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Proximate analysis and in vitro biological evaluations of *Misopates orontium* (L.)

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Original Research Article

ABSTRACT

Proximate analysis plays an important role in assessing the appropriateness of medicinal plants or their extracts orally taken by the marginal communities. Primary and secondary metabolites of *Misopates orontium (L.) belongs to Plantaginaceae family* were screened out and tested for therapeutic values through in-vitro biological assays. Quantitative analysis was done on powder of *Misopates orontium* by using standard methods for estimating the primary and secondary metabolites. The maximum value of total carbohydrates (11.04µg/ml), total starch (14.69µg/ml), total protein (14.34µg/ml), total amino acids(28.43µg/ml), total lipids (3.67 µg/ml), total glycosaponins (14.95%), total alkaloids (24.67%), total polyphenolics (28.40%) and total flavonoids (5.67%) were found in given plant samples. were found in the samples. Haemolytic and DNA protection studies of *Misopates orontium* were also performed. Solar protection factor was calculated of all fractions by adopting the standard procedure. By using concentration 200µg/ml and 400µg/ml methanolic fraction showed 4.57 and 4.86, hexane 1.54 and 5.0, aqueous 1.07 and 2.33, chloroform 0.94 and 3.87, ethyl acetate 18.56 and 28.41, butanol 4.34 and 7.91. Ethyl acetate fraction showed highest SPF value. Presence of polyphenols and flavonoids provide an evidence of this plant being traditionally used in the treatment of various ailments. The results of present study give an evidence for the existence of diverse primary and secondary metabolites in *Misopates orontium* and thus rationalizes its use in traditional medicines for the cure of different aliments

Keywords: *Misopates orontium*, Primary and Secondary metabolites, Haemolytic, DNA protection, Solar Protection Factor (SPF)

INTRODUCTION

Herbal medicines have been used in the ancient period for the ailment of different diseases. Every region of the world has established its own herbal list based on traditional knowledge of the ingredients and through experience (Al-Snafi 2015). Useful phytochemicals are found in almost every part of the plant; leaves, roots, stem and vegetables. They are classified as primary and secondary constituents. Primary constituents include chlorophyll, sugars and proteins while alkaloids, tri-terpenoids and phenolic substances are secondary compounds. Plants are the rich source of medicinally active organic compounds like tannins, steroids, tri-terpenoids, flavonoids, carbohydrates and alkaloids. The most important bioactive compounds are alkaloids, flavonoids, tannins and phenolic. Majority of phytochemicals compounds have been known to have valuable pharmacological activities such as anti-inflammatory, analgesic, anticancer, antimicrobial and antioxidant (Shamala *et al*., 2016; Kumari *et al*., 2016). All these ingredients possess definite pharmacological actions. Their importance remained great for the health of communities and individuals.

Misopates orontium L., family Scrophulariaceae, is an annual herbaceous plant found in hilly areas of Pakistan especially Margallah Hills National Park. The ethno-botanically this plant is used as fodder, fiber, fuel, timber, in tanning industry and in preparation of gum and medicines

Author Contribution: S.A., designed and performed the experimental work, wrote the complete manuscript and analyzed the data; **S.I.,** verified the analytical methods, investigate and supervised the findings of this work and did the proof reading of manuscript; **K.H.**, provided assistance in data analysis, manuscript reading and editing; **A.M**., helped in compiling the final results and also tabulate the results; **S.R.**, assisted in experimental work and helped in revision of experimental work.

(Jabeen *et al*.,2009). *M. orontium* is claimed to be used for a number of therapeutically assiduities such as, it has bitter and stimulant properties, and the whole plant has been employed for the treatment of tumors and ulcers. The scientific approval and the mechanisms of such claims are lacking in the literature (Lonnig *et al*., 2007). The present work has been carried out to quantify the phytochemical constituents and to investigate DNA damage protection, solar protection factor and safety profile of *M. orontium* L.

MATERIAL AND METHOD

Collection of plant material, authentication and extraction

The plant was collected from Bhimber (Bandiala), Kotli, Azad Kashmir and got authenticated by Dr. Uzma Hanif, Department of Botany, Government College University (GCU), Lahore, Pakistan. A specimen of plant was deposited in herbarium of GCU under voucher No: GC. Herb. Bot. 3458. The plant was dried under shade for about 15 days and then pulverized. Powder was stored in amber colored bottles at dry place. Methanolic extract made by maceration using rotary evaporator under reduced pressure at 45-50C˚. Fractionation was performed by different solvent of increasing order of polarity n-hexane, chloroform, ethyl acetate, n-butanol and aqueous. Each fractions of these solvents were dried and preserved for evaluation of different biological activities.

Determination of primary metabolites Carbohydrate contents

Carbohydrate content was determined by the method followed by Chandran *et al*., 2013 with certain modification.

Starch contents

Weigh 100 mg of sample powder and determined its contents in given sample by the methods followed by Chandran *et al*., 2012.

Protein contents

Protein content in the given plant sample was determined by the method Krishna *et al.,* 2014.

