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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(4): 2784-2790. **OPEN ACCESS**

Misopates orontium (L.) Raf: a promising ethnomedicinal plant with potent antimicrobial and antioxidant source

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The phytochemical analysis, antimicrobial and antioxidant activities of ethnobotanically important plant *Misopates orontium* (L.) Raf. were evaluated. Phytochemical analysis showed the presence of glycosides, carbohydrates, protein, saponins, alkaloids, sterols, lipids tannins and flavonoids. Antimicrobial activity of ethanolic extract of whole plant of *M. orontium* were evaluated against antibacterial and antifungal strains. The antibacterial activity of plant *M. orontium* showed maximum zone of inhibition 15 ± 0.6 mm against *P. aeruginosa* and *S. aureus* while the minimum zone of inhibition 9 ± 0.4 mm was obtained by *B. subtilis*. The antifungal activity of *M. orontium* found that highest zone of inhibition 12 ± 0.3 mm was formed by *A. oryzae* and 11± 0.6 mm against *A. niger*. Maximum result of total antioxidant activity (1.35 ± 0.04 mm) of plant *M. orontium* was observed by *n*-hexane extract

Keywords: *Misopates orontium* (L.) Raf., Antibacterial, Antifungal, Antioxidant

INTRODUCTION

The backbone of ancient and local medicine was medicinal plants and since last few decades' plants have been used for pharmacological investigations (Ajaib et al. 2020a). Almost 80% population depends entirely on plants for their health care system according to WHO (Maqbool et al. 2019. Drugs which were derived from plants were more effective and less toxic (Murugan et al. 2008; Ajaib et al. 2016). More than 50% plants produced modern medicinal drugs. Plants play important role in pharmaceutical industry and medicine development programmes (Kamran et al. 2016). In recent years' herbal medicines had a great importance because of their potential and efficacy (Prachayasittikul et al. 2008). More than 50% plants produced modern medicinal drugs (Stuffness, and Douros, 1982). Plants have potent contribution in medicine development and manufacturing drugs industry (Baker et al. 1995). In recent years herbal medicines had a great importance because of their potential and efficacy (Prachayasittikul et al. 2008). Usage of antimicrobial drugs is uncertain because struggle of drug and anxious unpleasant effects of antibiotics on the host (Ajaib et al. 2017).

Misopates orontium (L.) Raf. of family Plantaginaceae is an erect annual herb, with 50 cm height. Its flowering period was March to April. The Plantaginaceae is a multi-ethnic family mostly found in temperate zones (Costea et al. 2006). In this family, mostly herbaceous plants found.

Through research on this plant some important active components are explore in this and it is proved as antibacterial drugs (Wagner et al. 1999). Local people of Kotli and Bhimber Districts use this plants as pain killer and in wound healing Ajaib et al. 2020).

Figure 1:*M. orontium*

MATERIALS AND METHODS

The plant was obtained in March 2016 from District Bhimber AJK. The collected plant authenticated from herbarium in department of Botany, Mirpur University of Science and Technology, with a voucher number, i.e. MUST.BOT.5353.

Preparation of ethanolic extract

The plant *Misopates orontium* was washed, desiccated in shade and then grind with electric grinder and form fine powder of *M. orontium* (250 g) was subjected to 90% ethanol for seven days and then filtered. Ethanol extract obtained was after evaporation kept at temperature lower than 40 ˚C.

Phytochemical analysis (Qualitative tests)

Qualitative phytochemical analysis of ethanol extract of plant *M. orontium* was performed in order to find the phytochemical metabolites by following the methodology of Evans (2009); Shah and Sethi (2010).

Antimicrobial activity

For the investigation of antimicrobial activity *M. orontium* carried out against bacterial and fungal strains using agar well diffusion technique following Benzie, and Strain (1996). Standard antibiotics *i.e*. tetracycline, cefoperazone and erythromycin were used for antibacterial activity and terbinafine and griseofulvin for antifungal activity.

Determination of antioxidant activity

Extraction of crude extracts in non- polar and polar solvents

250 grams of powder plant of *M. orontium* were soaked in ethyl acteate for seven days then it is filtered, dehydrated and resoaked in chloroform for eight days and then filtered, dried and resoaked in ethanol, methanol, distilled water and *n*-hexane in same way. Finally, six extracts of plant were attained.

Total Phenolic Contents (TPC)

The Total Phenolic Contents of *M. orontium* was investigated by the procedure of Makkar et al. (1993).

Total Flavonoid Content (TFC)

Total Flavonoid contents (TFC) of *M. orontium* was investigated by the asssay of Dewanto et al. (2002).

DPPH Radical Scavenging activity

The DPPH radical scavenging activity was examined by using the method of Hassan et al. (2016).

