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IN-VITRO ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY OF *ONOSMA HISPIDUM* (RATANJOT) ROOTS

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ABSTRACT

Aims: *To evaluate the anti-inflammatory and antioxidant activity of methanolic root extract of onosma hispidum (Ratanjot) by in-vitro methods.* ***Methods:*** *DPPH free radical and ABTS radical scavenging activity estimation with total phenolic and flavonoid content methods were used to evaluate antioxidant activity while egg albumin denaturation method and HRBC membrane stabilization methods were used to estimate anti-inflammatory potential in-vitro.* ***Results:*** *Results concluded that methanolic root extract of O. hispidum possess highly significant anti-inflammatory and free radical scavenging property, having IC_{50} value 2.73 µg/ml by DPPH method while in ABTS radical scavenging activity the IC_{50} value was 2.74 µg/ml with the experimental value of standard ascorbic acid was 1.47 µg/ml. Folin- Ciocalteu method showed that extract exhibited significant total phenolic content 110.46 mg gallic acid equivalent/gm while total flavanoid content as rutin equivalents 114.83 mg per gm of extract.* ***Conclusion:*** *The plant exhibits significant anti-inflammatory and antioxidant activity as confirmed by IC_{50} values.*

KEY WORDS

Onosma, antioxidatnt, Ratanjot, ABTS, DPPH.

INTRODUCTION

The phytochemicals from plants leads to discovery of more efficient and less toxic bioactive medicinal drug against various diseases [1]. The process of inflammation is mediated by number of vasoactive, proliferative and chemotactic factors, provides many targets for anti-inflammatory action of various drugs [2]. As this process is a primary physiologic defence mechanism against infection, allergens, toxic chemicals, burns, anti-inflammatory drugs are used to treat, causes major problems by their side effects. Therefore, it is necessary to develop a potent anti-inflammatory herbal preparation with lesser side effects [3]. Free radicals are unstable, highly reactive, atoms or molecules with unpaired electron which may produce various disorders like cancer, alzemer's disease, neurogenrative disorders and inflammation. It is also believed that aging is also facilitated by reactive oxygen species (ROS), a free redical [4]. *Onosma hispidum* belongs to genus Onosma of family *Boregineaceae*, traditionally known as Ratanjot and used as a spice in various curries in India for colour and flavor. It is also used for many common health problems by tribals and local vaids in ayurvedic system of medicine. Ratanjot is a FDA approved colouring material for various pharmaceutical preparations. Onosmins A and B as 2-[(4-methylbenzyl) amino] benzoic acid and methyl 2-[(4-methylbenzyl) amino] benzoate, apigenin, 6, 4'-dimethoxy-3, 5, 7-trihydroxyflavone, 6, 7-dimethoxy-3, 5, 4'-trihydroxyflavone, apigenin 7-O-beta-D-glucoside, 4-hydroxy-3-methoxy cinnamic acid (ferulic acid), 4-hydroxy-3-methoxy benzoic acid (vanillic acid) and Hispidone ((2S)-5, 2'-dihydroxy-7, 4', 5'-

trimethoxyflavanone) have been isolated from *Onosma hispidum* [5, 6].

Onosma hispidum (family-*Boregineaceae*) is 70 cm tall, perinneal herb with prominent tap root distributed in India, Pakistan, Afghanistan and China, used traditionally for laxative, anthelmintic, disorder of blood, disease of eyes, bronchitis, abdominal pain, antibacterial and as wound healer under name Ratanjot [5]. This plant is scientifically validated for wound healing, antitussive, anticholinesterase inhibitor, lipoxygenase inhibitor, antimicrobial and antidiabetic activity [7]. On the basis of traditional uses and pharmacological screening, this study was selected to investigate the *in vitro* anti-inflammatory and antioxidant poteintial of methanolic root extract of *Onosma hispidum*.

MATERIALS AND METHODS

Collection and identification of plant

Dried roots of *O. hispidum* were purchased from local market and identified by Dr. S. Khatoon, Research Scientist, National Botanical Research Institute, Lucknow, India. Roots were ground into course powder with the aid of blender and stored in airtight containers at room temperature till use.

