Research Article

www.ijrap.net

IN VITRO PHARMACOLOGICAL STUDY OF EXTRACT OF *RAPHANUS SATIVUS* SPROUTS

P. Niharika [1]*, K. Arathi [2], Lilly Grace [3], Y. Elisha [2], S. Manohar Babu [4]

[1] Assistant Professor, Department of Pharmaceutical Chemistry, SIMS College of Pharmacy, Mangaldas Nagar, Guntur, Andhra Pradesh, India

[2] Student, Department of Doctor of Pharmacy, SIMS College of Pharmacy, Mangaldas Nagar, Guntur, Andhra Pradesh, India

[3] Student, Department of Pharmacy, SIMS College of Pharmacy, Mangaldas Nagar, Guntur, Andhra Pradesh, India

[4] Principal, Department of Pharmacology, SIMS College of Pharmacy, Mangaldas Nagar, Guntur, Andhra Pradesh, India

Received on: 11/06/19Accepted on: 17/07/19

***Corresponding author**
E-mail: niharika.mpharm12@gmail.com

DOI: 10.7897/2277-4343.100492

ABSTRACT

Ethyl acetate extract of *Raphanus sativus* sprouts belonging to *Brassicaceae,* produced by cold maceration reveal the presence of volatile oils. Dilutions of the extract 20, 40, 60, 80, 100 µg/ml prepared in DMSO were used for the *in vitro* study. The antimicrobial activity of the extract was assessed using *Bacillus subtilis* (NCIM 2698), *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (NCIM 2344), *Klebsiella pneumonia* (NCIM 2957) and *Aspergillus flavus* (NCIM 568) against the standards gentamycin and ketoconazole. *In vitro* pharmacological studies, include the reducing activity, antioxidant activity and anti inflammatory activity were evaluated using the ferric reducing assay, hydrogen peroxide scavenging assay and albumin denaturation method respectively. The extract had shown significant antibacterial activity on all organisms excluding *Klebsiella* and *Escherichia* than the standard. In vitro studies reveal significant reducing (36.015 ± 0.686), antioxidant (26.830 ± 0.240) and anti inflammatory activities (25.865 ± 0.191) against the standard butylated hydroxyl toluene (BHT) (31.879 ± 2.629), ascorbic acid (21.7245 ± 0.240) and Diclofenac (0.4825 ± 0.025) respectively.

Keywords: *Raphanus sativus* sprouts, anti microbial, reducing power, antioxidant activity, anti inflammatory activity.

INTRODUCTION

Raphanus sativus L. of *Brassicaceae* family commonly known as Radish, Wild Radish, White Charlock, Jointed Charlock which is an edible taproot generally found in America, Australia, Africa, and East Asia germinates both in fall and winter and matures from March to June. *Raphanus* produces a hairless cotyledon with 10-25 mm long petiole and as the plant matures, it has erect branches covered with prickly hairs contains flowers, seeds and also a taproot which helps the plant to survive during moisture stress and provides a reserve for regrowth. *Raphanus* requires a minimum temperature of 35ºC for germination[1-2]. Chemical constituents like Isothiocyanate[3], Cysteine-rich peptides, Pelargonidin-3-sophoroside-5-glucoside, Lipopolysaccharides, Sinapine[4], Glucosinolates and Phenols[6] have been recognized in *Raphanus sativus*.

From the literature review, anti-cancer activity of *Raphanus sativus* was because of isothiocyanates[3] in the edible part while various extracts have a nephroprotective[9] and peristaltic effect. Lipopolysaccharides and isothiocyanates have shown anti-herpes, analgesic and antiplatelet activities[4]. The vitamin-C in *Raphanus sativus* show powerful antioxidant[6]. The red pigment of radish has antioxidative and anti-viral activities[5], seeds possess anti-fungal[7] and hypotensive activities[5] while leaves possess the protective function against arsenic toxicity[8]. The present work is focused to determine the antimicrobial, *in vitro* pharmacological activities of sprouts of *Raphanus sativus*

MATERIALS AND METHODS

Chemicals and Instruments

All the chemicals of extraction and *in vitro* studies were from Merck. The organisms used for antimicrobial studies were from NCIMB Pune, India. Absorbance of the solutions during *in vitro* studies were measured using T60 UV-Visible Spectrophotometer (PG Instruments).

Collection and identification of plant

The seeds (about 1 kg) of *Raphanus sativus* (Family: *Brassicaceae*) were collected from the local market of Guntur, A.P India in January 2017. Seeds were authenticated by Dr.A. Kranthi, Botanist in Department of Botany and Microbiology, Acharya Nagarjuna University. Authenticate seeds of *Raphanus sativus* were used for the work.