Ferric reducing Antioxidant Power (FRAP) Assay

The FRAP assessment of *M. orontium* was carried out by using the methodology of Benzie and Strain (1996).

Phosphomolybdenum method

The assay of Prieto et al. (1999) was followed to evaluate the antioxidant activity

RESULTS AND DISCUSSION

The existing work was planned to appraise the phytochemical analysis, antimicrobial, and antioxidant effects of *M. orontium* (L.) Raf.

Qualitative phytochemical analysis of the ethanolic extract of whole plant of *M. orontium* (L) R. exposed the existence of different secondary metabolites. Phytochemical analysis showed that glycosides, flavonoids, carbohydrates, protein and saponin were moderately present but alkaloids, terpenoids, sterols, lipids and tannins were strongly present as studied by (Ajaib et al. (2019) while investigation phytochemicals in *Salsola imbricata*. (Table 1)

In this study, the antibacterial and antifungal activity of ethanolic extract of whole plant of *M. orontium* were evaluated. For the result assessment, different antibiotics (tetracycline,

cefoperazone, and erythromycin) were used in antibacterial activity while in antifungal activity the result were compared with antimycotics, griseofulvin and terbinafine. In antibacterial activity, whole plant of *M. orontium* extract showed activity against all bacteria strain. The minimum zone of inhibition 9.0 ± 0.05 mm was obtained by *B. subtilis* and the zone of inhibition 13 ± 0.08 mm was obtained by *E. coli*. The maximum zone of inhibition 15 ± 0.2 mm and 15 ± 0.06 mm was obtained by *S. aureus* and *P. aeruginosa* respectively similar findings also reported by Ajaib et al. (2014) during study of antibacterial activity of *Firmiana simplex* (Table 2).

In antifungal activity, ethanolic extract showed activity against *A. oryzae* with zone of inhibition 12 ± 0.3 mm and against *A. niger* with zone of inhibition 11± 0.65 mm. (Table 3).

The antioxidant activity was appraised by using five assays, *i.e.* total phenolic contents (TPC), total flavonoid contents (TFC), DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP) assay and phosphomolybdenum assay.

The results of DPPH radical scavenging activity were recognized that maximum radical scavenging potential 1.35 ± 0.02 at concentration of 1000 *µ*g/ml was observed by *n*-hexane extract of plant of *M. orontium*. BHT was used as a standard (Table 4).

Highest value of TFC was showed by *n*-hexane extract with absorbance ranges from 0.4 ± 0.2 to 0.8 ± 0.02 and ethyl acetate extract with absorbance ranges from 0.4 ± 0.01 to 0.8 ± 0.04 (Table 5).

The results of total phenolic content showed that the highest phenolic contents were given by methanolic extract, *i.e.,* 0.9 ± 0.02 of GAE at concentration of 1000 µg/ml and minimum phenolic content was showed by aqueous extract, *i.e.,* 0.5 ± 0.01 of GAE at concentration of 1000 *µ*g/ml (Table 6).

Table 1: Phytochemical Assessment of constituents of *Misopate orontium*

Group	Name of Test	Ethanolic extract of whole plant
Triterpenoids	Liebermann's Test	+
	Salkowaski test	+
Sterols	Sulphur test	+
	Liebermann's Test	+
	Salkowasti test	+
Glycosides	Bromine water test	+
	Legal's test	+
	Keller-killani test	_
Flavonoids	Ferric chloride test	_
	Alkaline reagent test	+
	Zinc-hydrochloride test	_
	Lead acetate test	_
Alkaloids	Mayer's test	+
	Wagner's test	+
	Hager's test	+
	Dragendroff's test	+
Proteins	Millon's test	+
	Xanthoprotic test	_
	Biuret test	+
	Ninhydrin test	+
Carbohydrates	Barfoed's test	_
	Benedict's test	+
	Molisch's test	_
Saponin	Legal's test	+
	Foam test	+
	Bromine water test	_
Lipids	Soap formation test	+
Tannins	Ferric chloride test	+

Table 2: Zone of inhibition produced by whole plant of *M. orontium* and Antibiotics against Bacterial strains (mm)

	M. orontium	Tetracycline	Cefoperazone	Erythromycin
P. aeruginosa	15 ± 0.06	20 ± 0.04	17 ± 0.07	10 ± 0.04
S. aureus	15 ± 0.2	21 ± 0.18	14 ± 0.26	9 ± 0.12
E. coli	13 ± 0.08	20 ± 0.17	15 ± 0.08	11 ± 0.21
B. subtilis	09 ± 0.05	20 ± 0.05	14 ± 0.12	12 ± 0.12

Table 3: Zone of inhibition produced by whole plant of *M. orontium* and antimycotics against fungal strains (mm)