Preparation of Extract

The course powder was extracted with methanol using hot soxhlet method [8] and extract was evaporated in rotary drum evaporator (Buchhi type) and finally dry it on water bath at 50^0C till dryness and stored in sterile bottle for further analysis [9, 10].

DPPH radical scavenging activity assay

The *in vitro* free radical scavenging activity of methanolic root extract of *O. hispidum* was estimated by 2, 2- diphenyl-1-picrylhydrazyl (DPPH) assay. The 24 mg DPPH was dissolved in 100 ml methanol and stock solution was prepared then stored at 20°C until use. Dilution of stock solution was done in such a way that resulting solution was attained an absorbance of about 0.98±0.02 at 517 nm. One 3 ml aliquot of this diluted solution was mixed with 100 µl of samples of various concentrations (10-500 µg/ml) then reaction mixture was incubated in dark for 15 min at room temperature and absorbance was taken at 517 nm [10]. The control was prepared as above without any sample. The DPPH free radical scavenging effect was analyzed by the equation:

[(control absorbance-sample absorbance/ (control absorbance)] ×100.

ABTS radical scavenging activity

The 2,2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) cation scavenging potential was performed by reacting ABTS solution (7 mM) with potassium persulfate (2.45 mM) solution and kept overnight in the dark to yield a dark colored solution containing ABTS radical cations. The ABTS radical cation was diluted with 50% methanol prior to use with an initial absorbance of about 0.70±0.02 at 745 nm and temperature not exceeded to 30°C. The 300 µl of test samples (10-500 µg/ml) mixed with 3.0 ml of ABTS working standard in a microcuvette and decrease in absorbance of samples was measured between 1 to 6 min. The percentage inhibition was calculated by the formula:

[(control absorbance-sample absorbance/ (control absorbance)] ×100.

The antioxidant potential of test samples was expressed as IC_{50}, the concentration necessary for 50% reduction of ABTS (anti-radical activity) estimated by dose response curve method [10].

Estimation of total flavonoid content

0.3 ml of extract of varying concentrations (100-500 µg/ml) diluted with 3.4 ml of 30% methanol then added 0.15 ml of $NaNO_2$ (0.5 M) with 0.15 ml of $AlCl_3.6H_2O$ of 0.3 M concentration then all were thoroughly mixed. After 5 min 1 ml of NaOH of 1 M was added. The solution was again mixed well, and absorbance was measured against the reagent blank at 506 nm. The standard curve for total flavonoids was made using rutin standard solution of varying cone from 100-500 µg/ml under the same procedure as test samples. The total flavonoids were expressed as milligrams of rutin equivalents per gm of dried sample [10].

Estimation of Total phenolic content

1 ml of properly diluted sample mixed with at least 60 ml of water and 5 ml of F-C reagent and between 0.5 to 8 min add 15 ml of 7% Na_2CO_3 then mixed well and made volume 100 mL with deionized water then incubate for 2h at 23°C then measured absorbance at 750 nm [11]. The TPC was determined from extrapolation of calibration curve which was made by preparing gallic acid solution and estimation of total phenolic compounds was carried out in triplicate and expressed as milligrams of gallic acid equivalents (GAE) per gm of dried sample [10].

Egg Albumin Denaturation Method

The reaction mixture of 5 ml consisted of 0.2 ml of egg albumin of fresh hen's egg and 2.8 ml of phosphate-buffered saline (PBS) at pH 6.4 with 2 ml of varying concentrations (10-500 µg/ml) of extract. A similar volume of double-distilled water served as the control. Mixtures were incubated at 37 ± 2°C in BOD incubator for 15 minutes then heated at 70°C for five minutes. After cooling, the absorbance was measured at 660 nm. 100 µg/ml of Indomethacin was used as standard drug. Percentage inhibition of protein denaturation was calculated by using the following formula: 100 × [Vt/Vc-1] Where, Vt= absorbance of the test sample, Vc= absorbance of control.

Each experiment was done in triplicate and the average was taken. The extract concentration for 50% inhibition (IC_{50}) was determined by dose-response curve by putting y= 50 in the line equation [12].