Amino acid contents

Sample powder of 100mgwas weighed and 10ml of 80% ethanol was added. Then, transfer the homogenate to centrifuge tube and centrifuge at 3000*g, collect the supernatant and make up final volume 10 ml with distilled water and use for the estimation. Prepared working standard solution into the series of test tubes marked S_1, S_2, S_3, S_4 and S_5 and marked T_1, T_2 sample into two other test tubes respectively. Another test tube marked 'B' with 1ml of distilled water serve as the blank and then, adds 1 ml of 'Ninhydrin reagent' to each test tube including blank. Vortex all the test tubes well and adds 5 ml of diluent solvent to all the test tubes including the blank. Measure the absorbance at 570 nm using spectrophotometer. Draw a standard curve by plotting the concentration of L-leucine on x-axis and the respective absorbance on y-axis. Finally calculate the amount of total free amino acids in the sample (More & Chaubal, 2016).

Total lipids

Fifteen gram plant powder was subjected to hot extraction with petroleum ether for 12-24 hours and temperature was maintained at 40-60 C. After 24 hours filtered the residue, dried it and difference between the weights before and after evaporation, weight of lipid content were calculated (Jadid *et al*., 2018).

Determination of secondary metabolites

Total glycosaponins, total glycosides, total phenols, total flavonoids and total alkaloids, were determined by using the following methods.

Saponins contents

Saponins quantitative determination was carried out using the method reported by Ejikeme *et al.,* and Obadoni & Ochuko. The saponins content was calculated as a percentage:

$$\%\, Saponin = \frac{Weight\ of\ saponin}{Weight\ of\ sample} \times 100$$

Glycoside contents

One gram 1gram of plant powder was weighed in 100ml volumetric flask then adds 10ml of 70% ethanol in it. Boiled it, filtered and filtrate was diluted with distilled water. Then add 3.5ml of 10% lead acetate. Filtered it and kept the filtrate into separating funnel with 15ml of chloroform. Two

layers were formed, lower organic layer was collected (chloroform); dried and weighed. Percentage of total glycosidal contents was determined (Ugwoke *et al*., 2017).

Alkaloidal Contents

Quantitative determination of alkaloid was done according to the method of Amiri *et al*., with little modification. The percentage of alkaloid is expressed mathematically as:

$$\%\ Alkaloid = \frac{Weight\ of\ alkaloid}{Weight\ of\ sample} \times 100$$

Total phenolic contents

Estimation of total poly phenolic contents in plant sample was done by applying methods as described by (Liaudanskas *et al*., 2017) with little modifications. Gallic acid was used as a standard.1mg/ml stock solutions of both standard and sample were made in methanol.

Total Flavonoid contents

Flavonoid determination was determined by method of Ejikeme *et al.* and Boham and Kocipai with little modifications. Quercetin (QTN) was used to draw standard curve. The flavonoid contents were determined by linear regression equation, obtained from calibration curve of standard. Total flavonoid contents were determined by following equation:
Total flavonoids= QTN equivalents (µg/mL) X extract volume/ Sample (g)

Solar Protection Factor (SPF)

Different fractions of *M. orontium* were dissolved in distilled water in such a way that concentration of each solution is 1mg/ml. These solutions were diluted in distilled water to get two concentrations (200µg/ml and 400µg/ml). Then spectrophotometer readings of these solutions were taken in wavelength ranging from 290-320nm at 5mint (290 to 320 at 5nm interval) and readings were noted. SPF was calculated from using formula;(Suva, 2014)

$$SPF = CF \times \sum_{290}^{320} EE \times I \times Abs$$

Here; EE= Erythemal effect spectrum, I =Solar intensity spectrum,
Abs = Absorbance of sample, CF=Correction factor (=10)
The values of EE×I are constant and are predetermined as shown in table.

Table I: Values of EE*I used in the calculation of SPF[1]

Wavelength(nm)	EE*I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

Cytotoxic studies

***In vitro* hemolytic activity**

Fresh human blood (3mL) was taken in EDTA vial and centrifuged for 5min at 850 rpm. Clear supernatant was decanted and sediment pellets were washed three times with chilled sterilized isotonic phosphate buffer saline (PBS) followed by the formation of suspension in 20mL of chilled sterilized PBS. Cells were counted using Haemocytometer. Triton-X was used as positive control and PBS served as negative control. Reaction mixture contain 20µL of plant extract and 180µL of blood cells suspension. They were incubated at 37°C for 40min.After incubation tubes were placed in ice cold PBS for 5min and then centrifuged at 1500 rpm for 5min. Collect carefully 100 µl of supernatant in eppondrof tubes and diluted with 900 µl of chilled sterilized PBS. All these samples including positive and negative control (200 µl) were loaded into 96well plate. By using ELISA microplate reader (Bio Tek, model µ-Quant TM Winooski, USA) took the absorbance at 630nm (Zubair et al., 2017).

$$\%\ \text{Hemolysis} = \frac{Abs(Sample\ absorbance)}{Abs\ (control\ absorbance)} \times 100$$