Preparation of plant material for Extraction

Seeds were graded to remove impurities, washed with hot water and soaked in water for 3 days. After germination, the sprouts produced on the 7th day were subjected to drying in the hot air oven at 45°C for 24 h. The dried sprouts were milled to a coarse powder transferred through the sieve and stored in air tight container.

Extraction Process

About 150 g of the coarse powder was taken into the conical flask and 500 ml of ethyl acetate (solvent) was added to the conical flask wrapped with the aluminum foil and allowed to macerate for about 5 days with periodical shakings. After cold maceration, the extract collected was filtrated and the excess solvent was reduced using a rotary evaporator. The semisolid extract was refrigerated at 4^0C for further analysis.

Phytochemical Analysis

Plants contain many therapeutically active or inactive constituents like Sugars, glycosides, tannins, flavonoids, gums, saponins, alkaloids and volatile oils. Ethyl acetate extract collected was analysed for phytochemicals. The results of various chemical tests of phytochemical investigation indicate the presence of volatile oils which were tabulated in Table 1.

Antimicrobial Activity

The antimicrobial activity of the extract was evaluated by agar disc diffusion method[10] using the 24 h and 48 h subcultures of bacterial and fungal strains *Staphylococcus aureus* (NCIM 2079), *Bacillus subtilis* (NCIM 2698), *Klebsiella pneumonia* (NCIM 2957), *Escherichia coli* (NCIM 2344), and *Aspergillus flavus* (NCIM 536) respectively. The media used for antibacterial and antifungal evaluation were nutrient agar and potato dextrose agar respectively. The antimicrobial activity of five dilutions of the extract of concentrations 20, 40, 60, 80 and 100 µg/ml prepared using DMSO was evaluated against the standards gentamycin and ketoconazole respectively. Discs soaked in the respective dilutions were placed on the inoculated agar plates and were incubated at 37^0C and 25^0C. The zone of inhibition produced after 24 and 48 h of incubation with bacterial and fungal cultures respectively, were measured and the diameters of zone of inhibition produced by the extract dilutions were tabulated in Table 2.

***In Vitro* Reducing Activity**

Reducing activity of the extract was determined by ferric reducing assay[11]. 2.5 ml of the extract dilutions (20, 40, 60, 80 and 100 µg/ml) were mixed with 2.5 ml of phosphate buffer (pH 6.6), 2.5 ml of 1% potassium ferrocyanide solution in the test tubes and were placed in the water bath for 20 minutes at 50^0C. The tubes were cooled rapidly and were mixed with 2.5 ml of 10% trichloroacetic acid solution. 2.5 ml of supernatant obtained by centrifugation of the above solution at 3000 RPM for 10 minutes was diluted with 2.5 ml of distilled water then, shaken with 0.5 ml of fresh 0.1% ferric chloride solution. The absorbance of the resulting mixture was colourimetrically read at 700 NM against the standard butylated hydroxytoluene (BHT) in triplicates. The percentage reducing activity was measured using the formula:

$$\% \text{ Reducing Activity} = (\text{Absorbance of test} / \text{Absorbance of control} - 1) \times 100$$

***In Vitro* Antioxidant Activity**

The antioxidant activity of the extract was evaluated *in vitro* by hydrogen peroxide radical scavenging method[12]. 1 ml of the dilutions of the extract (20, 40, 60, 80 and 100 µg/ml) were added to the tube containing 0.6 ml of 40 mM hydrogen peroxide solution. The resulting solutions were incubated for 10 minutes at 37^0 C and the absorbance of the solution was measured at 230 nm against phosphate buffer as a control and L-Ascorbic acid as standard. The whole experiment was repeated thrice. The percentage hydrogen peroxide radical scavenging activity was determined using the formula:

$$\% \text{ Radical Scavenging Activity} = (\text{Absorbance of test} / \text{Absorbance of control} - 1) \times 100$$

***In Vitro* Anti Inflammatory Activity**

In vitro Albumin denaturation method[13] was used to evaluate the anti-inflammatory activity of the extract. 1 ml of the extract dilution (20, 40, 60, 80 and 100 µg/ml) was added to 1 ml of 1% bovine albumin solution prepared in phosphate buffer (pH 7.4) and were incubated at 37^0C for 15 minutes. Denaturation was induced by placing the test tubes in a water bath at 60^0C for 10 minutes. After cooling, the turbidity produced by denaturation was measured using visible spectrophotometer at 660 nm against phosphate buffer. Diclofenac was used as a standard anti-inflammatory agent. All the experiments were performed in triplicates. The percentage inhibition of denaturation was measured using the formula:

$$\% \text{ Inhibition} = (\text{Absorbance of test} / \text{Absorbance of control} - 1) \times 100$$

