80	100
Control	100	100	100	100	100
% inhibition	0	15	25	20	0
RHYAM					
Sample	100	85	75	80	100
Control	100	100	100	100	100
% inhibition	0	15	25	20	0
STD	Miconaol 110.8	Amphotericin B 20.20	Miconazol 98.4	Miconazol 93.25	Miconazol 110.8

All samples shows non-significant activity

Table 4. *In vitro* phytotoxic bioassay *(prof. McLaughlin et al. 1991)*

Entry	Sample	Compound (µg/ml)	Fronds		Growth Regulation	Std. Drug
			Sample	Control		
1.	RHSAM	1000	04	19	78%	0.015
		100	08		5%	
		10	19		0%	
2.	RHSRD	1000	06	19	68%	0.015
		100	17		10%	
		10	18		5%	
3.	**RHSRM**	1000	13	19	31%	0.015
		100	15		21%	
		10	17		10%	
4.	RHSAD	1000	04	19	78%	0.015
		100	16		15%	
		10	18		5%	
5.	RHYRM	1000	06	19	68%	0.015
		100	08		57%	
		10	19		0%	
6.	RHYRD	1000	04	19	78%	0.015
		100	08		57%	
		10	14		26%	
7.	RHYAM	1000	10	19	47%	0.015
		100	16		15%	
		10	17		10%	
8.	**RHYAD**	1000	14	19	26%	0.015
		100	16		15%	
		10	18		5%	

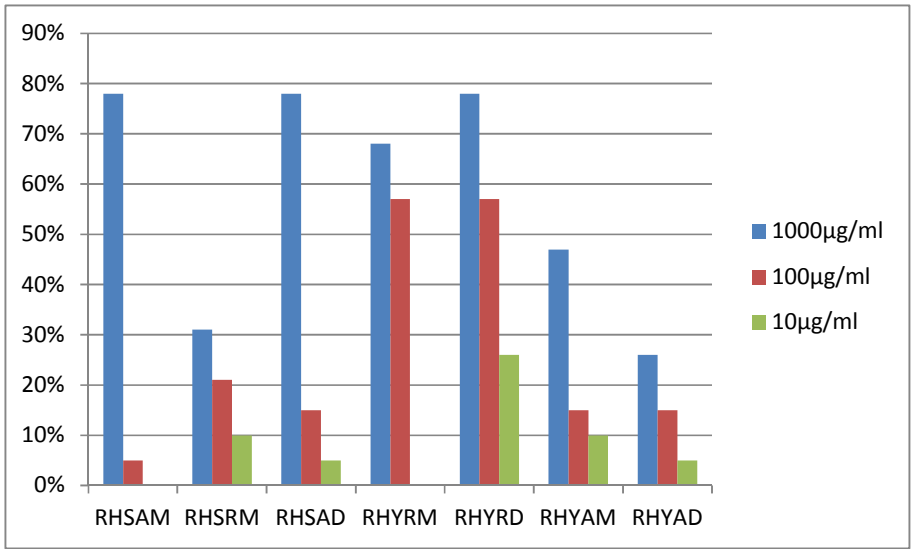

Fig. 1. Phytotoxic bioassay

All samples show significant activity at high dose only except RHYAD and RHSRM

Table 5. Anti oxidant activity

Code	DPPH scavenging activity		
	Conc. mg/ml	(%) inhibition	IC_{50} (µg/ml)
RHYRM	0.5	86.52±0.002	3.69±0.05
RHYRD	0.5	80.12±0.005	12.63±0.06
RHSAM	0.5	89.09±0.02	9.41±0.01
RHSAD	0.5	3.13±0.003	-
RHYAD	0.5	8.52±0.006	-
RHYAM	0.5	67.59±0.001	30.12±0.3
RHSRD	0.5	9.39±0.01	-
RHSRM	0.5	92.47±0.001	3.79±0.8