[1] SPF was calculated three times and their average is taken for final results

DNA damage protection assay

DNA was isolated from human blood using DNA isolation kit (QIAamp DNA Mini Kit 50, QIAGEN). Isolated DNA was quantified using nano drop technique. The ability of different fractions of plant extract to protect genomic DNA was determined using the previously described method with slight modifications (Kumar *et al.*, 2013). In this method, DNA damage was induced by hydroxyl radicals generated from Fenton's reagent. Reaction mixture contained 4 µl of genomic DNA, 3µl of Fenton's reagent and 4µl of different fractions of plant and make up the volume upto 20µl using deionized water. Positive and negative controls were also prepared. Reaction mixtures were incubated at 37°Cfor 30 min. Bromophenol dye was added in each sample after incubation. The reaction mixture (10 µl) was loaded in the wells of 1% Agarose gel and electrophoresis was allowed to run horizontally for 1hour at 100V followed by staining with ethidium bromide. DNA was under Gel documentation system (Syngene Model Gene Genius, Cambridge, UK).

FTIR spectroscopy

Powder root and fractions were analyzed in triplicates to get FTIR spectra using potassium bromide (KBr) disc.1mg crude drug and fractions were mixed with 100mg of KBr and transferred into die. The die was pressed under hydraulic press to produce the disc which were used to get the spectra in 4000-400cm^{-1}and different functional groups were observed (Sahayaraj *et al*., 2015).

RESULTS

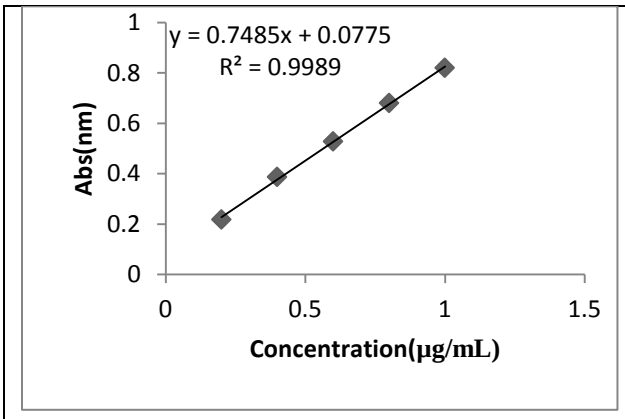
Results of quantitative analysis of primary and secondary metabolites were summarized in table. Total carbohydrate, total starch, total protein, total amino acid, total lipids, total glycosaponins, total alkaloids content, total polyphenols and total flavonoid content were calculated from linear regression curves.

Table II: Proximate analysis of primary metabolites of *Misopates orontium* L.

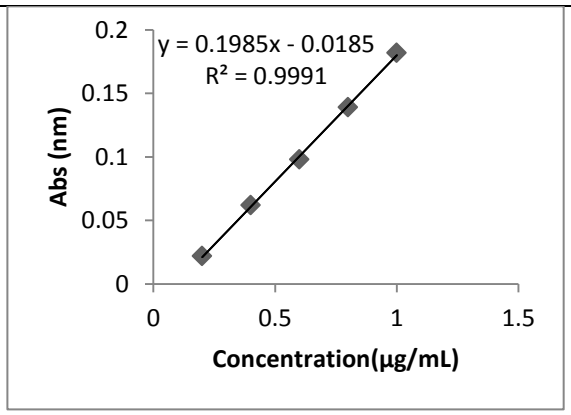
Sr. No.	Parameters	Mg mg/100ml values (w/w)	
		Mean	SD
1.	Total carbohydrates	11.04	±0.41
2.	Total starch	14.69	±0.28
3.	Total protein	14.34	±0.59
4.	Total amino acids	28.43	±1.32
5.	Total lipids	3.67	±0.73

Table III: Proximate analysis of secondary metabolites of *Misopates orontium* L.

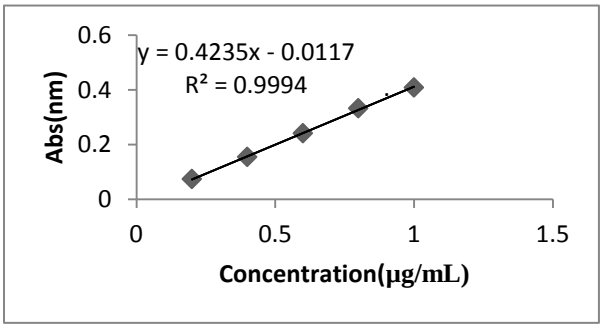
Sr. No.	Parameters	% values (w/w)	
		Mean	SD
1.	Total glycosaponin	14.95	±0.29
2.	Total alkaloid	24.67	±1.23
3.	Total phenols	28.40	±1.02
4.	Total flavonoid	5.67	±0.77