HRBC Membrane Stabilization Method

The blood sample (NSAID free) was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride). Blood sample was centrifuged at 3000 rpm and packed cells were washed with isosaline (0.9% w/v NaCl) and a 10% suspension was made with isosaline. Various concentrations of extract were prepared (10-500 µg/ml) using distilled water and in each concentration 1 ml phosphate buffer, 2 ml hyposaline, and 0.5 ml HRBC suspension were added and incubated at 37°C for 30 min after that centrifuged at 3000 rpm for 20 min. The hemoglobin content in supernatant solution was estimated by spectrophotometer at 560 nm. Indomethacin (100 µg/ml) was used as the reference drug and a control was prepared without extract. The percentage hemolysis was calculated by assuming that hemolysis produced by the control group as 100% [13]. The percentage of HRBC membrane stabilization or protection was calculated using the formula: 100 − ((OD of drug treated sample/OD of control) ×100).

Statistical analysis

Data are expressed as mean ± SD from three separate observations. For in vitro antioxidant assays one-way ANOVA test followed by Tukey's test ($P < 0.05$) was used to analyze the differences among IC_{50} of various fractions for different antioxidant assays. The IC_{50} values were determined using the standard line equation method [10].

RESULTS AND DISCUSSION

HRBC membrane stabilization

HRBC (Human red blood cell) membranes are similar to lysosomal membrane components and inhibition of hypotonicity and heat induced red blood cell membrane lysis was taken as a measure of potency of anti-inflammatory activity of extract. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Injury to red cell membrane will render the cell more susceptible to secondary damage through free radical induced lipid peroxidation. Membrane stabilization leads to prevention of leakage of serum protein and fluids into the tissues during a period of increased permeability caused by inflammatory mediators. The methanolic root extract of *O. hispidum* showed significant prevention of leakage of serum protein and fluids in a dose dependent manner with IC_{50} 3.30 µg/ml (Table 1).

Table No. 1: Effect of methanolic root extract of *Onosma hispidum* (Ratanjot) on HRBC membrane stabilization and protein denaturation

Concentration (µg/ml)		% inhibition of haemolysis	IC_{50} Value (µg/ml)	% inhibition of protein denaturation	IC_{50} Value (µg/ml)
Indomethacin	100	61.63	-	75.55	
Onosma hispidum	10	23.26	3.30	18.99	3.15
extract	50	37.95		38.68	
	100	48.97		51.53	
	200	62.44		68.43	
	300	71.42		75.00	
	400	73.46		77.51	
	500	77.14		81.00	

Egg albumin protein denaturation

Thermal treatments applied to egg white causes undesirable modifications to their physicochemical and functional properties. The results showed a sigmoidale evolution with negative slope of transmittance due to irreversible loss of solubility. Coagulation and/or precipitation of egg white protein has been observed at around 74°C for 20 min. The methanolic root extract of *O. hispidum* was used with concentration range of 10-500 µg/ml and showed a concentration dependent inhibition of protein denaturation (IC_{50} value 3.15 µg/ml) same as standard drug indomethacin.

DPPH free radical scavenging activity

DPPH is a crystalline organic chemical compound having stable free radical molecules and used as source of free radicals for *in vitro* study of free radical scavenging property of any compound or extract. The results of DPPH radical scavenging activity of methanolic root extract of *O. hispidum* is shown in Table 2. The scavenging effect was increased significantly with increase in concentration of extract with an IC_{50} 2.73 µg/ml when compared with standard ascorbic acid with IC_{50} value 1.47 µg/ml.

Table No. 2: Effect of methanolic root extract of *Onosma hispidum* (Ratanjot) on DPPH and ABTS free radical scavenging activity

Concentration (µg/ml)		% inhibition of DPPH free radical	IC_{50} Value (µg/ml)	%inhibition of ABTS radical	IC_{50} Value (µg/ml)
Standard	10	47.20	1.47	39.37	1.71
(Ascorbic acid)	50	55.43		48.28	
	100	59.80		64.64	
	200	67.12		80.84	
	300	75.74		85.30	
	400	81.51		90.00	
	500	93.21		92.65	
Onosma	10	66.24	2.73	37.90	2.74
hispidum	50	77.59		40.54	
extract	100	80.18		45.24	
	200	83.22		60.13	
	300	88.76		77.08	
	400	90.51		87.65	
	500	91.59		92.85	

Table No. 3: The total phenolic and flavonoid content in methanolic root extract of *Onosma hispidum* (Ratanjot)