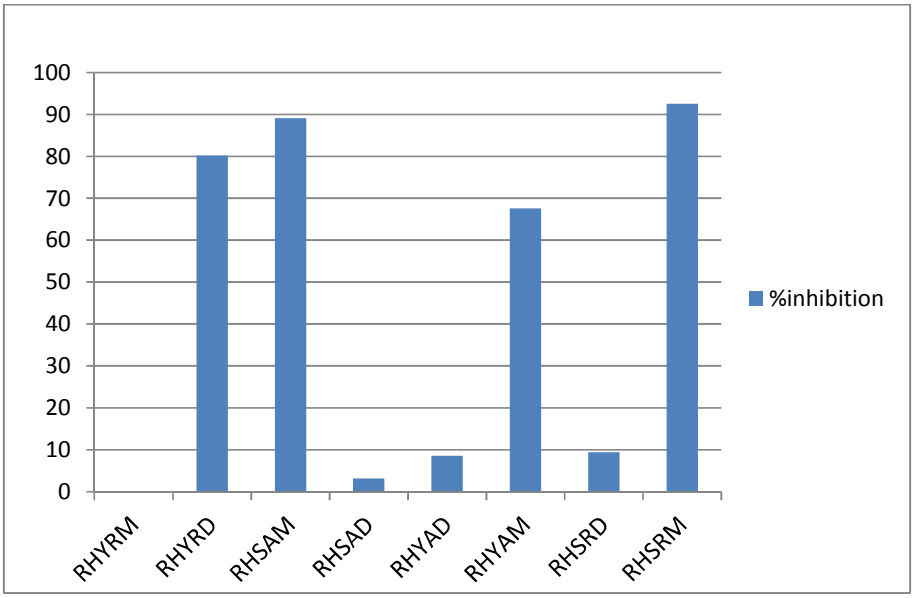

Fig. 2. DPPH scavenging activity

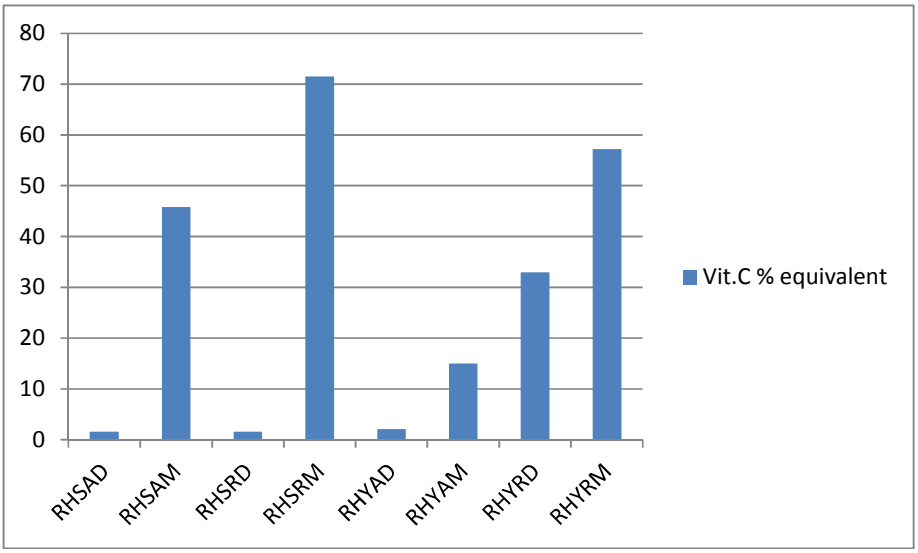

Fig. 3. Ferrous reducing power

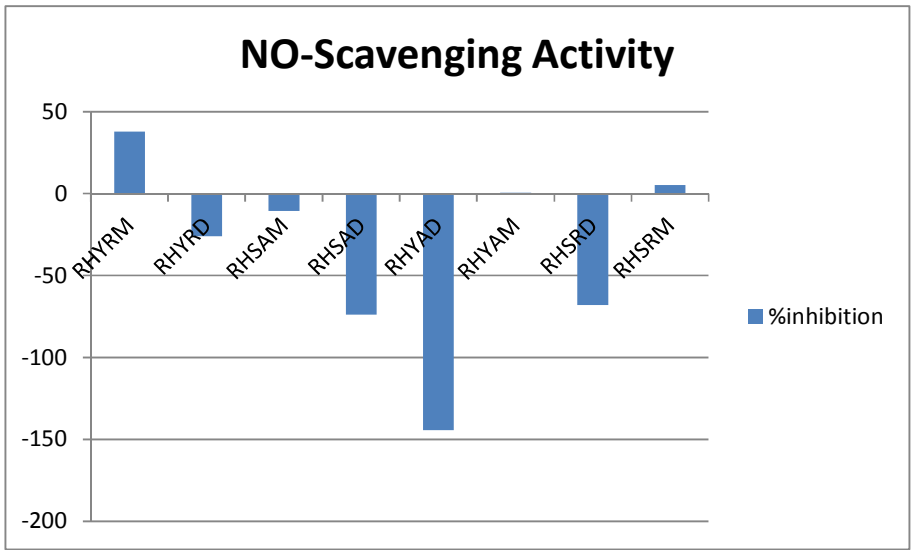

Fig. 4. No-scavenging activity

4. DISCUSSION

This project will be supportive in evaluating the nature of various constituents which could be helpful in the field of medicine. Depending upon the ethnopharmacological, biological and phytochemical investigation, *R. hastatus* and *R. himalyica* should be further investigated for therapeutic effects. Exhaustive phytochemical screening of the selected two plants will throw light on full plants utilization. Importance of medicinal plants is attracting increasing attention, to help meet; growing demands and reduces pressure on economy. It also contributes to poverty mitigation serving as subsistent, “safety nets” or low income “gap fillers” (Sher et. al, 2010).

Cardiac glycosides are present in aerial and root parts of *R. himalayica*. They have cytotoxic effects, expectorant, sedative, digestive, analgesic and moderate antitumor effects. Anthraquinone glycosides were found to be present in root parts of *R. hastatus.* Prescribed anthraquinones are sennoside A (5) and B (6). They have laxative effects in the body.

Flavonoids are present in all the samples specifically in root extract of *R. himalaiyca.*

Table 6. Ferrous reducing power

Code	Conc. mg/ml	Vit.C % equivalent
RHYRM	0.25	57.18±0.005
RHYRD	0.25	32.95±0.005
RHSAM	0.25	45.81±0.05
RHSAD	0.25	1.58±0.001
RHYAD	0.25	2.08±0.005
RHYAM	0.25	15.03±0.01
RHSRD	0.25	1.55±0.01
RHSRM	0.25	71.48±0.04

Table 7. NO-scavenging activity

Code	Conc. mg/ml	(%) inhibition
RHYRM	0.125	37.80±0.01
RHYRD	0.125	-26.12±0.01
RHSAM	0.125	-10.65±0.001
RHSAD	0.125	-73.88±0.005
RHYAD	0.125	-144.33±0.03
RHYAM	0.125	0.69±0.005
RHSRD	0.125	-68.04±0.005