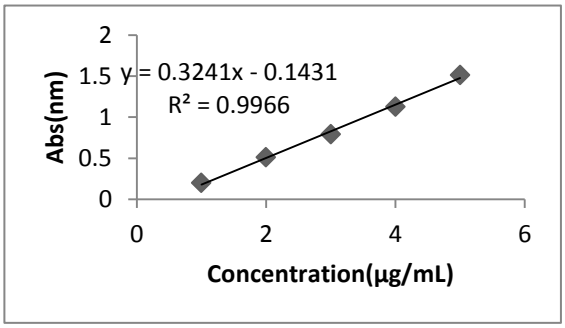
A- Total carbohydrate content



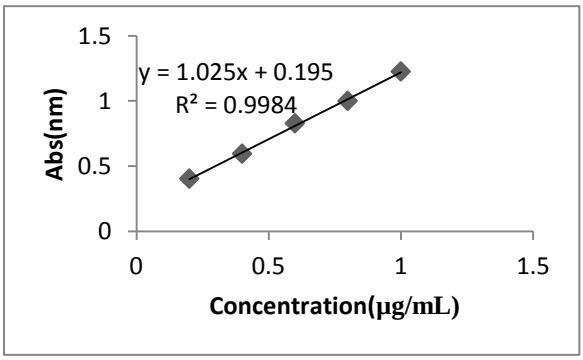
B- Total protein content



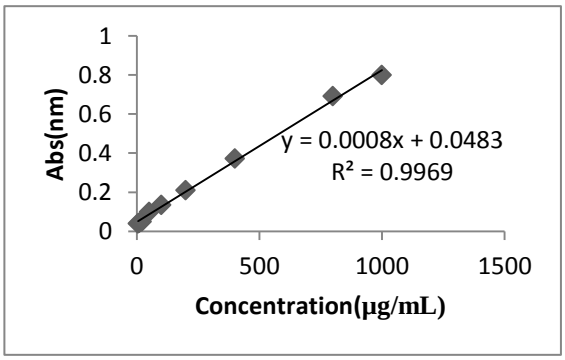
C- total starch content



D-Total amino acid content



E-total polyphenolic content



F-Total flavonoid content

Fig. 1: Standard curves of total carbohydrate, total starch, total protein, total amino acids, polyphenolic and flavonoid contents for *Misopates orontium* L.

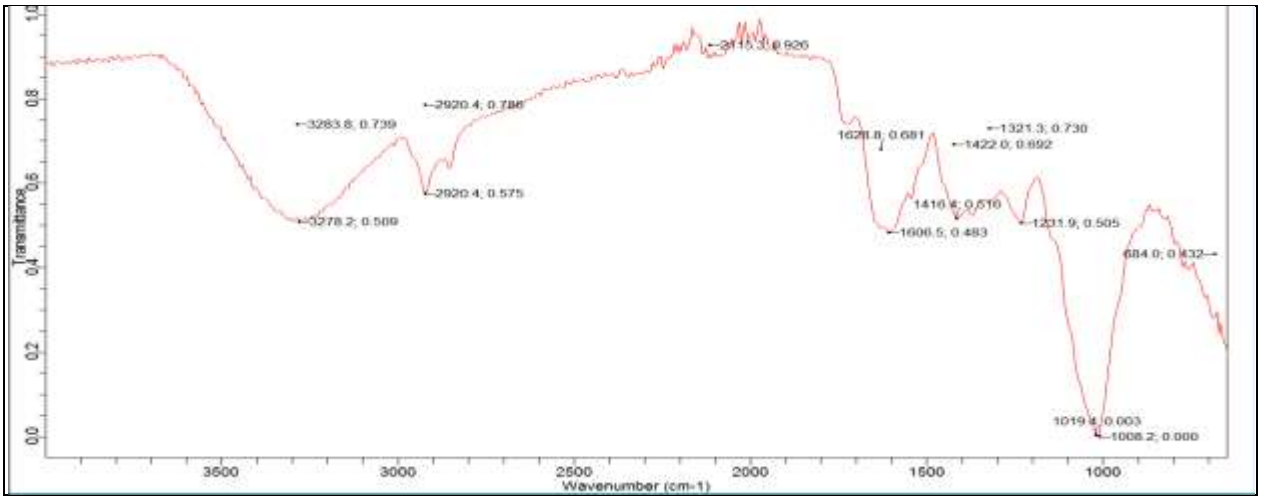
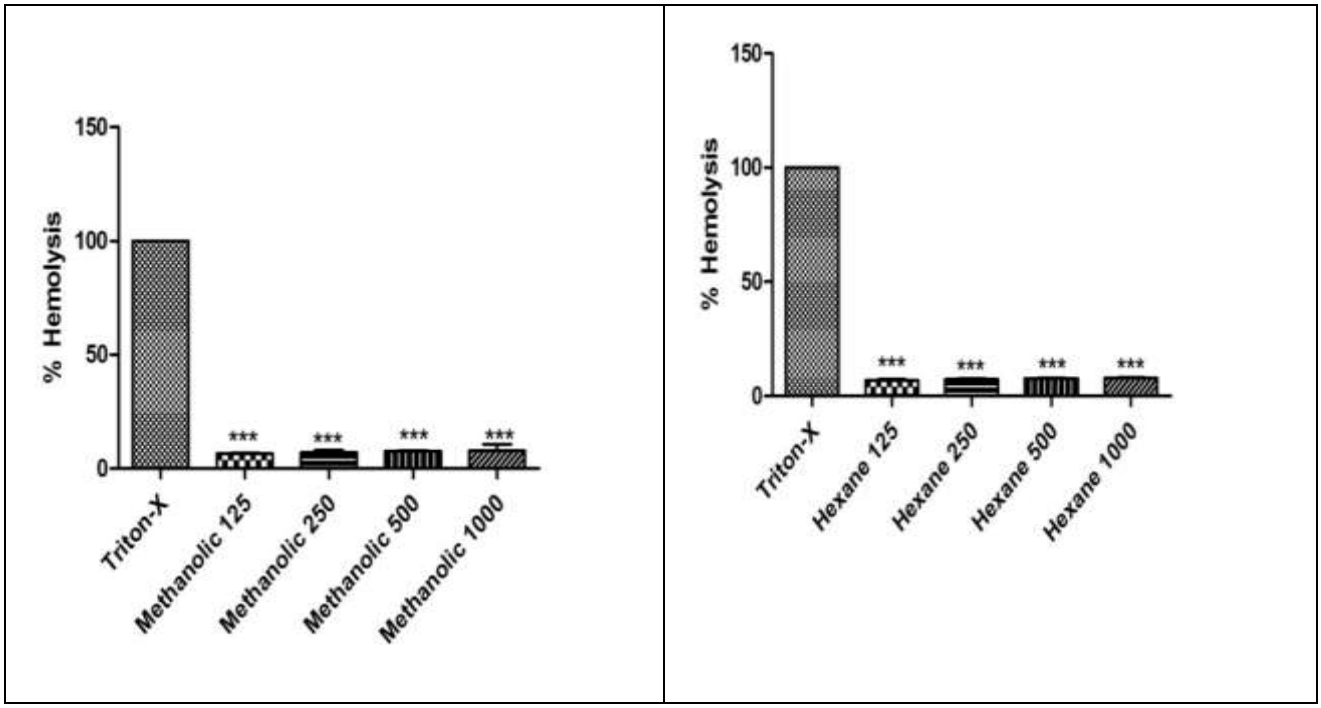