RESULTS AND DISCUSSION

Raphanus sativus an edible taproot of *Brassicaceae* family found to have many beneficial effects while, the sprouts were less studied. For the present study, sprouts of *Raphanus sativus* were made from three days soaking in distilled water and seven days incubation. Sprouts were minimized to coarse powder with homogenizer and were extracted with the solvent ethyl acetate by cold maceration. The extract collected after 5^{th} day was filtered and reduced to semisolid using Whatmann filter paper and rotary evaporator respectively. The collected extract was analyzed for phytochemicals with various chemical tests and the result of analysis indicating presence of volatile oils was tabulated in Table 1.

The extract was assessed for antimicrobial activity using different strains of bacteria and fungus by the agar disc diffusion method. The dilutions 20, 40, 60, 80, 100 µg/ml was used for the assessment against the standards gentamycin and ketoconazole. The dilution 100 µg/ml of the extract has significant activity on the *Bacillus subtilis, Staphylococcus aureus* and *Aspergillus flavus* while less significant activity on *Escherichia coli* and no activity on *Klebsiella pneumonia* compared to standard. The results of the antimicrobial activity were represented in Table 2.

In vitro study of the extract for reducing activity, antioxidant activity, anti inflammatory activity was done by ferric reducing assay, hydrogen peroxide scavenging assay and albumin denaturation method respectively. Same concentrations 20, 40, 60, 80, 100 µg/ml of the extract was used with standards butylated hydroxyl toluene (BHT) for reducing, Ascorbic acid for antioxidant and Diclofenac for anti-inflammatory assay. In the ferric reducing assay, 100 µg/ml dilution of the extract (36.015 $\pm$ 0.686) has shown significant activity compared to BHT (31.879 $\pm$ 2.629) which was represented in Figure 1. The antioxidant activity of the extract (26.830 $\pm$ 0.240) was significant compared to standard ascorbic acid (21.7245 $\pm$ 0.240) as shown in Figure 2 while there was a remarkable anti inflammatory activity (25.865 $\pm$ 0.191) than Diclofenac (0.4825 $\pm$ 0.025) as shown in Figure 3. The results of *in vitro* studies were tabulated in Table 3.

Table 1: Result of Phytochemical analysis of the ethyl acetate extract

Phytochemicals	Ethylacetate Extract of *Raphanus sativus*
Carbohydrates	+
Flavonoids	-
Tannins	-
Saponins	-
Glycosides	-
Alkaloids	-
Volatile Oils	+

(+): presence of constituent; (-): absence of constituents

Table 2: Results of antimicrobial studies of the ethyl acetate extract

Concentration of the extract (µg/ml)	Diameter of zone of inhibition in millimeters (mm)				
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumonia*	*Aspergillus flavus*
20	**-**	**-**	**-**	**-**	**-**
40	**12**	**15**	**-**	**-**	**-**
60	**15**	**17**	**-**	**-**	**18**
80	**20**	**25**	**10**	**-**	**19**
100	**30**	**32**	**16**	**-**	**22**
Gentamycin	**35**	**35**	**25**	**13**	**24**
Ketoconazole	**-**	**-**	**-**	**-**	**25**

Table 3: Results of *In vitro* Pharmacological Studies of the Ethyl acetate Extract

S. No.	Concentration of the extract µg/ml.	% Reducing power	% Radical Scavenging activity	% Inhibition of albumin denaturation
1	20	28.085 ± 0.474	1.492 ± 0.011	1.796 ± 0.006
2	40	33.100 ± 0.141	2.866 ± 0.049	1.995 ± 0.007
3	60	33.535 ± 0.544	18.3835 ± 0.380	5.965 ± 0.049
4	80	34.6395 ± 0.680	23.542 ± 0.455	22.030 ± 0.042
5	100	36.015 ± 0.686	26.830 ± 0.240	25.865 ± 0.191
6	Standard (BHT)	31.879 ± 2.629	-	-
7	Standard (Ascorbic acid)	-	21.7245 ± 0.240	-
8	Standard (Diclofenac)	-	-	0.4825 ± 0.025