	M. orontium	Terbinafine	Griseofulvin
A. niger	11.0 ± 0.65	17.0 ± 0.06	12.0 ± 0.03
A. oryzae	12.0 ± 0.3	16.0 ± 0.8	18.0 ± 0.26

Table 4: Absorbance and free radical scavenging of *M. orontium* by DPPH assay

Plant part	Absorbance at different concentrations (µg/ml)					
	Fractions	60	125	250	500	1000
Whole plant of *M. orontim*	Chloroform	0.45 ± 0.4	0.69 ± 0.6	0.76 ± 0.1	0.87 ± 0.01	0.98 ± 0.06
	Methanol	0.23 ± 0.2	0.3 ± 0.01	0.41 ± 0.74	0.53 ± 0.04	0.64 ± 0.05
	Ethyl acetate	0.6± 0.1	0.7 ± 0.002	0.82 ± 0.01	0.95 ± 0.02	1.23 ± 0.05
	Distilled water	0.26 ± 0.03	0.43 ± 0.08	0.54 ± 0.04	0.76 ± 0.03	0.98 ± 0.05
	n-hexane	0.87 ± 0.04	0.98 ± 0.03	1.17 ± 0.05	1.26 ± 0.4	1.35 ± 0.02
	Ethanol	0.25 ± 0.05	0.45 ± 0.06	0.53 ± 0.04	0.65 ± 0.03	0.76 ± 0.7
	BHT	0.45 ± 0.01	0.66 ± 0.03	0.845 ± 0.02	1.38 ± 0.4	2.43 ± 0.09

The result reported were run in triplicates and stated as Mean ± Standard error

Table 5: Total flavonoid content in the whole plant of *M. orontium*

Plant part	Absorbance at different concentrations (µg/ml)					
	Fractions	**60**	**125**	**250**	**500**	**1000**
Whole plant of *M. orontim*	Chloroform	0.1 ± 0.6	0.2 ± 0.8	0.3 ± 0.1	0.5 ± 0.4	0.6 ± 0.7
	Methanol	0.3 ± 0.4	0.4 ± 0.1	0.5 ± 0.3	0.6 ± 0.2	0.7 ± 0.1
	Ethyl acetate	0.4 ± 0.01	0.5 ± 0.08	0.6 ± 0.001	0.7 ± 0.03	0.8 ± 0.04
	Distilled Water	0.2 ± 0.08	0.3 ± 0.09	0.4 ± 0.07	0.5 ± 0.04	0.6 ± 0.03
	n-hexane	0.4 ± 0.2	0.5 ± 0.09	0.6 ± 0.09	0.7 ± 0.03	0.8 ± 0.02
	Ethanol	0.22 ± 0.1	0.28 ± 0.09	0.3 ± 0.05	0.4 ± 0.3	0.5 ± 0.4
	Ascorbic acid	0.36 ± 0.02	0.57 ± 0.05	0.89 ± 0.01	1.40 ± 0.03	2.42 ± 0.09

The reported results were run in triplicates and stated as Mean ± Standard error.

Table 6: Total phenolic content of *M. orontium*

Plant part	Absorbance at different concentrations (*µg)*/…)))ml)					
	Fractions	**60**	**125**	**250**	**500**	**1000**
Whole plant of *M. orontim*	Chloroform	0.1 ± 0.6	0.2 ± 0.8	0.3 ± 0.1	0.5 ± 0.4	0.6 ± 0.7
	Methanol	0.4 ± 0.03	0.5 ± 0.01	0.6 ± 0.03	0.8 ± 0.12	0.9 ± 0.02
	Ethyl acetate	0.3 ± 0.1	0.4 ± 0.07	0.5 ± 0.01	0.6 ± 0.02	0.8 ± 0.02
	Distilled water	0.2 ± 0.09	0.3 ± 0.08	0.3 ± 0.06	0.4 ± 0.03	0.5 ± 0.01
	n-hexane	0.22 ± 0.02	0.28 ± 0.06	0.36 ± 0.07	0.43 ± 0.02	0.59 ± 0.04
	Ethanol	0.3 ± 0.01	0.4 ± 0.009	0.5 ± 0.04	0.6 ± 0.2	0.7 ± 0.3
	(Gallic acid)	0.27 ± 0.03	0.54 ± 0.04	0.79 ± 0.02	1.44 ± 0.04	2.84 ±0.06