Fig. 2: FTIR analysis of Methanolic extract of *Misopates orontium* L.

Aqueous and ethyl acetate fraction showed 9.82% and 9.61% haemolysis of RBCs at higher concentration while all other fractions showed least haemolysis even at higher concentration. The result of all fractions were shown in figure



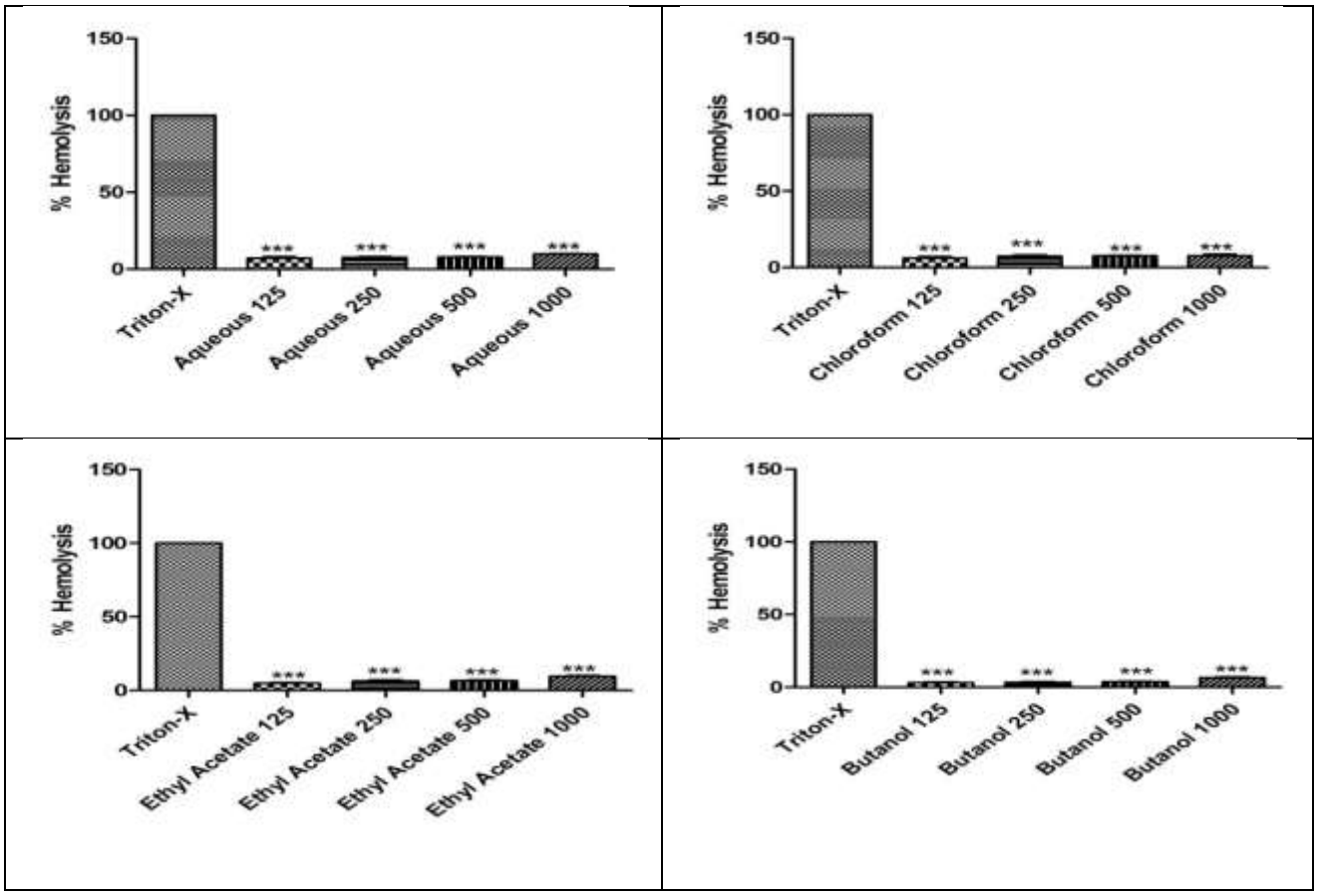


Fig. 3: Graphical representation of percentage hemolysis of different fraction of *Misopates orontium* L.

Hydroxyl radicals generated by Fenton's reagent causes DNA damage as DNA is absent in 3rd lane and only smear of degraded DNA can be observed. Methanolic and ethyl acetate extracts were found to be most effective, Hexane extract show little activity at high concentration, however, Aqueous and Butanol extract showed no activity at these concentrations.

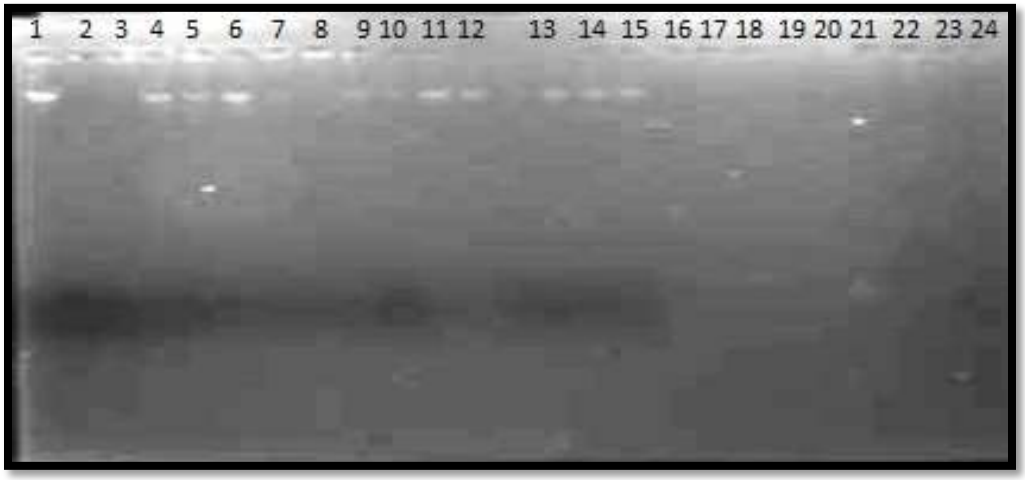


Fig. 4: DNA damage protection activity of *Misopates orontium* L. **Lane 1**: 4µl DNA+16µl DW, **Lane 2**: 3µl FR+17µl DW,

Lane 3: 4µl DNA+3µl FR+13µl DW, Lane 4: 4µl DNA+4µl Methanolic extract (50µg/µl) +3µl FR+9µl DW, Lane 5: 4µl DNA+4µl Methanolic extract (100µg/µl) +3µl FR+9µl DW, Lane 6: 4µl DNA+4µl Methanolic extract (200µg/µl) +3µl FR+9µl DW, Lane 7: 4µl