RHSRM	0.125	5.15±0.001

The research plants *R. hastatus* and *R. himalayica* were exhaustively evaluated by using three different assays like ferrous reducing power, DPPH-free radical scavenging activity and NO-Scavenging assay. In case of DPPH radical scavenging activity, the methanolic extract of aerial part of *R. hastatus* and methanolic extract of roots of *R. himalayica* (RHYRM and RHSAM) exhibited 86% and 89% inhibition, which were considered as excellent activity having IC_{50} value 3.69±0.05 and 9.41±0.01 respectively. In case of *R. himalayica* the crude extract of roots and aerial parts in both extract (methanolic and dichloromethane) shows significant to good and moderate activities. While in case of ferrous reducing assay, the samples RHSRM and RHYRM showed 71.48 % and 57.18% inhibition. Methanolic root extract of *R. himalayica* exhibited 37.80% inhibition and considered as low NO-Scavenging activity.

5. CONCLUSION

Root dichloromethane extract and root methanol extract of *R. hastatus and R. himalayica* exhibited significant cytotoxic activity showing %age growth regulation of 78% and 68% respectively at the highest dose (1000 µg/ml). Aerial parts dichloromethane extract and methanol extract of *Rumex hastatus* also exhibited significant cytotoxicity showing % age growth regulation of 78% at the highest dose (1000 µg/ml).

All plants samples exhibited antioxidant activities, but to a varied extent. It's relevant ethano medical uses, including astringent effects, pain killer, laxative effect and anti rheumatic properties, shows that phytotoxicity and antioxidant activity should be further evaluated and active constituents should be checked for the existence of possible synergism, if any.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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