The result reported were run in triplicates and stated as Mean ± Standard error

Table 7: Total antioxidant potential of *M. orontium* by Phosphomolybdenum assay

Plant part	Absorbance at different concentrations (µg/ml)					
	Fractions	**60**	**125**	**250**	**500**	**1000**
Whole plant of *M. orontim*	Chloroform	0.57 ± 0.02	0.65 ± 0.01	0.72 ± 0.4	0.84 ± 0.32	0.98 ± 0.7
	Methanol	0.64 ± 0.01	0.72 ± 0.03	0.85 ± 0.03	0.93 ± 0.042	0.99 ± 0.07
	Ethyl acetate	0.61 ± 0.1	0.75 ± 0.07	0.82 ± 0.01	0.95 ± 0.02	1.23± 0.02
	Distilled water	0.66 ± 0.09	0.74 ± 0.08	0.85 ± 0.06	0.96 ± 0.03	1.12 ± 0.01
	n-hexane	0.87 ± 0.02	0.95 ± 0.06	1.17 ± 0.07	1.26 ± 0.02	1.35 ± 0.04
	Ethanol	0.72 ± 0.01	0.85 ± 0.009	0.92 ± 0.04	0.99 ± 0.2	0.15±0.3
	BHA	0.55 ± 0.03	0.779 ± 0.04	1.04 ± 0.02	1.565 ± 0.04	2.43±0.06

Table 8: FRAP Assay of extracts of *M. orontium*

Plant part	Absorbance at different concentrations (µg/ml)					
	Fractions	**60**	**125**	**250**	**500**	**1000**
Whole plant of *M. orontim*	Chloroform	0.57 ± 0.6	0.65 ± 0.8	0.72 ± 0.1	0.84 ± 0.4	0.98 ± 0.7
	Methanol	0.23 ± 0.03	0.39 ± 0.01	0.47 ± 0.03	0.59 ± 0.12	0.68 ± 0.02
	Ethyl acetate	0.34 ± 0.1	0.48 ± 0.07	0.56 ± 0.01	0.62 ± 0.02	0.76 ± 0.02
	Distilled Water	0.45 ± 0.09	0.55 ± 0.08	0.67 ± 0.06	0.79 ± 0.03	0.86 ± 0.01
	n-hexane	0.72 ± 0.02	0.95 ± 0.06	1.17 ± 0.07	1.26 ± 0.02	1.35 ± 0.04
	Ethanol	0.65 ± 0.01	0.85 ± 0.009	0.92 ± 0.04	0.99 ± 0.2	1.15 ± 0.3

Maximum value of total antioxidant activity was shown by *n*- hexane 1.35 ± 0.04 which is nearly equal to standard BHA (Table 7).

Higher the FRAP value more significant the antioxidant capacity of plants. The maximum reduction potential was showed by the ethanol extracts *i.e.,* 0.65 ± 0.01 to 1.15 ± 0.3. Whereas the minimum activity was showed by methanolic extract, *i.e.,* from 0.23 ± 0.03 to 0.68 ± 0.02 (Table 8). The findings of the antioxidant activities were juxtaposed with Siddiqui et al. (2017) on *Lonicera quinqueloculari*s that conclude as antioxidant rich source.

CONCLUSION

Qualitative phytochemical analysis of ethanolic extract of whole plant of *M. orontium* show the presence of glycosides, flavonoids, carbohydrates, protein and saponin. In antimicrobial activity, the inhibitory result of ethanolic extract of whole plant of *M. orontium* was evaluated against antibacterial and antifungal strains. Maximum zone of inhibition (15 ± 0.6 and 15 ± 0.6 mm) were obtained by *P. aeruginosa* and *S. aureus* respectively while the minimum zone of inhibition 9 ± 0.4 mm was obtained by *B. subtilis*. In antifungal activity extract of whole plant of *M. orontium* showed activity against *A. oryzae* and *A. niger*. Highest inhibition zone was formed by *A. oryzae* 12 ± 0.3 mm and inhibition zone 11± 0.6

mm was observed against *A. niger*. Antioxidant effect was investigated by using five methods. *n*-Hexane extract of plant *M. orontium* showed maximum DPPH radical scavenging activity 1.35 ± 0.02 at concentration of 1000 µg/ml. Maximum TFC was showed by *n*- hexane extract (0.4 ± 0.2 to 0.8 ± 0.02) and ethyl acetate extract (0.4 ± 0.01 to 0.8 ± 0.04). The results of total phenolic content showed that the highest phenolic contents (0.9 ± 0.02 of GAE at concentration of 1000 µg/ml) were given by methanolic extract and minimum phenolic content was showed by aqueous extract 0.5 ± 0.01 of GAE at concentration of 1000 µg/ml. Maximum value (1.35 ± 0.04) of total antioxidant activity was shown by *n*- hexane extract which is nearly equal to standard BHA The maximum FRAP value (0.65 ± 0.01 to 1.15 ± 0.3) was observed by the ethanol extracts whereas the minimum activity was (0.23 ± 0.03 to 0.68 ± 0.02) showed by methanolic extract.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

MA designed and Z performleed the experiments and both also wrote the manuscript. SI, FS, SA, KHB and KMK reviewed the manuscript. All authors read and approved the final version.

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