DNA+4µl Hexane extract (50µg/µl) +3µl FR+9µl DW, Lane 8: 4µl DNA+4µl Hexane extract (100µg/µl) +3µl FR+9µl DW, Lane 9: 4µl DNA+4µl Hexane extract (200µg/µl) +3µl FR+9µl DW, Lane 10: 4µl DNA+4µl Chloroform extract (50µg/µl) +3µl FR+9µl DW, Lane 11: 4µl DNA+4µl Chloroform extract (100µg/µl) +3µl FR+9µl DW, Lane 12: 4µl DNA+4µl Chloroform extract (200µg/µl) +3µl FR+9µl DW, Lane 13: 4µl DNA+4µl Ethyl acetate extract (50µg/µl) +3µl FR+9µl DW, Lane 14: 4µl DNA+4µl Ethyl acetate extract (100µg/µl) +3µl FR+9µl DW, Lane 15: 4µl DNA+4µl Ethyl acetate extract (200µg/µl) +3µl FR+9µl DW, Lane 16: 4µl DNA+4µl Butanol extract (50µg/µl) +3µl FR+9µl DW, Lane 17: 4µl DNA+4µl Butanol extract (100µg/µl) +3µl FR+9µl DW, Lane 18: 4µl DNA+4µl Butanol extract (200µg/µl) +3µl FR+9µl DW, Lane 19: 4µl DNA+4µl Aqueous extract (50µg/µl) +3µl FR+9µl DW, Lane 20: 4µl DNA+4µl Aqueous extract (100µg/µl) +3µl FR+9µl DW, Lane 21: 4µl DNA+4µl Aqueous extract (200µg/µl) +3µl FR+9µl DW

Ethyl acetate fraction showed highest SPF value followed by n-butanol, n hexane and methanolic fraction while chloroform and aqueous fraction comparatively found to be least reactive. SPF value increase with increase in the concentration of these fractions .