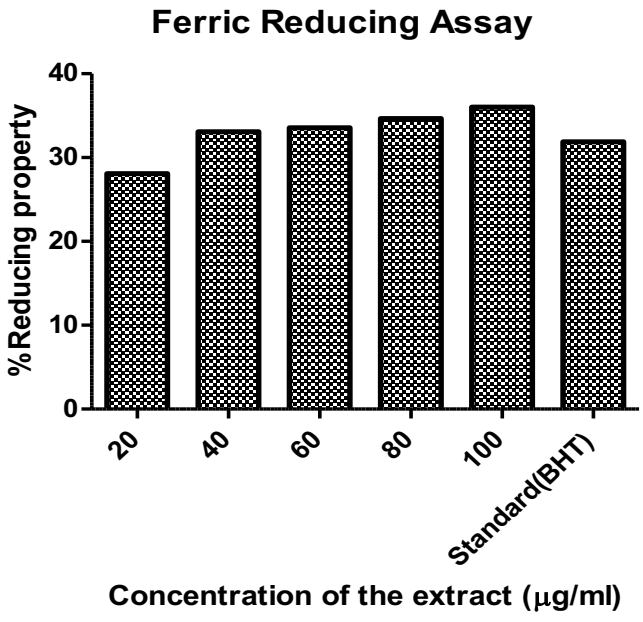

Figure 1: Graph of *in vitro* Reducing Assay of Extract dilutions against the standard BHT

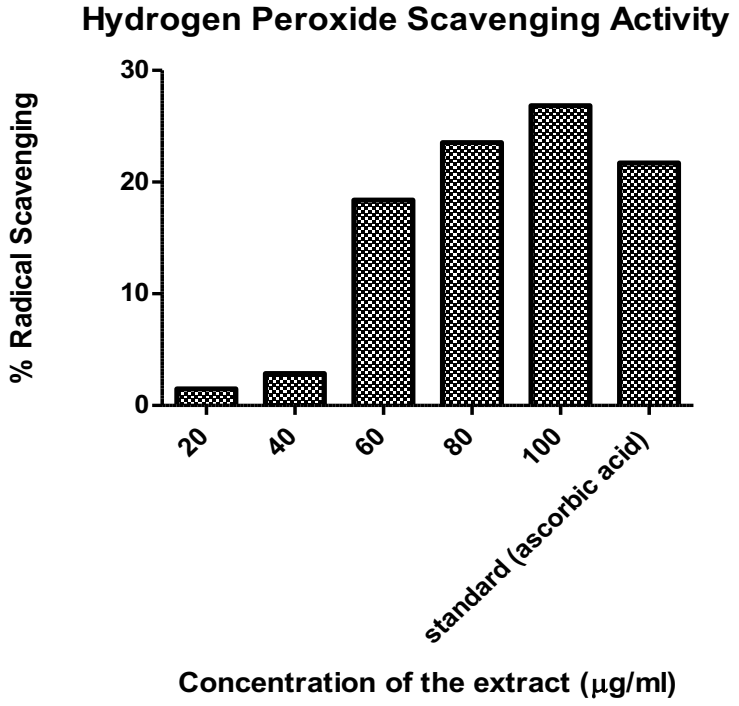

Figure 2: Graph of *in vitro* Anti Oxidant Assay of Extract dilutions against the standard Ascorbic acid

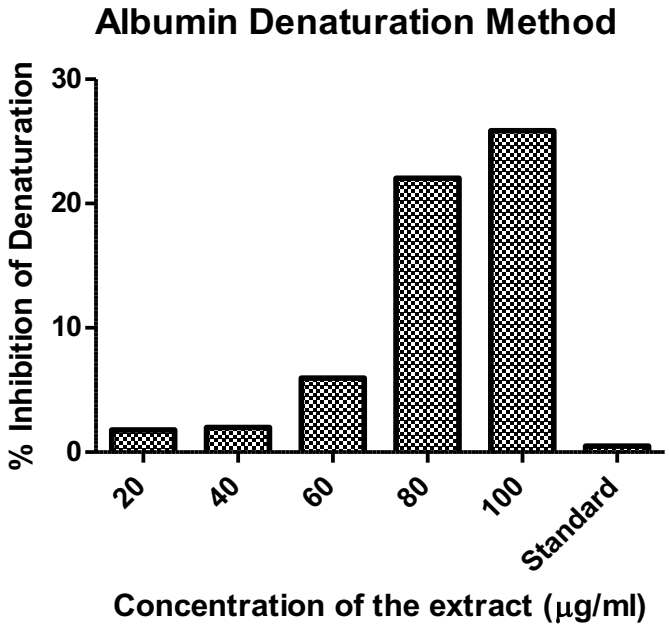

Figure 3: Graph of *in vitro*Anti Inflammatory Assay of Extract dilutions against the standard Diclofenac

CONCLUSION

The above study on *Raphanus sativus* sprouts is hypothesized to assess the antimicrobial, reducing, antioxidant and anti inflammatory activities. The *in vitro* study identified the potent antioxidant and anti inflammatory properties of the volatile oil present in the extract which can further be isolated and studied for it's anticancer, Cyclooxygenase (COX) inhibitory activities.