Concentration (µg/ml)		Milligram of GAE/g	Mg of GAE/g Mean±SD	Mg of RUE/gm	Mg of RUE/g Mean±SD
Onosma hispidum	100	131.66	110.46±12.86	120.00	114.83±9.37
	200	102.50		102.25	
	300	113.18		107.61	
	400	106.26		120.25	
	500	98.46		124.05	

ABTS free radical scavenging activity

ABTS is the chemical compound which produce radical cations with sodium persulfate which are blue in colour and react with most of antioxidant compound like phenols, thiols and vitamin C. The reaction of blue radicals of ABTS converted into colourless neutral ions. The results of DPPH radical scavenging activity of methanolic root extract of *O. hispidum* was showed significant free radical scavenging potential in a dose dependent manner with IC_{50} value 2.74 µg/ml when

compared with standard ascorbic acid with experimental IC_{50} 1.71 µg/ml as showed in Table No 2.

Total phenolic content

The phenols are aromatic secondary plant metabolites having property to scavenge free radicals and various active oxygen species like singlets hydroxyl radicals and superoxide free radicals. Methanolic root extract of *O. hispidum* have very prominent total phenolic content in terms of gallic acid equivalent (GAE) as 110.46±12.86 mg/gm of dried extract as showen in Table No 3.

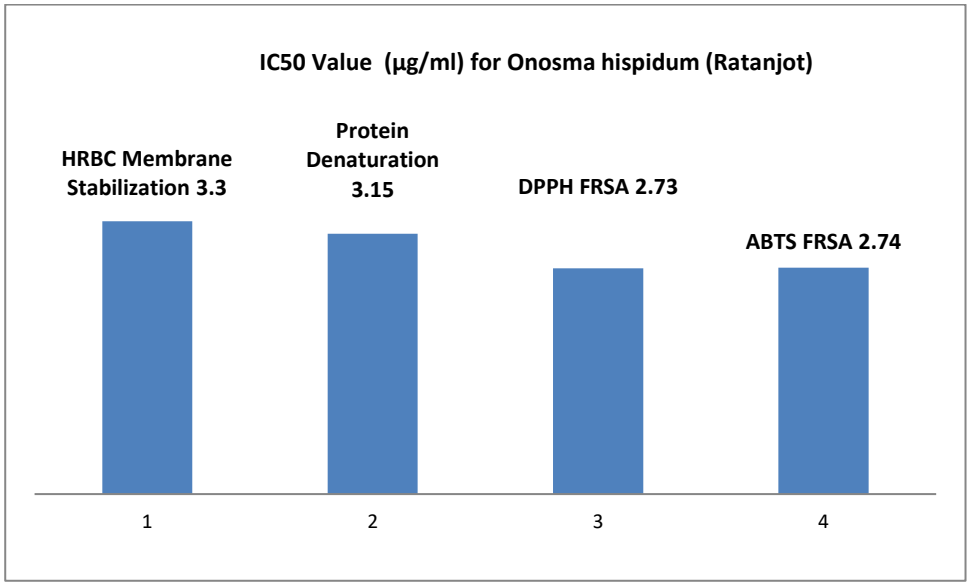
Total flavonoid content

The flavonoids are pigments having similar structure with flavones and responsible for flower colouration, physiological regulators, cell cycle inhibitors and chemical messengers. In humans, pharmacologically active in allergy, inflammation, antioxidant and microbial, fungal, viral infections with anti-cancer and anti-diarrheal activities. The methanolic root extract of *O. hispidum* showed significant quantity of flavonoid content in forms of rutin equivalents as 114.83±9.37 mg/gm of dried extract as shown in Table 3.

CONCLUSION

The Ratanjot (*O. hispidum*) roots exhibits excellent functional, nutritional and pharmacological importance. The present screening tests indicates that methanolic root extract possess moderately significant anti-inflammatory and antioxidant activity which also confirmed from IC_{50} values obtained from various *in vitro* studied by different methods (Figure 1). The plant can be further studied to explore the mechanism and constituent(s) involved in its antioxidant activity.

Figure No. 1: IC_{50} Values for different parameters in µg/ml of methanolic root extract of *Onosma hispidum* (Ratanjot)



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