Table IV: Absorbance of different fractions of *Misopates orontium* L. at concentration of 200µg/ml

Sr. No.	Wavelength (λnm)	EE*I (Normalize)	Absorbance					
			Methanolic	n-Hexane	Aqueous	Chloroform	Ethyl acetate	n-Butanol
1.	290	0.0150	0.437±0.01	0.34±0.05	0.225±0.06	0.324±0.05	2.51±0.2	0.648±0.10
2.	295	0.0817	0.475±0.12	0.184±0.03	0.175±0.02	0.128±0.0	2.1±0.05	0.523±0.03
3.	300	0.2874	0.460±0.01	0.146±0.01	0.132±0.02	0.096±0.0	1.91±0.3	0.447±0.09
4.	305	0.3278	0.457±0.06	0.175±0.01	0.101±0.07	0.092±0.01	1.85±0.01	0.43±0.05
5.	310	0.1864	0.453±0.02	0.123±0.03	0.073±0.01	0.078±0.02	1.76±0.09	0.394±0.01
6.	315	0.0839	0.444±0.05	0.112±0.01	0.045±0.01	0.062±0.04	1.62±0.12	0.382±0.07
7.	320	0.0180	0.437±0.03	0.102±0.04	0.04±0.04	0.057±0.03	1.51±0.04	0.39±0.00

Results are shown as Mean± SEM. Significant at P <0.05, P <0.01, P <0.001, ns=not significant

Table V: Absorbance of different fractions of *Misopates orontium* L. at concentration of 400µg/ml

Sr. No.	Wavelength (λnm)	EE*I (Normalize)	Absorbance					
			Methanolic	n-Hexane	Aqueous	Chloroform	Ethyl acetate	n-Butanol
1.	290	0.0150	0.47±0.07	0.767±0.02	0.464±0.02	0.703±0.01	3.45±0.02	1.108±0.01
2.	295	0.0817	0.499±0.01	0.556±0.01	0.293±0.02	0.476±0.06	3.13±0.01	0.885±0.01
3.	300	0.2874	0.487±0.06	0.514±0.01	0.262±0.00	0.424±0.00	2.98±0.04	0.808±0.06
4.	305	0.3278	0.49±0.01	0.502±0.05	0.219±0.01	0.346±0.01	2.77±0.00	0.782±0.04
5.	310	0.1864	0.484±0.01	0.462±0.03	0.195±0.04	0.366±0.03	2.7±0.01	0.749±0.01
6.	315	0.0839	0.47±0.04	0.442±0.08	0.187±0.03	0.342±0.02	2.61±0.00	0.724±0.00
7.	320	0.0180	0.463±0.02	0.43±0.02	0.174±0.02	0.325±0.01	2.58±0.02	0.727±0.03

Results are shown as Mean± SEM. Significant at P <0.05, P <0.01, P <0.001, ns=not significant

Table VI: SPF of different fractions of *Misopates orontium* L. at different concentrations

Sr. No.	Fraction	SPF	
		Concentration(200µg/ml)	Concentration(400µg/ml)
1.	Methanolic	4.57±0.11	4.86±0.18
2.	n-Hexane	1.54±0.06	5.00±0.26
3.	Aqueous	1.07±0.13	2.33±0.10
4.	Chloroform	0.94±0.04	3.87±0.06
5.	Ethyl acetate	18.56±0.51	28.41±0.82
6.	n-Butanol	4.34±0.21	7.91±0.07

Results are shown as Mean± SEM. Significant at P <0.05, P <0.01, P <0.001, ns=not significant

Statistical analysis

Values were expressed in triplicates with mean ± SD. Statistical significance differences between values of plant sample and respective control were determined by using graph pad prism. In each case *P < 0.05, **P < 0.01; ***P < 0.001 considered significant.

DISCUSSION

Phytochemicals present in plants based foods and herbs improve the quality of health. . These phytochemicals possess various pharmacological and biochemical actions when used by animals (Usunobun *et al.*, 2015). *Misopates orontium* was found to be a rich source of primary metabolites (carbohydrates, lipids, protein). Plant carbohydrates can be used as artificial sweeteners and help to rebuild the body of diabetic patients. Presence of carbohydrates, starch, proteins, free amino acids and lipids is an indication that plant has high nutritional values. Quantification of secondary metabolites is essential for extraction, purification, separation, crystallization and identification of different phytochemicals(Daniel &Krishnakumari, 2015). Plant contain a number of secondary metabolites including phenols, alkaloids, saponins, glycosides. Alkaloids contribute towards analgesic, antispasmodic and antimicrobial effect (Shukla *et al.*, 2015). Flavonoids are potent antioxidants that prevents the cell damage induced by oxidative stress and are used as anti-inflammatory agent, anticancer, in heart and skin diseases(Oyugi, 2016). Saponins are antimicrobial agents as well as regulate the blood lipids and glucose and also decrease risk of cancer (Achi *et al.*, 2017). Tannins have reported various medicinal properties i.e. antimicrobial, anti-inflammatory and wound healing (Daniel & Krishnakumari, 2015).