REFERENCES

1. Mayank, Malik, JK Norsworthy, AS Culpepper, MB Riley, W Bridges.Use of Wild Radish (*Raphanus raphanistrum*) and

Rye Cover Crops for Weed Suppression in Sweet Corn. Weed Science 2008; 56 (Suppl 4): 588-595.
2. Mihaela Ioana Georgescu, Vasilica Luchian, Octavian Groza. *Raphanus Raphanistrum* subsp. Landra (Moretti ex DC) Bonnier and Layens -Adventious species of Mediterranean origin adapted as a weed in crops-some considerations on morphological and anatomical peculiarities; Agriculture and Agricultural Science Procedia 2016; 10: 123-128.
3. Piman pocasap, Natthida weerapreeyakul. Sulforaphane in *Raphanus sativus* L.Var. Caudatus Alef increased in the late-bolting stage as well as anticancer activity. Asian Pacific Journal of Tropical Biomedicine 2017; 7(Suppl 11): 998-1004.
4. Tej singh, Kumari jyothi, Amar Patnaik, Ajeet Singh, Ranchan Chauhaan, S.S. Chande. Biosynthesis, characterization and antibacterial activity of silver nanoparticles using an endophytic fungal supernatant of *Raphanus sativus*. Journal of Genetic Engineering Biotechnology 2017; 15(Suppl1): 31-39.
5. Rosa Martha Perez Gutierrez, Rosalinda Lule Perez. *Raphanus sativus* (Radish): Their chemistry and Biology. Scientific World Journal 2004; 4: 811–837.
6. Rizwan Ashraf, BushraSultana. Variation in biochemical and antioxidant attributes of *Raphanus sativus* in response to foliar application of plant leaf extracts as plant growth regulator. Journal of Genetic Engineering and Biotechnology 2016; 14 (Suppl1): 1-8.
7. Ana L. Vilas Alves, Genoveva W. De Samblanx, Franky R.G. Terras, Bruno PA. Cammue, Willem F. Broekaert. Expression of functional *Raphanus sativus* antifungal protein in yeast. FEBS letters 1994; 348 (Suppl 3): 228-232.
8. Sayada Dilruba, MM Hasibuzzaman; Ameliorating effects of *Raphanus sativus* leaves on sodium arsenite-induced perturbation of blood indices in Swiss albino mice; Asian Pacific Journal of Tropical Biomedicine 2017; 7 (Suppl 10): 915-920.
9. Magesh salai Bojan, Rashmi rajappa, Dhana Rangesh Kumar, Vijayakumar, Jayathi Gopalan. The protective effect of *Raphanus sativus* on D-galactosamine induced nephrotoxicity in rats. Nutrition Cliniqueet Metabolism 2016; 30: 22-28.
10. P Parameswari, R Devika, P Vijayaraghavan. *In vitro* anti-inflammatory and antimicrobial potential of leaf extract from *Artemisia nilagirica* (Clarke) Pamp. Saudi Journal of Biological Sciences 2019; 26(Suppl 3): 460-463.
11. N. Subhashini, A. Thangathirupathi, N. Lavanya, Antioxidant Activity Of *Trigonella Foenum* Graecum Using Various *In Vitro* And *Ex Vivo* Models, Int J Pharm Pharm Sci 2011; 3 (Suppl 2): 96-102.
12. Jignasu P Mehta, Parvin H. Parmar, Sohil H. Vadia, Mahendra K. Patel, Chandrabhanu B Tripati. *In-vitro* antioxidant and *in-vivo* anti-inflammatory activities of aerial parts of *Cassia* species Arabian Journal of Chemistry 2017; 10: S1654-1662.
13. P. Padmanabhan, SN Jangle. Evaluation of *in vitro* anti inflammatory activity of herbal preparation, a combination of four medicinal plants. International Journal of Basic and Applied Medical Sciences 2012; 2 (Suppl 1): 109-116

ACKNOWLEDGEMENT

Authors are thankful to Dr. A. Kranthi, Botanist Acharya Nagarjuna University for her help with Authenification of the seeds.

Cite this article as:

P. Niharika. *In vitro* Pharmacological study of extract of *Raphanus sativus* sprouts. Int. J. Res. Ayurveda Pharm. 2019;10(4):87-91 http://dx.doi.org/10.7897/2277-4343.100492

Source of support: Nil, Conflict of interest: None Declared