FTIR analysis of *Misopates* powder shows broad band centered at 3278.2cm due to OH absorption. Broadening of this band is related to OH stretching vibration with intra molecular hydrogen bonded at OH. This is an indication that alcoholic or phenolic group is present in plant powder or it might be complex polyhydroxy group of tannins. The narrow band centered at 2920.4 cm-1 represent the CH stretching vibration of methyl, methylene and methoxy group. Weak band at 2115.3cm-1 due to C≡C stretching indicating the presence of mono substituted alkyne. Medium band at 1606.5 cm-1 is due to C=C stretching indicating the presence of conjugated alkene or the cyclic alkenes or aromatic ring in powder plant material. Peak at 1416.4 cm-1 is due to OH bending showing the presence of alcohols or carboxylic acid group. Peak at 1231.9 is due to CN stretching indicating the presence of amines. Strong peak at 1019 is due to C=C bending confirming the presence of unsaturation in compound

Red blood cells (RBCs) are the major cells in circulation and easy to isolate from blood. Their membrane resembles with structure of other membranes in the body and has complex structure that maintains the morphology of cell, elasticity and deformability. Exposure to toxic agents can change the membrane structure that results in hemolysis of

RBCs. Anything that causes haemolysis is cytotoxic to RBCs and other body cells. Hence, in order to assess the cytotoxicity of molecules. Erythrocytes are used as biological model(Farag & Alagawany, 2018). Phytochemicals present in plant extracts are responsible for haemolysis. Haemolysis depends on the dose of extract (Zohra & Fawzia, 2014).Treating cell with cytotoxic compound can cause various problems to human beings (Riaz *et al.*, 2012). A drug can be used for pharmacological applications after screening for cytotoxicity (Kannan *et al.*, 2013). A plant extract with low cytotoxicity can be safely used in various diseases except tumors (Rasool *et al.*, 2015). In the present study Triton X-100 was used as positive control and causes 100% lysis of RBCs and PBS was used negative control being nontoxic to RBCs. The percentage hemolysis of all fractions of *M. orontium* was within safe range i.e. below 10%. These results suggest that *M. orontium* fractions can be safely used for pharmacological applications.

DNA maintains the growth and repair by different metabolic reactions. Various factors like radiations, chemicals, hydroxyl radicals, and oxidative stress can damage DNA. Oxidative stress is one of the major causes of DNA damage in Human beings. This damage to DNA causes various diseases i.e. Alzheimer disease, cancer, Parkinson's disease, early aging and diabetic conditions. Medicinal plants are considered as a rich source of bioactive components. These bioactive components may have DNA damage protection activity. Hence they played an important role in providing better health to human beings(Kaur *et al.*, 2019). Considering DNA protective potential of medicinal plants, DNA damage protection activity of different fractions of *Misopates* was analyzed using Fenton's reagent as damaging agent. Except Butanol and aqueous extract, all the extracts lessen the oxidative stress and protect the DNA from hydroxyl radicals generated by Fenton's reaction. Methanol extract was comparatively found to be most effective to protect DNA bands followed by ethyl acetate, chloroform and hexane extract. Hexane and chloroform extract showed dose dependent protection. DNA damage protection activity of these fractions is due to phytochemicals like phenolic and flavonoids present in these fractions. These phytochemicals scavenge free radicals and protect DNA from damaging effects of these radicals. No significant activity shown by Butanol and aqueous extract is attributed to their inability to quench hydroxyl radicals.

UV radiations are one of major cause of skin damage. They are responsible for sunburns, wrinkles, early aging and allergies. UV radiation penetrate into skin cell and causes gene mutation first stage of development of skin cancer. Therefore everybody requires protection from damaging effects of UV radiation. Synthetic agents are no more suitable to use as photo protective agents due to their toxicity in humans. Natural substances have absorbance in UV region and are good antioxidants ,therefore, they can be used as photo protective agents(Korać & Khambholja, 2011; Mukherjee *et al.*, 2011). After obtaining significant DNA damage protection results from different fraction of *M. orontium,* they were analyzed for photo protective potential. The results revealed that *M. orontium* fractions can be used in skin formulation as they have good SPF value. Flavonoids present in plants are responsible for their solar protection factor (Ebrahimzadeh *et al.*, 2014). Therefore photo protective potential of *M. orontium* might be due to their high flavonoids content

CONCLUSION

The present research work conclude that crude Methanolic extract of *M. orontium* is a rich source of primary and secondary metabolites. All the fractions have acceptable safety profile against human red blood cells. Ethyl acetate fraction have more potential to be used in cosmetics as it protects the cells and DNA effectively against the damaging effect of free radicals and UV radiation. However, further studies are needed to isolate the phytochemicals responsible for its biological effects.

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